HSV 1&2 IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 1 and 2 in human serum and plasma

- for “in vitro” diagnostic use only -

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REF HSVG.CE
96 Tests

HSV IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 1 and 2 in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of crossreactive determinants and just a few of type-specific sequences.
The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.
The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.
Asymptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immunocompromised and suppressed patients in which the disease may evolve toward critical pathologies.
The determination of HSV specific antibodies has then become important in the monitoring of "risk" patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST

Microplates are coated with native inactivated HSV1 and HSV2.
The solid phase is first treated with the diluted sample and IgG to HSV are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti HSV IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HSV IgG antibodies present in the sample. A Calibration Curve, calibrated against an internal Gold Standard, makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
n° 1. 12 strips x 8 microwells coated with native UV inactivated HSV1 and HSV2 in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° …
Ready to use and color coded standard curve derived from human plasma positive for HSV IgG ranging:
4ml CAL1 = 0 arbU/ml
4ml CAL2 = 5 arbU/ml
2ml CAL3 = 10 arbU/ml
2ml CAL4= 20 arbU/ml
2ml CAL5 = 50 arbU/ml
4ml CAL6 = 100 arbU/ml.
Standards are calibrated in arbitrary units against an internal Gold Standard (or IGS).
It contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized. It contains fetal bovine serum proteins, human IgG antibodies to HSV at about 20 arbU/ml ± 20%, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300, 0.02% gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide, 0.045% ProClin 300 as preservatives. The reagent is blue colour coded.

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses.

The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control'' . The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor).

The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute Calibrators and the dissolved Control Serum as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION
If only a qualitative determination is required, proceed as described below:

Automated assay:

Proceed as described in section M1.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 arbU/ml and Calibrator 5 arbU/ml in duplicate and Calibrator 100 arbU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*

2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control (*)	100 µl
Samples diluted 1:101	100 µl
1st incubation	60 min
Temperature	+37°C

Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS(*)	S 7									
H	CAL3	CS(*)	S 8									

Legenda: BLK = Blank CAL = Calibrator
CS(*) = Control Serum - Not mandatory S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S1	S 9	S 17									
H	S2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm/620-630nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm/620-630nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm > OD450nm/620-630nm CAL1 + 0.100
CAL 6 100 arbU/ml	OD450nm/620-630nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.050 OD450nm/620-630nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 arbU/ml** > 0.150 OD450nm/620-630nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 2 **5 arbU/ml** OD450nm/620-630nm < OD450nm/620-630nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **100 arbU/ml** < 1.000 OD450nm/620-630nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm/620-630nm CAL 4 ± 20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from expected value	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control serum has occurred.

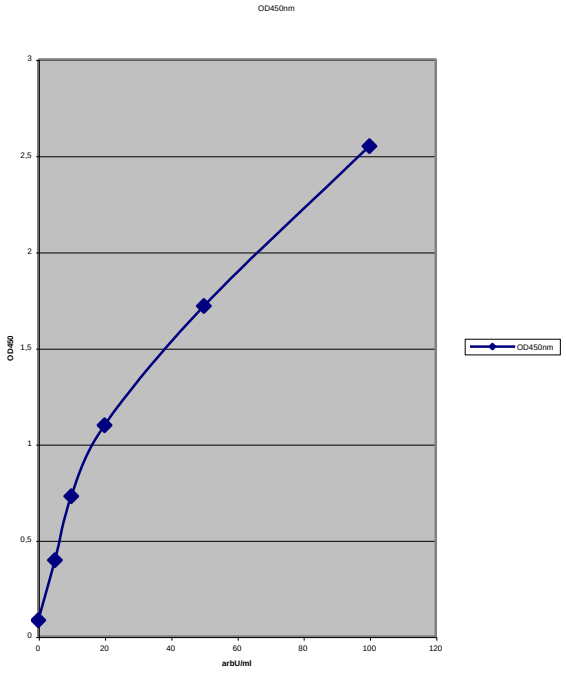
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL6), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method
If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at OD450nm/620-630nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Herpes Simplex Virus IgG antibody in samples.

An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.
P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm/620-630nm values for the Calibrators 0 and 5 arbU/ml and then check that the assay is valid.

Example of calculation:
The following data must not be used instead or real figures obtained by the user.

Calibrator 0 arbU/ml: 0.020 – 0.024
Mean Value: 0.022 OD450nm/620-630nm
Lower than 0.150 – Accepted

Calibrator 5 arbU/ml: 0.350 – 0.370
Mean Value: 0.360 OD450nm/620-630nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 100 arbU/ml: 2.245 OD450nm/620-630nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 5 arbU/ml are considered negative for anti HSV IgG antibody.
Samples with a concentration higher than 5 arbU/ml are considered positive for anti HSV IgG antibody.

Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for a primary infection of HSV due to the risk of neonatal malformations.

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *In the follow-up of pregnancy for HSV infection a positive result (presence of IgG antibody > 5 arbU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.*

R. PERFORMANCES

1. Limit of detection
The limit of detection of the assay has been calculated by means of an internal Gold Standard in absence of an international preparation to refer to.
The limit of detection has been calculated as mean OD450nm/620-630nm Calibrator 0 arbU/ml + 5 SD.
The table below reports the mean OD450nm/620-630nm values of this standard when diluted in negative plasma and then examined in the assay for three lots.

Mean OD450nm/620-630nm values (n = 2)

IgG arbU/ml	HSVG.PU Lot. 0203/2	HSVG Lot. 0403/M	HSVG.PU Lot. 0603
0	0.043	0..085	0.091
5	0.381	0.397	0.427
10	0.694	0.729	0.786
20	1.076	1.099	1.097
50	1.550	1.719	1.692
100	2.396	2.549	2.478

The assay shows a limit of detection far better than 5 arbU/ml.

In addition the preparation code Accurun n° 150, produced by Boston Biomedica Inc., BBI, USA, was tested in dilutions to determine the limit of its detection and provide a further value of analytical sensitivity

Mean OD450nm/620-630nm values (n = 2)

Dilution	HSVG.PU Lot. 0203/2	HSVG Lot. 0403/M	HSVG.PU Lot. 0603
1 X	1.694	1.719	1.708
2 X	1.085	1.117	1.100
4 X	0.730	0.751	0.744
8 X	0.446	0.464	0.453
16 X	0.301	0.314	0.306
32 X	0.150	0.165	0.158
0 arbU/ml	0.043	0.085	0.066
5 arbU/ml	0.381	0.397	0.395

2. Diagnostic sensitivity:
The diagnostic sensitivity has been tested in a performance evaluation study on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of HSV infection were tested.
The value, obtained from the analysis of more than 300 specimens, has been > 98%.
In addition the Performance panel PTH 201, supplied by BBI, was evaluated with the kit against a reference FDA approved kit.

BBI Panel PTH 201 (Performance)

Panel ID #	Dia.Pro OD450nm/620-630nm	Kit S/Co	REF HSV1 S/Co	REF HSV2 S/Co
01	1.064	**2.7**	**3.5**	**1.6**
02	2.525	**6.4**	**2.9**	**4.4**
03	0.860	**2.1**	**1.0**	**1.1**
04	2.391	**6.0**	**4.4**	**4.1**
05	1.793	**4.5**	**4.0**	**2.2**
06	1.093	**2.8**	0.8	**1.4**
07	0.801	**2.0**	0.9	**1.2**
08	2.180	**5.5**	**2.9**	**3.9**
09	2.086	**5.3**	**4.6**	**3.4**
10	0.029	0.1	0.3	0.3
11	1.900	**4.8**	**3.8**	**2.7**
12	0.995	**2.5**	**2.1**	**2.3**
13	1.833	**4.6**	**2.4**	**3.3**
14	0.153	0.4	0.4	0.5
15	2.130	**5.4**	**4.7**	**3.6**
16	1.320	**3.3**	**1.9**	**2.7**
17	3.008	**7.6**	**4.6**	**5.6**
18	1.042	**2.6**	**2.8**	**1.6**
19	0.097	0.2	0.3	0.3
20	0.414	**1.0**	0.6	0.8
21	1.682	**4.2**	**3.3**	**2.2**
22	2.364	**6.0**	**5.1**	**4.1**
23	1.926	**4.9**	**4.3**	**2.2**
24	1.556	**4.0**	**1.6**	**2.5**
25	2.372	**6.0**	**5.1**	**3.7**

Note: Cut-Off = 5 arbU/ml = 0.395

3. Diagnostic specificity:
The diagnostic specificity has been determined in the same study on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.

Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested.
No crossreaction was observed.
An overall value > 98% of specificity was found when examined on more than 100 specimens.
3. Precision:
It has been calculated on the Calibrator 5 arbU/ml, considered the cut-off of the assay, examined in 16 replicates in three separate runs for three lots.
Results are reported as follows:

HSVG: lot 0603/2

Mean values	1st run	2nd run	3rd run	Average value
OD450nm/620-630nm	0.450	0.438	0.449	0.446
Std.Deviation	0.020	0.021	0.026	0.022
CV %	4.4	4.8	5.7	5.0

HSVG.PU: lot 0603

Mean values	1st run	2nd run	3rd run	Average value
OD450nm/620-630nm	0.449	0.441	0.453	0.448
Std.Deviation	0.024	0.024	0.029	0.026
CV %	5.4	5.4	6.5	5.8

HSVG: Lot 0403/M

Mean values	1st run	2nd run	3rd run	Average value
OD450nm/620-630nm	0.405	0.406	0.405	0.405
Std.Deviation	0.027	0.031	0.030	0.029
CV %	6.6	7.6	7.4	7.2

The variability shown in the tables above did not result in sample misclassification.

5. Accuracy
The assay accuracy has been checked by the dilution and recovery tests. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled out up to 500 IU/ml.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

HSV 1&2 IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus types 1&2 in human plasma and sera

- for "in vitro" diagnostic use only -

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REF HSVM.CE
96 tests

HSV1&2 IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus types 1&2 in human plasma and sera with the "capture" system. The devise is intended for the follow-up of HSV infected patients and for the monitoring of risk of neonatal defects due to HSV infection during pregnancy. For “in vitro” diagnostic use only.

B. INTRODUCTION

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of cross-reactive determinants and just a few of type-specific sequences.

The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.

The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.

A-symptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immuno-compromised and suppressed patients in which the disease may evolve toward critical pathologies.

The determination of HSV specific antibodies has then become important in the monitoring of “risk” patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.

After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of inactivated HSV1&2, labeled with a specific antibody conjugated with peroxidase (HRP).

After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.

In the presence of bound conjugate the colorless substrate is hydrolyzed to a colored end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HSV1&2present in the sample.

A system is described how to control whether the positivity shown by a sample is true or not (Confirmation Test), helpful for the clinician to make a correct interpretation of results.

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE

12 strips x 8 microwells coated with anti human IgM affinity purified goat antibody, in presence of bovine proteins.

Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -

1x4.0 ml/vial. Ready to use control. It contains 1% human serum proteins, 2% casein, 10 mM citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is yellow colour coded.

3. Positive Control: CONTROL +

1x4.0 ml/vial. Ready to use control. It contains 1% citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.

The positive control is green colour coded.

4. Calibrator: CAL …ml

N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti HSV1&2 IgM, 4% Bovine proteins, 2% mannitol, 5mM tris base, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Lyophilized HSV1&2 Ag: AG HSV

N° 6 lyophilized vials. The vials contain lyophilized UV-light inactivated HSV1&2 in a protein buffer. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300. To be dissolved with 1.9 ml of Antigen Diluent as reported in the specific section.

6. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300. .

7. Enzyme conjugate: CONJ 20X

1x0.8 ml/vial. 20x concentrated solution of a HSV-specific antibody, labeled with HRP and diluted in a protein buffer containing 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives.

8. Antigen Diluent : AG DIL

n° 1 vial of 16 ml. Protein buffer solution for the preparation of the Immunocomplex. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code coloured with 0.01% red alimentary dye

9. Specimen Diluent : DILSPE

2x60.0 ml/vial. Proteic buffered solution for the dilution of samples. It contains 2% casein, 10 mM citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.

The reagent is color coded with 0.01% blue alimentary dye.

10. Chromogen/Substrate : SUBS TMB

1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide.

Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 0.3 M

1x15ml/vial. It contains 0.3 M H_2SO_4 solution.

Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control:

Ready to use. Mix well on vortex before use.

Positive Control:

Ready to use. Mix well on vortex before use.

Calibrator:

Add the volume of ELISA grade water reported on the label to the lyophilized powder. Let fully dissolve and then gently mix on vortex.
***Important Note**: The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Ag/Ab Immunocomplex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Conjugate/Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved HSV Ag and mix gently on vortex.

Important Notes*:*
1. *Dissolve and prepare only the number of vials necessary to the test. The Immunocomplex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.*
2. *The preparation of the Immucomplex has to be done **right before** the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.*

Specimen Diluent:
Ready to use. Mix well on vortex before use

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT
1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The **ELISA microplate reader** has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purpose. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Quality Control''. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
8. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS
1. Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.

2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

M.1 Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Specimen Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl diluted samples into the proper wells of the microplate.

This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Specimen Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.

Do not dilute controls/calibrator as they are ready to use.

Dispense 100 µl calibrators/control in the appropriate calibration/control wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

M. 2 Manual assay:

1. Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
2. Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
3. Dispense 100 µl of Negative Control in triplicate and 100 µl of Calibrator in the proper wells in duplicate. Dispense 100 µl of Positive Control in single into the proper well. Do not dilute controls and the calibrator as they are ready to use !
4. Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue colored and that controls and calibrator have been dispensed.
5. Incubate the microplate for **60 min at +37°C** .

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer as reported previously in section I.3.

7. Pipette 100 µl of the **Ag/Ab Immunocomplex** into each well, except the blanking well A1, and cover with the sealer. Check that all wells are red colored, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the* **Ag/Ab Immunocomplex***. Contamination might occur.*

8. Incubate the microplate for **60 min at +37°C** .

9. Wash microwells as in section I.3.

10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.
Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 (mandatory).

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Controls&calibrator(*) Samples diluted 1:101	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing	n° 5 with 20'' of soaking OR n° 6 cycles without soaking
Immunocomplex	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing	n° 5 with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL(*)	S6										
F	CAL(*)	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL(*) = Calibrator-Not Mandatory PC = Positive Control
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameter	Requirements
Blank well	< 0.050 OD450nm value
Negative Control mean value (NC)	< 0.200 OD450nm value after blanking coefficient of variation < 30%
Positive Control	≥ 0.750 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.05 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.200 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 0.750 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

**** Important Note:**

If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co ≥ 1.0

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator S/Co < 1.0	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

Cut-Off = NC + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 - 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient is not undergoing an acute infection of Herpes Simplex Virus.
Any patient showing an equivocal result, should be re-tested by examining a second sample taken from the patient after 1-2 weeks from first testing.
A positive result is indicative of a Herpes Simplex Virus infection.

An example of calculation is reported below:

Important Note: *The following data must not be used instead or real figures obtained by the user.*

Negative Control: 0.080 – 0.100 – 0.070 OD450nm
Mean Value: 0.090 OD450nm
Lower than 0.200 – Accepted
Positive Control: 1.850 OD450nm
Higher than 0.750 – Accepted

Cut-Off = 0.090+0.250 = 0.340

Calibrator: 0.800 - 0.840 OD450nm
Mean value: 0.820 OD450nm S/Co = 2.4
S/Co higher than 1.0 – Accepted
Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1 = negative
Sample 2 S/Co > 1.2 = positive

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of HSV due to the risk of severe neonatal malformations.*
3. *In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a different device for HSV IgM detection, before taking any preventive medical action.*
4. *Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, leading to a misinterpretation of the analytical result, can be revealed and then ruled out.*
5. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
6. *Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

No international standard for HSV1&2 IgM Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), calibrated on the preparation named “Accurun – Anti HSV2 IgM plasma” produced by Boston Biomedica Inc., USA, code 9106072, has been defined in order to provide the device with a constant and excellent sensitivity..
The limit of detection of the assay has been therefore calculated on the IGS. A limiting dilution curve was prepared in the Negative Control (NC).
Results of Quality Control are given in the following table:

OD450nm values

IGS	HSVM.CE Lot # RD1	HSVM.CE Lot # RD2	HSVM.CE Lot # RD3
1X	0.541	0.568	0.580
2X	0.272	0.298	0.300
4X	0.155	0.142	0.153
NC	0.095	0.100	0.128

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested in a clinical trial on panels of 40 samples classified positive by a CE marked kit. The value obtained from the analysis was > 98%.

3. Diagnostic specificity:

The diagnostic specificity has been determined in the clinical trial on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
A study conducted on more than 60 potentially cross-reactive samples has not revealed any interference in the system.
No cross reaction were observed.
The Performance Evaluation has provided a value > 98%.
False positive reactions may be anyway pointed out and then ruled out in the interpretation of results with the procedure reported in section T, able to verify whether or not a positive result is real.

3. Precision:

It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs.

Results are reported as follows:

HSVM.CE: lot # RD1

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.061	0.061	0.060	0.061
Std.Deviation	0.006	0.006	0.007	0.006
CV %	10.3	9.2	11.7	10.4

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.360	0.358	0.356	0.358
Std.Deviation	0.017	0.012	0.013	0.014
CV %	4.8	3.4	3.5	3.9

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.842	1.845	1.877	1.854
Std.Deviation	0.025	0.029	0.039	0.031
CV %	1.4	1.6	2.1	1.7

HSVM.CE: lot # RD2

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.122	0.122	0.122	0.122
Std.Deviation	0.012	0.009	0.011	0.011
CV %	9.9	7.5	8.9	8.8

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.432	0.431	0.462	0.442
Std.Deviation	0.023	0.019	0.025	0.022
CV %	5.3	4.4	5.5	5.0

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.858	1.852	1.841	1.850
Std.Deviation	0.031	0.035	0.039	0.035
CV %	1.7	1.9	2.1	1.9

HSVM.CE: lot # RD3

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.106	0.102	0.106	0.105
Std.Deviation	0.012	0.013	0.012	0.012
CV %	11.6	12.6	11.1	11.8

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.385	0.386	0.386	0.385
Std.Deviation	0.007	0.008	0.008	0.008
CV %	1.9	2.1	2.2	2.1

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.871	1.862	1.848	1.861
Std.Deviation	0.040	0.035	0.026	0.033
CV %	2.1	1.9	1.4	1.8

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of HSV is released to the doctor.

Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section. This reagent is called Solution A.
2. Then 25 ul concentrated Enzymatic Conjugate are diluted in 500 ul Antigen Diluent and mixed gently on vortex. Do not use any lyophilized antigen vial for this procedure ! This solution is called Solution B.
3. The well A1 of the strip is left empty for blanking.
4. The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and S/Co values.
5. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
6. The strip is incubated for 60 min at +37°C.
7. After washing, the blank well A1 is left empty.
8. 100 µl of Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl of Solution B are added to well E1.
10. The strip is incubated for 60 min at +37°C.
11. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
12. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 1.0 a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of HSV and a crossreaction with enzymatic conjugate has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value lower than 1.0 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of HSV and not due to any crossreaction.

The following table is reported for the interpretation of results

Well	**S/Co**		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.0
Interpretation	Problem of contam.	False positive	True positive

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Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l
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CE

HSV1 IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 1 in human serum and plasma

- for “in vitro” diagnostic use only -

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REF HSV1G.CE
96 Tests

HSV1 IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 1 in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of crossreactive determinants and just a few of type-specific sequences.
The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.
The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.
Asymptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immunocompromised and suppressed patients in which the disease may evolve toward critical pathologies.
The determination of HSV specific antibodies has then become important in the monitoring of "risk" patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST

Microplates are coated with native inactivated HSV1.
The solid phase is first treated with the diluted sample and IgG to HSV are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti HSV1 IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HSV1 IgG antibodies present in the sample. A Calibration Curve, calibrated against an internal Gold Standard, makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
n° 1. 12 strips x 8 microwells coated with native UV inactivated HSV1 in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° …
Ready to use and color coded standard curve derived from human plasma positive for HSV1 IgG ranging:
4ml CAL1 = 0 arbU/ml
4ml CAL2 = 5 arbU/ml
2ml CAL3 = 10 arbU/ml
2ml CAL4 = 20 arbU/ml
2mlCAL5 = 50 arbU/ml
4ml CAL6 = 100 arbU/ml.
Standards are calibrated in arbitrary units against an internal Gold Standard (or IGS).
It contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized. It contains fetal bovine serum proteins, human IgG antibodies to HSV1 at about 20 arbU/ml ±20%, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300, 0.02% gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide 0.1% and 0.045% ProClin 300 as preservatives. The reagent is blue colour coded.

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.

Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.

5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve

Ready to use component. Mix carefully on vortex before use.

Control Serum

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:

Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:

Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control".The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have

to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute Calibrators and the dissolved Control Serum as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION
If only a qualitative determination is required, proceed as described below:

Automated assay:

Proceed as described in section M1.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 arbU/ml and Calibrator 5 arbU/ml in duplicate and Calibrator 100 arbU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow .
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*

2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control (*) Samples diluted 1:101	100 µl 100 µl
1st incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS(*)	S 7									
H	CAL3	CS(*)	S 8									

Legenda: BLK = Blank CAL = Calibrator
CS(*) = Control Serum - Not mandatory S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 6 100 arbU/ml	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Substrate solution has not got contaminated during the assay
CAL 1 **0 arbU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 2 **5 arbU/ml** OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **100 arbU/ml** < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL 4 ± 20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from expected value	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control serum has occurred.

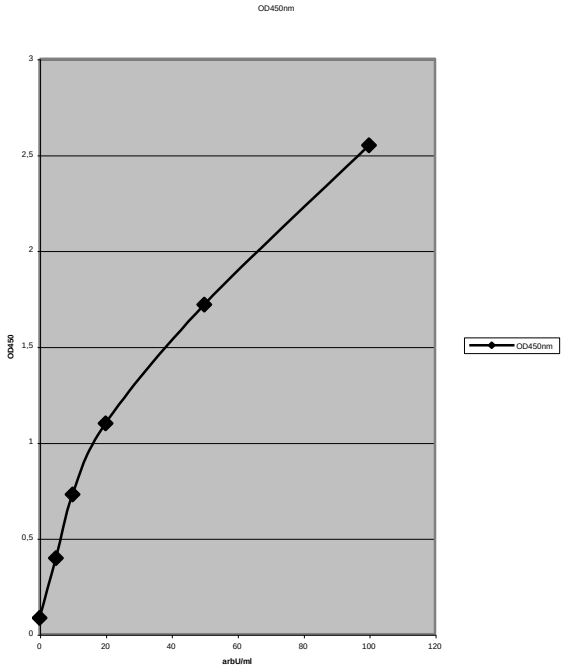
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL6), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method
If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Herpes Simplex Virus type 1 IgG antibody in samples.

An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 5 arbU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted

Calibrator 5 arbU/ml: 0.350 – 0.370 OD450nm
Mean Value: 0.360 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 100 arbU/ml: 2.245 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 5 arbU/ml are considered negative for anti HSV1 IgG antibody.
Samples with a concentration higher than 5 arbU/ml are considered positive for anti HSV1 IgG antibody.

Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for a primary infection of HSV due to the risk of neonatal malformations.

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *In the follow-up of pregnancy for HSV infection a positive result (presence of IgG antibody > 5 arbU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.*

R. PERFORMANCES

1. Limit of detection
The limit of detection of the assay has been calculated by means of an internal Gold Standard in absence of an international preparation to refer to.
The limit of detection has been calculated as mean OD450nm Calibrator 0 arbU/ml + 5 SD.
The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay for three lots.

Mean OD450nm values (n = 2)

IgG arbU/ml	HSV1G.PU Lot # 0703	HSV1G.PU Lot # 1203	HSV1G.PU Lot # 0204/2
0	0.077	0.034	0.043
5	0.355	0.404	0.318
10	0.742	0.713	0.516
20	1.254	1.216	0.944
50	1.952	1.928	1.728
100	2.623	2.261	2.072

The assay shows a limit of detection far better than 5 arbU/ml.

In addition the preparation code Accurun n° 150, produced by Boston Biomedica Inc., BBI, USA, was tested in dilutions to determine the limit of its detection and provide a further value of analytical sensitivity

Mean OD450nm values (n = 2)

Dilution	HSV1G.CE Lot # 1004	HSV1G.PU Lot # 1203	HSV1G.PU Lot # 0204/2
1 X	1.248	1.218	1.300
2 X	0.860	0.848	0.876
4 X	0.545	0.526	0.583
8 X	0.315	0.300	0.329
16 X	0.164	0.152	0.148
32 X	0.082	0.064	0.072
0 arbU/ml	0.057	0.050	0.047
5 arbU/ml	0.288	0.355	0.318

2. Diagnostic sensitivity:
The diagnostic sensitivity has been tested in a performance evaluation study on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of HSV infection were tested. The value, obtained from the analysis of more than 300 specimens, has been > 98%.

3. Diagnostic specificity:
The diagnostic specificity has been determined on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.
Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested.
No crossreaction was observed.
An overall value > 98% of specificity was found when examined on more than 100 specimens.

3. Precision:
It has been calculated on the Calibrator 5 arbU/ml, considered the cut-off of the assay, examined in 16 replicates in three separate runs for three lots.
Results are reported as follows:

HSV1G.CE Lot # 1004

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.292	0.290	0.285	0.289
Std.Deviation	0.024	0.024	0.027	0.025
CV %	8.24	8.28	9.42	8.65

HSV1G.PU: lot 1203

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.365	0.382	0.378	0.375
Std.Deviation	0.022	0.029	0.018	0.023
CV %	6.02	7.59	4.76	6.12

HSV1G.PU: Lot 0204/2

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.322	0.298	0.304	0.308
Std.Deviation	0.018	0.019	0.016	0.018
CV %	5.59	6.38	5.26	5.74

The variability shown in the tables above did not result in sample misclassification.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

HSV1 IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus type 1 in human plasma and sera

- for "in vitro" diagnostic use only -

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REF HSV1M.CE
96 tests

HSV1 IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus types 1 in human plasma and sera with the "capture" system. The device is intended for the follow-up of HSV1 infected patients and for the monitoring of risk of neonatal defects due to HSV infection during pregnancy.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of cross-reactive determinants and just a few of type-specific sequences.
The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.
The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.
A-symptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immuno-compromised and suppressed patients in which the disease may evolve toward critical pathologies.
The determination of HSV specific antibodies has then become important in the monitoring of “risk” patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a preparation of inactivated HSV1, labeled with a HSV1 specific antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of bound conjugate the colorless substrate is hydrolyzed to a colored end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HSV1 present in the sample.
A system is described how to control whether the positivity shown by a sample is true or not (Confirmation Test), helpful for the clinician to make a correct interpretation of results.

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with anti human IgM affinity purified goat antibody, in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -
1x4.0 ml/vial. Ready to use control. It contains 1% human serum proteins, 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The negative control is cpale yellow color coded..

3. Positive Control: CONTROL +
1x4.0 ml/vial. Ready to use control. It contains 1% human serum positive for HSV1 IgM, 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The positive control is green colour coded.

4. Calibrator: CAL …ml
N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti HSV1 IgM, fetal bovine serum, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Lyophilized HSV1 Ag: AG HSV1
N° 6 lyophilized vials. The vials contain gamma-ray inactivated HSV1 in protein buffer. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300. To be dissolved with 1.9 ml of Antigen Diluent as reported in the specific section.

6. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

7. Enzyme conjugate: CONJ 20X
1x0.8 ml/vial. 20x concentrated solution of a HSV1-specific antibody, labeled with HRP and diluted in a protein buffer containing 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives.

8. Antigen Diluent : AG DIL
n° 1 vial of 16 ml. Protein buffer solution for the preparation of the Immunocomplex. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code coloured with 0.01% red alimentary dye

9. Specimen Diluent : DILSPE
2x60.0 ml/vial. Proteic buffered solution for the dilution of samples. It contains 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The reagent is color coded with 0.01% blue alimentary dye.

10. Chromogen/Substrate : SUBS TMB
1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use.

Calibrator:
Add the volume of ELISA grade water reported on the label to the lyophilized powder. Let fully dissolve and then gently mix on vortex.
***Important Note**: The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before

use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Ag/Ab Immunocomplex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Conjugate/Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved HSV1 Ag and mix gently on vortex.

Important Notes*:*
1. *Dissolve and prepare only the number of vials necessary to the test. The Immunocomplex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.*
2. *The preparation of the Immucomplex has to be done **right before** the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.*

Specimen Diluent:
Ready to use. Mix well on vortex before use

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.

5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

M.1 Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Specimen Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl diluted samples into the proper wells of the microplate.

This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Specimen Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.

Do not dilute controls/calibrator as they are ready to use.

Dispense 100 µl calibrators/controls in the appropriate calibration/control wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

M. 2 Manual assay:

1. Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
2. Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
3. Dispense 100 µl of Negative Control and 100 µl of Calibrator in the proper wells in duplicate. Dispense 100 µl of Positive Control in single into the proper well. Do not dilute controls and the calibrator as they are ready to use !
4. Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue colored and that controls and calibrator have been dispensed.
5. Incubate the microplate for **60 min at +37°C** .

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer as reported previously (section I.3).
7. Pipette 100 µl of the **Ag/Ab Immunocomplex** into each well, except the blanking well A1, and cover with the sealer. Check that all wells are red colored, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the* **Ag/Ab Immunocomplex***. Contamination might occur.*

8. Incubate the microplate for **60 min at +37°C** .
9. Wash microwells as in step 6.
10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Controls&calibrator(*) Samples diluted 1:101	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Immunocomplex	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*

- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	NC	S4										
C	NC	S5										
D	CAL(*)	S6										
E	CAL(*)	S7										
F	PC	S8										
G	S1	S9										
H	S2	S10										

Legenda: BLK = Blank NC = Negative Control
CAL(*) = Calibrator-Not mandatory PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameter	Requirements
Blank well	< 0.05 OD450nm value
Negative Control mean value (NC)	< 0.200 OD450nm value after blanking coefficient of variation < 30%
Positive Control	> 1.000 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.05 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.200 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

**** Important Notes:**
The analysis must be done proceeding as the reading step described in the section M, point 12.

If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.2

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator S/Co < 1.2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of negative control instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm/620-630nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

Cut-Off = NC + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm/620-630nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 - 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient is not undergoing an acute infection of Herpes Simplex Virus type 1.
Any patient showing an equivocal result, should be re-tested by examining a second sample taken from the patient after 1-2 weeks from first testing.
A positive result is indicative of a Herpes Simplex Virus type 1 infection.

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 13).

Important Note: *The following data must not be used instead of real figures obtained by the user.*

Negative Control: 0.100 – 0.120 – 0.080 OD450nm
Mean Value: 0.100 OD450nm
Lower than 0.150 – Accepted
Positive Control: 1.850 OD450nm
Higher than 1.000 – Accepted

Cut-Off = 0.110+0.250 = 0.360

Calibrator: 1.000 - 0.900 OD450nm
Mean value: 0.950 OD450nm S/Co = 2.6
S/Co higher than 1.2 – Accepted

Sample 1: 0.075 OD450nm
Sample 2: 1.580 OD450nm
Sample 1 S/Co < 1 = negative
Sample 2 S/Co > 1.2 = positive

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of HSV due to the risk of severe neonatal malformations.*
3. *In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a different device for HSV IgM detection, before taking any preventive medical action.*
4. *Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, leading to a misinterpretation of the analytical result, can be revealed and then ruled out.*
5. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
6. *Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

No international standard for HSV1&2 IgM Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), calibrated on the preparation named "Accurun – Anti HSV2 IgM plasma" produced by Boston Biomedica Inc., USA, code 9106072, has been defined in order to provide the device with a constant and excellent sensitivity.
The limit of detection of the assay has been therefore calculated on the IGS. A limiting dilution curve was prepared in the Negative Control (NC).
Results of Quality Control are given in the following table:

OD450nm values

IGS	HSV1M.CE Lot # RD1	HSV1M.CE Lot # RD2	HSV1M.CE Lot # RD3
1X	0.450	0.460	0.455
2X	0.277	0.300	0.288
4X	0.216	0.198	0.185
NC	0.115	0.085	0.086

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested in a performance evaluation study on panels of 40 samples classified positive by a CE marked kit. The value obtained from the analysis was > 98%.

3. Diagnostic specificity:

The diagnostic specificity has been determined in the performance evaluation on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
A study conducted on more than 60 potentially cross-reactive samples has not revealed any interference in the system.
No cross reaction were observed.
The Performance Evaluation has provided a value > 98%.
False positive reactions may be anyway pointed out and then ruled out in the interpretation of results with the procedure reported in section T, able to verify whether or not a positive result is real.

4. Precision:

Results are reported as follows:

HSV1M.CE: lot # RD1

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.083	0.107	0.116	0.102
Std.Deviation	0.004	0.017	0.013	0.011
CV %	5.12	15.82	11.59	10.84

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.393	0.436	0.421	0.417
Std.Deviation	0.031	0.019	0.007	0.019
CV %	7.93	4.38	1.68	4.66

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.469	1.530	1.541	1.513
Std.Deviation	0.034	0.055	0.037	0.042
CV %	2.31	3.60	2.39	2.77

HSV1M.CE: lot # RD2

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.101	0.099	0.097	0.099
Std.Deviation	0.009	0.011	0.013	0.011
CV %	8.91	11.11	13.40	11.14

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.412	0.395	0.420	0.409
Std.Deviation	0.015	0.009	0.012	0.012
CV %	3.64	2.27	2.86	2.92

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.512	1.498	1.534	1.515
Std.Deviation	0.042	0.035	0.028	0.035
CV %	2.78	2.34	1.83	2.31

HSV1M.CE: lot # RD3

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.095	0.112	0.092	0.100
Std.Deviation	0.012	0.009	0.010	0.011
CV %	12.6	8.04	10.86	10.50

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.405	0.398	0.412	0.405
Std.Deviation	0.012	0.015	0.014	0.014
CV %	2.96	3.77	3.40	3.37

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.489	1.475	1.518	1.494
Std.Deviation	0.025	0.032	0.028	0.028
CV %	1.68	2.17	1.84	1.90

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 12.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of HSV is released to the doctor.
Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section. This reagent is called Solution A.
2. Then 25 ul concentrated Enzymatic Conjugate are diluted in 500 ul Antigen Diluent and mixed gently on vortex. Do not use any lyophilized antigen vial for this procedure ! This solution is called Solution B.
3. The well A1 of the strip is left empty for blanking.
4. The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and S/Co values.
5. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
6. The strip is incubated for 60 min at +37°C.
7. After washing, the blank well A1 is left empty.
8. 100 µl of Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl of Solution B are added to well E1.
10. The strip is incubated for 60 min at +37°C.
11. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
12. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 1.0 a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of HSV1 and a crossreaction with enzymatic conjugate has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value lower than 1.0 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of HSV and not due to any crossreaction.

The following table is reported for the interpretation of results

Well	S/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.0
Interpretation	Problem of contam.	False positive	True positive

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Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

HSV1 IgM

Ensayo inmunoenzimático (ELISA) de "captura" para la determinación de anticuerpos IgM al Virus Herpes Simplex tipo 1 en plasma y suero humanos

- Uso exclusivo para diagnóstico "in vitro"-

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REF HSV1M.CE
96 pruebas

HSV1 IgM

A. OBJETIVO DEL ESTUCHE.
Ensayo inmunoenzimático (ELISA) para la determinación de anticuerpos IgM al Virus Herpes Simplex tipo 1, en plasma y suero humanos, mediante un sistema de "captura".
El estuche ha sido concebido para el seguimiento de pacientes infectados con HSV y para el monitoreo de la infección durante el embarazo, causa de riesgo de malformaciones en el neonato.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
Los Virus del Herpes Simplex tipos 1 (HSV1) y 2 (HSV2) son grandes y complejos virus ADN que inducen la síntesis de diversas proteinas durante la infección, poseen un alto número de determinantes de reactividad cruzada y pocas secuencias tipo específicas.
La mayor parte de las infecciones herpéticas primarias y recurrentes son causadas por HSV2, mientras que aquellas infecciones no asociadas a los genitals son causadas fundamentalmente por HSV1.
La detección de anticuerpos IgG e IgM específicos al virus, es importante en el diagnóstico de las infecciones agudas/primarias, así como en las reactivaciones de una infección latente, en ausencia de síntomas clínicos evidentes.
En individuos aparentemente sanos y durante el embarazo, pueden aparecer infecciones asintomáticas debidas a HSV. En pacientes inmunocomprometidos se pueden presentar severas infecciones herpéticas, donde la enfermedad evoluciona hacia patologías clínicas.
La determinación de anticuerpos específicos al virus constituye un elemento importante para el seguimiento de pacientes en grupos de riesgo, así como para el monitoreo de las infecciones severas y agudas.

C. PRINCIPIOS DEL ENSAYO.
El ensayo se basa en el principio de "captura de IgM", donde los anticuerpos de esta clase presentes en la muestra son capturados por la fase sólida recubierta con un anticuerpo anti-IgM humano.
Luego del lavado, que elimina el resto de los componentes de la muestra en particular los anticuerpos IgG, se adiciona una preparación purificada de HSV 1, inactivado y marcado con un anticuerpo específico conjugado con Peroxidasa (HRP), lo cual permite detectar los anticuerpos IgM inmovilizados en la fase sólida.
Posteriormente a la incubación, los pocillos se lavan para eliminar cualquier traza de conjugado en exceso y se añade el substrato cromogénico. En presencia del conjugado el substrato es hidrolizado generándose una señal coloreada proporcional a la cantidad de anticuerpos IgM al HSV 1, presentes en la muestra.
La Prueba de Confirmación controla la ocurrencia de falsos positivos, lo cual permite a los clínicos una correcta interpretación de los resultados.

D. COMPONENTES.
Cada estuche contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
12 tiras de 8 pocillos recubiertos con anticuerpos de cabra anti-IgM humano, purificados por afinidad, en presencia de proteinas de bovino.
Las placas están en una bolsa sellada con desecante. Se deben poner las mismas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar entre 2 y 8ºC.

2. Control Negativo: CONTROL -
1x4.0 ml/vial. Listo para el uso. Contiene 1% de proteinas del suero humano, 2% de caseína, tampón Tris10 mM pH 6.0+/-0.1, 0.1% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como preservativos.
El control negativo está codificado con el color amarillo pálido.

3. Control Positivo: CONTROL +
1x4.0 ml/vial. Listo para el uso. Contiene 1% de suero humano positivo a IgM HSV1, 2% de caseína, tampón Tris10 mM pH 6.0+/-0.1, 0.1% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como preservativos.
El control positivo está codificado con el color verde.

4. Calibrador: CAL …ml
n° 1 vial. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene anticuerpos IgM a HSV1, suero fetal bovino, además de sulfato de gentamicina 0.2 mg/ml y ProClin 300 0.045% como preservativos.
Nota: El volumen necesario para disolver el contenido del frasco varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Antígenos liofilizados HSV1 Ag: AG HSV
N° 6 viales liofilizados. Contienen antígenos de HSV1 en un tampón proteico, inactivados por radiaciones gamma, 2% de proteinas de bovino, tampón Tris HCl 10 mM pH 6.8+/-0.1 además de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 al 0.045%.
Debe disolverse con 1.9 ml de Diluente de Antígeno, según se indica más adelante.

6. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x. Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/-0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%.

7. Conjugado: (CONJ 20X)
1x0.8 ml/vial. Solución concentrada 20x. Contiene un anticuerpo específico anti-HSV1 conjugado con peroxidasa (HPR) diluído en un tampón proteico, tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, además de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como preservativos.

8. Diluente de Antígeno: (AG DIL)
n° 1 vial de 16 ml. Solución tamponada proteica para la preparación del inmunocomplejo. Contiene tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, además de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 al 0.045% como preservativos. El reactivo está codificado con el color rojo (0.01% de colorante rojo).

9. Diluente de muestras : (DILSPE)
2x60ml/vial. Solución tamponada proteica para la dilución de las muestras. Contiene 2% de caseína, tampón Tris 10 mM a pH 6.0 +/- 0.1, 0.2% de Tween 20, además de azida sódica al 0.09% y 0.1% de ProClin 300 al 0.045% como preservativos.
El reactivo está codificado con el color azul (0.01% de colorante azul).

10. Cromógeno/Substrato: (SUBS TMB)
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02% así como dimetilsulfóxido 4%.
Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

11. Ácido Sulfúrico: (H2SO4 0.3 M)
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Sellador adhesivo, n° 2

13. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (1000 ul, 100 ul and 10 ul) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (+/-0.5°C tolerancia).
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El estuche debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: “Biosafety in Microbiological and Biomedical Laboratories”, ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los estuches, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el estuche a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes estuches.
7. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al supervisor para realizar el procedimiento pertinente y reemplazar el estuche.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas luego de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del estuche usando puntas desechables y cambiándolas luego de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el estuche e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en estuches abiertos, en uso por un período de hasta 3 meses.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: “Biosafety in Microbiological and Biomedical Laboratories”, ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del estuche deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del estuche (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Se recomienda el uso del código de barras.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante almenos 12 meses. Evitar congelar/descongelar cada muestra más de una vez, ya que pueden generarse partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Estudios de estabilidad realizados en estuches en uso (hasta 6 veces) no han arrojado pérdida de actividad significativa en un período de 3 meses.

Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de concervación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Control Negativo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Control Positivo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Calibrador:
Añadir al polvo liofilizado, el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver completamente y luego mezclar cuidadosamnete con el vórtex antes de usar.
Nota: *Para preservar la reactividad se recomienda mantenerla congelada en alícuotas a –20ºC. No recongelar.*

Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada debe diluirse 20x con agua bidestilada hasta alcanzar 1200 ml y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

Inmunocomplejo Ag/Ab:
Proceder cuidadosamente según se indica:
1. Disolver el contenido de un vial liofilizado utilizando 1.9 ml de Diluente Antígeno. Dejar disolver completamente y luego mezclar cuidadosamente con el vórtex.
2. Mezclar el Conjugado concentrado con ayuda del vórtex. Añadir luego 0.1 ml del mismo al vial del Ag HSV1 disuelto y mezclar suavemente en el vórtex.

Notas Importantes*:*
1. *Disolver y preparar solamente los viales necesarios para la prueba. El inmunocomplejo obtenido no es estable. Almacenar la solución sobrante en alícuotas a –20°C.*
2. *La preparación del inmunocomplejo debe realizarse **justo antes** de dispensar las muestras y los controles en la placa. Mezclar nuevamente en vórtex justo antes de usar.*

Diluente de muestras :
Listo para el uso. Mezclar bien con un vórtex antes de usar.

Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL ESTUCHE.
1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (acohol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periodicamente los residuos de los componentes del estuche.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC de tolerancia) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10nm b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Control de calidad interno”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de

sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.

7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el estuche, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del estuche (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen del mismo con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
4. Disolver el Calibrador como se ha descrito anteriormente y mezclar suavemente.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
11. En caso de surgir algún problema, se debe detener el ensayo y avisar al supervisor.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

M.1 Ensayo automatizado:

En el caso de que el ensayo se realice de manera automatizada con un sistema ELISA, se recomienda programar al equipo para aspirar 1000µl de Diluente de Muestras, y posteriormente 10µl de muestra (factor de dilución 1:101).

La mezcla debe ser dispensada cuidadosamente en un tubo de dilución. Antes de aspirar la muestra siguiente, las agujas deben lavarse debidamente para evitar cualquier contaminación cruzada entre las muestras. Cuando todas las muestras han sido diluídas, programar el equipo para dispensar 100 µl de las mismas en los pocillos correspondientes.

Este procedimiento puede realizarse en dos pasos de dilución de 1:10 cada uno (90 µl de Diluente de Muestras + 10 µl de muestra) en una segunda plataforma de dilución. Programar el equipo para aspirar primeramente 100 µl de Diluente de Muestras, luego 10 µl de la primera dilución en la plataforma y finalmente dispensar todo el contenido en los pocillos apropiados de la microplaca.

No diluir el Calibrador ni los controles, ya que están listos para el uso.

Dispensar 100ul de controles/calibrador en los pocillos correspondientes.

Para las operaciones siguientes, consulte las instrucciones que aparecen debajo para el Ensayo Manual.

Es muy importante comprobar que el tiempo entre el dispensado de la primera y la última muestra sea calculado por el instrumento y considerado para los lavados.

M.2 Ensayo Manual.

1. Diluir las muestras 1:101 dispensando primeramente 10 µl de muestra y luego 1 ml de Diluente de Muestra en un tubo de dilución, mezclar bien con vórtex.
2. Poner el número de tiras necesarias en el soporte plástico. Dejar el pocillo A1 vacío para el blanco.
3. Dispensar 100 µl del Control Negativo y 100µl de Calibrador por duplicado. Luego dispensar 100µl del Control Positivo (sencillo) en los respectivos pocillos. No diluir los controles ni el calibrador ya que están listos para el uso.
4. Dispensar 100 µl de las muestras diluídas en los pocillos correspondientes y chequear luego que estos pocillos son de color azul y que los controles y el calibrador han sido añadidos.
5. Incubar la microplaca **60 min a +37°C**.

***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el test manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Lavar la microplaca según se indica (section I.3).
7. Dispensar 100uL del **Inmunocomplejo Ag/Ab** en todos los pocillos, excepto en el A1 y cubrir con el sellador. Compruebe que este reactivo de color rojo ha sido añadido en todos los pocillos excepto el A1.

***Nota importante**: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el **Inmunocomplejo Ag/Ab**. Podría producirse contaminación.*

8. Incubar la microplaca **60 min a +37°C**.
9. Lavar la microplaca, de igual forma que en el paso 6.
10. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1. Incubar la microplaca a **temperatura ambiente (18-24°C) durante 20 minutos**.

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

11. Dispensar 100µl de Ácido Sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición del ácido cambia el color de los controles positivos y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas generales importantes:

1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*

2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*

N. ESQUEMA DEL ENSAYO.

Controles&Calibrador (*) Muestras diluídas 1:101	100 ul 100 ul
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Inmunocomplejo	100 ul
2da incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H_2O_2	100 ul
3ra incubación	**20 min**
Temperatura	t.a.°
Ácido Sulfúrico	100 ul
Lectura D.O.	450nm / 620-630nm

t.a.°temperatura ambiente

(*) Notas importantes:

- *El calibrador (CAL) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba.*
- *El calibrador (CAL) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

A continuación se describe un ejemplo del esquema de dispensado:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 3										
B	CN	M 4										
C	CN	M 5										
D	CAL(*)	M 6										
E	CAL(*)	M 7										
F	CP	M 8										
G	M 1	M 9										
H	M 2	M10										

Leyenda: BL = Blanco CN = Control Negativo
CAL(*) = Calibrador - No obligatorio CP = Control Positivo M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza una validación sobre los controles cada vez que se usa el estuche, para verificar si el performance del ensayo es el esperado.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	< 0.050 DO450nm
Control Negativo, valor medio (CN)	< 0.200 DO450nm valor después de leer el blanco Coeficiente de variación < 30%
Control Positivo	> 1000 DO450nm

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.
En caso contrario, detenga el ensayo y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.050DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control Negativo (CN) > 0.200 DO450nm después de leer el blanco Coefficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Control Positivo < 1000 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, luego de comprobar, informe al supervisor para tomar las medidas pertinentes.

***** Notas importantes:***

El análisis debe seguir el paso de lectura descrito en la sección M, punto 12.

Si se ha usado el Calibrador, comprobar los siguientes datos:

Parámetro	Exigencia
Calibrador	M/Co > 1.2

Si los resultados de la prueba no se corresponden con los requisitos indicados anteriormente, proceder del siguiente modo:

Problema	Compruebe que
Calibrador M/Co < 1.2	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.

En cualquier caso, si todos los demás parámetros (blanco, control negativo, control positivo) se corresponden con los requisitos establecidos, la prueba puede considerarse válida.

P. CÁLCULO DEL VALOR DE CORTE.

Los resultados de la prueba se calculan a partir de un valor medio de DO450nm / 620-630nm del control Negativo (CN), mediante un valor de corte (Co) hallado con la siguiente fórmula:

Valor de corte = CN + 0.250

El valor encontrado en la prueba es utilizado para la interpretación de los resultados, según se describe a continuación.

***Nota Importante**: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS.

La interpretación de los resultados se realiza mediante la razón entre las DO a 450nm / 620-630nm de las muestras (M) y el Valor de corte (Co).
Los resultados se interpretan según la siguiente tabla:

(M/Co)	Interpretación
< 1.0	Negativo
1.0 – 1.2	Equívoco
> 1.2	Positivo

Un resultado negativo indica que el paciente no está padeciendo infección aguda por el Virus Herpes Simplex tipo 1.
Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial.
Un resultado positivo es indicativo de infección por el Virus Herpes Simplex tipo 1.

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.
Control Negativo: 0. 100 – 0.120 – 0.080 DO 450nm
Valor medio: 0.100 DO 450nm
Menor de 0.150 – Válido
Control Positivo: 1.850 DO 450nm
Mayor de 1000 – Válido
Valor de corte = 0.110+0.250 = 0.360
Calibrador: 1.000 - 0.900 DO 450nm
Valor medio: 0.950 DO 450nm M/Co = 2.6
M/Co Mayor de 1.2 – Válido

Muestra 1: 0.075 DO 450nm
Muestra 2: 1.580 DO 450nm
Muestra 1 M/Co < 1 = negativa
Muestra 2 M/Co > 1.2 = positiva

Notas importantes:

1. *La interpretación de los resultados debe hacerse bajo la vigilancia del supervisor del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
2. *Debe ponerse particular atención a la interpretación de los resultados ante sospecha de infección primaria por HSV en el embarazo, debido a las posibilidades de malformaciones del neonato.*
3. *En el monitoreo de infección por HSV durante el embarazo, se recomienda, antes de tomar cualquier decisión médica preventiva, confirmar cualquier resultado positivo, primero con el procedimiento descrito y luego con un sistema de detección de IgM anti-HSV.*
4. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
5. *El diagnóstico de infección debe ser evaluado y comunicado al paciente por un médico calificado.*

R. PERFORMANCES.

1. Límite de detección.

Hasta el momento no ha sido definido por la Comunidad Europea, un estándar internacional para la detección de anticuerpos IgM a HSV1. En ausencia del mismo y para garantizar una óptima sensibilidad, el límite de detección del ensayo ha sido calculado por medio de un Gold Standard Interno (IGS), a partir de una preparación "Accurun–Anti HSV 2 IgM Plasma", producida por Boston Biomedica Inc., Estados Unidos, código 9106072.
Se construyó una curva de dilución limitante utilizando el Control Negativo (CN).
La siguiente tabla muestra los resultados del Control de Calidad:

Valores DO 450nm

IGS	HSV1M.CE Lote # RD1	HSV1M.CE Lote # RD2	HSV1M.CE Lote# RD3
1X	0.450	0.460	0.455
2X	0.277	0.300	0.288
4X	0.216	0.198	0.185
CN	0.115	0.085	0.086

2. Sensibilidad Diagnóstica:

La sensibilidad diagnóstica se ha estudiado en un ensayo clínico utilizando paneles de 40 muestras, clasificadas como positivas mediante un estuche marcado CE. El valor obtenido del análisis fue > 98%.

3. Especificidad Diagnóstica :

La especificidad diagnóstica ha sido determinada en un ensayo clínico, utilizando paneles de más de 300 muestras provenientes de individuos sanos de origen europeo, clasificadas como negativas mediante un estuche de referencia.
Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos para determinar la especificidad. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.
Las muestras congeladas han sido probadas para comprobar si la colección y el almacenamiento interfiere con el procedimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.
Se realizó un estudio con más de 60 muestras que pudieran introducir reacción cruzada y no se observó interferencia alguna en el sistema. No se detectó reacción cruzada.
El estudio para evaluar el performance reveló un valor > 98%.
El procedimiento reportado en la sección T, permite verificar los resultados falsos positivos y de esta forma lograr una correcta interpretación de los resultados.

4. Precisión:

Ha sido calculada a partir de tres muestras, una negativa, una debilmente positiva y una positiva, examinadas en 16 réplicas en tres corridas separadas.
Los resultados son los siguientes:

HSV1M.CE: lote # RD1

Negativa (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.083	0.107	0.116	0.102
Desviación estándar	0.004	0.017	0.013	0.011
CV %	5.12	15.82	11.59	10.84

Débil reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.393	0.436	0.421	0.417
Desviación estándar	0.031	0.019	0.007	0.019
CV %	7.93	4.38	1.68	4.66

Altamente reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	1.469	1.530	1.541	1.513
Desviación estándar	0.034	0.055	0.037	0.042
CV %	2.31	3.60	2.39	2.77

HSV1M.CE: lote # RD2

Negativa (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.101	0.099	0.097	0.099
Desviación estándar	0.009	0.011	0.013	0.011
CV %	8.91	11.11	13.40	11.14

Débil reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.412	0.395	0.420	0.409
Desviación estándar	0.015	0.009	0.012	0.012
CV %	3.64	2.27	2.86	2.92

Altamente reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	1.512	1.498	1.534	1.515
Desviación estándar	0.042	0.035	0.028	0.035
CV %	2.78	2.34	1.83	2.31

HSV1M.CE: lote # RD3

Negativa (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.095	0.112	0.092	0.100
Desviación estándar	0.012	0.009	0.010	0.011
CV %	12.6	8.04	10.86	10.50

Débil reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.405	0.398	0.412	0.405
Desviación estándar	0.012	0.015	0.014	0.014
CV %	2.96	3.77	3.40	3.37

Altamente reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	1.489	1.475	1.518	1.494
Desviación estándar	0.025	0.032	0.028	0.028
CV %	1.68	2.17	1.84	1.90

Nota importante:
Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 12.

S. LIMITACIONES.
La contaminación bacteriana o la inactivación por calor de la muestra pueden afectar los valores de DO y por tanto alterar los niveles del analito.

Las muestras que luego de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.
El ensayo es útil solo para probar muestras independientes y no mezclas.
El diagnóstico de una enfermedad infecciosa no debe establecerse en base a un solo resultado, sino que deben tenerse en consideración la historia clínica del paciente, la sintomatología, así como otros datos diagnósticos.

T. PRUEBA DE CONFIRMACIÓN.
Se ejecuta esta prueba con el propósito de garantizar la mayor precision del ensayo en el seguimiento del embarazo, donde un resultado falso positivo puede conducir a un aborto. La misma debe realizarse a cada una de las muestras positivas, antes de emitir un diagnóstico de infección por HSV.
Proceder para la confirmación como sigue:

1. Preparar el complejo Antígeno/Conjugado como se describe anteriormente. Este reactivo se denomina Solución A.
2. Diluir el Conjugado concentrado, 1:20 en el Diluente de Antígeno (ej: 25 ul de Conjugado concentrado en 500 ul de Diluente de Antígeno) y mezclar suavemente con ayuda del vórtex. No usar ningún vial de Ag liofilizado para este procedimiento! Este reactivo se denomina Solución B.
3. Dejar vacío el pocillo A1 para el blanco.
4. Dispensar el Control Negativo en las posiciones B1+C1, se utiliza para calcular el valor de corte y los valores M/Co.
5. Diluir 1:101 la muestra positiva para confirmar y dispensarla en las posiciones D1+E1.
6. Incubar la tira 60 minutos a +37°C.
7. Luego del lavado, el pocillo A1 para el blanco queda vacío.
8. Dispensar 100 µl de la Solución A en los pocillos B1+C1+D1.
9. Dispensar 100 µl de la Solución B en el pocillo E1.
10. Incubar la tira 60 minutos a +37°C.
11. Luego del lavado, adicionar 100 µl del Cromógeno/Substrato en todos los pocillos e incubar la tira 20 minutos a temperatura ambiente.
12. Dispensar 100µl del Acido Sulfúrico en todos los pocillos y medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo), calibrando el instrumento con el pocillo A1 (blanco).

La interpretación de los resultados se realiza de la siguiente forma:

1. Si la muestra en posición D1 tiene un valor de M/Co menor de 1.0, probablemente en el primer ensayo haya ocurrido un error en el dispensado o alguna contaminación. Debe repetirse el Procedimiento del Ensayo, sección M.
2. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en posición E1 el valor de M/Co es todavía mayor de 1.2, la muestra se considera un **falso positivo**. La reactividad de la muestra, en este caso, no depende de la presencia específica de HSV1, por lo tanto ha ocurrido una reacción cruzada con el conjugado.
3. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en la posición E1 el valor M/Co es menor de 1.0 se considera **realmente positiva**. La reactividad de la muestra, en este caso se debe a la presencia específica de HSV1 y no a reacciones cruzadas.

En la siguiente tabla se muestra la interpretación de los resultados:

Pocillo	M/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.0
Interpretación	Probl. de contam.	Falso positivo	Realmente positivo

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad conforme a la norma ISO 13485. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovannni (Milán) – Italia

CE

HSV2 IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 2 in human serum and plasma

- for “in vitro” diagnostic use only -

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REF HSV2G.CE
96 Tests

HSV2 IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 2 in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of crossreactive determinants and just a few of type-specific sequences.
The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.
The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.
Asymptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immunocompromised and suppressed patients in which the disease may evolve toward critical pathologies.
The determination of HSV specific antibodies has then become important in the monitoring of "risk" patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST

Microplates are coated with synthetic HSV2 specific glycoprotein G or gG.
The solid phase is first treated with the diluted sample and IgG to HSV2 are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti HSV2 IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HSV2 IgG antibodies present in the sample. A Calibration Curve, calibrated against an internal Gold Standard, makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
n° 1. 12 strips x 8 microwells coated with synthetic HSV2-specific gG in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° …
Ready to use and colorcoded standard curve derived from human plasma positive for HSV2 IgG ranging:
4ml CAL1 = 0 arbU/ml
4ml CAL2 = 5 arbU/ml
2ml CAL3 = 10 arbU/ml
2ml CAL4 = 20 arbU/ml
2ml CAL5 = 50 arbU/ml
4ml CAL6 = 100 arbU/ml.
Standards are calibrated in arbitrary units against an internal Gold Standard (or IGS).
It contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and ProClin 300 0.045% as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized. It contains fetal bovine serum proteins, human IgG antibodies to HSV2 at about 20 arbU/ml ± 20%, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300, 0.02% gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide 0.1% and 0.045% ProClin 300 as preservatives. The reagent is blue colour coded.

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses.

The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storage.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, mandatory) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control''. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first

100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute Calibrators and the dissolved Control Serum as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION
If only a qualitative determination is required, proceed as described below:

Automated assay:
Proceed as described in section M1.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 arbU/ml and Calibrator 5 arbU/ml in duplicate and Calibrator 100 arbU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

General Important notes:

1. *Ensure that no fingerprints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*

2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control (*) Samples diluted 1:101	100 µl 100 µl
1st incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS(*)	S 7									
H	CAL3	CS(*)	S 8									

Legenda: BLK = Blank CAL = Calibrator
CS(*) = Control Serum - Not mandatory S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 2	S 10									
B	CAL1	S 3	S 11									
C	CAL1	S 4	S 12									
D	CAL2	S 5	S 13									
E	CAL2	S 6	S 14									
F	CAL6	S 7	S 15									
G	S1	S 8	S 16									
H	S2	S 9	S 17									

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm ≥ OD450nm CAL1 + 0.100
CAL 6 100 arbU/ml	OD450nm ≥ 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 arbU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 2 **5 arbU/ml** OD450nm ≤ OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **100 arbU/ml** ≤ 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL 4 ± 20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from expected value	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control serum has occurred.

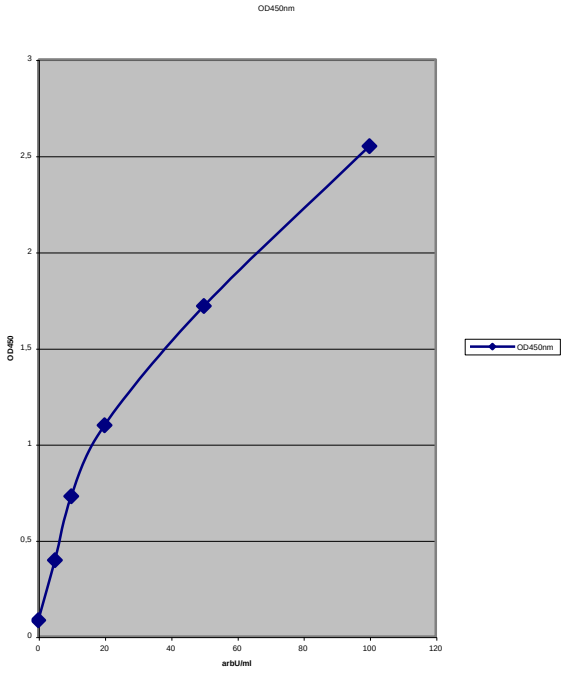
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL6), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method
If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Herpes Simplex Virus type 2 IgG antibody in samples.

An example of Calibration curve is reported in the next page.

Example of Calibration Curve:



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 5 arbU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted

Calibrator 5 arbU/ml: 0.350 – 0.370 OD450nm
Mean Value: 0.360 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 100 arbU/ml: 2.245 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 5 arbU/ml are considered negative for anti HSV2 IgG antibody.
Samples with a concentration higher than 5 arbU/ml are considered positive for anti HSV2 IgG antibody.

Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for a primary infection of HSV due to the risk of neonatal malformations.

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *In the follow-up of pregnancy for HSV infection a positive result (presence of IgG antibody > 5 arbU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.*

R. PERFORMANCES

1. Limit of detection
The limit of detection of the assay has been calculated by means of an internal Gold Standard in absence of an international preparation to refer to.
The limit of detection has been calculated as mean OD450nm Calibrator 0 arbU/ml + 5 SD.
The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay for three lots.

Mean OD450nm values (n = 2)

IgG arbU/ml	HSV2G.PU Lot # 1203	HSV2G.PU Lot # 1103	HSV2G Lot # 0304/2
0	0.022	0.030	0.014
5	0.353	0.384	0.269
10	0.596	0.606	0.557
20	1.169	1.471	0.895
50	2.030	2.276	1.776
100	3.102	3.353	2.893

The assay shows a limit of detection far better than 5 arbU/ml.

The NIBSC sample QCRHSV2QC1 (anti HSV2 Quality Control Reagent sample 1 code 13/B642) is detected positive with a mean S/Co of about 4.

2. Diagnostic sensitivity:
The diagnostic sensitivity has been tested in a performance evaluation study on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of HSV infection were tested. The value, obtained from the analysis of more than 300 specimens, has been ≥ 98%.

3. Diagnostic specificity:
The diagnostic specificity has been determined on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.
Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested.
No crossreaction was observed.
An overall value > 98% of specificity was found when examined on more than 100 specimens.

3. Precision:
It has been calculated on the Calibrator 5 arbU/ml, considered the cut-off of the assay, examined in 16 replicates in three separate runs for three lots.

Results are reported as follows:

HSV2G.CE: lot 1004

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.286	0.303	0.256	0.282
Std.Deviation	0.022	0.037	0.020	0.026
CV %	7.7	12.4	7.74	9.28

HSV2G.PU: lot 1103

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.375	0.384	0.394	0.384
Std.Deviation	0.019	0.022	0.015	0.019
CV %	5.07	5.73	3.81	4.87

HSV2G.PU: lot 1203

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.352	0.345	0.332	0.343
Std.Deviation	0.017	0.020	0.024	0.020
CV %	4.83	5.78	7.23	5.95

The variability shown in the tables above did not result in sample misclassification.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history,

symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

HSV2 IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus type 2 in human plasma and sera

- for "in vitro" diagnostic use only -

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REF HSV2M.CE
96 tests

HSV2 IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus types 2 in human plasma and sera with the "capture" system. The devise is intended for the follow-up of HSV2 infected patients and for the monitoring of risk of neonatal defects due to HSV infection during pregnancy.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of cross-reactive determinants and just a few of type-specific sequences.
The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.
The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.
A-symptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immuno-compromised and suppressed patients in which the disease may evolve toward critical pathologies.
The determination of HSV specific antibodies has then become important in the monitoring of “risk” patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a preparation of inactivated HSV2, labeled with a HSV2 specific antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of bound conjugate the colorless substrate is hydrolyzed to a colored end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HSV2 present in the sample.
A system is described how to control whether the positivity shown by a sample is true or not (Confirmation Test), helpful for the clinician to make a correct interpretation of results.

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with anti human IgM affinity purified goat antibody, in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -
1x4.0 ml/vial. Ready to use control. It contains 1% human serum proteins, 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The negative control is pale yellow color coded.

3. Positive Control: CONTROL +
1x4.0 ml/vial. Ready to use control. It contains 1% human serum positive for HSV2 IgM, 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The positive control is green colour coded.

4. Calibrator: CAL …ml
N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti HSV2 IgM, fetal bovine serum, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Lyophilized HSV2 Ag: AG HSV2
N° 6 lyophilized vials. The vials contain lyophilized gamma-ray inactivated HSV2 in protein buffer. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300. To be dissolved with 1.9 ml of Antigen Diluent as reported in the specific section.

6. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

7. Enzyme conjugate: CONJ 20X
1x0.8 ml/vial. 20x concentrated solution of a HSV2-specific antibody, labeled with HRP and diluted in a protein buffer containing 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives.

8. Antigen Diluent : AG DIL
n° 1 vial of 16 ml. Protein buffer solution for the preparation of the Immunocomplex. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code coloured with 0.01% red alimentary dye

9. Specimen Diluent : DILSPE
2x60.0 ml/vial. Proteic buffered solution for the dilution of samples. It contains 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The reagent is color coded with 0.01% blue alimentary dye.

10. Chromogen/Substrate : SUBS TMB
1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use.

Calibrator:
Add the volume of ELISA grade water reported on the label to the lyophilized powder. Let fully dissolve and then gently mix on vortex.
Important Note*: The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before

use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Ag/Ab Immunocomplex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Conjugate/Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved HSV2 Ag and mix gently on vortex.

Important Notes*:*
1. *Dissolve and prepare only the number of vials necessary to the test. The Immunocomplex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.*
2. *The preparation of the Immucomplex has to be done **right before** the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.*

Specimen Diluent:
Ready to use. Mix well on vortex before use

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT
1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter de 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS
1. Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.

4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

M.1 Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Specimen Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl diluted samples into the proper wells of the microplate.

This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Specimen Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.

Do not dilute controls/calibrator as they are ready to use.

Dispense 100 µl calibrators/control in the appropriate calibration/control wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

M. 2 Manual assay:

1. Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
2. Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
3. Dispense 100 µl of Negative Control and 100 µl of Calibrator in the proper wells in duplicate. Dispense 100 µl of Positive Control in single into the proper well. Do not dilute controls and the calibrator as they are ready to use !
4. Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue colored and that controls and calibrator have been dispensed.
5. Incubate the microplate for **60 min at +37°C** .

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic as reported previously (section I.3).
7. Pipette 100 µl of the **Ag/Ab Immunocomplex** into each well, except the blanking well A1, and cover with the sealer. Check that all wells are red colored, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the* **Ag/Ab Immunocomplex***. Contamination might occur.*

8. Incubate the microplate for **60 min at +37°C** .
9. Wash microwells as in step 6.
10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to yellow .
12. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Controls&calibrator (*) Samples diluted 1:101	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Immunocomplex	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	NC	S4										
C	NC	S5										
D	CAL(*)	S6										
E	CAL(*)	S7										
F	PC	S8										
G	S1	S9										
H	S2	S10										

Legenda: BLK = Blank NC = Negative Control
CAL(*) = Calibrator–Not Mandatory PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameter	Requirements
Blank well	< 0.05 OD450nm value
Negative Control mean value (NC)	< 0.200 OD450nm value after blanking coefficient of variation < 30%
Positive Control	> 1.000 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.05 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.200 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

**** Important Notes:**
The analysis must be done proceeding as the reading step described in the section M, point 12.

If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.2

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator S/Co < 1.2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of negative control instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm/620-630nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

Cut-Off = NC + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm/620-630nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 - 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient is not undergoing an acute infection of Herpes Simplex Virus type 2.
Any patient showing an equivocal result, should be re-tested by examining a second sample taken from the patient after 1-2 weeks from first testing.
A positive result is indicative of a Herpes Simplex Virus type 2 infection.

An example of calculation is reported below (data obtained proceeding as the reading step described in the section M, point 12).

Important Note: *The following data must not be used instead of real figures obtained by the user.*

Negative Control: 0.090 – 0.110 – 0.070 OD450nm
Mean Value: 0.100 OD450nm
Lower than 0.200 – Accepted
Positive Control: 1.850 OD450nm
Higher than 1.000 – Accepted

Cut-Off = 0.100+0.250 = 0.350

Calibrator: 0.900 – 1.100 OD450nm
Mean value: 1.000 OD450nm S/Co = 2.8
S/Co higher than 1.2 – Accepted

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1 = negative
Sample 2 S/Co > 1.2 = positive

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of HSV due to the risk of severe neonatal malformations.*
3. *In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a different device for HSV IgM detection, before taking any preventive medical action.*
4. *Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, leading to a misinterpretation of the analytical result, can be revealed and then ruled out.*
5. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
6. *Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

No international standard for HSV1&2 IgM Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), calibrated on the preparation named "Accurun – Anti HSV2 IgM plasma" produced by Boston Biomedica Inc., USA, code 9106072, has been defined in order to provide the device with a constant and excellent sensitivity..
The limit of detection of the assay has been therefore calculated on the IGS. A limiting dilution curve was prepared in Negative Control (NC).
Results of Quality Control are given in the following table:

OD450nm values

IGS	HSV2M.CE Lot # RD1	HSV2M.CE Lot # RD2	HSV2M.CE Lot # RD3
1X	0.560	0.572	0.590
2X	0.343	0.324	0.348
4X	0.239	0.218	0.225
NC	0.145	0.132	0.139

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested in a clinical trial on panels of 40 samples classified positive by a kit US FDA approved. The value obtained from the analysis was > 98%.

3. Diagnostic specificity:

The diagnostic specificity has been determined in a performance evaluation study on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
A study conducted on more than 60 potentially cross-reactive samples has not revealed any interference in the system.
No cross reaction were observed.
The Performance Evaluation has provided a value > 98%.
False positive reactions may be anyway pointed out and then ruled out in the interpretation of results with the procedure reported in section T, able to verify whether or not a positive result is real.

4. Precision:

Results are reported as follows:

HSV2M.CE: lot # RD1

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.092	0.113	0.097	0.101
Std.Deviation	0.011	0.019	0.010	0.013
CV %	12.25	16.83	10.24	13.11

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.451	0.471	0.435	0.452
Std.Deviation	0.018	0.000	0.033	0.017
CV %	3.92	0.00	7.48	3.8

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.530	1.574	1.527	1.543
Std.Deviation	0.023	0.052	0.006	0.027
CV %	1.48	3.33	0.37	1.73

HSV2M.CE: lot # RD2

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.095	0.101	0.097	0.098
Std.Deviation	0.006	0.008	0.005	0.006
CV %	6.30	7.92	5.15	6.45

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.431	0.428	0.453	0.437
Std.Deviation	0.023	0.018	0.023	0.021
CV %	5.3	4.2	5.10	4.9

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.558	1.552	1.541	1.550
Std.Deviation	0.031	0.025	0.039	0.032
CV %	1.98	1.61	2.53	2.04

HSV2M.CE: lot # RD3

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.104	0.108	0.099	0.104
Std.Deviation	0.015	0.010	0.011	0.012
CV %	14.4	9.2	11.11	11.57

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.425	0.436	0.440	0.434
Std.Deviation	0.008	0.006	0.009	0.008
CV %	1.8	1.4	2.0	1.7

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.571	1.562	1.558	1.564
Std.Deviation	0.040	0.034	0.024	0.033
CV %	2.54	2.17	1.54	2.08

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 12.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of HSV is released to the doctor.
Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section. This reagent is called Solution A.
2. Then 25 ul concentrated Enzymatic Conjugate are diluted in 500 ul Antigen Diluent and mixed gently on vortex. Do not use any lyophilized antigen vial for this procedure ! This solution is called Solution B.
3. The well A1 of the strip is left empty for blanking.
4. The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and S/Co values.
5. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
6. The strip is incubated for 60 min at +37°C.
7. After washing, the blank well A1 is left empty.
8. 100 µl of Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl of Solution B are added to well E1.
10. The strip is incubated for 60 min at +37°C.
11. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
12. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 1.0 a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of HSV2 and a crossreaction with enzymatic conjugate has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value lower than 1.0 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of HSV and not due to any crossreaction.

The following table is reported for the interpretation of results

Well	S/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.0
Interpretation	Problem of contam.	False positive	True positive

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Manufacturer:
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CE

HSV2 IgM

Ensayo inmunoenzimático (ELISA) de "captura" para la determinación de anticuerpos IgM al Virus Herpes Simplex tipo 2 en plasma y suero humanos

- Uso exclusivo para diagnóstico "in vitro"-

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REF HSV2M.CE
96 pruebas

HSV2 IgM

A. OBJETIVO DEL ESTUCHE.
Ensayo inmunoenzimático (ELISA) para la determinación de anticuerpos IgM al Virus Herpes Simplex tipo 2, en plasma y suero humanos, mediante un sistema de "captura".
El estuche ha sido concebido para el seguimiento de pacientes infectados con HSV y para el monitoreo de la infección durante el embarazo, causa de riesgo de malformaciones en el neonato.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
Los Virus del Herpes Simplex tipos 1 (HSV1) y 2 (HSV2) son grandes y complejos virus ADN que inducen la síntesis de diversas proteinas durante la infección, poseen un alto número de determinantes de reactividad cruzada y pocas secuencias tipo específicas.La mayor parte de las infecciones herpéticas primarias y recurrentes son causadas por HSV2, mientras que aquellas infecciones no asociadas a los genitales son causadas fundamentalmente por HSV1.
La detección de anticuerpos IgG e IgM específicos al virus, es importante en el diagnóstico de las infecciones agudas/primarias, así como en las reactivaciones de una infección latente, en ausencia de síntomas clínicos evidentes.
En individuos aparentemente sanos y durante el embarazo, pueden aparecer infecciones asintomáticas debidas a HSV. En pacientes inmunocomprometidos se pueden presentar severas infecciones herpéticas, donde la enfermedad evoluciona hacia patologías clínicas.
La determinación de anticuerpos específicos al virus constituye un elemento importante para el seguimiento de pacientes en grupos de riesgo, así como para el monitoreo de las infecciones severas y agudas.

C. PRINCIPIOS DEL ENSAYO.
El ensayo se basa en el principio de "captura de IgM", donde los anticuerpos de esta clase presentes en la muestra, son capturados por la fase sólida recubierta con un anticuerpo anti-IgM humano.
Luego del lavado, que elimina el resto de los componentes de la muestra en particular los anticuerpos IgG, se adiciona una preparación purificada de HSV 2, inactivado y marcado con un anticuerpo específico conjugado con Peroxidasa (HRP), lo cual permite detectar los anticuerpos IgM inmovilizados en la fase sólida. Posteriormente a la incubación, los pocillos se lavan para eliminar cualquier traza de conjugado en exceso y se añade el substrato cromogénico. En presencia del conjugado, el substrato es hidrolizado generándose una señal coloreada proporcional a la cantidad de anticuerpos IgM al HSV 2, presentes en la muestra.
La Prueba de Confirmación controla la ocurrencia de falsos positivos, lo cual permite a los clínicos una correcta interpretación de los resultados.

D. COMPONENTES.
Cada estuche contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
12 tiras de 8 pocillos recubiertos con anticuerpos de cabra anti-IgM humano, purificados por afinidad, en presencia de proteinas de bovino.
Las placas están en una bolsa sellada con desecante. Se deben poner las mismas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar entre 2 y 8ºC.

2. Control Negativo: CONTROL -
1x4.0 ml/vial. Listo para el uso. Contiene 1% de proteinas del suero humano, 2% de caseína, tampón Tris10 mM pH 6.0+/-0.1, 0.1% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como preservativos.
El control negativo está codificado con el color amarillo pálido.

3. Control Positivo: CONTROL +
1x4.0 ml/vial. Listo para el uso. Contiene 1% de suero humano positivo a IgM HSV2, 2% de caseína, tampón Tris10 mM pH 6.0+/-0.1, 0.1% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como preservativos.
El control positivo está codificado con el color verde.

4. Calibrador: CAL …ml
n° 1 vial. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene anticuerpos IgM a HSV2, suero fetal bovino, además de sulfato de gentamicina 0.2 mg/ml y ProClin 300 0.045% como preservativos.
Nota: El volumen necesario para disolver el contenido del frasco varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Antígenos liofilizados HSV2 Ag: AG HSV2
N° 6 viales liofilizados. Contienen antígenos de HSV2 en un tampón proteico, inactivados por radiaciones gamma, 2% de proteinas de bovino, tampón Tris HCl 10 mM pH 6.8+/-0.1 además de 0.2 mg/ml de sulfato de gentamicina y 0.045% de ProClin 300. Debe disolverse con 1.9 ml de Diluente de Antígeno, según se indica más adelante.

6. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x. Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/-0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%.

7. Conjugado: CONJ 20X
1x0.8 ml/vial. Solución concentrada 20x. Contiene un anticuerpo específico anti-HSV2 conjugado con peroxidasa (HPR) diluído en un tampón proteico, tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, además de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como preservativos.

8. Diluente de Antígeno: AG DIL
n° 1 vial de 16 ml. Solución tamponada proteica para la preparación del inmunocomplejo. Contiene tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, además de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como preservativos. El reactivo está codificado con el color rojo (0.01% de colorante rojo).

9. Diluente de muestras : DILSPE
2x60ml/vial. Solución tamponada proteica para la dilución de las muestras. Contiene 2% de caseína, tampón Tris 10 mM a pH 6.0 +/- 0.1, 0.2% de Tween 20, además de azida sódica al 0.09% y 0.045 de ProClin 300 como preservativos.
El reactivo está codificado con el color azul (0.01% de colorante azul).

10. Cromógeno/Substrato: SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02% así como dimetilsulfóxido al 4%.
Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

11. Ácido Sulfúrico: H_2SO_4 0.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Sellador adhesivo, n° 2

13. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (1000 ul, 100 ul and 10 ul) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (+/-0.5°C tolerancia).
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El estuche debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: “Biosafety in Microbiological and Biomedical Laboratories”, ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los estuches, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el estuche a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes estuches.
7. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al supervisor para realizar el procedimiento pertinente y reemplazar el estuche.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas luego de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del estuche usando puntas desechables y cambiándolas luego de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el estuche e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en estuches abiertos, en uso por un período de hasta 3 meses.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: “Biosafety in Microbiological and Biomedical Laboratories”, ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del estuche deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del estuche (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Se recomienda el uso del código de barras.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante almenos 12 meses. Evitar congelar/descongelar cada muestra más de una vez, ya que pueden generarse partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Estudios de estabilidad realizados en estuches en uso (hasta 6 veces) no han arrojado pérdida de actividad significativa en un período de 3 meses.

Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de concervación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Control Negativo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.
Control Positivo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Calibrador:
Añadir al polvo liofilizado, el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver completamente y luego mezclar cuidadosamnete con el vórtex antes de usar.
Nota: *Para preservar la reactividad se recomienda mantenerla congelada en alícuotas a –20ºC. No recongelar.*
Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada debe diluirse 20x con agua bidestilada y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

Inmunocomplejo Ag/Ab:
Proceder cuidadosamente según se indica:
1. Disolver el contenido de un vial liofilizado utilizando 1.9 ml de Diluente Antígeno. Dejar disolver completamente y luego mezclar cuidadosamente con el vórtex.
2. Mezclar el Conjugado concentrado con ayuda del vórtex. Añadir luego 0.1 ml del mismo al vial del Ag HSV2 disuelto y mezclar suavemente en el vórtex.

Notas Importantes*:*
1. *Disolver y preparar solamente los viales necesarios para la prueba. El inmunocomplejo obtenido no es estable. Almacenar la solución sobrante en alícuotas a –20°C.*
2. *La preparación del inmunocomplejo debe realizarse **justo antes** de dispensar las muestras y los controles en la placa. Mezclar nuevamente en vórtex justo antes de usar.*

Diluente de muestras :
Listo para el uso. Mezclar bien con un vórtex antes de usar.

Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL ESTUCHE.
1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (acohol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periodicamente los residuos de los componentes del estuche.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC de tolerancia) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10nm b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Control de calidad interno”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en la sección “Control de calidad interno”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente de Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el estuche, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del estuche (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen del mismo con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la Solución de Lavado Concentrada 20X, como se ha descrito anteriormente.
4. Disolver el Calibrador como se ha descrito anteriormente y mezclar suavemente.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
11. En caso de surgir algún problema, se debe detener el ensayo y avisar al supervisor.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

M.1 Ensayo automatizado:

En el caso de que el ensayo se realice de manera automatizada con un sistema ELISA, se recomienda programar al equipo para aspirar 1000µl de Diluente de Muestras, y posteriormente 10µl de muestra (factor de dilución 1:101).

La mezcla debe ser dispensada cuidadosamente en un tubo de dilución. Antes de aspirar la muestra siguiente, las agujas deben lavarse debidamente para evitar cualquier contaminación cruzada entre las muestras. Cuando todas las muestras han sido diluídas, programar el equipo para dispensar 100 µl de las mismas en los pocillos correspondientes.

Este procedimiento puede realizarse en dos pasos de dilución de 1:10 cada uno (90 µl de Diluente de Muestras + 10 µl de muestra) en una segunda plataforma de dilución. Programar el equipo para aspirar primeramente 100 µl de Diluente de Muestras, luego 10 µl de la primera dilución en la plataforma y finalmente dispensar todo el contenido en los pocillos apropiados de la microplaca.

No diluir el Calibrador ni los controles, ya que están listos para el uso.

Dispensar 100ul de controles/calibrador en los pocillos correspondientes.

Para las operaciones siguientes, consulte las instrucciones que aparecen debajo para el Ensayo Manual.

Es muy importante comprobar que el tiempo entre el dispensado de la primera y la última muestra sea calculado por el instrumento y considerado para los lavados.

M.2 Ensayo Manual.

1. Diluir las muestras 1:101 dispensando primeramente 10 µl de muestra y luego 1 ml de Diluente de Muestra en un tubo de dilución, mezclar bien con vórtex.
2. Poner el número de tiras necesarias en el soporte plástico. Dejar el pocillo A1 vacío para el blanco.
3. Dispensar 100 µl del Control Negativo y 100µl del Calibrador por duplicado. Luego dispensar 100µl del Control Positivo (sencillo) en los respectivos pocillos. No diluir los controles ni el calibrador ya que están listos para el uso.
4. Dispensar 100 µl de las muestras diluídas en los pocillos correspondientes y chequear luego que estos pocillos son de color azul y que los controles y el calibrador han sido añadidos.
5. Incubar la microplaca **60 min a +37°C**.

***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el test manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Lavar la microplaca según se indica en la seción I.3.
7. Dispensar 100uL del **Inmunocomplejo Ag/Ab** en todos los pocillos, excepto en el A1 y cubrir con el sellador. Compruebe que este reactivo de color rojo ha sido añadido en todos los pocillos excepto el A1.

***Nota importante**: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el **Inmunocomplejo Ag/Ab**. Podría producirse contaminación.*

8. Incubar la microplaca **60 min a +37°C**.
9. Lavar la microplaca, de igual forma que en el paso 6.
10. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1. Incubar la microplaca a **temperatura ambiente (18-24°C) durante 20 minutos**.

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

11. Dispensar 100µl de Ácido Sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición del ácido cambia el color de los controles positivos y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas generales importantes:

1. *Segurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*

N. ESQUEMA DEL ENSAYO.

Controles&Calibrador (*) Muestras diluídas 1:101	100 ul 100 ul
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Inmunocomplejo	100 ul
2da incubación	**60 min**

Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H_2O_2	100 ul
3ra incubación	**20 min**
Temperatura	t.a.*
Ácido Sulfúrico	100 ul
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

(*) Notas importantes:

- *El calibrador (CAL) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba.*
- *El calibrador (CAL) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

A continuación se describe un ejemplo del esquema de dispensado:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 3										
B	CN	M 4										
C	CN	M 5										
D	CAL(*)	M 6										
E	CAL(*)	M 7										
F	CP	M 8										
G	M 1	M 9										
H	M 2	M10										

Leyenda: BL = Blanco CN = Control Negativo
(*) CAL = Calibrador - No Obligadorio CP = Control Positivo
M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza una validación sobre los controles y el calibrador cada vez que se usa el estuche, para verificar si el performance del ensayo es el esperado.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	< 0.050 DO450nm
Control Negativo, valor medio (CN)	< 0.200 DO450nm valor después de leer el blanco Coeficiente de variación < 30%
Control Positivo	> 1000 DO450nm

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.

En caso contrario, detenga el ensayo y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.050DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control Negativo (CN) > 0.200 DO450nm después de leer el blanco Coefficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Control Positivo < 1000 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, luego de comprobar, informe al supervisor para tomar las medidas pertinentes.

***** Notas importantes:***

El análisis debe seguir el paso de lectura descrito en la sección M, punto 12.

Si se ha usado el Calibrador, comprobar los siguientes datos:

Parámetro	Exigencia
Calibrador	M/Co > 1.2

Si los resultados de la prueba no se corresponden con los requisitos indicados anteriormente, proceder del siguiente modo:

Problema	Compruebe que
Calibrador M/Co < 1.2	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.

En cualquier caso, si todos los demás parámetros (blanco, control negativo, control positivo) se corresponden con los requisitos establecidos, la prueba puede considerarse válida.

P. CÁLCULO DEL VALOR DE CORTE.

Los resultados de la prueba se calculan a partir de un valor medio de DO450nm/620-630nm del control Negativo (CN), mediante un valor de corte (Co) hallado con la siguiente fórmula:

Valor de corte = CN + 0.250

El valor encontrado en la prueba es utilizado para la interpretación de los resultados, según se describe a continuación.

***Nota Importante**: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS.
La interpretación de los resultados se realiza mediante la razón entre las DO a 450nm de las muestras (M) y el Valor de corte (Co).
Los resultados se interpretan según la siguiente tabla:

(M/Co)	Interpretación
< 1.0	Negativo
1.0 – 1.2	Equívoco
> 1.2	Positivo

Un resultado negativo indica que el paciente no está padeciendo infección aguda por el Virus Herpes Simplex tipo 2.
Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial.
Un resultado positivo es indicativo de infección por el Virus Herpes Simplex tipo 2.

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Control Negativo: 0.090 – 0.110 – 0.070 DO 450nm
Valor medio: 0.100 DO 450nm
Menor de 0.150 – Válido
Control Positivo: 1.850 DO 450nm
Mayor de 1000 – Válido
Valor de corte = 0.100+0.250 = 0.350

Calibrador: 0.900 – 1.100 DO 450nm
Valor medio: 1.000 DO 450nm M/Co = 2.8
M/Co Mayor de 1.2 – Válido

Muestra 1: 0.070 DO 450nm
Muestra 2: 1.690 DO 450nm
Muestra 1 M/Co < 1 = negativa
Muestra 2 M/Co > 1.2 = positiva

Notas importantes:
1. La interpretación de los resultados debe hacerse bajo la vigilancia del supervisor del laboratorio para reducir el riesgo de errores de juicio y de interpretación.
2. Debe ponerse particular atención a la interpretación de los resultados ante sospecha de infección primaria por HSV en el embarazo, debido a las posibilidades de malformaciones del neonato.
3. En el monitoreo de infección por HSV durante el embarazo, se recomienda, antes de tomar cualquier decisión médica preventiva, confirmar cualquier resultado positivo, primero con el procedimiento descrito y luego con un sistema de detección de IgM anti-HSV.
4. Antes de emitir un resultado positivo, cada muestra reactiva debe someterse al exámen de confirmación reportado en la sección T, lo cual permite una correcta interpretación de los resultados ya que descarta los falsos positivos.
5. Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
6. El diagnóstico de infección debe ser evaluado y comunicado al paciente por un médico calificado.

R. PERFORMANCES.

1. Límite de detección.
Hasta el momento no ha sido definido por la Comunidad Europea, un estándar internacional para la detección de anticuerpos IgM a HSV1&2. En ausencia del mismo y para garantizar una óptima sensibilidad, el límite de detección del ensayo ha sido calculado por medio de un Gold Standard Interno (IGS), a partir de una preparación "Accurun–Anti HSV 2 IgM Plasma", producida por Boston Biomedica Inc., Estados Unidos, código 9106072. Se construyó una curva de dilución limitante utilizando el Control Negativo (CN).
La siguiente tabla muestra los resultados del Control de Calidad:

Valores DO 450nm

IGS	HSV2M.CE Lote # RD1	HSV2M.CE Lote # RD2	HSV2M.CE Lote # RD3
1X	0.560	0.572	0.590
2X	0.343	0.324	0.348
4X	0.239	0.218	0.225
CN	0.145	0.132	0.139

2. Sensibilidad diagnóstica :
La sensibilidad diagnóstica se ha estudiado en un ensayo clínico utilizando paneles de 40 muestras, clasificadas como positivas mediante un estuche aprovado US FDA. El valor obtenido del análisis fue > 98%.

3. Especificidad diagnóstica :
La especificidad diagnóstica ha sido determinada en un ensayo clínico, utilizando paneles de más de 300 muestras provenientes de individuos sanos de origen europeo, clasificadas como negativas mediante un estuche de referencia.
Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos para determinar la especificidad. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.
Las muestras congeladas han sido probadas para comprobar si la colección y el almacenamiento interfiere con el procedimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.
Se realizó un estudio con más de 60 muestras que pudieran introducir reacción cruzada y no se observó interferencia alguna en el sistema. No se detectó reacción cruzada.
El estudio para evaluar el performance reveló un valor > 98%.
El procedimiento reportado en la sección T, permite verificar los resultados falsos positivos y de esta forma lograr una correcta interpretación de los resultados.

4. Precisión :
Ha sido calculada a partir de tres muestras, una negativa, una debilmente positiva y una positiva, examinadas en 16 réplicas en tres corridas separadas.
Los resultados son los siguientes:

HSV2M.CE: lote # RD1

Negativa (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.092	0.113	0.097	0.101
Desviación estándar	0.011	0.019	0.010	0.013
CV %	12.25	16.83	10.24	13.11

Débil reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.451	0.471	0.435	0.452
Desviación estándar	0.018	0.000	0.033	0.017
CV %	3.92	0.00	7.48	3.8

Altamente reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	1.530	1.574	1.527	1.543
Desviación estándar	0.023	0.052	0.006	0.027
CV %	1.48	3.33	0.37	1.73

HSV2M.CE: lote # RD2

Negativa (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.095	0.101	0.097	0.098
Desviación estándar	0.006	0.008	0.005	0.006
CV %	6.30	7.92	5.15	6.45

Débil reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.431	0.428	0.453	0.437
Desviación estándar	0.023	0.018	0.023	0.021
CV %	5.3	4.2	5.10	4.9

Altamente reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	1.558	1.552	1.541	1.550
Desviación estándar	0.031	0.025	0.039	0.032
CV %	1.98	1.61	2.53	2.04

HSV2M.CE: lote # RD3

Negativa (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.104	0.108	0.099	0.104
Desviación estándar	0.015	0.010	0.011	0.012
CV %	14.4	9.2	11.11	11.57

Débil reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.425	0.436	0.440	0.434
Desviación estándar	0.008	0.006	0.009	0.008
CV %	1.8	1.4	2.0	1.7

Altamente reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	1.571	1.562	1.558	1.564
Desviación estándar	0.040	0.034	0.024	0.033
CV %	2.54	2.17	1.54	2.08

Nota importante:
Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 12.

S. LIMITACIONES.
La contaminación bacteriana o la inactivación por calor de la muestra pueden afectar los valores de DO y por tanto alterar los niveles del analito.

Las muestras que luego de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.
El ensayo es útil solo para probar muestras independientes y no mezclas.
El diagnóstico de una enfermedad infecciosa no debe establecerse en base a un solo resultado, sino que deben tenerse en consideración la historia clínica del paciente, la sintomatología, así como otros datos diagnósticos**.**

T. PRUEBA DE CONFIRMACIÓN.
Se ejecuta esta prueba con el propósito de garantizar la mayor precision del ensayo en el seguimiento del embarazo, donde un resultado falso positivo puede conducir a un aborto. La misma debe realizarse a cada una de las muestras positivas, antes de emitir un diagnóstico de infección por HSV.
Proceder para la confirmación como sigue:

1. Preparar el complejo Antígeno/Conjugado como se describe anteriormente. Este reactivo se denomina Solución A.
2. Diluir el Conjugado concentrado, 1:20 en el Diluente de Antígeno (ej: 25 ul de Conjugado concentrado en 500 ul de Diluente de Antígeno) y mezclar suavemente con ayuda del vórtex. No usar ningún vial de Ag liofilizado para este procedimiento! Este reactivo se denomina Solución B.
3. Dejar vacío el pocillo A1 para el blanco.
4. Dispensar el Control Negativo en las posiciones B1+C1, se utiliza para calcular el valor de corte y los valores M/Co.
5. Diluir 1:101 la muestra positiva para confirmar y dispensarla en las posiciones D1+E1.
6. Incubar la tira 60 minutos a +37°C.
7. Luego del lavado, el pocillo A1 para el blanco queda vacío.
8. Dispensar 100 µl de la Solución A en los pocillos B1+C1+D1.
9. Dispensar 100 µl de la Solución B en el pocillo E1.
10. Incubar la tira 60 minutos a +37°C.
11. Luego del lavado, adicionar 100 µl del Cromógeno/Substrato en todos los pocillos e incubar la tira 20 minutos a temperatura ambiente.
12. Dispensar 100µl del Acido Sulfúrico en todos los pocillos y medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, recomendado), calibrando el instrumento con el pocillo A1 (blanco).

La interpretación de los resultados se realiza de la siguiente forma:

1. Si la muestra en posición D1 tiene un valor de M/Co menor de 1.0, probablemente en el primer ensayo haya ocurrido un error en el dispensado o alguna contaminación. Debe repetirse el Procedimiento del Ensayo, sección M.
2. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en posición E1 el valor de M/Co es todavía mayor de 1.2, la muestra se considera un **falso positivo**. La reactividad de la muestra, en este caso, no depende de la presencia específica de HSV2, por lo tanto ha ocurrido una reacción cruzada con el conjugado.
3. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en la posición E1 el valor M/Co es menor de 1.0 se considera **realmente positiva**. La reactividad de la muestra, en este caso se debe a la presencia específica de HSV y no a reacciones cruzadas.

En la siguiente tabla se muestra la interpretación de los resultados:

Pocillo	M/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.0
Interpretación	Probl. de contam.	Falso positivo	Realmente positivo

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad conforme a la norma ISO 13485. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
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516_4

ORG 516 AMA-M2

INTENDED PURPOSE

AMA-M2 is an ELISA test system for the quantitative measurement of IgG class autoantibodies against mitochondrial M2 subtype antigen in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

The test is used as an aid in the differential diagnosis of primary biliary cirrhosis (PBC). In patients with other autoimmune diseases occurrence of AMA antibodies may be related to the development or association of PBC. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
516_4	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified mitochondrial M2 subtype (PDC-E2, BCOADC-E2, OGDC-E2) antigen is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color
correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

CONTENTS OF THE KIT

ORG 516	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***AMA***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 IU/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 12.5 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 25 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the international reference preparation WHO 67/183 for AMA-M2 as 100 IU/ml.

Measuring range

The calculation range of this ELISA assay is 0 - 200 IU/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 IU/ml

Interpretation of results

Negative: < 10 IU/ml
Positive: ≥ 10 IU/ml

Linearity

Samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed IU/ml	Expected IU/ml	O/E [%]
WHO	1:100	108.5	100.0	109
.	1:200	51.2	50.0	102
.	1:400	25.2	25.0	101
.	1:800	12.8	12.5	102
.	1:1600	6.1	6.3	98
.	1:3200	3.1	3.1	99
1	1:100	49.5	49.5	100
.	1:200	25.0	24.8	101
.	1:400	12.2	12.4	99
.	1:800	5.9	6.2	95

Limit of detection

Functional sensitivity was determined to be: 1 IU/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean IU/ml	CV %
1	39.8	7.0
2	81.3	3.8
3	177.3	3.6

Inter-Assay		
Sample	Mean IU/ml	CV %
1	40.1	6.2
2	84.6	11.8
3	180.4	3.8

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Primary biliary cirrhosis (PBC)	143	139	97.2
Rheumatoid Arthritis	60	1	1.7
Normal human sera	267	18	6.7

		Clinical Diagnosis POS	NEG	
ORG 516	POS	139	19	
	NEG	4	308	
		143	327	470

Sensitivity: 97.2 %
Specificity: 94.2 %
Overall agreement: 95.1 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.
The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control

Former version: *ORG 516_IFU_EN_QM113145_2013-12-16_2.1* Reason for revision: *Introduction electronic IFU on homepage*

1. Pipet **100 µl** calibrator, control or patient sample
   → Incubate for **30 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
2. Pipet **100 µl** enzyme conjugate
   → Incubate for **15 minutes** at room temperature
   → Discard the contents of the wells and wash 3 times with **300 µl** wash solution
3. Pipet **100 µl** substrate solution
   → Incubate for **15 minutes** at room temperature
4. Add **100 µl** stop solution
   → Leave untouched for **5 minutes**
   → Read at **450 nm**

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Anti-SARS-CoV-2 S1-RBD IgG Test System

Product Codes: 12525-300

1.0 INTRODUCTION

Intended Use: The Qualitative Determination of Anti-SARS-CoV-2 Specific Antibodies of the IgG type in Human Serum or Plasma by Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), discovered at the end of 2019, is the cause of the disease COVID-19.[1-2] Both SARS-CoV-2 and SARS-CoV, the cause of the 2002 SARS epidemic, are of the genus betacoronavirus and are closely related.[2] Transmission of SARS-CoV-2 is primarily through close contact with infected patients via expelled respiratory droplets, usually from coughing or sneezing.[1-2]

Due to its high transmission rate and severeness, COVID-19 has emerged as a global pandemic that has forced lockdowns and quarantine protocols from countries all over the world.[3] Though diagnoses are primarily conducted using viral nucleic acid detection via real-time reverse transcriptase PCR, many false negatives have been reported and there is urgent need for serological antibody screening as a more robust and reliable test methodology.[4-5]

Tests for immunoglobulin G (IgG) antibodies are of particular interest since they are produced in high amounts and indicate previous or recovering infection of pathogens. High levels of IgG are also known to mark immunity to a pathogen.[6] Additionally, IgG antibodies can be a good marker for efficacy of treatment of COVID-19 and successful immunization against SARS-CoV-2. However, IgG antibodies to SARS-CoV-2 do not usually appear in detectable levels until 10-20 days after symptom onset.[7-8] Therefore it is recommended that patient samples be repeated on a weekly basis to monitor the increase and stabilization of anti-SARS-CoV-2 S1-RBD IgG antibodies.

The Anti-SARS-CoV-2 (COVID-19) S1-RBD IgG AccuBind® ELISA test kit is a qualitative test designed to produce highly sensitive and specific results with a simple and brief protocol. The test utilizes a recombinant receptor binding domain (RBD) from the spike region of SARS-CoV-2 coated on microwells to capture native antibodies in the sample. In the first step, prediluted samples are added directly to the wells. After the first incubation, excess sample material is washed out and an anti-human IgG (anti-hIgG) antibody labeled with an enzyme is added to the wells. After the second incubation, excess material is washed out again and substrate is added to produce a measurable color through the reaction with the enzyme and hydrogen peroxide.

3.0 PRINCIPLE

Sequential Sandwich ELISA Method (TYPE 10):

The reagents required for the sequential ELISA assay include immobilized antigen, circulating antibody to SARS-CoV-2, and enzyme-linked human IgG-specific antibody.

Upon adding a sample containing the anti-SARS-CoV-2 antibody, reaction results between the antigen that has been immobilized on the microwell and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$h\text{-}Ab_{(X\text{-}SARS\text{-}CoV\text{-}2)} + Ag_{(RBD)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} h\text{-}Ab_{(X\text{-}SARS\text{-}CoV\text{-}2)} - Ag_{(RBD)}$$

$Ag_{(RBD)}$ = Immobilized Antigen (Constant Quantity)
$h\text{-}Ab_{(X\text{-}SARS\text{-}CoV\text{-}2)}$= Human Antibody (Variable Quantity)
$h\text{-}Ab_{(X\text{-}SARS\text{-}CoV\text{-}2)} - Ag_{(rRBD)}$= Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG,) is then added to the microwells. This conjugate binds to the immune complex that formed.

$$IC_{(h\text{-}IgG,)} + {}^{ENZ}Ab_{(X\text{-}h\text{-}IgG)} \Rightarrow {}^{ENZ}Ab_{(X\text{-}h\text{-}IgG)} - IC_{(h\text{-}IgG)}$$

$IC_{(h\text{-}IgG)}$ = Immobilized Immune complex (Variable Quantity)
${}^{ENZ}Ab_{(X\text{-}h\text{-}IgG)}$ = Enzyme-antibody Conjugate (Constant Quantity)
${}^{ENZ}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$ = Ag-Ab Complex (Variable)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing a serum reference equivalent to the positive-negative cut-off value, the absorbance value can be compared to the cut-off to determine a positive or negative result.

4.0 REAGENTS

Materials provided:

A. Anti-SARS-CoV-2 IgG Controls – 1ml/vial - Icons PC, NC, CC
Three (3) vials of ready-to-use references for anti-SARS-CoV-2 at positive, negative, and cut-off levels of IgG. Store at 2-8°C. A preservative has been added.
Note: The Cut-Off Control is traceable to the WHO 1st International Standard NIBSC Code 20/136. The cut-off level of the Anti-SARS-CoV-2 S1-RBD IgG Test System equals 110 IU/ml.

B. Anti-hIgG Enzyme Reagent – 12 ml/vial - Icon
One (1) vial of anti-human IgG-horseradish peroxides (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8°C.

C. SARS-CoV-2 RBD Coated Plate – 96 wells – Icon
One 96-well microplate coated with recombinant spike receptor binding domain from SARS-CoV-2 and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Serum Diluent Concentrate – 20ml
One (1) vial of concentrated serum diluent containing buffer salts and a dye. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C

F. Substrate – 12ml/vial - Icon S^N
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial – Icon
One (1) vial contains a strong acid (0.5 M H_2SO_4). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Fixed volume or variable volume pipette capable of delivering volumes ranging from 10 to 1000 µl with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050 ml, 0.100 ml, and 0.350 ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

Any components containing human serum from COVID-19 patients have been heat inactivated prior to handling and manufacturing. All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Please note that there has been no evidence of COVID-19 transmission through blood handling, but technicians should always exercise caution and treat all patient samples as potentially hazardous.[9]

Samples may be refrigerated at 2-8°C for a maximum period of seven (7) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.200ml of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Serum Diluent**
   Dilute contents of Serum Diluent Concentrate to 200ml (1:10 Dilution) in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer**
   Dilute contents of wash solution concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
3. **Patient Sample Dilution (1/100)**
   For example, dispense 0.010ml (10µl) of each patient specimen into 0.990 ml (990 µl) of serum diluent or 0.0101 ml (10.1 µl) into 1 ml (1000 µl). Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note : Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each control sample and patient specimen to be assayed in duplicate. Dilute the patient or any external control samples 1/100 (see Reagent Preparation **Section 8.0)** **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.100 ml (100µl) of the appropriate control or diluted patient specimen into the assigned well for IgG determination.
   DO NOT SHAKE THE PLATE AFTER SAMPLE ADDITION
3. Cover and incubate 30 minutes at room temperature**.**
4. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
5. Add 350µl of wash buffer (see Reagent Preparation Section 8.0), decant (blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
6. Add 0.100 ml (100µl) of Anti-hIgG Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
7. Cover and incubate for thirty (30) minutes at room temperature.
8. Wash the wells three (3) times with 350 µl wash buffer by repeating steps (4 & 5) as explained above.
9. Add 0.100 ml (100µl) of Substrate Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells. Do not use the Substrate Reagent if it looks blue.**
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
10. Incubate at room temperature for fifteen (15) minutes.
11. Add 0.050ml (50µl) of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix. **Always add reagents in the same order to minimize reaction time differences between wells.**
12. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: The relationship of absorbance to cut-off value is not necessarily linear so samples need not be diluted further if the absorbance is higher than the plate reader's capability (usually 3.0). However, these samples should be interpreted as strongly positive.

10.0 INTERPRETATION OF RESULTS

A Cut-Off Control (CC) and kit specific Cut-Off Factor is used to ascertain the positivity or negativity of samples. Follow the following procedure to interpret the sample results.

1. Record the absorbance of all samples obtained from the printout of the microplate reader as outlined in Example 1.
2. Multiply the average absorbance of the Cut-Off Control by the Cut-Off Factor to obtain the Cut-Off Value.
3. Divide the average absorbance of each sample by the Cut-Off Value and multiply by 10 to obtain the relative value unit (RV).
4. If RV <9, the sample is negative for Anti-SARS-CoV-2 S1-RBD IgG and if RV >10, the sample is positive for Anti-SARS-CoV-2 S1-RBD IgG
5. Samples with RV that fall within the range of 9-10 are considered borderline and should be retested with a new blood draw within 4-7 days for reevaluation.
6. To convert RV to IU/ml, multiply RV by 11. This calculation is accurate up to 25 RV or 275 IU/ml. Patients higher than 25 RV may not dilute linearly with respect to the cut-off value.

Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1
(Cut Off Factor = 1.0)

COV = MeanCC x COF
COV = Cut-Off Value
MeanCC = Mean Absorbance of Cut-Off Control
COF = Cut-Off Factor (See Certificate of Analysis)
COV = 0.667 x 1.0 = 0.667

Sample I.D.	Abs	Mean Abs	RV	Pos/Neg
Negative	0.178	0.173	÷0.667 x 10 = 2.6	Negative
	0.167			
Cut-Off	0.668	0.667	÷0.667 x 10 = 10	Cut-Off
	0.667			
Positive	2.805	2.845	÷0.667 x 10 = 42.6	Positive
	2.884			
Patient 1	0.177	0.176	***÷0.667 x 10 = 2.6***	***Negative***
	0.175			
Patient 2	1.534	1.603	***÷0.667 x 10 = 24.0***	***Positive***
	1.671			
Patient 3	0.621	0.628	***÷0.667 x 10 = 9.4***	***Borderline***
	0.635			

*The data presented in Example 1 is for illustration only and **should not** be used in lieu of a Cut-Off Control run and Cut-Off Factor with each assay. **In this example, since the Cut-Off Factor = 1.0, the average absorbance of the Cut-Off Control = Cut-Off Value**

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. Maximum Absorbance (Positive control) > 1.8
2. Positive control RV > 15
3. Negative control RV < 6

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the Cut-Off control.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-SARS-CoV-2 in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. The Anti-SARS-CoV-2 (COVID-19) S1-RBD IgG AccuBind® ELISA Test System is a qualitative assay and does not necessarily give an indication of quantities of IgG antibodies.
11. Samples, which are contaminated microbiologically, should not be used.
12. Any patient samples used in manufacturing have been heat inactivated prior to handling. However, treat all samples, including the control samples, as potentially hazardous or infectious.
13. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
14. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
15. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
16. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The clinical significance of the result should be used in evaluating the possible presence of SARS-CoV-2 infection or COVID-19. However, **clinical inferences should not be solely based on this test** but rather as an adjunct to the clinical manifestations of the patient and other relevant tests such as Histology, nasophyrangeal swab, etc. A positive result does not indicate active COVID-19 infection and does not distinguish between infection or contagiousness of COVID-19. Similarly, a negative result does not eliminate the absence COVID-19 infection but rather a very low titer of antibody that may be related to the early stages of disease.
7. A positive result on the Anti-SARS-CoV-2 S1-RBD IgG AccuBind® ELISA test system does not necessarily predict immunity to the SARS-CoV-2. There has not yet been a conclusive study to indicate that the presence of IgG antibodies confirms immunity to the SARS-CoV-2 virus.
8. There have not been sufficient studies to determine the longevity of Anti-SARS-CoV-2 S1-RBD IgG in human patients. Therefore, it is possible that a positive IgG may decrease to a negative result over the course of several months or years on some patients.
9. If the Anti-SARS-CoV-2 S1-RBD IgG AccuBind® ELISA Test System is used to monitor antibody response in vaccinated patients, samples should be taken two weeks after the full course of vaccine doses have been administered. It is not uncommon to observe a negative result on a sample with only one dose of a vaccine regimen that requires two or more doses.

13.0 EXPECTED RANGES OF VALUES

A study of apparently healthy population (>150) from prior to December 2019 was undertaken to determine expected values for the Anti-SARS-CoV-2 Accubind® ELISA test system. Based on the data, the following cut-off point was established.

Presence of SARS-CoV-2 antibodies Confirmed

IgG > 10 RV

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Anti-SARS-CoV-2 (COVID-19) S1-RBD AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented below.

TABLE 1

Within Assay Precision (Values in RV)

Sample	N	X	σ	C.V.
Negative	20	3.3	0.13	3.95%
Borderline	20	9.5	0.29	2.64%
Positive	20	19.3	0.32	1.65%

TABLE 2*

Between Assay Precision (Values in RV)

Sample	N	X	σ	C.V.
Negative	16	1.6	0.14	8.75%
Borderline	16	9.1	0.35	3.50%
Positive	16	29.8	1.45	4.85%

*As measured in eight experiments in duplicate.

14.2 Sensitivity

The sensitivity of the Anti-SARS-CoV-2 S1-RBD IgG AccuBind® ELISA Test System was determined by testing samples from 60 patients who had previously tested positive for SARS-CoV-2 via RT-PCR. The patient samples were sourced from three different blood banks. 59 out of the 60 patients tested positive indicating that the sensitivity of the test is at least 98.3% Positive Percent Agreement (PPA).

14.3 Accuracy

The Anti-SARS-CoV-2 (COVID-19) S1-RBD IgG AccuBind® ELISA test system was used to test samples drawn at subsequent time intervals from 60 patients who tested PCR and IgG positive for SARS-CoV-2. The data is shown in Table 3 below.

TABLE 3

		Candidate Test Results		
Days from Symptom Onset	Number of Subjects Tested	IgG Positive Results	IgG PPA	95% CI
0-7 days	17	14	82.4%	59.0%-93.8%
8-14 days	23	22	95.7%	79.0%-99.2%
≥15 days	21	20	95.2%	77.3%-99.2%
Unknown	16	16	100%	80.6%-100%
Total Subjects	77	N/A	N/A	N/A

Overall IgG PPA: (93.5% 72/77); [95% CI (85.7% - 97.2%)]

14.4 Specificity

>150 different patient samples drawn prior to December 2019 were assayed to determine the prevalence of false positives. No false positive samples were detected indicating the Anti-SARS-CoV-2 (COVID-19) S1-RBD IgG AccuBind® ELISA Test System has a 100% Specificity.

16.0 REFERENCES

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Effective Date: 2021-SEP-22 Rev 1 **DCO: 1508**
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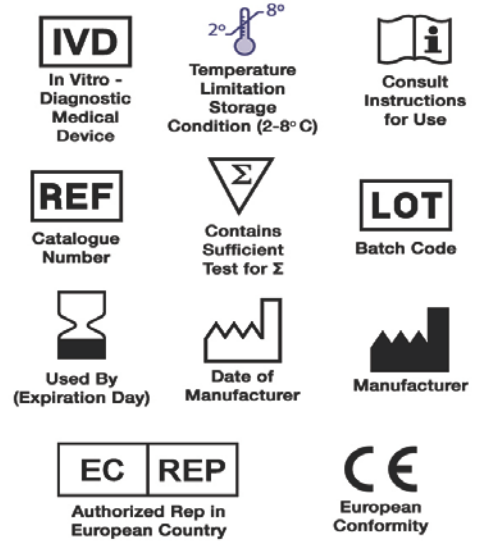
Glossary of Symbols
(EN 980/ISO 15223)



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AccuBind
ELISA Microwells

Anti-SARS-CoV-2 (COVID-19) IgM Test System

Product Codes: 11725-300

1.0 INTRODUCTION

Intended Use: The Qualitative Determination of Anti-SARS-CoV-2 Specific Antibodies of the IgM type in Human Serum or Plasma by Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), discovered at the end of 2019, is the cause of the disease COVID-19.[1-2] Both SARS-CoV-2 and SARS-CoV, the cause of the 2002 SARS epidemic, are of the genus betacoronavirus and are closely related.[2] Transmission of SARS-CoV-2 is primarily through close contact with infected patients via expelled respiratory droplets, usually from coughing or sneezing.[1-2]

Due to its high transmission rate and severeness, COVID-19 has emerged as a global pandemic that has forced lockdowns and quarantine protocols from countries all over the world.[3] Though diagnoses are primarily conducted using viral nucleic acid detection via real-time reverse transcriptase PCR, many false negatives have been reported and there is urgent need for serological antibody screening as a more robust and reliable test methodology.[4-5]

Tests for immunoglobulin M (IgM) antibodies are of importance as as an early detection of infection.[6] The body's primary defense against a pathogen (antigen) is to produce antibodies. Specifically, IgM appears first and wanes over time as IgG antibodies begin to rise and appear at detectable levels 10-20 days after symptom onset.[7-8]

The Anti-SARS-CoV-2 (COVID-19) IgM AccuBind® ELISA test kit is a qualitative test designed to produce highly sensitive and specific results with a simple and brief protocol. The test utilizes a recombinant nucleocapsid protein (rNCP) in the Enzyme Reagent and Anti-human IgM antibodies coated on microwells to capture native antibodies in the sample. In the first step, prediluted samples are added directly to the wells. After the first incubation, excess sample material is washed out and a rNCP labeled with an enzyme is added to the wells to detect IgM against SARS-CoV-2. After the second incubation, excess material is washed out again and substrate is added to produce a measurable color through the reaction with the enzyme and hydrogen peroxide.

3.0 PRINCIPLE

Sequential Sandwich ELISA Method (TYPE 10):

The reagents required for the sequential ELISA assay include immobilized antibody, circulating antibody to SARS-CoV-2, and enzyme-linked SARS-CoV-2 antigen.

Upon adding a sample containing the anti-SARS-CoV-2 antibody, reaction results between the antibody that has been immobilized on the microwell and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$h\text{-}Ab_{(IgM)} + Ab_{(x\text{-}IgM)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} h\text{-}Ab_{(IgM)} - Ab_{(x\text{-}IgM)}$$

$Ab_{(x\text{-}IgM)}$ = Immobilized Antibody (Constant Quantity)
$h\text{-}Ab_{(IgM)}$ = Human Antibody (Variable Quantity)
$h\text{-}Ab_{(IgM)} - Ab_{(x\text{-}IgM)}$ = Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked SARS-CoV-2 antigen is then added to the microwells. This conjugate binds to the immune complex that formed.

$$IC_{(h\text{-}IgM.)} + {}^{ENZ}Ag_{(SARS\text{-}CoV\text{-}2)} \Rightarrow {}^{ENZ}Ag_{(X\text{-}SARS\text{-}CoV\text{-}2)} - IC_{(h\text{-}IgM)}$$

$IC_{(h\text{-}IgM)}$ = Immobilized Immune complex (Variable Quantity)

${}^{ENZ}Ab_{(X\text{-}SARS\text{-}CoV\text{-}2)}$ = Enzyme-antibody Conjugate (Constant Quantity)

${}^{ENZ}Ab_{(X\text{-}SARS\text{-}CoV\text{-}2)} - I.C._{(h\text{-}IgM)}$ = Ag-Ab Complex (Variable)

The anti-h-IgM enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing a serum reference equivalent to the positive-negative cut-off value, the absorbance value can be compared to the cut-off to determine a positive or negative result.

4.0 REAGENTS

Materials provided:

A. Anti-SARS-CoV-2 IgM Controls – 1ml/vial - Icons PC, NC, CC
Three (3) vials of ready-to-use references for anti-SARS-CoV-2 at positive, negative, and cut-off levels of IgM. Store at 2-8°C. A preservative has been added.

B. SARS-CoV-2 IgM Enzyme Reagent – 12 ml/vial - Icon
One (1) vial of nucleocapsid protein from SARS-CoV-2 labeled with horseradish peroxides (HRP) in a buffering matrix. A preservative has been added. Store at 2-8°C.

C. Anti hIgM Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with anti-human IgM antibody and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Serum Diluent Concentrate – 20ml
One (1) vial of concentrated serum diluent containing buffer salts and a dye. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C

F. Substrate – 12ml/vial - Icon S^N
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (0.5 M H_2SO_4). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Fixed volume or variable volume pipette capable of delivering volumes ranging from 10 to 1000 µl with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050 ml, 0.100 ml, and 0.350 ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

Any components containing human serum from COVID-19 patients have been heat inactivated prior to handling and manufacturing. All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Please note that there has been no evidence of COVID-19 transmission through blood handling, but technicians should always exercise caution and treat all patient samples as potentially hazardous.[9]

Samples may be refrigerated at 2-8°C for a maximum period of seven (7) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.200ml of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Serum Diluent**
   Dilute contents of Serum Diluent Concentrate to 200ml (1:10 Dilution) in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer**
   Dilute contents of wash solution concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
3. **Patient Sample Dilution (1/100)**
   For example, dispense 0.010ml (10µl) of each patient specimen into 0.990 ml (990 µl) of serum diluent or 0.0101 ml (10.1 µl) into 1 ml (1000 µl). Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note : Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each control sample and patient specimen to be assayed in duplicate. Dilute the patient or any external control samples 1/100 (see Reagent Preparation **Section 8.0)** **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.100 ml (100µl) of the appropriate control or diluted patient specimen into the assigned well for IgM determination.
   DO NOT SHAKE THE PLATE AFTER SAMPLE ADDITION
3. Cover and incubate 30 minutes at room temperature.
4. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
5. Add 350µl of wash buffer (see Reagent Preparation Section 8.0), decant (blot) or aspirate. Repeat two (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
6. Add 0.100 ml (100µl) of SARS-CoV-2 IgM Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
7. Cover and incubate for thirty (30) minutes at room temperature.
8. Wash the wells five (5) times with 350 µl wash buffer by repeating steps (4 & 5) as explained above.
9. Add 0.100 ml (100µl) of Substrate Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells. Do not use the Substrate Reagent if it looks blue.**
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
10. Incubate at room temperature for twenty (20) minutes to develop sufficient color.
11. Add 0.050ml (50µl) of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix. **Always add reagents in the same order to minimize reaction time differences between wells.**
12. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: The relationship of absorbance to cut-off value is not necessarily linear so samples need not be diluted further if the absorbance is higher than the plate reader's capability (usually 3.0). However, these samples should be interpreted as strongly positive.

10.0 INTERPRETATION OF RESULTS

A Cut-Off Control is used to ascertain the positivity or negativity of samples. Follow the following procedure to interpret the sample results.

1. Record the absorbance of all samples obtained from the printout of the microplate reader as outlined in Example 1.
2. Multiply the average absorbance of the Cut-Off Control by the Cut-Off Factor to obtain the Cut-Off Value.
2. Divide the average absorbance of each sample by the Cut-Off Value and multiply by 10 to obtain the relative value unit (RV).
3. If RV <9, the sample is negative for Anti-SARS-CoV-2 IgM and if RV >10, the sample is positive for Anti-SARS-CoV-2 IgM
4. Samples with RV that fall within the range of 9-10 are considered borderline and should be retested with a new blood draw within 4-7 days for reevaluation.

Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1
(Cut-Off Factor = 1.0)

COV = MeanCC x COF
COV = Cut-Off Value
MeanCC = Mean Absorbance of Cut-Off Control
COF = Cut-Off Factor (See Certificate of Analysis)
COV = 0.230 x 1.0 = 0.230

Sample I.D.	Abs	Mean Abs	RV	Pos/Neg
Negative	0.059	0.060	÷0.230 x 10 =2.6	Negative
	0.061			
Cut Off	0.216	0.230	÷0.230 x 10 =10	Cut-Off
	0.244			
Positive	2.805	2.845	÷0.230 x 10 =124	Positive
	2.884			
Patient 1	0.104	0.105	***÷0.230 x 10 =4.6***	***Negative***
	0.106			
Patient 2	1.534	1.603	***÷0.230 x 10 =69.7***	***Positive***
	1.671			
Patient 3	0.225	0.217	***÷0.230 x 10 =9.4***	***Borderline***
	0.209			

*The data presented in Example 1 is for illustration only and **should not** be used in lieu of a Cut-Off sample run with each assay. **In this example, since the Cut-Off Factor = 1.0, the average absorbance of the Cut-Off Control = Cut-Off Value**

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. Maximum Absorbance (Positive control) > 1.5
2. Positive control RV > 15
3. Negative control RV < 6

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the Cut-Off control.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-SARS-CoV-2 in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. The Anti-SARS-CoV-2 (COVID-19) IgM AccuBind® ELISA Test System is a qualitative assay and does not necessarily give an indication of quantities of IgM antibodies.
11. Samples, which are contaminated microbiologically, should not be used.
12. Any patient samples used in manufacturing have been heat inactivated prior to handling. However, treat all samples, including the control samples, as potentially hazardous or infectious.
13. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
14. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
15. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
16. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The clinical significance of the result should be used in evaluating the possible presence of SARS-CoV-2 infection or COVID-19. However, **clinical inferences should not be solely based on this test** but rather as an adjunct to the clinical manifestations of the patient and other relevant tests such as Histology, nasophyrangeal swab, etc. A positive result does not indicate COVID-19 and does not distinguish between infection or contagiousness of COVID-19. Similarly, a negative result does not eliminate the absence COVID-19 infection but rather a very low titer of antibody that may be related to the early stages of disease.
7. Since this test utilizes the nucleocapsid protein of SARS-CoV-2, antibodies against any part of the spike protein are not detected. The nucleocapsid protein is produced in high levels during infection and is very immunogenic. Therefore, a positive result confirms a current or previous *contraction* of COVID-19. Patients who have been vaccinated against the spike protein of SARS-CoV-2 but have not been exposed to the live virus will not react with the test.

13.0 EXPECTED RANGES OF VALUES

A study of apparently healthy population (>150) from prior to December 2019 was undertaken to determine expected values for the Anti-SARS-CoV-2 Accubind® ELISA test system. Based on the data, the following cut-off point was established.

Presence of SARS-CoV-2 antibodies Confirmed

IgM > 10 RV

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Anti-SARS-CoV-2 (COVID-19) AccuBind® ELISA Test System were determined by analyses on two different levels of pool control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented below.

TABLE 1
Within Assay Precision (Values in RV)

Sample	N	X	σ	C.V.
Negative	20	2.1	0.11	5.24%
Borderline	20	9.2	0.23	2.50%
Positive	20	30.5	0.54	1.77%

TABLE 2*
Between Assay Precision (Values in RV)

Sample	N	X	σ	C.V.
Negative	16	1.9	0.16	8.42%
Borderline	16	9.3	0.45	4.84%
Positive	16	29.6	1.38	4.66%

*As measured in eight experiments in duplicate.

14.2 Sensitivity

The sensitivity of the Anti-SARS-CoV-2 IgM AccuBind® ELISA Test System was determined by testing samples from 41 patients who had previously tested positive for SARS-CoV-2 via RT-PCR. The patient samples were sourced from three different blood banks. 40 out of the 41 patients tested positive indicating that the sensitivity of the test is at least 97.6% Positive Percent Agreement.

14.3 Accuracy

The Anti-SARS-CoV-2 (COVID-19) IgM AccuBind® ELISA test system was used to test samples drawn at various time intervals from 41 patients who tested PCR and IgM positive for SARS-CoV-2. The data is shown in Table 3 below.

TABLE 3

Days from Symptom Onset	Number of Subjects Tested	Candidate Test Results: Total Antibody Positive results	Candidate Test Results: Total Antibody PPA	Candidate Test Results: 95% CI
0-7 days	7	7	100%	64.6%-100%
8-14 days	14	14	100%	78.5%-100%
15-30 days	9	9	100%	70.1%-100%
Unknown	11	10	90.9%	62.3%-98.4%
Total Subjects	41	N/A	N/A	N/A

Overall IgM PPA: (97.6% 40/41); [(95% CI (87.4% - 99.6%)]

14.4 Specificity

>150 different patient samples drawn prior to December 2019 were assayed to determine the prevalence of false positives. No false positive samples were detected indicating the Anti-SARS-CoV-2 (COVID-19) IgM AccuBind® ELISA Test System has a 100% Specificity.

16.0 REFERENCES

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Effective Date: 2021-FEB-15 Rev 1 **DCO: 1474**
MP11725 **Product Code: 11725-300**

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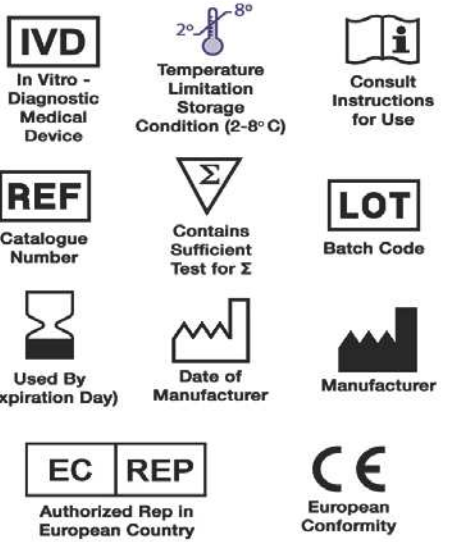
Glossary of Symbols
(EN 980/ISO 15223)



TOXO IgG

Enzyme Immunoassay for the quantitative/qualitative determination of IgG antibodies to Toxoplasma gondii in human serum and plasma

- for “in vitro” diagnostic use only -

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Code: TOXOG.CE
96 Tests

TOXO IgG

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Toxoplasma gondii in plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Toxoplasma gondii is an obligate intracellular protozoan parasite that is probably capable of infecting all species of mammals, including man. The detection of IgM antibodies to T.gondii is particularly helpful for the diagnosis of acute infections in "risk" individuals, in association with AIDS, organ transplantation and pregnancy. As most of T.gondii infections are mild or asymptomatic in otherwise healthy individuals, the detection of T.gondii specific IgM antibodies, in absence of detectable specific IgG, has become important for the monitoring of acute infections in pregnant women, as the parasite can lead to severe birth defects. Moreover, as T.gondii infections are most severe in immunocompromised patients, where the disease can be fatal, acute infections due to this parasite have to be distinguished from other disorders.
Recently developed IgM capture assays provide the clinician with a helpful and reliable test, not affected by the rheumatoid factor as it happens to be in classic sandwich tests.

C. PRINCIPLE OF THE TEST
Microplates are coated with native T. gondii antigens, highly purified by sucrose gradient centrifugation and inactivated.
The solid phase is first treated with the diluted sample and IgG to T. gondii are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti Toxoplasma gondii IgG are detected by the addition of polyclonal specific anti human IgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti Toxoplasma gondii IgG antibodies present in the sample. A Calibration Curve, calibrated against the W.H.O 3nd international standard , makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS
Each kit contains sufficient reagents to perform 96 tests.

1. : **Microplate**: MICROPLATE
12 strips x 8 microwells coated with purified and gamma-irradiation inactivated Toxoplasma gondii in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° ….
Ready to use and colour coded, calibrated against the 3nd international standard produced by the World Health Organization (WHO). The calibration curve range is as follows:
4ml CAL 1 = 0 WHO IU/ml
4ml CAL 2 = 50 WHO IU/ml
2ml CAL 3 = 100 WHO IU/ml
2ml CAL 4 = 250 WHO IU/ml
2ml CAL 5 = 500 WHO IU/ml
4ml CAL 6 = 1000 WHO IU/ml.
It contains Toxo IgG positive plasma titrated against WHO 3rd international standard code TOXM, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 2% casein, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. Standards are blue colored.

3. Control Serum: CONTROL ….
n° 1 vial - Lyophilized. To be dissolved with the volume of EIA grade water reported on the label. It contains fetal bovine serum, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives and human plasma positive to T.gondii calibrated at 250 IU/ml +/-10%, whose content is calibrated on 3nd international standard produced by the World Health Organization (WHO - TOXM).
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 10 mM Tris buffer pH 6.8+/-0.1,5% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives. Coded with 0.01% red alimentary dye

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide or H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vialIt contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. To be used to dilute the sample.

9. Plate sealing foils n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices

should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-T.gondii IgG antibody concentration is expected to be higher than 1000 IU/ml should be diluted before use, either 1:10 or 1:100 in the Calibrator 0 IU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M.1 Quantitative analysis

Automated assay:
In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute controls/calibrator as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:
1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the 1st and 2nd wells (positions A1 and B1 of the microplate) empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of 1:101 diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the 1st and the 2nd blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important notes:
1. *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*
2. *Mix thoroughly the Enzyme Conjugate on vortex before its use !!!*
7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M.2 QUALITATIVE ANALYSIS

If only a qualitative determination is required, proceed as described below:

Automated assay:
Proceed as described in section M1.

Manual assay:
1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the 1st well (positions A1 of the microplate) empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 IU/ml and 100 µl of Calibrator 50 IU/ml in duplicate, and 100 µl of Calibrator 1000 IU/ml in single. Then dispense 100 µl of 1:101 diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important notes:
1. *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*
2. *Mix thoroughly the Enzyme Conjugate on vortex before its use !!!*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *The Control Serum (CS) does not affect the test results calculation. The Control Serum may be used only when a laboratory internal quality control a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control Samples diluted 1:101	100 µl 100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS	S 7									
H	CAL3	CS	S 8									

Legenda: BLK = Blank CAL = Calibrator CS = Control Serum S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrators
CS = Control Serum S = Sample

O. INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether the performances of the assay are as expected and required by the IVDD directive 98/79/EC. Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
Calibrator 0 IU/ml (CAL1)	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
Calibrator 50 IU/ml	OD450nm > OD450nm CAL1 + 0.100
Calibrator 1000 IU/ml	OD450nm > 1.000
Control Serum	250 WHO IU/ml +/-10%

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not got contaminated during the assay
Calibrator 0 IU/ml > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

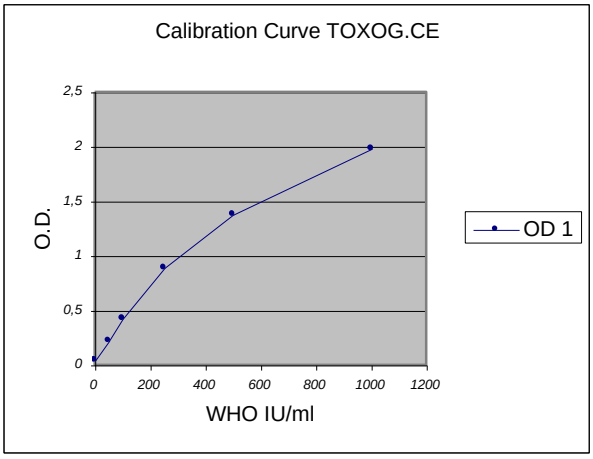
Calibrator 50 IU/ml OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 1000 IU/ml < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (e.g..: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 11.

P. RESULTS

P.1 Quantitative method
If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Toxoplasma gondii IgG antibody in samples.
An example of Calibration curve is reported in this page.



Important Notes:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 50 IU/ml and then check that the assay is valid.

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 11).

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 IU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 50 IU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted
Calibrator 1000 IU/ml: 2.845 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS
Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of Toxoplasma gondii due to the risk of severe neonatal malformations.
The cut-off of the device has been set at 50 IU/ml, and not lower as some other devices present on the market do, in order to assure the highest diagnostic value to the test, in particular when the assay is applied in pregnancy monitoring.
Upon infection, in fact, a part from the very first time of seroconversion, patients develop a strong immunological response to Toxoplasma gondii, far exceeding 50 IU/ml.
Low titer antibodies (below 50 IU/ml) mostly show low avidity to the infective agent and may represent a diagnostic marker of a recent infection, in combination with IgM.
Pregnant women, with antibodies concentrations below 50 IU/ml are by the devise considered negative in order to make the clinician consider them "risk" patients and follow them up for both IgG and IgM along pregnancy.
Samples with a concentration higher than 50 WHO IU/ml are considered positive for anti Toxoplasma gondii IgG antibody, surely able to provide immunity against the infection.
This titer is considered the lowest concentration of IgG to provide an effective immunological protection against a second infection of Toxoplasma gondii by NCCLS, USA.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *In the follow-up of pregnancy for Toxoplasma Gondii infection a positive result (presence of IgG antibody > 50 IU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what indicated in the standard prEN 13612.

1. Limit of detection

The limit of detection of the assay has been calculated by means of the 3nd international standard produced by the World Health Organization (WHO).

The limit of detection has been calculated as mean OD450nm Calibrator 0 IU/ml + 5 SD.

The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay.

OD450nm values

WHO IU/ml	TOXOG.CE Lot # 0503	TOXOG.CE Lot # 0403	TOXOG.CE Lot # 0303
250	0.816	0.853	0.974
100	0.365	0.398	0.445
50	0.209	0.244	0.246
10	0.094	0.125	0.108
Std 0	0.033	0.031	0.056

The assay shows a limit of detection better than 10 IU/ml.

2. Diagnostic Sensitivity:

The diagnostic sensitivity has been tested in a Performance Evaluation trial on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of Toxoplasma gondii Virus infection were tested.

The value, obtained from the analysis of more than 300 specimens, has been > 98%.

3. Diagnostic Specificity:

The diagnostic specificity has been determined on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.

Frozen specimens have been tested, as well, to check for interferences due to collection and storage.

No interference was observed.

Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested. No crossreaction was observed.

An overall value > 98% of specificity was found when examined on more than 100 specimens.

4. Precision:

It has been calculated on three Calibrators, examined in 16 replicates in three separate runs with three lots.

Results are reported as follows

TOXOG.CE: lot 0503

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.067	0.066	0.070	0.067
Std.Deviation	0.006	0.005	0.006	0.006
CV %	9.3	7.7	9.0	8.7

Calibrator 50 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.276	0.259	0.268	0.267
Std.Deviation	0.025	0.006	0.010	0.014
CV %	9.1	2.4	3.6	5.0

Calibrator 1000 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.768	2.657	2.707	2.711
Std.Deviation	0.118	0.098	0.101	0.106
CV %	4.3	3.7	3.7	3.9

TOXOG.CE: lot # 0403

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.067	0.065	0.068	0.066
Std.Deviation	0.003	0.004	0.006	0.004
CV %	5.2	6.3	8.3	6.6

Calibrator 50 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.270	0.262	0.265	0.265
Std.Deviation	0.012	0.009	0.008	0.010
CV %	4.5	3.4	3.1	3.7

Calibrator 1000 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.765	2.652	2.718	2.712
Std.Deviation	0.115	0.101	0.092	0.103
CV %	4.2	3.8	3.4	3.8

TOXOG.CE: lot # 0303

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.068	0.067	0.069	0.068
Std.Deviation	0.004	0.004	0.006	0.004
CV %	5.1	6.1	8.0	6.4

Calibrator 50 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.268	0.261	0.265	0.265
Std.Deviation	0.012	0.009	0.008	0.010
CV %	4.6	3.3	3.2	3.7

Calibrator 1000 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.766	2.651	2.719	2.712
Std.Deviation	0.115	0.100	0.091	0.102
CV %	4.2	3.8	3.3	3.8

The variability shown in the tables above did not result in sample misclassification.

5. Accuracy
The assay accuracy has been checked by the dilution and recovery tests. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled out up to 4.000 IU/ml.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 11.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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TOXO IgG

Ensayo inmunoenzimático para la determinación cualitativa/cuantitativa de anticuerpos IgG frente a *Toxoplasma gondii* en plasma y suero humano

Uso exclusivo para diagnóstico “in vitro”

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96 pruebas

TOXO IgG

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos IgG frente a *Toxoplasma gondii*, en plasma y suero humanos.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
Toxoplasma gondii es un protozoo, parásito intracelular obligado, que puede infectar probablemente a todas las especies de mamíferos, incluido el hombre.
La detección de anticuerpos IgM contra *T. gondii* es particularmente útil en el diagnóstico de la infección aguda, ya sea en los individuos "de riesgo", durante el embarazo, en personas sometidas a trasplante de órganos, o en pacientes con SIDA.
Gran parte de las infecciones por *T. gondii* en individuos sanos son leves o asintomáticas. La detección de anticuerpos IgM al mismo, en ausencia de anticuerpos detectables de clase IgG, es de gran importancia en el seguimiento de infecciones agudas durante el embarazo ya que el parásito puede ocasionar severos trastornos en el noenato. Por otra parte, como las infecciones agudas por *T. gondii* son severas en pacientes inmunocomprometidos, deben ser diferenciadas de otros tipos de trastornos.
El sistema ELISA de captura de IgM constituye una prueba diagnóstica potente y confiable, sobretodo porque no se ve afectada en presencia del factor reumatoideo como ha sucedido en los ensayos clásicos tipo "sandwich".

C. PRINCIPIOS DEL ENSAYO.
Los pocillos de la placa están recubiertos con antígenos nativos de *T. gondii*, purificados por gradiente de centrifugación con sacarosa e inactivados.
Se añade la muestra diluída, y los anticuerpos IgG contra *T. gondii* presentes en la misma son capturados por los antígenos de la fase sólida.
Después del lavado, en la 2ª incubación, los anticuerpos IgG anti *Toxoplasma gondii* son detectados mediante anticuerpos policlonales específicos anti-IgG humana, conjugados con Peroxidasa (HPR).
La enzima capturada en la fase sólida, combinada con la mezcla substrato/cromógeno, genera una señal óptica proporcional a la cantidad de anticuerpos IgG anti-*T. gondii* presentes en la muestra. Posteriormente, con la ayuda de una Curva de Calibración contra el 3er estándar internacional de la Organización Mundial de la Salud (O.M.S), es posible determinar cuantitativamente los anticuerpos IgG contenidos en la muestra.

D. COMPONENTES.
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
12 tiras de 8 pocillos recubiertos con *T. gondii*, altamente purificado e inactivado por radiaciones gamma en presencia de proteínas del suero bovino.
Las placas están almacenadas en bolsas selladas con desecante. Se deben poner las mismas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y conservar a 2-8ºC.

2. Curva de Calibración: CAL N° …
6x2.0 ml/vial. Listo para el uso y curva con código estándar de color, calibrada contra el 3er estándar International producido por la O.M.S. con rangos:

4ml CAL 1 = 0 O.M.S IU/ml
4ml CAL 2 = 50 O.M.S IU/ml
2ml CAL 3 = 100 O.M.S IU/ml
2ml CAL 4 = 250 O.M.S IU/ml
2ml CAL 5 = 500 O.M.S IU/ml
4ml CAL 6 = 1000 O.M.S IU/ml.
Contiene plasma positivo a Toxo IgG, titulado contra el 3er estándar internacional O.M.S. (código: TOXM), caseína al 2%, tampón Citrato de sodio 10 mM pH 6.0+/-0.1, 0.1% de Tween 20, así como azida sódica 0.09% y ProClin 300 0.045% como conservantes. Los estándar son de color azul.

3. Suero Control: CONTROL …ml
1 vial. Liofilizado. Para ser disuelto en el volumen de agua de calidad EIA indicado en la etiqueta. Contiene proteínas del suero bovino fetal, plasma humano positivo a *T. gondii a* 250 IU/ml +/-10% calibrado contra el 3er estándar internacional O.M.S. (código: TOXM), contiene además sulfato de gentamicina 0.2 mg/ml y ProClin 300 0.045% como conservantes.
Nota: El volumen necesario para disolver el contenido del frasco varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

4. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%

5. Conjugado: CONJ
2x8ml/vial. Solución lista para el uso. Codificado con el color rojo. Contiene anticuerpos policlonales anti-IgG humanos conjugados con Peroxidasa (HRP), BSA 5%, tampón Tris 10 mM pH 6.8+/-0.1, además sulfato de gentamicina 0.02% y ProClin 300 0.045% como conservantes.

6. Cromógeno/Substrato. SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM pH 3.5-3.8, dimetilsulfóxido 4%, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02%.
Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

7. Ácido Sulfúrico: H_2SO_4 O.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Diluente de muestras: DILSPE
2x60ml/vial. Contiene proteínas del suero de cabra, 2% de caseína, tampón Citrato de sodio 10 mM pH 6.0 +/-0.1, 0.1% de Tween 20, azida sódica al 0.09% y ProClin 300 0.045% como conservantes. Se utiliza para diluir las muestras.

9. Sellador adhesivo, n° 2

10. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.
1. Micropipetas calibradas (1000µl, 100µl y 10µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (tolerancia+/-1°C).
6. Lector calibrado de microplacas de ELISA con filtros de 450 nm (lectura) y filtros de 620-630 nm.

7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar los indumentos protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbicas cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contengan precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas depués de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de vencimiento indicada en el equipo e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 3 meses.
11. Tratar todas las muestras como potencialmente infectivas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infectivos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según la técnica estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben ser identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante al menos 12 meses, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.
6. Aquellas muestras, cuya concentración de IgG anti-*T. gondii* se sospeche mayor de 1000 IU/ml, deben diluirse a 1:10 o 1:100 antes del uso, con ayuda del Calibrador 0 IU/ml. Las diluciones deben efectuarse en tubos limpios desechables añadiendo 50 µl de la muestra y 450 µl del Cal 0 (1:10), después 50 µl de la dilución 1:10 y 450 µl del Cal 0 (1:100). Mezclar los tubos en el vortex y después proseguir con los pasos indicados en la sección M.

PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 3 meses.

Microplaca:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Curva de Calibración:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Suero Control:

Añadir al polvo liofilizado el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver totalmente y mezclar delicadamente en el vórtex.

Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada y mezclarse delicadamente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*
Conjugado:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Diluente de muestras :
Solución lista para el uso. Mezclar bien con un vórtex antes de usar.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra (etanol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450 nm y de un segundo filtro de 620-630 nm obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10 b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medida de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y las de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no sean contaminados con partículas o agregados visibles.
3. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico.
4. Compruebe que no han ocurrido roturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
5. Disolver el Suero Control liofilizado, como se ha descrito anteriormente.
6. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
7. Dejar los componentes restantes hasta alcanzar la temperatura ambiente (aprox. 1 hora), mezclar después suavemente en el vórtex todos los reactivos líquidos.

8. Ajustar la incubadora de ELISA a 37ºC y alimentar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
9. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
10. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
11. Comprobar que las micropipetas estén fijadas en el volumen requerido.
12. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
13. En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

El equipo puede usarse tanto para la determinación cuantitativa como cualitativa.

M1. DETERMINACIÓN CUANTITATIVA:

Ensayos Automatizados.

En caso de que el ensayo se realice de manera automatizada con un sistema ELISA, se recomienda programar el equipo para aspirar 1000µl de Diluente de Muestras, y posteriormente 10µl de muestra (factor de dilución 1:101).

La mezcla debe ser dispensada cuidadosamente en un tubo de dilución. Antes de aspirar la muestra siguiente, las agujas deben lavarse debidamente para evitar cualquier contaminación cruzada entre las muestras. Una vez diluídas las mismas, programar el equipo para dispensar 100 µl de cada una en los pocillos correspondientes.

Este procedimiento puede realizarse además en dos pasos de dilución 1:10 (90 µl Diluente de Muestras + 10 µl Muestra) en una segunda plataforma de dilución. Después, se recomienda programar el equipo para aspirar 100µl de Diluente de Muestras y 10µl de la primera dilución en la plataforma, posteriormente dispensar el contenido total en los pocillos correspondientes.

No es necesario diluir el Calibrador ni el Suero Control (ya diluído) pues están listos para el uso.

Dispensar 100µl de controles/calibradores en los pocillos correspondientes.

Para las operaciones siguientes, consulte las instrucciones que aparecen a continuación para el Ensayo Manual.

Es muy importante comprobar que el tiempo entre el dispensado de la primera y la última muestra sea calculado por el instrumento y considerado para los lavados.

Ensayo Manual.

1. Diluir las muestras 1:101 en un tubo de dilución apropiado (ejemplo: 1000 de Diluente de Muestras+10µl de muestra). No diluir el Panel de Calibración, ya que los calibradores están listos para el uso. Mezclar cuidadosamente, con ayuda de un vórtex, todos los componentes líquidos y continuar como se describe a continuación.
2. Poner el número de tiras necesarias en el soporte de plástico. Dejar vacíos los pocillos A1 y B1 para el blanco.
3. Dispensar 100 µl de Calibradores y 100 µl de Suero Control, por duplicado, después dispensar 100 µl de cada muestra diluída en su pocillo correspondiente.
4. Incubar la microplaca **60 min a +37°C**.

***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el ensayo manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

5. Lavar la microplaca con el lavador automático dispensando y aspirando 350µl/pocillo de solución de lavado diluída, segun según se indica (section I.3).
6. Dispensar 100µl del Conjugado en todos los pocillos, excepto en A1 y B1, después cubrir con el sellador. Compruebe que este reactivo de color rojo ha sido añadido en todos los pocillos excepto A1 y B1.

***Notas importantes**:*
Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.
Mezclar el Conjugado en el vórtex antes de usarlo!

7. Incubar la microplaca durante **60 minutos a +37ºC.**
8. Lavar los pocillos de igual forma que en el paso 5.
9. Dispensar 100µl de TMB/H_2O_2 en todos los pocillos, incluidos los del blanco. Controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca por **20 minutos a temperatura ambiente (18-24°C).**

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

10. Dispensar 100µl de Ácido Sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición del ácido cambia el color de los calibradores positivos, el suero control y las muestras positivas de azul a amarillo.
11. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco B1 (blanco).

Notas generales importantes:

1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de stop y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*
3. *El suero de control (CS) no afecta al cálculo de los resultados de la prueba. El suero de control (CS) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

M2. DETERMINACIÓN CUALITATIVA:

Si se requiere solamente un análisis cualitativo, proceda como se indica a continuación.

Ensayo automatizado:

Proceder según la sección M1.

Ensayo Manual.

1. Diluir las muestras 1:101 en un tubo de dilución apropiado (ejemplo: 1000 de Diluente de Muestras+10µl de muestra). No diluir el Panel de Calibración, ya que los calibradores están listos para el uso. Mezclar cuidadosamente, con ayuda de un vórtex, todos los componentes líquidos y continuar como se describe a continuación.
2. Poner el número de tiras necesarias en el soporte de plástico. Dejar vacío el pocillo A1 para el blanco.
3. Dispensar 100 µl del Calibrador 0 IU/ml y 100 µl del Calibrador 50 IU/ml por duplicado, y 100 µl del Calibrador 1000 IU/ml sencillo. Después dispensar 100 µl de cada muestra diluída 1:101 en su pocillo correspondiente.
4. Incubar la microplaca **60 min a +37°C**.***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo*

cuando se hace el ensayo manualmente. No sellar cuando se emplean equipos automatizados de ELISA.

5. Lavar la microplaca con el lavador automático dispensando y aspirando 350µl/pocillo de solución de lavado diluída, según se indica (section I.3).
6. Dispensar 100µl del Conjugado en todos los pocillos, excepto en A1, después cubrir con el sellador. Compruebe que este reactivo de color rojo haya sido añadido en todos los pocillos excepto A1.

***Notas importantes**:*
Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.
Mezclar el Conjugado en el vórtex antes de usarlo!

7. Incubar la microplaca durante **60 minutos a +37ºC.**
8. Lavar los pocillos de igual forma que en el paso 5.
9. Dispensar 100µl de TMB/H_2O_2 en todos los pocillos, incluido el del blanco. Controlar que los reactivos hayan sido correctamente añadidos. Incubar la microplaca durante **20 minutos a temperatura ambiente (18-24°C).**

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

10. Dispensar 100µl de Ácido Sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición del ácido cambia el color de los calibradores positivos, el suero control y las muestras positivas de amarillo a azul.
11. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

N. ESQUEMA DEL ENSAYO.

Método	**Operaciones**
Calibradores & Control Muestras diluídas 1:101	100 µl 100 µl
1ra incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2da incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
TMB/H2O2	100 µl
3ra incubación	20 min
Temperatura	t.a.*
Ácido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado en el análisis cuantitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	CAL4	M 1									
B	BL	CAL4	M 2									
C	CAL1	CAL5	M 3									
D	CAL1	CAL5	M 4									
E	CAL2	CAL6	M 5									
F	CAL2	CAL6	M 6									
G	CAL3	SC	M 7									
H	CAL3	SC	M 8									

Leyenda: BL = Blanco // CAL = Calibradores // M = Muestra // SC = Suero Control

A continuación se describe un ejemplo del esquema de dispensado en el análisis cualitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 3	M 11									
B	CAL1	M 4	M 12									
C	CAL1	M 5	M 13									
D	CAL2	M 6	M 14									
E	CAL2	M 7	M 15									
F	CAL6	M 8	M 16									
G	M 1	M 9	M 17									
H	M 2	M 10	M 18									

Leyenda: BL = Blanco // CAL = Calibradores //M = Muestra

O. CONTROL DE CALIDAD INTERNO.
Se realiza un grupo de pruebas de validación con los controles cada vez que se usa el equipo para verificar si el funcionamiento del ensayo es correcto, según las directivas IVDD 98/79/EC.
Asegurar el cumplimiento de los siguientes parámetros :

Parámetro	**Exigencia**
Pocillo Blanco	< 0.100 DO450nm
Calibrador 0 IU/ml (CAL1)	< 0.150 valor medio DO450nm después de leer el blanco Coeficiente de variación < 30%
Calibrador 50 IU/ml	DO450nm > DO450nm CAL1 + 0.100
Calibrador 1000 IU/ml	DO450nm > 1.000
Suero Control	250 O.M.S. IU/ml +/-10%

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.
En caso contrario, detenga el ensayo y compruebe:

Problema	**Compruebe que**
Pocillo blanco > 0.100 DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Calibrador 0 IU/ml > 0.150 DO450nm después de leer el blanco Coeficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido alimentado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensado de un calibrador positivo en lugar del negativo). 4. no ha existido contaminación del Cal negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.

Calibrador 50 IU/ml DO450nm < DO450nm CAL1 + 0.100	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Calibrador 1000 IU/ml < 1.000 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Suero Control Valor distinto al esperado	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar una muestra equivocada). 3. el proceso de lavado y los parámetros del lavador son correctos. 4. no ha ocurrido contaminación externa de los controles. 5. el Suero Control ha sido disuelto con el volumen correcto indicado en la etiqueta Si se indica un error, el ensayo debe repetirse tras eliminar la causa del mismo. En caso de no encontrar un error, procédase como sigue: a) si se obtiene un valor hasta +/-20%: la precisión global del laboratorio podría no permitir alcanzar +/-10% del valor esperado. Comunicar el problema al responsable para aceptar ó rechazar este resultado. b) si se obtiene un valor superior a +/-20%: en este caso el test es inválido y hay que avisar al servicio de atención al cliente de DiaPro

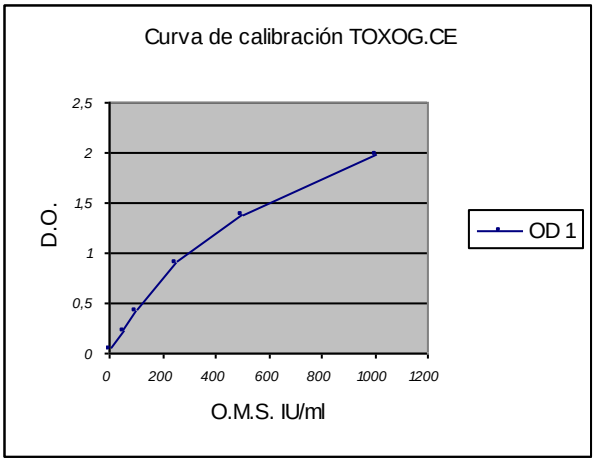
De presentarse alguno de los problemas anteriores, después de comprobar, avisar al responsable para tomar las medidas pertinentes.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 11.

P. RESULTADOS.

P.1 Método cuantitativo.
Si el ensayo resulta válido, usar para el método cuantitativo un sistema de ajuste de curva para diseñar la curva de calibración con los valores obtenidos en la lectura a 450nm (se sugiere interpolar 4 parámetros).
Posteriormente, calcular sobre la curva de calibración la concentración de anticuerpos IgG contra el *T. gondii* presentes en la muestra.

A continuación, un ejemplo de curva de calibración:



Nota Importante:
No usar la curva anterior para formular los cálculos.

P.2 Método cualitativo.
En el método cualitativo, calcular los valores medios de DO450nm para los Calibradores 0 y 50 IU/ml, después comprobar que el ensayo es válido.

A continuación, un ejemplo de los cálculos a realizar para el método cualitativo: (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 11).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Calibrador 0 IU/ml: 0.020 – 0.024 DO450nm
Valor medio : 0.022 DO 450nm
Menor de 0.150 – Válido

Calibrador 50 IU/ml: 0.250 – 0.270 DO 450nm
Valor medio : 0.260 DO 450nm
Mayor que Cal 0 + 0.100 – Válido

Calibrador 250 IU/ml: 2.845 DO 450nm
Mayor que 1.000 – Válido

Q. INTERPRETACIÓN DE LOS RESULTADOS.
Debe ponerse particular atención a la interpretación de los resultados en el seguimiento del embarazo, debido a que la infección por *T. gondii* puede provocar malformaciones en el neonato.
El cut-off del producto ha sido posizionado a 50 IU/ml, y no debajo de esta, en modo de assigurar al test un mayor valor diagnostico, en particular sobre todo cuando il dosaje es aplicado durante il monitorage de las mujeres embarazadas.
Debido a la infecciòn, de echo a parte del primerisimo periodo di sieroconversiòn, el paciente desarrolla una fuerte respuesta immunologica contra l'agente infectante, que sobrepasa di bastante las 50 IU/ml.
Anticuerpos con titulo bajo(debajo de 50 IU/ml) muestran prevalentemente una baja reactividad contra el Toxoplasma gondii y pueden asi representar un marcador diagnostico de una reciente infecciòn, en combinaziòn con los IgM.
Las muestras con una concentración menor de 50 OMS IU/ml son consideradas negativas a anticuerpos IgG contra *Toxoplasma gondii,* en modo de inducir el medico a considerar tales sujetos 'a riesgo' y de proseguir el monitorage sea de los IgG que de los IgM durante el embarazo.

Las muestras con una concentración mayor de 50 OMS IU/ml se consideran positivas a anticuerpos IgG contra el Virus Toxoplasma gondii y del punto de vista inmunológico protejidos contra la infecciòn. Este título es considerado, según NCCLS Estados Unidos, la menor concentración de IgG que ofrece una protección inmunológica efectiva contra una segunda infección por *Toxoplasma gondii*.

Notas importantes:

1. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
2. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
3. *En el monitoreo de infección por Toxoplasma Gondii durante el embarazo, un resultado positivo (presencia de anticuerpos IgG > 50 IU/ml) debe ser confirmado para eliminar cualquier riesgo de falso positivo o falsa definición de protección.*

R. FUNCIONAMIENTO.

La evaluación del funcionamiento ha sido realizada según lo establecido en el estándar prEN 13612.

1. Límite de detección.

El límite de detección del ensayo ha sido calculado por medio del 3er estándar internacional producido por la Organización Mundial de la Salud (O.M.S).

El límite de detección ha sido calculado como valor medio de DO450nm del Calibrador 0 OMS U/ml + 5 SD.

La siguiente tabla muestra los valores medios de DO450nm del estándar, diluído en plasma negativo y examinado en el ensayo.

Valores DO450nm

O.M.S. IU/ml	TOXOG.CE Lote # 0503	TOXOG.CE Lote # 0403	TOXOG.CE Lote # 0303
250	0.816	0.853	0.974
100	0.365	0.398	0.445
50	0.209	0.244	0.246
10	0.094	0.125	0.108
Est 0	0.033	0.031	0.056

El ensayo demuestra un límite de detección superior a 10 IU/ml.

2. Sensibilidad Diagnóstica:

La sensibilidad diagnóstica se ha estudiado en un ensayo clínico externo utilizando paneles de muestras, clasificadas como positivas mediante un equipo de referencia US FDA. Se probaron muestras positivas correspondientes a diferentes etapas de la infección por *Toxoplasma gondii.*

El valor obtenido del análisis de más de 300 muestras fue > 98%.

3. Especificidad Diagnóstica::

La especificidad diagnóstica ha sido determinada en el mismo centro, utilizando paneles de muestras provenientes de individuos sanos, clasificadas como negativas mediante un equipo de referencia US FDA.

Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos para determinar la especificidad. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

Las muestras congeladas han sido analizadas para comprobar si la colección y la conservacion interfiere con el procedimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.

Se analizaron muestras de potencial interferencia derivadas de pacientes con diversas patologías (mayormente positivos a ANA, AMA y RF) y de mujeres embarazadas. No se observaron reacciones cruzadas.

Se obtuvo un valor de especificidad total > 98% al examinar más de 100 muestras.

4. Precisión:

Ha sido calculada a partir de tres Calibradores examinados en 16 réplicas en tres corridas separadas, para 3 lotes.

Los resultados son los siguientes:

TOXOG.CE: lote 0503

Calibrador 0 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.067	0.066	0.070	0.067
Desviación estándar	0.006	0.005	0.006	0.006
CV %	9.3	7.7	9.0	8.7

Calibrador 50 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.276	0.259	0.268	0.267
Desviación estándar	0.025	0.006	0.010	0.014
CV %	9.1	2.4	3.6	5.0

Calibrador 1000 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	2.768	2.657	2.707	2.711
Desviación estándar	0.118	0.098	0.101	0.106
CV %	4.3	3.7	3.7	3.9

TOXOG.CE: lote # 0403

Calibrador 0 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.067	0.065	0.068	0.066
Desviación estándar	0.003	0.004	0.006	0.004
CV %	5.2	6.3	8.3	6.6

Calibrador 50 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.270	0.262	0.265	0.265
Desviación estándar	0.012	0.009	0.008	0.010
CV %	4.5	3.4	3.1	3.7

Calibrador 1000 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	2.765	2.652	2.718	2.712
Desviación estándar	0.115	0.101	0.092	0.103
CV %	4.2	3.8	3.4	3.8

TOXOG.CE: lote # 0303

Calibrador 0 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.068	0.067	0.069	0.068
Desviación estándar	0.004	0.004	0.006	0.004
CV %	5.1	6.1	8.0	6.4

Calibrador 50 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.268	0.261	0.265	0.265
Desviación estándar	0.012	0.009	0.008	0.010
CV %	4.6	3.3	3.2	3.7

Calibrador 1000 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	2.766	2.651	2.719	2.712
Desviación estándar	0.115	0.100	0.091	0.102
CV %	4.2	3.8	3.3	3.8

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

5. Exactitud.
La exactitud del ensayo ha sido comprobada mediante diluciones y pruebas de recuperación. Cualquier "efecto gancho", estimación errónea que puede presentarse a elevadas dosis del analita, no se manifiesta hasta 4.000 IU/ml.

Nota importante:
Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 11.

S. LIMITACIONES DEL PROCEDIMIENTO.
La contaminación bacterica o la inactivación por calor de la muestra pueden afectar los valores de DO y por tanto alterar los niveles del analita.
Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.
El ensayo es útil solo para probar muestras independientes y no mezclas.
El diagnóstico de una enfermedad infecciosa no debe establecerse en base a un solo resultado, sino que deben tenerse en consideración la historia clínica del paciente, la sintomatología, así como otros datos diagnósticos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni
(Milán) - Italia

CE
0318

Toxo IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM antibodies to Toxoplasma gondii in human plasma and sera

- for "in vitro" diagnostic use only -

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REF TOXOM.CE
96 tests

Toxo IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Toxoplasma gondii or T.gondii in human plasma and sera with the "capture" system.
The devise is intended for the follow-up of T.gondii infected patients and for the monitoring of risk of neonatal defects due to T.gondii infection during pregnancy.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that is probably capable of infecting all species of mammals, including man.
The detection of IgM antibodies to T.gondii is particularly helpful for the diagnosis of acute infections in “risk” individuals, in association with AIDS, organ transplantation and pregnancy.
As most of T.gondii infections are mild or asymptomatic in otherwise healthy individuals, the detection of T.gondii specific IgM antibodies, in absence of detectable specific IgG, has become important for the monitoring of acute infections in pregnant women, as the parasite can lead to severe birth defects.
Moreover, as T.gondii infections are most severe in immunocompromised patients, where the disease can be fatal, acute infections due to this parasite have to be distinguished from other disorders.
Recently developed IgM capture assays provide the clinician with a helpful and reliable test, not affected by the rheumatoid factor as it happens to be in classic sandwich tests.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a preparation of inactivated T.gondii, labeled with a specific monoclonal antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of peroxidase the colorless substrate is hydrolysed to a colored end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to T.gondii present in the sample.
A system is described how to control whether the positivity shown by a sample is true or not (Confirmation Test), helpful for the clinician to make a correct interpretation of results.

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with anti human IgM affinity purified goat antibody, in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -
1x4.0 ml/vial. Ready to use control. It contains 1% human plasma negative for T.gondii IgM, 2% casein, 10 mM Tris-citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The negative control is colorless.

3. Positive Control: CONTROL +
1x4.0 ml/vial. Ready to use control. It contains 1% human plasma positive for T.gondii IgM, 2% casein, 10 mM Tris-citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The positive control is green colour coded.

4. Calibrator: CAL …ml
N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti T.gondii IgM at 200 WHO IU/ml +/-10% (3rd WHO International Standard for T.gondii IgG&IgM), fetal bovine serum, 0.2 mg/ml gentamicine sulphate and ProClin 300 0.045% as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Lyophilized T.gondii Ag: AG TOXO
N° 6 lyophilized vials.
The vials contain lyophilized gamma ray inactivated Toxoplasma gondii in a protein buffer. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300. To be dissolved with 1.9 ml of Antigen Diluent as reported in the specifi section.

6. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300

7. Enzyme conjugate: CONJ 20X
1x0.8 ml/vial. 20x concentrated solution of a T.gondii-specific monoclonal antibody, labeled with HRP and diluted in a protein buffer containing 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives.

8. Antigen Diluent : AG DIL
n° 1 vial of 16 ml. Protein buffer solution for the preparation of the Immunocomplex. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code colored with 0.01% red alimentary dye

9. Specimen Diluent : DILSPE
2x60.0 ml/vial. Proteic buffered solution for the dilution of samples. It contains 2% casein, 10 mM citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The reagent is color coded with 0.01% blue alimentary dye.

10. Chromogen/Substrate : SUBS TMB
1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use.

Calibrator:
Add the volume of ELISA grade water reported on the label to the lyophilized powder. Let fully dissolve and then gently mix on vortex.

***Important Note**: The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
***Note:** Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Ag/Ab Immunocomplex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved T.gondii antigen and mix gently on vortex.

***Important Notes**:*
1. *Dissolve and prepare only the number of vials necessary to the test. The Immunocomplex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.*
2. *The preparation of the Immucomplex has to be done **right before** the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.*

Specimen Diluent:
Ready to use. Mix well on vortex before use

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check

that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

M.1 Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Specimen Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl diluted samples into the proper wells of the microplate.

This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Specimen Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.

Do not dilute controls/calibrator as they are ready to use.

Dispense 100 µl calibrators/control in the appropriate calibration/control wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

M. 2 Manual assay:

1. Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
2. Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
3. Dispense 100 µl of Negative Control in triplicate and 100 µl of Calibrator in the proper wells in duplicate. Dispense 100 µl of Positive Control in single into the proper well. Do not dilute controls and the calibrator as they are ready to use !
4. Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue colored and that controls and calibrator have been dispensed.
5. Incubate the microplate for **60 min at +37°C** .

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer as reported previously (section I.3).
7. Pipette 100 µl Ag/Ab Immunocomplex into each well, except the blanking well A1, and cover with the sealer. Check that all wells are red colored, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Ag/Ab Immunocomplex . Contamination might occur.*

8. Incubate the microplate for **60 min at +37°C** .
9. Wash microwells as in step 6.
10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 (mandatory).

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Controls&calibrator Samples diluted 1:101	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Immunocomplex	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A quality control check is performed on the controls/calibrator any time the kit is used in order to verify whether the performance of the assay matches the requirements reported in table below.

Parameter	Requirements
Blank well	< 0.050 OD450nm value
Negative Control mean value (NC)	< 0.150 OD450nm value after blanking coefficient of variation < 30%
Calibrator	S/Co > 1.5
Positive Control	> 0.750 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 1.5	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 0.750 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

Cut-Off = NC + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 - 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient is not undergoing an acute infection of Toxoplasma gondii.
Any patient showing an equivocal result, should be re-tested by examining a second sample taken from the patient after 1-2 weeks from first testing.
A positive result is indicative of a Toxoplasma gondii infection.

An example of calculation is reported below:

***Important Note:** The following data must not be used instead or real figures obtained by the user.*

Negative Control: 0.050 – 0.060 – 0.070 OD450nm
Mean Value: 0.060 OD450nm
Lower than 0.150 – Accepted
Positive Control: 1.850 OD450nm
Higher than 0.750 – Accepted

Cut-Off = 0.060+0.250 = 0.310

Calibrator: 0.550 - 0.530 OD450nm
Mean value: 0.540 OD450nm S/Co = 1.7
S/Co higher than 1.0 – Accepted

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1 = negative
Sample 2 S/Co > 1.2 = positive

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of Toxoplasma gondii due to the risk of severe neonatal malformations.*
3. *Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, leading to a misinterpretation of the analytical result, can be revealed and then ruled out.*
4. *In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a different device for T.gondii IgM detection, before taking any preventive medical action.*
5. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
6. *Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

Dia.Pro Diagnostic BioProbes s.r.l. has defined the 3rd WHO International Standard for TOXO IgG (Coded TOXG), positive also for IgM anti Toxoplasma Gondii, as an Internal Gold Standard (or IGS).
Results of Quality Control are given in the following table:

The limits of detection of this material, when diluted first in negative serum and then in the sample diluent to generate dilutions tested in four replicates, are reported in the following table for three lots of the device:

OD450nm values

WHO (IGS) IU/ml	TOXOM.CE Lot # 0703	TOXOM.CE Lot # 0603	TOXOM.CE Lot # 0503
3000 IU/ml	2.936	3.005	2.983
1500 IU/ml	2.547	2.615	2.589
750 IU/ml	2.350	2.433	2.378
375 IU/ml	1.368	1.452	1.377
188 IU/ml	0.911	1.125	0.968
94 IU/ml	0.522	0.637	0.561
47 IU/ml	0.271	0.338	0.285
23 IU/ml	0.176	0.171	0.115
Negative	0.060	0.055	0.052

In addition the preparation Accurun n° 136 supplied by Boston Biomedica Inc., USA, has been also used to detect the sensitivity of the devise. The preparation was examined on three lots in 4 replicates. Results, expressed as S/Co values, are reported in table below:

ACCURUN # 136	TOXOM.CE Lot # 0703	TOXOM.CE Lot # 0603	TOXOM.CE Lot # 0503
1X	0.808	0.957	0.796
2X	0.389	0.468	0.369
4X	0.169	0.228	0.188
8X	0.065	0.078	0.059
Negative	0.051	0.063	0.044

2. Diagnostic Sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from patients carrying T.gondii acute infection, confirmed by clinical symptoms and analysis.
An overall value > 98% has been found in the study conducted on a total number of more than 60 samples.
The Performance Panel code PTT 201, supplied by Boston Biomedica Inc. USA, has been also evaluated. Data are reported below:

BBI Performance Panel code PTT 201

Sample ID	TOXOM.CE OD450nm	S/Co	REF BioMerieux VIDAS S/Co	Sample ID	TOXOM.CE OD450nm	S/Co	REF BioMerieux VIDAS S/Co
1	0.052	0.1	0.3	14	0.082	0.2	0.2
2	0.048	0.1	0.1	15	0.121	0.3	0.2
3	0.078	0.2	0.1	16	0.049	0.1	0.1
4	0.072	0.2	0.4	17	0.476	**1.4**	**1.5**
5	0.048	0.1	0.1	18	0.057	0.1	0.1
6	0.044	0.1	0.1	19	0.185	0.5	0.2
7	0.045	0.1	0.1	20	0.092	0.2	0.4
8	1.134	**3.5**	**3.5**	21	0.165	0.5	0.1
9	0.126	0.3	0.1	22	0.084	0.2	0.1
10	0.047	0.1	0.1	23	3.181	**9.8**	**10.3**
11	1.232	**3.8**	**2.4**	24	0.137	0.4	0.2
12	0.088	0.2	0.1	25	1.007	**3.1**	**1.8**
13	3.166	**9.8**	**7.3**				

3. Diagnostic Specificity:

The diagnostic specificity has been determined on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
A study conducted on more than 60 potentially cross-reactive samples has not revealed any interference in the system.
No cross reaction were observed.
The Performance Evaluation study conducted in a qualified external reference center on more than 400 total samples has provided a value > 98%.
False positive reactions may be anyway pointed out and then ruled out in the interpretation of results with the procedure reported in section T, able to verify whether or not a positive result is real.

4. Precision:
It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs. Results are reported as follows:

TOXOM.CE: lot # 0703

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.058	0.072	0.076	0.069
Std.Deviation	0.005	0.006	0.007	0.006
CV %	8.9	8.3	9.1	8.7

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.583	0.567	0.579	0.576
Std.Deviation	0.040	0.049	0.056	0.048
CV %	6.8	8.6	9.7	8.4

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.754	2.625	2.625	2.668
Std.Deviation	0.247	0.214	0.126	0.196
CV %	9.0	8.2	4.8	7.3

TOXOM.CE: lot # 0603

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.063	0.064	0.061	0.063
Std.Deviation	0.008	0.012	0.009	0.010
CV %	13.2	18.2	15.3	15.6

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.641	0.651	0.644	0.645
Std.Deviation	0.038	0.042	0.042	0.041
CV %	5.9	6.5	6.6	6.3

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.889	2.830	2.879	2.866
Std.Deviation	0.122	0.123	0.074	0.106
CV %	4.2	4.4	2.6	3.7

TOXOM.CE: lot # 0403

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.057	0.060	0.060	0.059
Std.Deviation	0.006	0.007	0.006	0.007
CV %	11.1	12.4	10.5	11.3

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.544	0.556	0.520	0.540
Std.Deviation	0.040	0.078	0.058	0.058
CV %	7.3	14.0	11.1	10.8

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.850	2.866	2.846	2.854
Std.Deviation	0.139	0.122	0.126	0.129
CV %	4.9	4.3	4.4	4.5

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of Toxoplasma gondii is released to the doctor.
Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section. This reagent is called Solution A.
2. Then 25 ul concentrated Enzymatic Conjugate are diluted in 500 ul Antigen Diluent and mixed gently on vortex. Do not use any lyophilized vial of T.gondii for this procedure ! This solution is called Solution B.
3. The well A1 of the strip is left empty for blanking.
4. The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and S/Co values.
5. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
6. The strip is incubated for 60 min at +37°C.
7. After washing, the blank well A1 is left empty.
8. 100 µl of Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl of Solution B are added to well E1.
10. The strip is incubated for 60 min at +37°C.
11. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
12. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 1.0 a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of T.gondii and a crossreaction with the monoclonal antibody, labeled with HRP, has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value lower than 1.2 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of T.gondii and not due to any crossreaction.

The following table is reported for the interpretation of results:

Well	S/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.2
Interpretation	Problem of contam.	False positive	True positive

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
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CE
0318

Toxo IgM

Ensayo inmunoenzimático (ELISA) de "captura" para la determinación de anticuerpos IgM frente a *Toxoplasma gondii* en plasma y suero humanos

- Uso exclusivo para diagnóstico "in vitro"-

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REF TOXOM.CE
96 pruebas

Toxo IgM

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático (ELISA) para la determinación de anticuerpos IgM frente a *Toxoplasma gondii* en plasma y suero humanos, mediante un sistema de "captura".
El equipo ha sido concebido para el seguimiento de pacientes infectados por *T. gondii* y de la infección durante el embarazo, causa de riesgo de malformaciones en el neonato.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
Toxoplasma gondii es un protozoo, parásito intracelular obligado, que puede infectar probablemente a todas las especies de mamíferos, incluido el hombre.
La detección de anticuerpos IgM frente a *T. gondii* es particularmente útil en el diagnóstico de la infección aguda, ya sea en los individuos "de riesgo", durante el embarazo, en personas sometidas a trasplante de órganos, o en pacientes con SIDA.
Gran parte de las infecciones por *T. gondii* en individuos sanos son leves o asintomáticas. La detección de anticuerpos IgM al mismo, en ausencia de anticuerpos detectables de clase IgG, es de gran importancia en el seguimiento de infecciones agudas durante el embarazo ya que el parásito puede ocasionar severos trastornos en el neonato. Por otra parte, como las infecciones agudas por *T. gondii* son graves en pacientes inmunocomprometidos, deben ser diferenciadas de otros tipos de trastornos.
El sistema ELISA de captura de IgM constituye una prueba diagnóstica potente y segura, sobretodo porque no se ve afectada en presencia del factor reumatoide como ha sucedido en los ensayos clásicos tipo "sandwich".

C. PRINCIPIOS DEL ENSAYO.
El ensayo se basa en el principio de "captura de IgM", donde los anticuerpos de esta clase presentes en la muestra son capturados por la fase sólida recubierta con un anticuerpo anti-IgM humana.
Depués del lavado, que elimina el resto de los componentes de la muestra en particular los anticuerpos IgG, se adiciona una preparación inactivada de *T. gondii*, marcado con un anticuerpo monoclonal conjugado con Peroxidasa (HRP), lo cual permite detectar los anticuerpos IgM inmovilizados en la fase sólida.
Posteriormente a la incubación, los pocillos se lavan para eliminar cualquier traza de conjugado en exceso y se añade el substrato cromogénico. En presencia del conjugado el substrato es hidrolizado generándose una señal de color proporcional a la cantidad de anticuerpos IgM a *Toxoplasma gondii* presentes en la muestra.
La Prueba de Confirmación controla la presencia de falsos positivos, lo cual permite a los clínicos una correcta interpretación de los resultados.

D. COMPONENTES.
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
12 tiras de 8 pocillos recubiertos con anticuerpos monoclonales de cabra anti-IgM humana, purificados por afinidad, en presencia de proteinas de bovino.
Las placas están en una bolsa sellada con desecante. Se deben poner las mismas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y conservar entre 2 y 8ºC.

2. Control Negativo: CONTROL -
1x4.0 ml/vial. Listo para el uso. Contiene 1% de plasma humano negativo a IgM-*T. gondii*, 2% de caseína, tampón Tris-citrato 10 mM pH 6.0+/-0.1, 0.1% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como conservantes.
El control negativo es incoloro.

3. Control Positivo: CONTROL +
1x4.0 ml/vial. Listo para el uso. Contiene 1% de plasma humano positivo a IgM-*T. gondii*, 2% de caseína, tampón Tris-citrato 10 mM pH 6.0+/-0.1, 0.1% de Tween 20, y tambien azida sódica 0.09% y ProClin 300 0.045% como conservantes.
El control positivo está codificado con el color verde.

4. Calibrador: CAL …ml
n° 1 vial. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene IgM anti *T. gondii* a 200 O.M.S. IU/ml +/-10% (3er Estándar Internacional de la O.M.S. para *T. gondii* IgG&IgM), contiene además suero fetal bovino, sulfato de gentamicina 0.2 mg/ml y ProClin 300 0.045% como conservantes.
Nota: El volumen necesario para disolver el contenido del frasco varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Antígenos liofilizados de *T.gondii* : AG TOXO
N° 6 viales liofilizados. Contienen *T. gondii* liofilizado, inactivado por radiaciones gamma, diluído en un tampón proteico. Contienen además 2% de proteinas de bovino, tampón Tris HCl 10 mM pH 6.8+/-0.1, sulfato de gentamicina 0.2 mg/ml y ProClin 300 0.045% como conservantes.
Debe disolverse con 1.9 ml de Diluente de Antígeno, según se indica más adelante.

6. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%

7. Conjugado: CONJ 20X
1x0.8 ml/vial. Solución concentrada 20x de un anticuerpo monoclonal anti-*T. gondii*, conjugado con peroxidasa (HPR) diluído en un tampón proteico. Contiene tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, y tambien 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservantes.

8. Diluente de Antígeno: AG DIL
n° 1 vial de 16 ml. Solución tamponada proteica para la preparación del Inmunocomplejo. Contiene tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, además 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservantes. El reactivo está codificado con el color rojo (0.01% de colorante rojo).

9. Diluente de muestras DILSPE
2x60ml. Solución tamponada proteica para la dilución de las muestras. Contiene 2% de caseína, tampón Citrato 10mM a pH 6.8 +/- 0.1, 0.1% de Tween 20, además azida sódica al 0.09% y 0.045% de ProClin 300 como conservantes.
El reactivo está codificado con el color azul (0.01% de colorante azul).

10. Cromógeno/Substrato SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02% así como dimetilsulfóxido 4%.
Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

11. Ácido Sulfúrico: H_2SO_4 0.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Sellador adhesivo, n° 2

13. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (1000µl, 100µl y 10µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (+/-0.5°C tolerancia).
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar los indumentos protectores adecuados de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbicos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contengan precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente y reemplazar el equipo.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas depués de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de vencimiento indicada en el equipo e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 3 meses.
11. Tratar todas las muestras como potencialmente infectivas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infectivos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según la técnica estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben ser identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Se recomienda el uso del código de barras.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante varios meses, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Estudios de estabilidad realizados en equipos en uso no han arrojado pérdida de actividad significativa en un período de hasta 3 meses.

Microplacas:
Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.
Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el

desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Control Negativo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Control Positivo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Calibrador:
Añadir al polvo liofilizado, el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver completamente y después mezclar cuidadosamente con el vórtex antes de usar.
Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada y mezclarse delicadamente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

Inmunocomplejo Antígeno/Anticuerpo :
Proceder cuidadosamente según se indica:
1. Disolver el contenido de un vial liofilizado utilizando 1.9 ml de Diluente de Antígeno. Dejar disolver completamente y después mezclar cuidadosamente con el vórtex.
2. Mezclar el Conjugado concentrado con ayuda del vórtex. Añadir después 0.1 ml del mismo al vial del Ag de *T. gondii* disuelto y mezclar suavemente en el vórtex.

Notas Importantes*:*
1. *Disolver y preparar solamente los viales necesarios para la prueba. El inmunocomplejo obtenido no es estable. Almacenar la solución sobrante en alícuotas a –20°C.*
2. *La preparación del inmunocomplejo debe realizarse **justo antes** de dispensar las muestras y los controles en la placa. Mezclar nuevamente en vórtex justo antes de usar.*

Diluente de muestras :
Listo para el uso. Mezclar bien con un vórtex antes de usar.

Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.
1. Las micropipetas deben estar calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (acohol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periodicamente los residuos de los componentes del equipo.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC de tolerancia) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10nm b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medida de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por

muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.

7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no seancontaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido roturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Disolver el Calibrador como se ha descrito anteriormente y mezclar suavemente.
4. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
5. Dejar los componentes restantes hasta alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37ºC y alimentar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
11. En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

M.1 Ensayo automatizado:

En caso de que el ensayo se realice de manera automatizada con un sistema ELISA, se recomienda programar el equipo para aspirar 1000µl de Diluente de Muestras, y posteriormente 10µl de muestra (factor de dilución 1:101).

La mezcla debe ser dispensada cuidadosamente en un tubo de dilución. Antes de aspirar la muestra siguiente, las agujas deben lavarse debidamente para evitar cualquier contaminación cruzada entre las muestras. Cuando todas las muestras han sido diluídas, programar el equipo para dispensar 100 µl de las mismas en los pocillos correspondientes.

Este procedimiento puede realizarse en dos pasos de dilución de 1:10 cada uno (90 µl de Diluente de Muestras + 10 µl de muestra) en una segunda plataforma de dilución. Programar el equipo para aspirar primeramente 100 µl de Diluente de Muestras, después 10 µl de la primera dilución en la plataforma y finalmente dispensar todo el contenido en los pocillos apropiados de la microplaca.

No diluir el Calibrador ni los controles, ya que están listos para el uso.

Dispensar 100µl de controles/calibrador en los pocillos correspondientes.

Para las operaciones siguientes, consulte las instrucciones que aparecen debajo para el Ensayo Manual.

Es muy importante comprobar que el tiempo entre el dispensado de la primera y la última muestra sea calculado por el instrumento y considerado para los lavados.

M. 2 Ensayo Manual.

1. Diluir las muestras 1:101 dispensando primeramente 10 µl de muestra y después 1 ml de Diluente de Muestras en un tubo de dilución, mezclar bien con vórtex.
2. Poner el número de tiras necesarias en el soporte plástico. Dejar el pocillo A1 vacío para el blanco.
3. Dispensar 100 µl del Control Negativo por triplicado y 100µl de Calibrador por duplicado, después dispensar 100µl del Control Positivo, sencillo, en los respectivos pocillos. No diluir los controles ni el calibrador ya que están listos para el uso!
4. Dispensar 100 µl de las muestras diluídas en los pocillos correspondientes y chequear después que estos pocillos son de color azul y que los controles y el calibrador han sido añadidos.
5. Incubar la microplaca **60 min a +37°C**.

***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el ensayo manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Lavar la microplaca con el lavador automático según se indica (section I.3).
7. Dispensar 100µl del **Inmunocomplejo Antígeno/Anticuerpo** en todos los pocillos, excepto en el A1 y cubrir con el sellador. Compruebe que este reactivo de color rojo hyaa sido añadido en todos los pocillos excepto el A1.

***Nota importante**: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el **Inmunocomplejo Antígeno/Anticuerpo**. Podría producirse contaminación.*

8. Incubar la microplaca **60 min a +37°C**.
9. Lavar la microplaca, de igual forma que en el paso 6.
10. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1. Incubar la microplaca a **temperatura ambiente (18-24°C) por 20 minutos**.

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

11. Dispensar 100µl de Ácido Sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición del ácido cambia el color de los controles positivos y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo), calibrando el instrumento con el pocillo A1 (blanco, obligatorio).

Notas generales importantes:

1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de stop y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo*

3. *El calibrador (CAL) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba. El calibrador (CAL) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

N. ESQUEMA DEL ENSAYO.

Controles&Calibrador Muestras diluídas 1:101	100 µl 100 µl
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Inmunocomplejo	100 µl
2da incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H2O2	100 µl
3ra incubación	**20 min**
Temperatura	t.a.*
Ácido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 2										
B	CN	M 3										
C	CN	M 4										
D	CN	M 5										
E	CAL	M 6										
F	CAL	M 7										
G	CP	M 8										
H	M 1	M9										

Leyenda: BL = Blanco CN = Control Negativo
CAL = Calibrador CP = Control Positivo M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un control de validación sobre los controles y el calibrador cada vez que se usa el equipo, para verificar si el performance del ensayo es el esperado.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	< 0.050 DO450nm
Control Negativo, valor medio (CN)	< 0.150 DO450nm valor después de leer el blanco Coeficiente de variación < 30%
Calibrador	M/Co > 1.5
Control Positivo	> 0.750 DO450nm

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.
En caso contrario, detenga el ensayo y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.050DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control Negativo (CN) > 0.150 DO450nm después de leer el blanco Coefficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido alimentado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Calibrador M/Co < 1.5	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Control Positivo < 0.750 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, después de comprobar, informe al responsable para tomar las medidas pertinentes.

P. CÁLCULO DEL VALOR DE CORTE.

Los resultados de la prueba se calculan a partir de un valor medio de DO450nm del control Negativo (CN), mediante un valor de corte (Co) hallado con la siguiente fórmula:

Valor de corte = CN + 0.250

El valor encontrado en la prueba es utilizado para la interpretación de los resultados, según se describe a continuación.

***Nota Importante**: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS.

La interpretación de los resultados se realiza mediante la relaión entre las DO a 450nm de las muestras (M) y el Valor de corte (Co).
Los resultados se interpretan según la siguiente tabla:

(M/Co)	Interpretación
< 1.0	Negativo
1.0 – 1.2	Equívoco
> 1.2	Positivo

Un resultado negativo indica que el paciente no está padeciendo infección aguda por *T. gondii*.
Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial.
Un resultado positivo es indicativo de infección por *T. gondii*.

A continuación, un ejemplo de los cálculos a realizar:

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Control Negativo: 0.050 – 0.060 – 0.070 DO 450nm
Valor medio: 0.060 DO 450nm
Menor de 0.150 – Válido
Control Positivo: 1.850 DO 450nm
Mayor de 0.750 – Válido
Valor de corte = 0.060+0.250 = 0.310

Calibrador: 0.550 - 0.530 DO 450nm
Valor medio: 0.540 DO 450nm M/Co = 1.7
M/Co Mayor de 1.0 – Válido

Muestra 1: 0.070 DO 450nm
Muestra 2: 1.690 DO 450nm
Muestra 1 M/Co < 1 = negativa
Muestra 2 M/Co > 1.2 = positiva

Notas importantes:
1. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
2. *Debe ponerse particular atención a la interpretación de los resultados ante sospecha de infección primaria por* T. gondii *en el embarazo, debido al riesgo de malformaciones en el neonato.*
3. *Antes de emitir un criterio de positividad, cada muestra positiva debe ser sometida a la Prueba Confirmatoria reportada en la sección T. Mediante la misma es posible descartar cualquier error en la interpretación del resultado analítico producido por una falsa reactividad de la muestra.*
4. *En el monitoreo de infección por* T. gondii *durante el embarazo, se recomienda, antes de tomar cualquier decisión médica preventiva, confirmar cualquier resultado positivo, primero con el procedimiento descrito y después con un sistema de detección de IgM.*
5. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
6. *El diagnóstico de infección debe ser evaluado y comunicado al paciente por un médico calificado.*

R. CARACTERíSTICAS DEL PERFORMANCE.

1. Límite de detección.

Dia.Pro Diagnostic BioProbes s.r.l. ha definido como Gold Standard Interno (IGS) el 3er Estándar Internacional O.M.S. para TOXO IgG (Código TOXG), positivo además para IgM anti *Toxoplasma Gondii*.
En la siguiente tabla se muestran los resultados, para tres lotes del producto, después de una primera dilución en suero negativo y posteriormente en Diluente de Muestras con el objetivo de generar un sistema de diluciones a probar en cuatro réplicas.

Valores DO450nm

O.M.S (IGS) IU/ml	TOXOM.CE Lote # 0703	TOXOM.CE Lote # 0603	TOXOM.CE Lote # 0503
3000 IU/ml	2.936	3.005	2.983
1500 IU/ml	2.547	2.615	2.589
750 IU/ml	2.350	2.433	2.378
375 IU/ml	1.368	1.452	1.377
188 IU/ml	0.911	1.125	0.968
94 IU/ml	0.522	0.637	0.561
47 IU/ml	0.271	0.338	0.285
23 IU/ml	0.176	0.171	0.115
Negativo	0.060	0.055	0.052

Para detectar la sensibilidad del equipo se probó además la preparación Accurun n° 136 suministrada por Boston Biomedical Inc., Estados Unidos. La preparación ha sido examinada en tres lotes en cuatro réplicas. En la siguiente tabla se relacionan los resultados expresados como valores de M/Co:

ACCURUN # 136	TOXOM.CE Lote # 0703	TOXOM.CE Lote # 0603	TOXOM.CE Lote # 0503
1X	0.808	0.957	0.796
2X	0.389	0.468	0.369
4X	0.169	0.228	0.188
8X	0.065	0.078	0.059
Negativo	0.051	0.063	0.044

2. Sensibilidad Diagnóstica

La sensibilidad diagnóstica se ha estudiado utilizando paneles de muestras, clasificadas como positivas mediante un equipo de referencia US FDA.
Las muestras positivas se obtuvieron de pacientes con infección aguda por *T. gondii*, confirmada mediante análisis clínicos y la observación de los síntomas.
El valor del análisis obtenido después del estudio de más de 60 muestras, fue > 98%.
Se evaluaron además el Performance Panel PTT 201, suministrado por BBI, Estados Unidos.
Los valores se muestran a continuación.

BBI Performance Panel PTT 201

Muestra ID	TOXOM.CE DO450nm	M/Co	REF BioMerieux VIDAS M/Co	Muestra ID	TOXOM.CE DO450nm	M/Co	REF BioMerieux VIDAS M/Co
1	0.052	0.1	0.3	14	0.082	0.2	0.2
2	0.048	0.1	0.1	15	0.121	0.3	0.2
3	0.078	0.2	0.1	16	0.049	0.1	0.1
4	0.072	0.2	0.4	17	0.476	**1.4**	**1.5**
5	0.048	0.1	0.1	18	0.057	0.1	0.1
6	0.044	0.1	0.1	19	0.185	0.5	0.2
7	0.045	0.1	0.1	20	0.092	0.2	0.4
8	1.134	**3.5**	**3.5**	21	0.165	0.5	0.1
9	0.126	0.3	0.1	22	0.084	0.2	0.1
10	0.047	0.1	0.1	23	3.181	**9.8**	**10.3**
11	1.232	**3.8**	**2.4**	24	0.137	0.4	0.2
12	0.088	0.2	0.1	25	1.007	**3.1**	**1.8**
13	3.166	**9.8**	**7.3**				

3. Especificidad Diagnóstica:

La especificidad diagnóstica ha sido determinada utilizando paneles de más de 300 muestras provenientes de individuos sanos de origen europeo, clasificadas como negativas mediante un equipo de referencia.

Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos para determinar la especificidad. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

Las muestras congeladas han sido probadas para comprobar si la colección y la concervacion interfiere con el procedimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.

Un estudio realizado con más de 60 muestras que pudieran presentar potencialmente reactividad cruzada, no reveló interferencia alguna en el sistema. No se detectó reacción cruzada.

El estudio para evaluar el performance, realizado en un centro de referencia externo con más de 400 muestras totales, reveló un valor > 98%.

El procedimiento reportado en la sección T permite detectar y descartar los falsos positivos en la interpretación de los resultados y por tanto verificar si un resultado positivo es real.

La Prueba de Confirmación es un sistema que permite estimar, con un 100% de confiabilidad, la especificidad de una prueba (ya que en ausencia de un antígeno específico, un resultado positivo no es posible).

4. Precisión:

Ha sido calculada a partir de tres muestras, una negativa, una debilmente positiva y una positiva, examinadas en 16 réplicas en tres series separadas.

Los resultados son los siguientes:

TOXOM.CE: lote # 0703

Negativa (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.058	0.072	0.076	0.069
Desviación estándar	0.005	0.006	0.007	0.006
CV %	8.9	8.3	9.1	8.7

Débil reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.583	0.567	0.579	0.576
Desviación estándar	0.040	0.049	0.056	0.048
CV %	6.8	8.6	9.7	8.4

Altamente reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	2.754	2.625	2.625	2.668
Desviación estándar	0.247	0.214	0.126	0.196
CV %	9.0	8.2	4.8	7.3

TOXOM.CE: lote # 0603

Negativa (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.063	0.064	0.061	0.063
Desviación estándar	0.008	0.012	0.009	0.010
CV %	13.2	18.2	15.3	15.6

Débil reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.641	0.651	0.644	0.645
Desviación estándar	0.038	0.042	0.042	0.041
CV %	5.9	6.5	6.6	6.3

Altamente reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	2.889	2.830	2.879	2.866
Desviación estándar	0.122	0.123	0.074	0.106
CV %	4.2	4.4	2.6	3.7

TOXOM.CE: lote # 0403

Negativa (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.057	0.060	0.060	0.059
Desviación estándar	0.006	0.007	0.006	0.007
CV %	11.1	12.4	10.5	11.3

Débil reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.544	0.556	0.520	0.540
Desviación estándar	0.040	0.078	0.058	0.058
CV %	7.3	14.0	11.1	10.8

Altamente reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	2.850	2.866	2.846	2.854
Desviación estándar	0.139	0.122	0.126	0.129
CV %	4.9	4.3	4.4	4.5

S. LIMITACIONES.

La contaminación bacterica o la inactivación por calor de la muestra pueden afectar los valores de DO y por tanto alterar los niveles del analito.

Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.

El ensayo es útil solo para probar muestras independientes y no mezclas.

El diagnóstico de una enfermedad infecciosa no debe establecerse en base a un solo resultado, sino que deben tenerse en consideración la historia clínica del paciente, la sintomatología, así como otros datos diagnósticos.

T. PRUEBA DE CONFIRMACIÓN.

Se realiza esta prueba con el propósito de garantizar la mayor precision del ensayo en el seguimiento del embarazo, donde un resultado falso positivo puede conducir a un aborto. La misma debe realizarse a cada una de las muestras positivas, antes de emitir un diagnóstico de infección por *Toxoplasma gondii*.

Proceder para la confirmación como sigue:

1. Preparar el Inmunocomplejo Antígeno/Anticuerpo según se describe en la sección. Este reactivo se denomina Solución A.
2. Diluir 25 µl del Conjugado concentrado en 500 µl de Diluente de Antígeno, mezclar sdelicadamente con ayuda del vórtex. No usar para este procedimiento ningún vial liofilizado de *T. gondii*! Este reactivo se denomina Solución B.
3. Dejar vacío el pocillo A1 para el blanco.

4. Dispensar el Control Negativo en las posiciones B1+C1, el mismo se usa para calcular el valor de corte y los valores M/Co.
5. La muestra positiva a confirmar, diluída 1:101, se añade en las posiciones D1+E1.
6. Incubar la tira durante 60 min a +37°C.
7. después del lavado el pocillo A1 queda vacío.
8. Dispensar 100 µl de la Solución A en los pocillos B1+C1+D1.
9. Dispensar 100 µl de la Solución B en el pocillo E1.
10. Incubar la tira durante 60 min a +37°C.
11. Después del lavado, adicionar a todos los pocillos 100 µl del Cromógeno/Substrato, posteriormente incubar la tira durante 20 minutos a t.a.
12. Dispensar 100 µl de Ácido sulfúrico en todos los pocillos, medir después la intensidad del color utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

La interpretación de los resultados se realiza de la siguiente forma:

1. Si la muestra en posición D1 tiene un valor de M/Co menor de 1.0, probablemente en el primer ensayo haya ocurrido un error en el dispensado o alguna contaminación. Debe repetirse el Procedimiento del Ensayo, sección M.
2. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en posición E1 el valor de M/Co es todavía mayor de 1.2, la muestra se considera un **falso positivo**. La reactividad de la muestra, en este caso, no depende de la presencia específica de *T.gondii*, por lo tanto ha ocurrido una reacción cruzada con el anticuerpo monoclonal conjugado con HRP.
3. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en la posición E1 el valor M/Co es menor de 1.2 se considera **realmente positiva**. La reactividad de la muestra, en este caso se debe a la presencia específica del protozoo y no a reacciones cruzadas.

En la siguiente tabla se muestra la interpretación de los resultados:

Pocillo	**M/Co**		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.2
Interpretación	Probl. de contam.	Falso positivo	Realmente positivo

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
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ВЕКТОР

БЕСТ

Набор реагентов для иммуноферментного выявления иммуноглобулинов класса А к антигенам *Ureaplasma urealyticum*

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 23.10.2009
Приказом Росздравнадзора № 8459-Пр/09

Ureaplasma urealyticum – IgA – ИФА – БЕСТ

НАБОР РЕАГЕНТОВ
D-2258

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов предназначен для выявления иммуноглобулинов класса А (IgA) к антигенам *Ureaplasma urealyticum* в сыворотке *(плазме)* крови человека и может быть использован в клинических и эпидемиологических исследованиях.

1.2. Набор реагентов рассчитан на проведение 96 анализов, включая контроли. Возможны 12 независимых постановок ИФА, при каждой из которых 3 лунки используют для постановки контролей.

2. ХАРАКТЕРИСТИКИ НАБОРА

2.1. Принцип действия.

Метод определения основан на твёрдофазном иммуноферментном анализе с применением рекомбинантных антигенов. Во время первой инкубации, при наличии в исследуемых образцах иммуноглобулинов класса А к антигенам *Ureaplasma urealyticum,* происходит их связывание с иммобилизованными на поверхности лунок планшета рекомбинантными антигенами *Ureaplasma urealyticum*. Не связавшийся материал удаляют отмывкой.

На второй стадии антитела к IgA человека, меченные пероксидазой хрена *(конъюгат)*, свя-

зываются с комплексом «антиген-антитело». Не связавшийся конъюгат удаляют отмывкой.

Во время третьей инкубации с раствором тетраметилбензидина происходит окрашивание раствора в лунках, содержащих комплексы «антиген-антитело».

Реакцию останавливают добавлением стоп-реагента. Результаты ИФА регистрируют с помощью спектрофотометра, измеряя **оптическую плотность (ОП)** в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допустима регистрация результатов только с фильтром 450 нм. Интенсивность жёлтого окрашивания пропорциональна количеству содержащихся в исследуемом образце иммуноглобулинов класса А к антигенам *Ureaplasma urealyticum*.

После измерения ОП раствора в лунках на основании рассчитанного значения $ОП_{крит}$ анализируемые образцы оцениваются как положительные, сомнительные или отрицательные.

2.2. Состав набора:

Набор содержит все необходимые для проведения анализа реагенты, кроме дистиллированной воды:

- планшет разборный с иммобилизованными рекомбинантными антигенами *Ureaplasma urealyticum* – 1 шт.;

- положительный контрольный образец ($К^+$), инактивированный – 1 фл., 0,5 мл;
- отрицательный контрольный образец ($К^-$), инактивированный – 1 фл., 1 мл;
- конъюгат – 1 фл.;
- раствор для предварительного разведения (РПР) – 1 фл., 3 мл;
- раствор для разведения конъюгата (РК) – 1 фл., 13 мл.
- раствор для разведения сывороток (РС) – 1 фл., 13 мл;
- концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Тх25) – 1 фл., 28 мл;
- раствор тетраметилбензидина (ТМБ) – 1 фл., 13 мл;
- стоп-реагент – 1 фл., 12 мл;

Набор дополнительно комплектуется:

- плёнками для заклеивания планшета – 3 шт.;
- ванночками для реагентов – 2 шт.;
- наконечниками для пипеток на 4–200 мкл – 16 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Результат качественного определения набором иммуноглобулинов класса А к антигенам *Ureaplasma urealyticum* должен соответствовать требованиям СПП *(рег. № 05-2-202 от 27.03.08)*, включающей образцы сывороток, содержащие специфические IgA к антигенам *Ureaplasma urealyticum*: **чувствительность**

по иммуноглобулинам класса А к антигенам *Ureaplasma urealyticum* – 100%.

3.2. Результат качественного определения набором иммуноглобулинов класса А к антигенам *Ureaplasma urealyticum* должен соответствовать требованиям СПП *(рег. № 05-2-202 от 27.03.08),* включающей образцы сывороток, не содержащие IgA к антигенам *Ureaplasma urealyticum*: **специфичность** по иммуноглобулинам класса А к антигенам *Ureaplasma urealyticum* – 100%.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а *(ГОСТ Р 51609-2000)*.

При подготовке к проведению анализа следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом:

– работать в резиновых перчатках;
– не пипетировать растворы ртом;
– все использованные материалы дезинфицировать в соответствии с требованиями с СП 1.3.2322-08 и МУ-287-113.

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ:

– спектрофотометр, позволяющий проводить измерения ОП растворов в лунках планшета при длине волны 450 нм и/или в двухволновом режиме при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–650 нм;
– термостат, поддерживающий температуру (37±1) °С;
– холодильник бытовой;
– пипетки полуавтоматические одноканальные с переменным или фиксированным объёмом со сменными наконечниками, позволяющие отбирать объёмы жидкости от 5 до 1000 мкл;
– пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объёмы жидкостей от 5 до 300 мкл;
– промывочное устройство для планшета;
– перчатки резиновые хирургические;
– бумага фильтровальная лабораторная;
– цилиндр вместимостью 1000 мл;
– вода дистиллированная;
– дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

Допускается использование образцов, хранившихся не более 5 суток при (2–8) °С, либо при минус (20±3) °С, если необходимо более длительное хранение.

Сыворотки, содержащие взвешенные частицы, могут дать неправильный результат. Такие образцы перед использованием следует центрифугировать при 3000 об/мин 10–15 минут.

Нельзя использовать проросшие, гемолизированные, гиперлипидные сыворотки или подвергавшиеся многократному замораживанию и оттаиванию.

7. ПРОВЕДЕНИЕ ИММУНОФЕРМЕНТНОГО АНАЛИЗА

7.1. **ВНИМАНИЕ! Тщательное соблюдение описанных ниже требований позволит избежать искажения результатов ИФА.**

– Перед постановкой реакции все компоненты набора необходимо выдержать при температуре (18–25) °С не менее 30 минут.

– Для приготовления растворов и проведения ИФА следует использовать чистую мерную посуду и автоматические пипетки с погрешностью измерения объёмов не более 5%.

– Лиофилизированные компоненты должны

быть восстановлены, как минимум, за 15 минут до их использования.

– После отбора необходимого количества стрипов оставшиеся сразу упаковать в пакет с осушителем. Упакованные стрипы, плотно закрытые флаконы с исходными компонентами хранить при (2–8) °С.
– Раствор конъюгата в рабочем разведении готовить непосредственно перед использованием.
– Раствор ТМБ готов для использования. Необходимо исключить воздействие прямого света на раствор ТМБ.
– При промывке лунки *(стрипа, планшета)* заполнять полностью, не допуская переливания промывочного раствора через края лунок, и не касаясь лунок наконечником пипетки. Время между заполнением и опорожнением лунок должно быть не менее 30 секунд.
– При использовании автоматического или ручного промывателя необходимо следить за состоянием ёмкости для промывочного раствора и соединительных шлангов: в них не должно быть «заростов». Раз в неделю желательно емкость для промывочного раствора и шланги промывать 70% спиртом.
– Не допускать высыхания лунок планшета между отдельными операциями.

– При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов *(ФСБ-Т×25, раствор ТМБ, стоп-реагент)*, которые взаимозаменяемы в наборах АО «Вектор-Бест».

– При приготовлении растворов и проведении ИФА следует использовать **одноразовые** наконечники для дозаторов.

– Посуду *(ванночки)*, используемые для работы с растворами конъюгата и ТМБ, не обрабатывать дезинфицирующими растворами и моющими средствами.

– В случае повторного использования посуду *(ванночки)* для раствора конъюгата промыть проточной водой и тщательно ополоснуть дистиллированной водой, посуду *(ванночки)* для раствора ТМБ сразу после работы необходимо промыть 50% раствором этилового спирта, а затем дистиллированной водой.

– Для дезинфекции посуды и материалов, контактирующих с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и

хлор, например, комбинированные средства на основе ЧАС *(четвертичных аммониевых соединений)*, спиртов, третичных аминов.

– Пипетки и рабочие поверхности обрабатывать только 70% раствором этилового спирта. Не использовать перекись водорода, хлорамин и т.д.

7.2. Приготовление реагентов.

7.2.1. Промывочный раствор.

Взболтать содержимое флакона с ФСБ-Т×25. При выпадении осадка солей в концентрате прогреть его перед разведением до полного растворения осадка.

В соответствии с числом используемых стрипов отобрать необходимое количество ФСБ-Т×25 *(см. таблицу)* и развести дистиллированной водой до указанного в таблице объёма или содержимое 1 флакона – до **700 мл**.

Хранение: при температуре (2–8) °С до 72 часов.

7.2.2. Контрольные образцы.

Контрольные образцы ($К^+$ и $К^-$) готовы к использованию.

Хранение: при температуре (2–8) °С в течение всего срока годности набора.

Таблица расхода реагентов

	Количество используемых стрипов											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Промывочный раствор												
ФСБ-Т×25, мл	2	4	6	8	10	12	14	16	18	20	22	24
Дистиллированная вода, мл	до 50	до 100	до 150	до 200	до 250	до 300	до 350	до 400	до 450	до 500	до 550	до 600
Раствор конъюгата в рабочем разведении												
Конъюгат (концентрат), мкл	α*	2×α	3×α	4×α	5×α	6×α	7×α	8×α	9×α	10×α	11×α	12×α
РК, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0
Раствор ТМБ												
Раствор ТМБ, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0

α = ▲▲▲ **мкл**

7.2.3. Растворы конъюгата.

Внимание! *Для работы с конъюгатом рекомендуем использовать одноразовые наконечники для пипеток.*

Приготовить концентрированный раствор конъюгата путём растворения содержимого флакона с конъюгатом в **1,0 мл РПР**.

Хранение: концентрированный раствор конъюгата при температуре (2–8) °С до 1 месяца.

Внимание! *Раствор конъюгата в рабочем разведении готовить в пластиковой ванночке, входящей в состав набора, непосредственно перед использованием!*

Перед приготовлением раствора конъюгата в рабочем разведении необходимо аккуратно перемешать, не допуская вспенивания, содержимое флаконов с концентратом конъюгата и с РК.

В пластиковую ванночку отобрать необходимое количество *(см. таблицу)* концентрированного раствора конъюгата, добавить соответствующее количество РК и аккуратно перемешать пипетированием до получения равномерного окрашивания.

7.2.4. Раствор ТМБ.

Внимание! *Раствор ТМБ готов к применению.*

Необходимо исключить воздействие света на раствор ТМБ.

В пластиковую ванночку отобрать только необходимое в соответствии с числом используемых стрипов количество раствора ТМБ *(см. таблицу)*. Остатки раствора ТМБ из ванночки утилизировать *(не сливать во флакон с исходным раствором ТМБ)*.

7.3. Проведение анализа

7.3.1. Подготовить необходимое количество стрипов к работе. Оставшиеся – сразу упаковать во избежание губительного воздействия влаги. Для этого стрипы поместить в цефленовый пакет с влагопоглотителем, тщательно закрыть пакет пластиковой застёжкой. Упакованные таким образом стрипы хранить при (2–8) °С до конца срока годности набора.

Приготовить промывочный раствор *(п. 7.2.1)*, концентрированный раствор конъюгата *(п. 7.2.3)*.

Внимание! *Концентрированный раствор конъюгата должен быть приготовлен, как минимум, за 15 минут до постановки ИФА и выдержан при температуре (18–25) °С.*

7.3.2. Перед постановкой ИФА лунки стрипов промыть один раз промывочным раствором, заливая в каждую лунку по 400 мкл промывочного раствора. По истечении 5 минут

раствор аккуратно удалить в сосуд с дезинфицирующим раствором.

По окончании промывки необходимо тщательно удалить влагу из лунок, постукивая перевёрнутыми стрипами по сложенной в несколько слоёв фильтровальной бумаге. Не допускать высыхания лунок стрипов между отдельными операциями при постановке реакции.

7.3.3. Во все лунки стрипов внести по **80 мкл РС**. В одну лунку внести **20 мкл К$^{+}$**, в две другие лунки по **20 мкл К$^{-}$**, в остальные лунки – по **20 мкл исследуемых образцов**, получая таким образом, разведение 1:5. Внесение образцов должно сопровождаться аккуратным перемешиванием *(пипетирование не менее 4 раз)*. Не допускать вспенивания и касания наконечником дна и стенок лунки.

Лунки заклеить плёнкой и инкубировать при температуре (37±1) °С **30 минут**.

За 5 минут до окончания инкубации приготовить раствор конъюгата в рабочем разведении.

7.3.4. По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором, промыть лунки стрипов 5 раз промывочным раствором и тщательно удалить влагу.

Внимание! *Каждую лунку при промывке необходимо заполнять полностью (**400 мкл***

***промывочного раствора**). Необходимо добиваться полного опорожнения лунок после каждого их заполнения. Время между заполнением и опорожнением лунок должно быть не менее 30 секунд.*

7.3.5. Во все лунки планшета внести по **100 мкл раствора конъюгата в рабочем разведении.**

Внимание! *Для внесения раствора конъюгата использовать пластиковую ванночку и одноразовые наконечники, входящие в состав набора.*

Заклеить лунки плёнкой и инкубировать при температуре (37±1) °С **30 минут**.

По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором, лунки промыть 5 раз промывочным раствором и удалить влагу, как описано выше.

7.3.6. Во все лунки внести по **100 мкл раствора ТМБ.**

Внимание! *Для внесения раствора ТМБ использовать пластиковую ванночку и одноразовые наконечники, входящие в состав набора.*

Стрипы поместить в защищённое от света место при температуре (18–25) °С на **30 минут**.

7.3.7. Остановить реакцию добавлением в каждую лунку по **100 мкл стоп-реагента** и через 2–3 минуты измерить ОП.

Следует избегать попадания стоп-реагента на одежду и открытые участки тела. При попадании – промыть большим количеством воды.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Результаты ИФА регистрировать с помощью спектрофотометра, измеряя ОП в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допускается регистрация результатов только с фильтром 450 нм.

Выведение спектрофотометра на нулевой уровень *(«бланк»)* осуществлять по воздуху.

9. УЧЁТ РЕЗУЛЬТАТОВ АНАЛИЗА

9.1. Результаты исследований учитывать только при соблюдении следующих условий:

– среднее значение ОП в лунках с $К^-$ не более 0,25 ($ОП_{ср}К^- \leq 0{,}25$);

– значение ОП в лунке с $К^+$ не менее 0,6 ($ОПК^+ \geq 0{,}60$).

Вычислить **критическое значение ОП ($ОП_{крит}$)** по формуле:

$$\mathbf{ОП_{крит} = ОП_{ср}(К^-) + 0{,}25},$$

где $ОП_{ср}К^{-}$ – среднее значение ОП для отрицательного контрольного образца.

Исследуемый образец оценить как:

– **отрицательный**, т.е. не содержащий IgA к антигенам *Ureaplasma urealyticum*, если полученное для него значение $ОП_{обр} \leq ОП_{крит} - 0{,}05$;

– **положительный**, т.е. содержащий IgA к антигенам *Ureaplasma urealyticum*, если значение $ОП_{обр} \geq ОП_{крит} + 0{,}05$;

– сомнительный, если $ОП_{крит} - 0{,}05 < ОП_{обр} < ОП_{крит} + 0{,}05$.

Пациентам с сомнительными и положительными результатами рекомендуется дополнительное обследование *(выявление возбудителя, обследование парных сывороток).* Все клинические и лабораторные данные должны быть рассмотрены в совокупности.

10. УСЛОВИЯ ХРАНЕНИЯ И ЭКСПЛУАТАЦИИ НАБОРА

10.1. Транспортирование набора должно проводиться при температуре (2–8) °С. Допускается транспортирование при температуре до 25 °С не более 10 суток. Замораживание не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя должно производиться при температуре (2–8) °С. Замораживание не допускается.

10.3. Срок годности набора реагентов – 12 месяцев со дня выпуска.

По вопросам, касающимся качества набора, обращаться в ***АО «Вектор-Бест»*** *по адресу:*

630559, Новосибирская область, Новосибирский район, п. Кольцово, а/я 121;
тел.: (383) 332-92-49, 227-60-30;
тел./факс: (383) 332-94-47, 332-94-44;
E-mail: plkobtk@vector-best.ru

и в ***Институт стандартизации и контроля лекарственных средств ФГУ «НЦ ЭСМП» Росздравнадзора*** *по адресу: 117246, Москва, Научный проезд, д. 14А, тел. (495) 120-60-95; 120-60-96.*

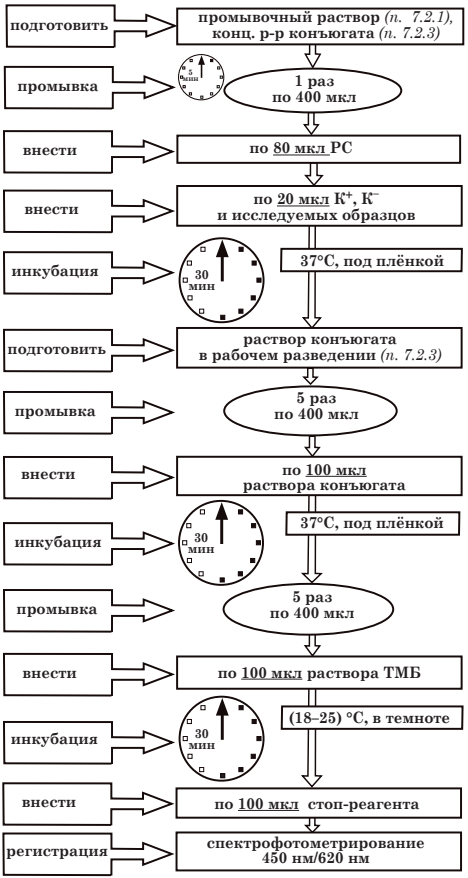
ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ:

– Набор реагентов предназначен для профессионального применения и должен использоваться обученным персоналом;

– При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются *(утилизируются)* в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов следует проводить по МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения»;

– Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007;
– Не применять набор реагентов по назначению после окончания срока годности;
– Транспортирование должно проводиться всеми видами крытого транспорта в соответствии с правилами перевозок, действующими на транспорте данного вида.
– Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации;
– Безопасность и качество изделия гарантируются в течение всего срока годности;
– Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.
– Производитель обязуется за свой счёт заменить изделие, технические и функциональные характеристики *(потребительские свойства)* которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

Схема анализа D-2258



ГРАФИЧЕСКИЕ СИМВОЛЫ

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ/n	Содержимого достаточно для проведения n количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Дата изготовления: XXXX-XX-XX Формат даты: год-месяц-число		Изготовитель
	Использовать до: XXXX-XX-XX Формат даты: год-месяц-число		Обратитесь к Инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению		

Консультацию специалиста по работе с набором можно получить по тел.: (383) 332-81-44.

20.04.16

АКЦИОНЕРНОЕ ОБЩЕСТВО
«ВЕКТОР-БЕСТ»

Международный сертификат
ISO 13485

Наш адрес: 630117, Новосибирск-117, а/я 492

Тел.: (383) 332-37-58, 332-37-10, 332-36-34,
332-67-49, 332-67-52

Тел./факс: (383) 227-73-60 (многоканальный)

E-mail: vbmarket@vector-best.ru
Internet: www.vector-best.ru

ВЕКТОР

БЕСТ

Набор реагентов для иммуноферментного выявления иммуноглобулинов класса G к антигенам *Ureaplasma urealyticum*

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 23.10.2009
Приказом Росздравнадзора № 8458-Пр/09

Ureaplasma urealyticum – IgG – ИФА – БЕСТ

НАБОР РЕАГЕНТОВ
D-2254

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов предназначен для выявления иммуноглобулинов класса G (IgG) к антигенам *Ureaplasma urealyticum* в сыворотке (плазме) крови человека и может быть использован в клинических и эпидемиологических исследованиях.

1.2. Набор реагентов рассчитан на проведение 96 анализов, включая контрольные образцы. Возможны 12 независимых постановок ИФА, при каждой из которых 3 лунки используют для постановки контролей.

2. ХАРАКТЕРИСТИКИ НАБОРА

2.1. Принцип действия.

Метод определения основан на твёрдофазном иммуноферментном анализе с применением рекомбинантных антигенов. Во время первой инкубации, при наличии в исследуемых образцах иммуноглобулинов класса G к антигенам *Ureaplasma urealyticum*, происходит их связывание с иммобилизованными на поверхности *лунок планшета рекомбинантными антигенами Ureaplasma urealyticum*. Не связавшийся материал удаляют отмывкой.

На второй стадии антитела к IgG человека, меченные пероксидазой хрена *(конъюгат),* свя-

зываются с комплексом «антиген-антитело». Не связавшийся конъюгат удаляют отмывкой.

Во время третьей инкубации с раствором тетраметилбензидина происходит окрашивание раствора в лунках, содержащих комплексы «антиген-антитело».

Реакцию останавливают добавлением стоп-реагента. Результаты ИФА регистрируют с помощью спектрофотометра, измеряя **оптическую плотность (ОП)** в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допустима регистрация результатов только с фильтром 450 нм. Интенсивность жёлтого окрашивания пропорциональна количеству содержащихся в исследуемом образце иммуноглобулинов класса G к антигенам *Ureaplasma urealyticum*.

После измерения ОП раствора в лунках на основании рассчитанного значения $ОП_{крит}$ анализируемые образцы оцениваются как положительные, сомнительные или отрицательные.

2.2. Состав набора.

Набор содержит все необходимые для проведения анализа реагенты, кроме дистиллированной воды:

- планшет разборный с иммобилизованными рекомбинантными антигенами *Ureaplasma urealyticum* – 1 шт.;
- положительный контрольный образец ($К^+$), инактивированный – 1 фл., 0,5 мл;
- отрицательный контрольный образец ($К^-$), инактивированный – 1 фл., 1 мл;
- конъюгат – 1 фл.;
- раствор для предварительного разведения (РПР) – 1 фл., 3 мл;
- раствор для разведения конъюгата (РК) – 1 фл., 13 мл.
- разводящий буфер для сывороток (РБС) – 1 фл., 13 мл;
- концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Тх25) – 1 фл., 28 мл;
- раствор тетраметилбензидина (ТМБ) – 1 фл., 13 мл;
- стоп-реагент – 1 фл., 12 мл;

Набор дополнительно комплектуется:

- плёнками для заклеивания планшета – 3 шт.;
- ванночками для реагентов – 2 шт.;
- наконечниками для пипеток на 4–200 мкл – 16 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Результат качественного определения набором иммуноглобулинов класса G к антигенам *Ureaplasma urealyticum* должен соответствовать требованиям СПП *(рег. № 05-2-107 от 29.05.08)*, включающей образцы сывороток, содержащие специфические IgG к антигенам *Ureaplasma urealyticum:* **чувствительность** по иммуноглобулинам класса G к антигенам *Ureaplasma urealyticum* – 100%.

3.2. Результат качественного определения набором иммуноглобулинов класса G к антигенам *Ureaplasma urealyticum* должен соответствовать требованиям СПП *(рег. № 05-2-107 от 29.05.08*), включающей образцы сывороток, не содержащие IgG к антигенам *Ureaplasma urealyticum*: **специфичность** по иммуноглобулинам класса G к антигенам *Ureaplasma urealyticum* – 100%.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а *(ГОСТ Р 51609-2000).*

При подготовке к проведению анализа следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом:

* работать в резиновых перчатках;

* не пипетировать растворы ртом;

* все использованные материалы дезинфицировать в соответствии с требованиями с СП 1.3.2322-08 и МУ-287-113.

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ:

– Спектрофотометр, позволяющий проводить измерения оптической плотности растворов в лунках планшета при длине волны 450 нм и/или в двухволновом режиме при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–650 нм;

– термостат, поддерживающий температуру (37±1) °С;

– холодильник бытовой;

– пипетки полуавтоматические одноканальные с переменным или фиксированным объёмом со сменными наконечниками, позволяющие отбирать объёмы жидкости от 5 до 1000 мкл;

– пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объёмы жидкостей от 5 до 300 мкл;

– промывочное устройство для планшета;

– перчатки резиновые хирургические;

– бумага фильтровальная лабораторная;
– цилиндр вместимостью 1000 мл;
– вода дистиллированная;
– дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

Допускается использование образцов, хранившихся при (2–8)°С не более 5 суток, либо при минус (20±3) °С, если необходимо более длительное хранение.

Сыворотки, содержащие взвешенные частицы, могут дать неправильный результат. Такие образцы перед использованием следует центрифугировать при 3000 об/мин 10–15 минут.

Нельзя использовать проросшие, гемолизированные, гиперлипидные сыворотки или подвергавшиеся многократному замораживанию и оттаиванию.

7. ПРОВЕДЕНИЕ ИММУНОФЕРМЕНТНОГО АНАЛИЗА

7.1. **ВНИМАНИЕ! Тщательное соблюдение описанных ниже требований позволит избежать искажения результатов ИФА.**

– Перед постановкой реакции все компоненты набора необходимо выдержать при температуре (18–25) °С не менее 30 минут.

– Для приготовления растворов и проведения ИФА следует использовать чистую мерную посуду и автоматические пипетки с погрешностью измерения объёмов не более 5%.

– Лиофилизированные компоненты должны быть восстановлены, как минимум, за 15 минут до их использования.

– После отбора необходимого количества стрипов оставшиеся сразу упаковать в пакет с осушителем. Упакованные стрипы, плотно закрытые флаконы с исходными компонентами хранить при (2–8) °С.

– Раствор конъюгата в рабочем разведении готовить непосредственно перед использованием.

– Раствор ТМБ готов для использования. Необходимо исключить воздействие прямого света на раствор ТМБ.

– При промывке лунки *(стрипа, планшета)* заполнять полностью, не допуская переливания промывочного раствора через края лунок, и не касаясь лунок наконечником пипетки. Время между заполнением и опорожнением лунок должно быть не менее 30 секунд.
– При использовании автоматического или ручного промывателя необходимо следить за состоянием ёмкости для промывочного раствора и соединительных шлангов: в них не должно быть «заростов». Раз в неделю желательно ёмкость для промывочного раствора и шланги промывать 70% спиртом.
– Не допускать высыхания лунок планшета между отдельными операциями.
– При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов *(ФСБ-Т×25, раствор ТМБ, стоп-реагент),* которые взаимозаменяемы в наборах АО «Вектор-Бест».
– При приготовлении растворов и проведении ИФА следует использовать **одноразовые** наконечники для дозаторов.
– Посуду *(ванночки)*, используемые для работы с растворами конъюгата и ТМБ, не обрабаты-

вать дезинфицирующими растворами и моющими средствами.

– В случае повторного использования посуду *(ванночки)* для раствора конъюгата промыть проточной водой и тщательно ополоснуть дистиллированной водой, посуду *(ванночки)* для раствора ТМБ сразу после работы необходимо промыть 50% раствором этилового спирта, а затем дистиллированной водой.
– Для дезинфекции посуды и материалов, контактирующих с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе ЧАС *(четвертичных аммониевых соединений)*, спиртов, третичных аминов.
– Пипетки и рабочие поверхности обрабатывать только 70% раствором этилового спирта. Не использовать перекись водорода, хлорамин и т.д.

7.2. Приготовление реагентов.

7.2.1. Промывочный раствор.

Взболтать содержимое флакона с ФСБ-Т×25. При выпадении осадка солей в концентрате прогреть его перед разведением до полного растворения осадка.

Таблица расхода реагентов

	Количество используемых стрипов											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Промывочный раствор												
ФСБ-Т×25, мл	2	4	6	8	10	12	14	16	18	20	22	24
Дистиллированная вода, мл	до 50	до 100	до 150	до 200	до 250	до 300	до 350	до 400	до 450	до 500	до 550	до 600
Раствор конъюгата в рабочем разведении												
Конъюгат (концентрат), мкл	α*	2×α	3×α	4×α	5×α	6×α	7×α	8×α	9×α	10×α	11×α	12×α
РК, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0
Раствор ТМБ												
Раствор ТМБ, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0

α = ▲▲▲ **мкл**

В соответствии с числом используемых стрипов отобрать необходимое количество ФСБ-Т×25 *(см. таблицу)* и развести дистиллированной водой до указанного в таблице объёма или содержимое 1 флакона – до **700 мл**.

Хранение: при температуре (2–8) °С 72 часа.

7.2.2. Контрольные образцы.

Контрольные образцы *($К^+$ и $К^-$)* готовы к использованию.

Хранение: при температуре (2–8) °С в течение всего срока годности набора.

7.2.3. Растворы конъюгата.

Внимание! *Для работы с конъюгатом рекомендуем использовать одноразовые наконечники для пипеток.*

Приготовить концентрированный раствор конъюгата путём растворения содержимого флакона с конъюгатом в **1,0 мл РПР.**

Хранение: концентрированный раствор конъюгата – при температуре (2–8) °С до 1 месяца.

Внимание! *Раствор конъюгата в рабочем разведении готовить в пластиковой ванночке, входящей в состав набора, непосредственно перед использованием!*

Перед приготовлением раствора конъюгата в рабочем разведении необходимо аккуратно перемешать, не допуская вспенивания, содержимое флаконов с концентратом конъюгата и с РК.

В пластиковую ванночку отобрать необходимое количество *(см. таблицу)* концентрированного раствора конъюгата, добавить соответствующее количество РК и аккуратно перемешать пипетированием до получения равномерного окрашивания.

7.2.4. Раствор ТМБ.

Внимание! *Раствор ТМБ готов к применению.*

Необходимо исключить воздействие света на раствор ТМБ.

В пластиковую ванночку отобрать только необходимое в соответствии с числом используемых стрипов количество раствора ТМБ *(см. таблицу)*. Остатки раствора ТМБ из ванночки утилизировать *(не сливать во флакон с исходным раствором ТМБ)*.

7.3. Проведение анализа.

7.3.1. Подготовить необходимое количество стрипов к работе. Оставшиеся – сразу упаковать во избежание губительного воздействия влаги. Для этого стрипы поместить в цефленовый пакет с влагопоглотителем, тщательно закрыть

пакет пластиковой застёжкой. *Упакованные таким образом стрипы хранить при (2–8)°С до конца срока годности набора.*

Приготовить промывочный раствор *(п. 7.2.1)*, концентрированный раствор конъюгата *(п. 7.2.3)*.

Внимание! *Концентрированный раствор конъюгата должен быть приготовлен, как минимум, за 15 минут до постановки ИФА и выдержан при температуре (18–25) °С.*

7.3.2. Перед постановкой ИФА лунки стрипов промыть один раз промывочным раствором, заливая в каждую лунку по 400 мкл промывочного раствора. По истечении 5 минут раствор аккуратно удалить в сосуд с дезинфицирующим раствором.

По окончании промывки необходимо тщательно удалить влагу из лунок, постукивая перевернутыми стрипами по сложенной в несколько слоёв фильтровальной бумаге. Не допускать высыхания лунок стрипов между отдельными операциями при постановке реакции.

7.3.3. Во все лунки стрипов внести по **80 мкл РБС**. В одну лунку внести **20 мкл $К^+$,** в две другие лунки по **20 мкл $К^-$**, в остальные лунки – по **20 мкл исследуемых образцов**, получая таким образом, разведение 1:5. Внесение образцов должно сопровождаться аккуратным

перемешиванием *(пипетирование не менее 4 раз)*. Не допускать вспенивания и касания наконечником дна и стенок лунки.

Лунки заклеить плёнкой и инкубировать при температуре (37±1) °С **30 минут**.

За 5 минут до окончания инкубации приготовить раствор конъюгата в рабочем разведении.

7.3.4. По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором, промыть лунки стрипов 5 раз промывочным раствором и тщательно удалить влагу.

Внимание*! Каждую лунку при промывке необходимо заполнять полностью* ***(400 мкл промывочного раствора)****. Необходимо добиваться полного опорожнения лунок после каждого их заполнения. Время между заполнением и опорожнением лунок должно быть не менее 30 секунд.*

7.3.5. Во все лунки планшета внести по **100 мкл раствора конъюгата в рабочем разведении**.

Внимание! *Для внесения раствора конъюгата использовать пластиковую ванночку и одноразовые наконечники, входящие в состав набора.*

Заклеить лунки плёнкой и инкубировать при температуре (37±1) °С **30 минут**.

По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором, лунки промыть 5 раз промывочным раствором и удалить влагу, как описано выше.

7.3.6. Во все лунки внести по **100 мкл раствора ТМБ.**

Внимание! *Для внесения раствора ТМБ использовать пластиковую ванночку и одноразовые наконечники, входящие в состав набора.*

Стрипы поместить в защищённое от света место при температуре (18–25) °С на **30 минут**.

7.3.7. Остановить реакцию добавлением в каждую лунку по **100 мкл стоп-реагента** и через 2–3 минуты измерить ОП.

Следует избегать попадания стоп-реагента на одежду и открытые участки тела. При попадании – промыть большим количеством воды.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Результаты ИФА регистрировать с помощью спектрофотометра, измеряя ОП в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допускается регистрация результатов только с фильтром 450 нм.

Выведение спектрофотометра на нулевой уровень *(«бланк»)* осуществлять по воздуху.

9. УЧЁТ РЕЗУЛЬТАТОВ АНАЛИЗА

9.1. Результаты исследований учитывать только при соблюдении следующих условий:

– среднее значение ОП в лунках с К$^-$ не более 0,25 ($ОП_{ср}К^- \leq 0,25$);

– значение ОП в лунке с К$^+$ не менее 0,6 ($ОПК^+ \geq 0,60$).

Вычислить **критическое значение ОП ($ОП_{крит}$)** по формуле:

$$\mathbf{ОП_{крит} = ОП_{ср}\ К^- + 0,25},$$

где $ОП_{ср}\ К^-$ – среднее значение ОП для К–.

Исследуемый образец оценить как:

– отрицательный, т.е. не содержащий IgG к антигенам *Ureaplasma urealyticum*, если полученное для него значение $ОП_{обр} \leq О\ ОП_{крит} - 0,05$;

– положительный, т.е. содержащий IgG к антигенам *Ureaplasma urealyticum*, если значение $ОП_{обр} \geq ОП_{крит} + 0,05$;

– сомнительный, если ОПкрит – 0,05 < $ОП_{обр} < ОП_{крит} + 0,05$.

9.2. Интерпретация результатов.

ОП сыворотки	Результат	Титр IgG
$\mathbf{ОП_{обр}} \leq ОП_{крит} - 0{,}05$	отрицательный	–
$ОП_{крит} - 0{,}05 < \mathbf{ОП_{обр}} < ОП_{крит} + 0{,}05$	сомнительный	–
$ОП_{крит} + 0{,}05 \leq \mathbf{ОП_{обр}} \leq 1{,}5 \times ОП_{крит}$	слабоположительный	1:5
$1{,}5 \times ОП_{крит} < \mathbf{ОП_{обр}} \leq 2 \times ОП_{крит}$	положительный	1:10
$2 \times ОП_{крит} < \mathbf{ОП_{обр}} \leq 3 \times ОП_{крит}$	сильноположительный	1:20
$3 \times ОП_{крит} < \mathbf{ОП_{обр}} \leq 4 \times ОП_{крит}$	сильноположительный	1:40
$\mathbf{ОП_{обр}} > 4 \times ОП_{крит}$	сильноположительный	1:80

Пациентам с сомнительными и положительными результатами рекомендуется дополнительное обследование (выявление возбудителя, обследование парных сывороток). Все клинические и лабораторные данные должны быть рассмотрены в совокупности.

10. УСЛОВИЯ ХРАНЕНИЯ И ЭКСПЛУАТАЦИИ НАБОРА

10.1. Транспортирование набора должно проводиться при температуре (2–8) °С. Допускается транспортирование при температуре до 25 °С не более 10 суток. Замораживание не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя должно производиться при температуре (2–8) °С. Замораживание не допускается.

10.3. Срок годности набора реагентов – 12 месяцев со дня выпуска.

По вопросам, касающимся качества набора, обращаться в ***АО «Вектор-Бест»*** *по адресу:*
630559, п. Кольцово, Новосибирской обл, Новосибирского района, а/я 121,
тел.: (383) 332-92-49, 227-60-30;
тел./факс: (383), 332-94-47, 332-94-44.;
E-mail: plkobtk@vector-best.ru

и в ***Институт стандартизации и контроля лекарственных средств ФГУ «НЦ ЭСМП» Росздравнадзора*** *по адресу: 117246, Москва, Научный проезд, д.14А, тел.: (495) 120-60-95, 120-60-96.*

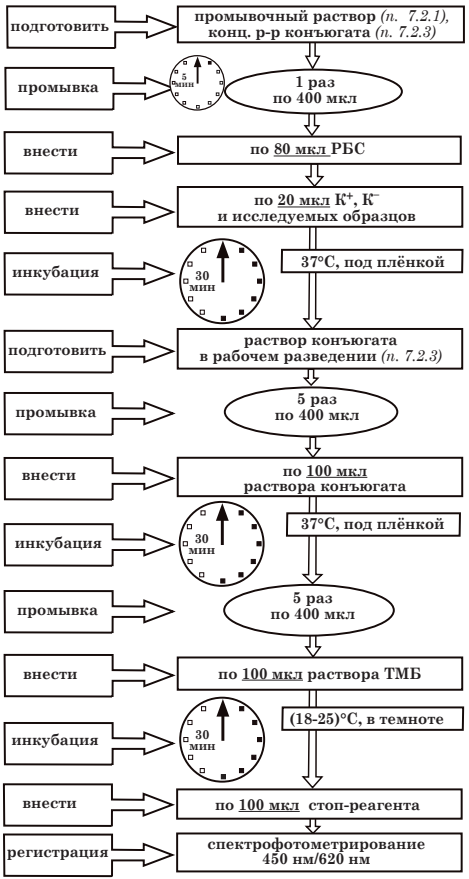
ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ:

– Набор реагентов предназначен для профессионального применения и должен использоваться обученным персоналом;
– При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются *(утилизируются)* в соответствии с

СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов следует проводить по МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения»;

– Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007;
– Не применять набор реагентов по назначению после окончания срока годности;
– Транспортирование должно проводиться всеми видами крытого транспорта в соответствии с правилами перевозок, действующими на транспорте данного вида.
– Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации;
– Безопасность и качество изделия гарантируются в течение всего срока годности;
– Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.
– Производитель обязуется за свой счёт заменить изделие, технические и функциональные характеристики *(потребительские свойства)* которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

Схема анализа D-2254



ГРАФИЧЕСКИЕ СИМВОЛЫ

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Дата изготовления: XXXX-XX-XX Формат даты: год-месяц-число		Изготовитель
	Использовать до: XXXX-XX-XX Формат даты: год-месяц-число		Обратитесь к Инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению		

Консультацию специалиста по работе с набором можно получить по тел.: (383) 332-81-44.

20.04.16

АКЦИОНЕРНОЕ ОБЩЕСТВО
«ВЕКТОР-БЕСТ»

Международный сертификат
ISO 13485

Наш адрес: 630117, Новосибирск-117, а/я 492

Тел.: (383) 332-37-58, 332-37-10, 332-36-34,
332-67-49, 332-67-52

Тел./факс: (383) 227-73-60 (многоканальный)

E-mail: vbmarket@vector-best.ru
Internet: www.vector-best.ru

HBc IgM

"Capture" Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM class antibody to Hepatitis B Virus core Antigen in human plasma and sera

- for "in vitro" diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
Via G. Carducci n° 27
20099 Sesto San Giovanni (Milano) - Italy
Phone +39 02 27007161
Fax +39 02 44386771
e-mail: info@diapro.it

REF BCM.CE
96 Tests

HBc IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM class antibodies to Hepatitis B Virus core Antigen in human plasma and sera with the "capture" system.
The kit is intended for the classification of the viral agent and for the follow-up of chronic patients under therapy.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of Hepatitis B virus (or HBV).
Particles have a size of 27nm and contain a circular double-stranded DNA molecule, a specific DNA-polymerase and HBcAg. HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregation of the core particles ; the antigen contains at least one immunological determinant.
Upon primary infection, anti HBcAg IgM antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, together or slightly later than HBsAg, the viral surface antigen.
Anti HBcAg IgM titers, very high during the acute phase, decrease along the illness, as IgG antibodies appear, down to undetectable levels in convalescent patients.
In chronic hepatitis, however, spikes of anti HBcAg IgM synthesis are present, confirming reactivation of HBV in hepatocites and giving origin to permanent IgM low titers.
The determination of anti HBcAg IgM antibodies has become very important for the fast classification of the virus, of the phase of the illness and for the monitoring of patients under treatment with interferon.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of recombinant HBcAg, labelled with a monoclonal antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of peroxidase the colourless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with purified anti human IgM specific mouse monoclonal antibody, post-coated with bovine serum proteins and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
6x2.0 ml/vial. Ready to use and color coded standard curve calibrated on the HBcIgM reference preparation supplied by Paul Erlich Institute (HBc-Referenzserum-IgM 84), ranging: CAL1 = 0 U/ml // CAL2 = 5 U/ml // CAL3 = 10 U/ml // CAL4 = 20 U/ml // CAL 5 = 50 U/ml // CAL 6 = 100 U/ml.
It contains chemical inactivated HBcIgM positive human plasma, 100 mM Tris buffer pH 7.4+/-0.1, 0.5% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The Calibration Curve is coded with blue alimentary dye.
Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

4. Enzyme Conjugate (Immunocomplex) : CONJ
1x16.0 ml/vial. Ready-to-use solution. Contains an immunocomplex formed by a specific mouse monoclonal antibody, labelled with HRP, and a purified recombinant HBcAg.
The reagent is dissolved into a buffer solution 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives. The component is red colour coded.

5. Specimen Diluent : DILSPE
2x60.0 ml/vial. Buffered solution for the dilution of samples; it contains 100 mM Tris buffer pH 7.4+/-0.1, 0.5% Tween 20, 2% Casein, 0.045% ProClin 300 and 0.09% sodium azide as preservatives. The component is blue color coded.

6. Control Serum : CONTROL …ml
1 vial. Lyophilized. Contains fetal bovine serum, human HBcIgM positive human plasma calibrated at 20 ± 10% PEI U/ml. 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Important Notes
1. The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .
2. Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

7. Chromogen/Substrate : SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Plate sealing foils: n° 2

10. Package insert: n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.

Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.

6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve:

Ready to use. Mix well on vortex before use.

Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200ml and mixed gently end-over-end before use.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

Enzyme conjugate:

Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

Specimen Diluent

Ready to use. Mix on vortex before use.

Control Serum

Dissolve the content of the vial with EIA grade water as reported in the label. Mix well on vortex before use. The dissolved control serum is ready to use.
Note: The control after dissolution is not stable. Store frozen in aliquots at –20°C.

Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of ±5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Control Serum as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two procedures can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples.

2. Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the Calibrators and the dissolved Control Serum as they are ready-to-use.
3. Leave the A1+B1 wells empty for blanking purposes.
4. Pipette 100 µl of the Calibrators in duplicate, 100 µl dissolved Control Serum in duplicate followed by 100 µl of diluted samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators, Control Serum and samples have been correctly added.
5. Incubate the microplate **for 60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

6. When the first incubation is finished, wash the microwells as previously described (section I.3)
7. In all the wells except A1+B1, pipette 100 µl Enzyme Conjugate. Incubate the microplate **for 60 min at +37°C**.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

8. When the second incubation is finished, wash the microwells as previously described (section I.3)
9. Pipette 100 µl Chromogen/Substrate into all the wells, A1+B1 included.

Important note: *Do not expose to strong direct light. as a high background might be generated.*

10. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.
11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to block the enzymatic reaction.. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
12. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M.2 Qualitative analysis

1 Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples.
2 Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the Calibrators as they are ready-to-use.
3 Leave the A1 well empty for blanking purposes.
4 Pipette 100 µl Calibrator 0 U/ml in duplicate, 100 µl Calibrator 10 U/ml in duplicate and 100 µl Calibrator 100 U/ml in single. Then dispense 100 µl diluted samples in proper sample wells. Check that Calibrators and samples have been correctly added.
5 Incubate the microplate **for 60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

6 When the first incubation is finished, wash the microwells as previously described (section I.3)
7 In all the wells except A1, pipette 100 µl Enzyme Conjugate. Incubate the microplate **for 60 min at +37°C**.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

8 When the second incubation is finished, wash the microwells as previously described (section I.3)
9 Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light. as a high background might be generated.*

10 Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.
11 Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to block the enzymatic reaction. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
12 Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management*

N. ASSAY SCHEME

The assay protocol can be summarized in the table below:

Calibrators & diluted samples & dissolved Control Serum	100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	100ul
3rd incubation	**20 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm /620-630nm

An example of dispensation scheme in quantitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S1									
B	BLK	CAL4	S2									
C	CAL1	CAL5	S3									
D	CAL1	CAL5	S4									
E	CAL2	CAL6	S5									
F	CAL2	CAL6	S6									
G	CAL3	CS	S7									
H	CAL3	CS	S8									

Legenda: BLK = Blank // CAL = Calibrators
CS = Control Serum // S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL3	S 6	S 14									
E	CAL3	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

Legenda: BLK = Blank // CAL = Calibrators// S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameter	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 PEI U/ml coefficient of variation	< 0.150 OD450nm after blanking < 30%
Calibrator 5 PEI U/ml	OD450nm > OD450nm Cal 0 U/ml + 5SD and anyway > OD450nm Cal 0 U/ml + 0.100
Calibrator 10 PEI U/ml	OD450nm > OD450nm Cal 0 U/ml + 0.200
Calibrator 100 PEI U/ml	> 1.000 OD450nm
Control Serum	OD450nm = OD450nm of the Calibrator 20 U/ml ± 10%

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 U/ml > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive calibrators instead of Cal 0); 4. that no contamination of the Cal O, or of the wells where this was dispensed, has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator 5 U/ml < CAL 0 + 5SD or < CAL 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 10 U/ml < CAL 0 + 0.200	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 100 U/ml < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the calibrator; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (ex.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

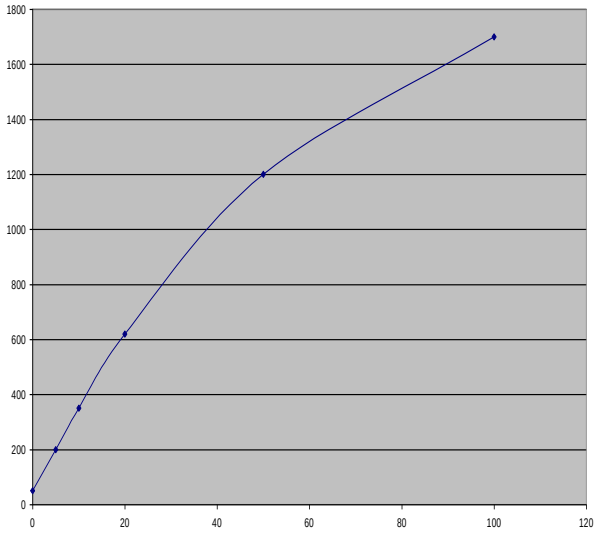
If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 12.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm/620-630nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti HBc IgM antibody in samples.
An example of Calibration curve is reported below.



Important Note: *Do not use this example to make real calculations on samples.*

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm/620-630nm values for the Calibrators 0 and 10 U/ml and then check that the assay is valid.

Example of calculation (data obtained proceeding as the the reading step described in the section M, point 12).

The following data must not be used instead of real figures obtained by the user.

Calibrator 0 U/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 10 U/ml: 0.350 – 0.330 OD450nm
Mean Value: 0.340 OD450nm
Higher than Cal 0 + 0.200 – Accepted
Calibrator 100 U/ml: 2.845 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS

Q.1 Qualitative results
For qualitative interpretations, the medical literature generally considers positive samples showing a concentration of HBc IgM ≥ 10 PEI U/ml.
Test results are therefore interpreted as a ratio of the sample OD450nm and the OD450nm/620-630nm of the Cal 10 PEI U/ml (or S/Co) according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

Q.2 Quantitative results
The calibration curve is used to determine the concentration of IgM antibodies to HBcAg in samples.
Samples with a concentration lower than 5 PEI U/ml are considered negative for HBcIgM.
Samples with a concentration between 5 and 10 PEI U/ml are considered in a gray-zone.
In the follow up of chronic hepatitis, however, values higher of 5 PEI U/ml may be considered positive for HBcIgM, when in presence of other clinical signs.
Samples with a concentration higher than 10 PEI U/ml are considered positive for HBcIgM.

Important general notes:
1. *When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to produce the calibration curve, calculate sample concentration and generate the correct interpretation of results.*
2. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
3. *A positive result is indicative of HBV infection and therefore the patient should be treated accordingly.*
4. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
5. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCES
Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. Limit of detection
The limit of detection of the assay has been calculated by means of :

1.1 the HBcIgM reference preparation supplied by Paul Erlich Institute, Germany (HBc-Referenzserum-IgM 84), on which the Standard Curve has been calibrated.
1.2 Accurun 113 (cat. N° A113-5001) supplied by Boston Biomedica Inc., USA

Results of Quality Control for three lots are given in the following tables:

BCM.CE	Lot #	0103	Lot #	0103/2	Lot #	0303
PEI U/ml	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
100	2.752	**8.9**	2.883	**9,7**	2.911	**9.1**
50	1.917	**6.2**	1.972	**6.7**	2.053	**6.4**
20	0.980	**3.2**	0.914	**3.1**	1.095	**3.4**
10	0.544	**1.8**	0.513	**1.7**	0.592	**1.8**
5	0.310	**1.0**	0.296	**1.0**	0.321	**1.0**
2.5	0.155	0.5	0.149	0.5	0.161	0.5
1.25	0.084	0.3	0.084	0.3	0.093	0.3
negative	0.040		0.035		0.044	

BBI Accurun # 113 lot # 48-9999-0621

BCM.CE	Lot #	0103	Lot #	0103/2	Lot #	0303
BBI 113	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1 x	3.336	**10.8**	3.195	**10.4**	3.269	**10.3**
2 x	2.472	**8.0**	2.385	**7.8**	2.385	**7.5**
4 x	1.467	**4.7**	1.413	**4.6**	1.429	**4.5**
8 x	0.865	**2.8**	0.807	**2.6**	0.856	**2.7**
16 x	0.430	**1.4**	0.427	**1.4**	0.410	**1.3**
32 x	0.234	0.8	0.234	0.8	0.248	0.8
64 x	0.129	0.4	0.133	0.4	0.122	0.4
128 x	0.086	0.3	0.082	0.3	0.089	0.3
negative	0.040		0.040		0.052	

Moreover the BBI's panel # PHE 102 was also examined in three lots of product; data are reported below with reference to a European kit (BBI's results).

BBI – Panel code PHE 102

	Lot # 0103	Lot # 0103/2	Lot # 0303	Sorin EIA
Member	S/Co	S/Co	S/Co	S/Co
01	**6.7**	**6.3**	**6.5**	**2.0**
02	**11.3**	**10.0**	**10.7**	**6.1**
03	**9.5**	**7.2**	**8.4**	**3.0**
04	**5.8**	**3.4**	**4.1**	**2.1**
05	**11.3**	**11.4**	**11.2**	**3.1**
06	**12.1**	**11.6**	**11.8**	**4.1**
07	**0.1**	**0.1**	**0.1**	**0.2**
08	**9.2**	**8.5**	**8.8**	**2.3**
09	**12.2**	**11.7**	**11.9**	**4.2**
10	**11.7**	**10.2**	**10.8**	**2.8**
11	**5.9**	**5.8**	**5.8**	**2.1**
12	**12.7**	**11.4**	**11.7**	**5.2**
13	**11.6**	**11.0**	**11.3**	**3.6**
14	**7.0**	**6.3**	**6.6**	**2.3**
15	**12.4**	**11.5**	**11.8**	**4.5**

2. Diagnostic Sensitivity:

It is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

The diagnostic sensitivity has been tested internally and externally in a qualified Clinical Laboratory on panels of samples classified positive by a US FDA approved kit.

Positive samples were collected from different patients and from different HBV pathologies (acute and chronic hepatitis).

An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.

A Seroconversion panel produced by BBI, USA, code # PHM 935A, havs also been studied; results are reported below with reference to two commercial kits (BBI's results).

BBI Panel PHM 935A

	Lot # 0103	Abbott EIA	DiaSorin EIA
Member #	S/Co	S/Co	S/Co
01	0.2	0.1	0.1
02	0.2	0.1	0.1
03	0.2	0.1	0.1
04	0.1	0.1	0.1
05	0.2	0.1	0.1
06	0.2	0.1	0.1
07	0.2	0.1	0.1
08	0.1	0.1	0.1
09	0.1	0.1	0.1
10	0.1	0.1	0.1
11	0.2	0.1	0.1
12	0.2	0.1	0.1
13	**2.8**	**3.7**	0.7
14	**5.0**	**6.4**	0.9
15	**> 12**	**6.2**	**4.5**
16	**> 12**	**5.6**	**4.5**
17	**> 12**	**5.5**	**4.3**
18	**> 12**	**4.8**	**4.3**
19	**> 12**	**> 6.6**	**4.4**
20	**> 12**	**> 6.6**	**5.2**

3. Diagnostic Specificity:

It is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

The diagnostic specificity has been determined internally and externally in a qualified Clinical Laboratory on panels of negative samples from normal individuals and blood donors, classified negative with a US FDA approved kit.

A total number of more than 400 negative specimens were tested. A diagnostic specificity > 98% has been found.

Moreover, the diagnostic specificity was assessed by testing more than 50 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.).

No interference was observed in the study.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.

4. Precision:

It has been calculated on three samples examined in 16 replicate in three different runs, carried out on three different lots. The values found were as follows:

BCM.CE: lot # 0103

Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.055	0.053	0.051	0.053
Std.Deviation	0.005	0.006	0.005	0.006
CV %	9.9	12.3	10.7	10.9

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.324	0.308	0.321	0.318
Std.Deviation	0.022	0.018	0.024	0.021
CV %	6.8	5.7	7.5	6.7

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.109	2.048	2.052	2.070
Std.Deviation	0.101	0.088	0.136	0.109
CV %	4.8	4.3	6.7	5.2

BCM.CE: lot # 0103/2

Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.057	0.053	0.054	0.055
Std.Deviation	0.005	0.005	0.004	0.004
CV %	8.3	9.0	7.3	8.2

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.332	0.331	0.322	0.328
Std.Deviation	0.017	0.018	0.016	0.017
CV %	5.0	5.5	4.9	5.1

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.311	2.208	2.212	2.244
Std.Deviation	0.110	0.090	0.095	0.098
CV %	4.7	4.1	4.3	4.4

BCM.CE: lot # 0303

Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.043	0.042	0.040	0.042
Std.Deviation	0.004	0.005	0.004	0.004
CV %	10.3	11.1	10.9	10.8

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.320	0.326	0.314	0.320
Std.Deviation	0.023	0.024	0.026	0.024
CV %	7.1	7.4	8.2	7.6

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.150	2.163	2.092	2.135
Std.Deviation	0.057	0.067	0.076	0.067
CV %	2.6	3.1	3.6	3.1

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 12.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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CE
0318

HBc IgM

Ensayo inmunoenzimático de "Captura" (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos clase IgM al Antígeno *core* del virus de la Hepatitis B en plasma y suero humanos

- Uso exclusivo para diagnóstico "in vitro"-

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REF BCM.CE
96 pruebas

HBc IgM

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos clase IgM al Antígeno *core* del virus de la Hepatitis B (HBV) en plasma y suero humanos mediante el sistema de "Captura".
El equipo ha sido diseñado para la clasificación del agente viral y para el seguimiento de pacientes crónicos sometidos a terapia.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
El antígeno *core* del virus de la Hepatitis B (HBcAg) es el elemento principal de las partículas del núcleo del virus.
Las partículas tienen un tamaño de 27nm y contienen una mólecula de ADN circular de doble cadena, una ADN polimerasa específica y HBcAg. El antígeno del *core* está compuesto por un polipéptido simple de 17 kD, el cual es liberado en el proceso de desagregación de la partícula viral. Este antígeno contiene al menos un determinante inmunogénico. Durante la infección primaria, los anticuerpos IgM anti-HBcAg, son unos de los primeros marcadores del HBV que aparecen en el suero, conjuntamente o ligeramente antes de que aparezca el antígeno de superficie (HBsAg).
Los títulos de anticuerpos IgM al HBcAg, bastante altos durante la fase aguda, descienden en el transcurso de la enfermedad hasta alcanzar niveles no detectables en pacientes convalescientes. Sin embargo, en el caso de la hepatitis crónica, se aprecian picos de anticuerpos IgM anti-HBcAg, lo cual confirma la reactivación del virus en los hepatocitos y origina bajos títulos permanentes de IgM.
La determinación de anticuerpos IgM anti-HBcAg es de gran importancia para la rápida clasificación del virus, de las fases de la enfermedad, así como para el seguimiento de pacientes sometidos a tratamiento con interferón.

C. PRINCIPIOS DEL ENSAYO.
El ensayo se basa en el principio de "captura de IgM" donde esta clase de anticuerpos, si están presentes en la muestra, quedan capturados por la fase sólida, recubierta por anticuerpos anti-IgM humanos.
Después del lavado, mediante el cual se eliminan los restantes componentes de la muestra fundamentalmente los anticuerpos IgG, se detectan los anticuerpos IgM unidos a la fase sólida mediante la adición de una preparación de HBcAg recombinante purificada, marcada con un anticuerpo monoclonal conjugado con peroxidasa (HRP).
Después de la incubación y previo lavado, se añade la mezcla cromógeno/substrato, la cual se combina con la enzima conjugada unida a la fase sólida. El substrato es hidrolizado, en presencia de peroxidasa, a un producto coloreado final cuya densidad óptica es detectable y es proporcional a la cantidad de anticuerpos IgM al HBcAg presentes en la muestra.

D. COMPONENTES
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
8x12 tiras de pocillos recubiertos con un anticuerpo monoclonal de ratón anti-IgM humano, post-recubiertos con proteínas del suero bovino y almacenados en bolsas selladas con desecante. Se deben poner las placas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar a 4ºC.

2. Curva de Calibración: CAL N° …
6x2.0 ml/vial. Listo para el uso y curva estándar con código de color, calibrada a partir de una preparación de HBcIgM de referencia, suministrada por el Instituto Paul Erlich (HBc-Referenzserum-IgM 84), con rangos: CAL1 = 0 U/ml // CAL2 = 5 U/ml // CAL3 = 10 U/ml // CAL4 = 20 U/ml // CAL 5 = 50 U/ml // CAL 6 = 100 U/ml. Contiene plasma humano HBcIgM positivo sometido a inactivación química, tampón Tris 100 mM pH 7.4+/- 0.1, 0.5% de Tween 20, así como azida sódica 0.09% y ProClin 300 0.045% como conservantes. La Curva de Calibración está codificada con el color azul.
Nota importante: *Aunque el plasma esté inactivado por métodos químicos, se debe manipular como potencialmente infeccioso.*

3. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10mM a pH 7.0+/- 0.1, Tween 20 al 0.05% y ProClin 300 al 0.045%.

4. Conjugado (Inmunocomplejo) : CONJ
1x16.0 ml/vial. Solución lista para el uso. Contiene un Inmunocomplejo formado por un anticuerpo monoclonal de ratón marcado con HRP y HBcAg recombinante purificado. El reactivo está disuelto en tampón Tris 10 mM pH 6.8+/-0.1, BSA 2%, además de sulfato de gentamicina 0.2 % y ProClin 300 0.045% como conservantes. El reactivo está codificado con el color rojo.

5. Diluente de muestras :DILSPE
2x60.0 ml/vial. Solución tamponada para disolver las muestras. Contiene tampónTris 100 mM pH 7.4 +/- 0.1, 0.5% de Tween 20, caseína al 2%, 0.045% de ProClin 300 y azida sódica al 0.09% como conservantes. El reactivo está codificado con el color azul.

6. Suero Control: CONTROL …ml
1 vial. Liofilizado.
Contiene suero bovino fetal, plasma humano positivo a HBcIgM, concentrado a 20 ±10% PEI U/ml, 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservantes.
Notas importantes:
1. El volumen necesario para disolver el contenido del vial varía en cada lote. Se recomienda usar el volumen correcto reportado en la etiqueta.
2. Aunque el plasma esté inactivado por métodos químicos, se debe manipular como potencialmente infeccioso.

7. Cromógeno/Substrato. SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM pH 3.5-3.8, dimetilsulfóxido 4%, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02%.
Nota: Evitar la exposición a la luz, ya que la substancia es fotosensible.

8. Ácido Sulfúrico: H_2SO_4 O.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Sellador adhesivo, n° 2

10. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (150µl, 100µl y 50µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (tolerancia+/-1°C).
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el equipo e internamente en los reactivos.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben de ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según la técnica estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Evitar la adición de conservantes, especialmente azida sódica ya que puede afectar la actividad enzimática del conjugado, generando resultados falsos negtivos.
3. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados.
4. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
5. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante almenos 12 meses. Evitar congelar/descongelar cada muestra más de una vez, ya que pueden generarse partículas que podrían afectar al resultado de la prueba.
6. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos utilizados hasta 6 veces, durante un período de hasta 3 meses.

Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: Atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Curva de Calibración:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada hasta alcanzar 1200ml y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.

Conjugado:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Diluente de muestras :
Solución lista para el uso. Mezclar bien con un vórtex antes de usar.

Suero Control:
Añadir al polvo liofilizado el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver totalmente y mezclar suavemente en el vórtex. El suero disuelto está listo para el uso.
Nota: Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.

Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra (etanol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de $\pm$5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10 b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y las de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos e instrumentos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase

primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la Solución de Lavado Concentrada 20X, como se ha descrito anteriormente y mezclar suavemente.
4. Disolver el Suero Control como se ha descrito anteriormente.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar después suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.

En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.
Pueden realizarse dos procedimientos acorde a los requerimientos del clínico.

M.1 Análisis Cuantitativo

1. Poner el número necesario de tiras en el soporte plástico e identificar los pocillos de las muestras y de los estándares.
2. Diluir las muestras **1:101** dispensando en un tubo desechable 1 ml de Diluente de Muestras y 10 µl de muestra, mezclar con ayuda de un vórtex, antes de usar. No diluir los Calibradores y el Suero Control disuelto ya que están listos para el uso.
3. Dejar los pocillos A1 y B1 vacíos para el blanco.
4. Dispensar 100µl de los Calibradores por duplicado, 100µl del Suero Control disuelto por duplicado y después 100µl de las muestras diluídas. El Suero Control se emplea para verificar que el sistema analítico funcione como es debido. Comprobar que el Suero Control, los Calibradores y las muestras han sido añadidos adecuadamente.
5. Incubar la microplaca durante **60 minutos a +37ºC.**

Nota importante*: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace la prueba manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Después de la primera incubación, lavar los pocillos según lo descrito previamente (sección I.3).
7. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1 y B1, controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **60 minutos a +37°C.**

Nota importante*: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta y no sumergir la parte superior de la misma en los controles o muestras. Podría producirse contaminación.*

8. Después de la segunda incubación, lavar los pocillos según lo descrito previamente (sección I.3).
9. Dispensar 100µl de Cromógeno/Substrato en todos los pocillos, incluidos los del blanco.

Nota importante*: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

10. Incubar la microplaca, protegida de la luz, durante **20 minutos a temperatura ambiente (18-24°C).** Los pocillos correspondientes a las muestras positivas, el Suero Control y los Calibradores positivos deben cambiar de color claro a azul.
11. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición de la Solución de parada cambia el color del control positivo y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con los pocillos A1 y B1 (blanco).

M.2 Análisis Cualitativo

1. Poner el número necesario de tiras en el soporte plástico e identificar los pocillos de las muestras y de los estándares.
2. Diluir las muestras **1:101** dispensando en un tubo desechable 1 ml de Diluente de Muestras y 10 µl de muestra, mezclar con ayuda de un vórtex, antes de usar. No diluir los Calibradores disuelto ya que están listos para el uso.
3. Dejar el pocillo A1 vacío para el blanco.
4. Dispensar 100 µl del Calibrador 0 U/ml por duplicado, 100 µl del Calibrador 10 U/ml por duplicado, 100 µl del Calibrador 100 U/ml simple. Dispensar después 100 µl de las muestras diluídas en los pocillos correspondientesComprobar que el Suero Control, los Calibradores y las muestras han sido añadidos adecuadamente.
5. Incubar la microplaca durante **60 minutos a +37ºC.**

Nota importante*: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace la prueba manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Después de la primera incubación, lavar los pocillos según lo descrito previamente (sección I.3).
7. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1. Incubar la microplaca durante **60 minutos a +37°C.**

Nota importante*: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta y no sumergir la parte superior de la misma en los controles o muestras. Podría producirse contaminación.*

8. Después de la segunda incubación, lavar los pocillos según lo descrito previamente (sección I.3).
9. Dispensar 100µl de Cromógeno/Substrato en todos los pocillos, incluido el A1.

Nota importante*: No exponer directamente a fuerte iluminación, de lo contrario pudieran generarse interferencias.*

10. Incubar la microplaca, protegida de la luz, durante **20 minutos a temperatura ambiente (18-24°C).** Los pocillos correspondientes a las muestras positivas, el Suero Control y los Calibradores positivos deben cambiar de color claro a azul.
11. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición de la Solución de parada cambia el color del control positivo y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm

(lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1.

Notas generales importantes:

1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*

2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*

3. *El suero de control (CS) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba. El suero de control (CS) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

N. ESQUEMA DEL ENSAYO

El protocolo del ensayo se resume en la siguiente tabla:

Calibradores & Muestras diluídas & Suero Control Disuelto	100 µl
1ra incubación	**60 min**
Temperatura	+37°C
Lavados	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Conjugado	100 µl
2da incubación	**60 min**
Temperatura	+37°C
Lavados	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Cromógeno/Substrato	100µl
3ra incubación	**20 min**
Temperatura	t.a.*
Acido Sµlfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado en el análisis cuantitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	CAL4	M1									
B	BL	CAL4	M2									
C	CAL1	CAL5	M 3									
D	CAL1	CAL5	M 4									
E	CAL2	CAL6	M 5									
F	CAL2	CAL6	M 6									
G	CAL3	SC	M 7									
H	CAL3	SC	M 8									

Leyenda: BL = Blanco // CAL = Calibradores // SC= Suero Control // M = Muestra

A continuación se describe un ejemplo del esquema de dispensado en el análisis cualitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 3	M 11									
B	CAL1	M 4	M 12									
C	CAL1	M 5	M 13									
D	CAL3	M 6	M 14									
E	CAL3	M 7	M 15									
F	CAL6	M 8	M 16									
G	M1	M 9	M 17									
H	M2	M 10	M 18									

Leyenda: BL = Blanco // CAL = Calibradores // M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un grupo de pruebas con los controles cada vez que se usa el equipo para verificar si el procedimiento durante el ensayo se ha realizado correctamente.

Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	< 0.100 DO450nm
Calibrador 0 PEI U/ml Coeficiente de variación	< 0.150 DO450nm después de leer el blanco < 30%
Calibrador 5 PEI U/ml	DO450nm > DO450nm Cal 0 U/ml + 5DS y > DO450nm Cal 0 U/ml + 0.100
Calibrador 10 PEI U/ml	DO450nm > DO450nm Cal 0 U/ml + 0.200
Calibrador 100 PEI U/ml	> 1.000 DO450nm
Suero Control	DO450nm = DO450nm Calibrador 20 U/ml +/-10%

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.

En caso contrario, detenga el ensayo y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.100DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Calibrador 0 U/ml > 0.150 DO 450nm después de leer el blanco Coeficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento del ensayo (dispensado de un Calibrador positivo en lugar del Cal 0) 4. no ha existido contaminación del Cal 0 o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.

Calibrador 5 U/ml < CAL 0 + 5 DS or < CAL 0 + 0.100	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Calibrador 10 U/ml < CAL 0 + 0.200	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Calibrador 100 U/ml < 1.000 DO 450nm	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución. 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.

Suero Control Valor distinto al esperado	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar una muestra equivocada). 3. el proceso de lavado y los parámetros del lavador son correctos. 4. no ha ocurrido contaminación externa de los controles. 5. el Suero Control ha sido disuelto con el volumen correcto indicado en la etiqueta Si se indica un error, el ensayo debe repetirse tras eliminar la causa del mismo. En caso de no encontrar un error, procédase como sigue: a) si se obtiene un valor hasta +/-20%: la precisión global del laboratorio podría no permitir alcanzar +/-10% del valor esperado. Comunicar el problema al responsable para aceptar ó rechazar este resultado. b) si se obtiene un valor superior a +/-20%: en este caso el test es inválido y hay que avisar al servicio de atención al cliente de DiaPro

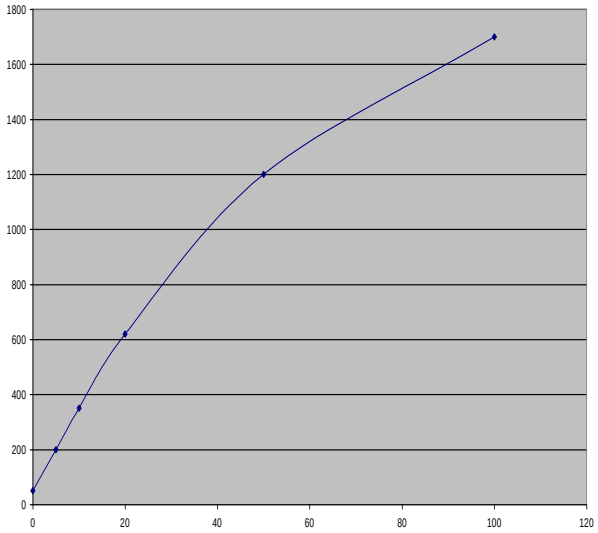
Si se presenta alguno de los problemas anteriores, avisar al responsable para tomar las medidas pertinentes.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 12.

P. RESULTADOS.

P.1 Método cuantitativo.
Si el ensayo resulta válido, usar para el método cuantitativo un programa de ajuste de curva para diseñar la curva de calibración con los valores obtenidos en la lectura a 450nm/620-630nm (se sugiere interpolar 4 parámetros). Después calcular sobre la curva de calibración la concentración de anticuerpos IgM anti-HBc presentes en la muestra.

A continuación, un ejemplo de curva de calibración:



Nota Importante:
No usar la curva anterior para formular los cálculos.

P.2 Método cualitativo.
En el método cualitativo, calcular los valores medios de DO450nm/620-630nm para los Calibradores 0 y 10 U/ml, después comprobar que el ensayo es válido.

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12)*:*

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Calibrador 0 U/ml: 0.020 – 0.024 DO 450nm
Valor medio: 0.022 DO 450nm
Menor de 0.150 – Válido

Calibrador 10 U/ml: 0.350 – 0.330 DO 450nm
Valor medio: 0.340 DO 450nm
Mayor de Cal 0 + 0.200 – Válido

Calibrador 100 U/ml: 2.845 DO 450nm
Mayor de 1.000 – Válido

Q. INTERPRETACIÓN DE LOS RESULTADOS.
Q.1 Resultados cualitativos:
Para el método cualitativo, la literatura médica generalmente considera positivas aquellas muestras con una concentración de HBc IgM $\geq$ 10 PEI U/ml.
Los resultados se interpretan como la razón entre la DO 450nm /620-630nm de la muestra y la DO 450nm del Cal 10 PEI U/ml (M/Co), como se indica en la tabla:

M/Co	Interpretación
< 0.9	Negativo
0.9 - 1.1	Equívoco
> 1.1	Positivo

Q.2 Resultados Cuantitativos:
La Curva de Calibración se emplea para determinar la concentración de anticuerpos IgM anti-HBcAg, presentes en la muestra.
Las muestras con una concentración menor de 5 PEI U/ml se consideran negativas a HBcIgM.
Las muestras con una concentración entre 5 y 10 PEI U/ml se consideran en la zona gris.

En el seguimiento de hepatitis crónica, sin embargo, valores superiores a 5 PEI U/ml pueden considerarse positivos a HBcIgM si están presentes otros signos clínicos.
Las muestras con una concentración mayor de 10 PEI U/ml se consideran positivas a HBcIgM.

Notas generales importantes:

1. *Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*
2. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
3. *Un resultado positivo indica infección por HBV por lo tanto el paciente debe ser tratado adecuadamente.*
4. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
5. *El diagnóstico de infección con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.*

R. FUNCIONAMIENTO
La evaluación del funcionamiento ha sido realizada según lo reportado en las Especificaciones Técnicas Comunes (ETC) (art. 5, Capítulo 3 de las Directivas IVD 98/79/EC).

1. Límite de detección.
El límite de detección del ensayo ha sido calculado por medio de:

1.3 La preparación de referencia para HBcIgM suministrada por el Instituto Paul Erlich, Alemania (HBc-Referenzserum-IgM 84), a partir de la cual se ha calibrado la Curva Estándar.
1.4 Accurun 113 (cat. N° A113-5001) suministrada por Boston Biomedica Inc., Estados Unidos.

La siguiente tabla muestra los resultados del Control de Calidad para tres lotes analizados:

BCM.CE	Lote #	0103	Lote #	0103/2	Lote #	0303
PEI U/ml	DO450nm	M/Co	DO450nm	M/Co	DO450nm	M/Co
100	2.752	**8.9**	2.883	**9,7**	2.911	**9.1**
50	1.917	**6.2**	1.972	**6.7**	2.053	**6.4**
20	0.980	**3.2**	0.914	**3.1**	1.095	**3.4**
10	0.544	**1.8**	0.513	**1.7**	0.592	**1.8**
5	0.310	**1.0**	0.296	**1.0**	0.321	**1.0**
2.5	0.155	0.5	0.149	0.5	0.161	0.5
1.25	0.084	0.3	0.084	0.3	0.093	0.3
Negativo	0.040		0.035		0.044	

BBI Accurun # 113

BCM.CE	Lote #	0103	Lote #	0103/2	Lote #	0303
BBI 113	DO450nm	M/Co	DO450nm	M/Co	DO450nm	M/Co
1 x	3.336	**10.8**	3.195	**10.4**	3.269	**10.3**
2 x	2.472	**8.0**	2.385	**7.8**	2.385	**7.5**
4 x	1.467	**4.7**	1.413	**4.6**	1.429	**4.5**
8 x	0.865	**2.8**	0.807	**2.6**	0.856	**2.7**
16 x	0.430	**1.4**	0.427	**1.4**	0.410	**1.3**
32 x	0.234	0.8	0.234	0.8	0.248	0.8
64 x	0.129	0.4	0.133	0.4	0.122	0.4
128 x	0.086	0.3	0.082	0.3	0.089	0.3
Negativo	0.040		0.040		0.052	

Además se ha examinado el panel # PHE 102 de BBI en tres lotes del producto, los datos se reportan a continuación con referencia a un equipo europeo (resultados de BBI).

BBI – Panel código PHE 102

	Lote# 0103	Lote # 0103/2	Lote # 0303	Sorin EIA
Miembro	M/Co	M/Co	M/Co	M/Co
01	**6.7**	**6.3**	**6.5**	**2.0**
02	**11.3**	**10.0**	**10.7**	**6.1**
03	**9.5**	**7.2**	**8.4**	**3.0**
04	**5.8**	**3.4**	**4.1**	**2.1**
05	**11.3**	**11.4**	**11.2**	**3.1**
06	**12.1**	**11.6**	**11.8**	**4.1**
07	**0.1**	**0.1**	**0.1**	**0.2**
08	**9.2**	**8.5**	**8.8**	**2.3**
09	**12.2**	**11.7**	**11.9**	**4.2**
10	**11.7**	**10.2**	**10.8**	**2.8**
11	**5.9**	**5.8**	**5.8**	**2.1**
12	**12.7**	**11.4**	**11.7**	**5.2**
13	**11.6**	**11.0**	**11.3**	**3.6**
14	**7.0**	**6.3**	**6.6**	**2.3**
15	**12.4**	**11.5**	**11.8**	**4.5**

2. Sensibilidad Diagnóstica:
Se define como la probabilidad del ensayo de detectar positivos en presencia del analito específico.
La sensibilidad diagnóstica ha sido probada interna y externamente en un Laboratorio Clínico calificado, a partir de paneles de muestras clasificadas como positivas según un equipo certificado US FDA.
Las muestras positivas se obtuvieron de diferentes pacientes y a partir de diversas patologías producidas por HBV (hepatitis aguda y crónica). En un estudio realizado a más de 200 muestras, se encontró un valor > 98%.
También se realizó un estudio con un panel de Seroconversión producido por BBI, Estados Unidos, código # PHM 935A cuyos resultados se reportan a continuación con referencia a dos equipos comerciales (resultados BBI).

BBI Panel PHM 935A

	Lote # 0103	Abbott EIA	DiaSorin EIA
Miembro#	M/Co	M/Co	M/Co
01	0.2	0.1	0.1
02	0.2	0.1	0.1
03	0.2	0.1	0.1
04	0.1	0.1	0.1
05	0.2	0.1	0.1
06	0.2	0.1	0.1
07	0.2	0.1	0.1
08	0.1	0.1	0.1
09	0.1	0.1	0.1
10	0.1	0.1	0.1
11	0.2	0.1	0.1
12	0.2	0.1	0.1
13	**2.8**	**3.7**	0.7
14	**5.0**	**6.4**	0.9
15	**> 12**	**6.2**	**4.5**
16	**> 12**	**5.6**	**4.5**
17	**> 12**	**5.5**	**4.3**
18	**> 12**	**4.8**	**4.3**
19	**> 12**	**> 6.6**	**4.4**
20	**> 12**	**> 6.6**	**5.2**

3. Especificidad Diagnóstica:
Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico.
La especificidad diagnóstica ha sido determinada interna y externamente en un Laboratorio Clínico calificado, a partir de paneles de muestras provenientes de individuos sanos y donantes de sangre, las mismas fueron clasificadas como negativas según un equipo certificado US FDA.
Se examinaron más de 400 muestras negativas, la especificidad diagnóstica encontrada fue > 98%.
También se analizaron más de 50 muestras que pudieran provocar interferencia (por ejemplo: otras enfermedades infecciosas, pacientes afectados por hepatitis no virales, pacientes sometidos a diálisis, mujeres embarazadas, hemofílicos, lipémicos, etc.). No se observaron interferencias en el estudio.
Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

Las muestras congeladas han sido probadas para comprobar si la congelación interfiere con el procedimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.

4. Precisión:
Se realizó un estudio con 3 muestras, examinadas en 16 réplicas, en tres corridas separadas utilizando 3 lotes diferentes. Los valores obtenidos se reportan a continuación :

BCM.CE: lote # 0103

Cal 0 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.055	0.053	0.051	0.053
Desviación estándar	0.005	0.006	0.005	0.006
CV %	9.9	12.3	10.7	10.9

Cal 5 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.324	0.308	0.321	0.318
Desviación estándar	0.022	0.018	0.024	0.021
CV %	6.8	5.7	7.5	6.7

Cal 50 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.109	2.048	2.052	2.070
Desviación estándar	0.101	0.088	0.136	0.109
CV %	4.8	4.3	6.7	5.2

BCM.CE: lote # 0103/2

Cal 0 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.057	0.053	0.054	0.055
Desviación estándar	0.005	0.005	0.004	0.004
CV %	8.3	9.0	7.3	8.2

Cal 5 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.332	0.331	0.322	0.328
Desviación estándar	0.017	0.018	0.016	0.017
CV %	5.0	5.5	4.9	5.1

Cal 50 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.311	2.208	2.212	2.244
Desviación estándar	0.110	0.090	0.095	0.098
CV %	4.7	4.1	4.3	4.4

BCM.CE: lote # 0303

Cal 0 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.043	0.042	0.040	0.042
Desviación estándar	0.004	0.005	0.004	0.004
CV %	10.3	11.1	10.9	10.8

Cal 5 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.320	0.326	0.314	0.320
Desviación estándar	0.023	0.024	0.026	0.024
CV %	7.1	7.4	8.2	7.6

Cal 50 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.150	2.163	2.092	2.135
Desviación estándar	0.057	0.067	0.076	0.067
CV %	2.6	3.1	3.6	3.1

Nota importante:
Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 12.

S. LIMITACIONES DEL PROCEDIMIENTO.
Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.
La contaminación bacteriana de las muestras o la inactivación por calor pueden modificar los valores de absorbancia con la consiguiente alteración de los niveles del analito.
Este ensayo es adecuado solo para el análisis de muestras individuales y no para mezclas.
El diagnóstico de una enfermedad infecciosa no se debe formular en base al resultado de un solo ensayo, sino que es necesario tomar en consideración la historia clínica y la sintomatología del paciente así como otros datos diagnósticos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni
Milán – Italia

CE
0318

HBcAb

Competitive Enzyme Immunoassay for the determination of antibodies to Hepatitis B core Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

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REF. BCAB.CE
96 Tests

HBcAb

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis B core Antigen in human plasma and sera.
The kit is intended for the screening of blood units and the follow-up of HBV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of HBV.
HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregating the core particles; the antigen contains at least one immunological determinant.
Upon primary infection, anti HBcAg antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, slightly later than HBsAg, the viral surface antigen.
Anti HBcAg antibodies are produced usually at high titers and their presence is detectable even years after infection. Isolated HBcAb, in absence of other HBV markers, have been observed in infected blood units, suggesting the use of this test for screening HBV, in addition of HBsAg.
The determination of HBcAb has become important for the classification of the viral agent, together with the detection of the other markers of HBV infection, in sera and plasma.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of competition where the antibodies in the sample compete with a monoclonal antibody for a fixed amount of antigen on the solid phase.
A purified recombinant HBcAg is coated to the microwells.
The patient's serum/plasma is added to the microwell together with an additive able to block interferences present in the sample.
In the second incubation after washing, a monoclonal antibody, conjugated with Horseradish Peroxidase (HRP) and specific for HBcAg is added and binds to the free rec-HBcAg coated on the plastic.
After incubation, microwells are washed to remove any unbound conjugate and then the chromogen/substrate is added. In the presence of peroxidase enzyme the colorless substrate is hydrolyzed to a colored end-product.
The color intensity is inversely proportional to the amount of antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate MICROPLATE
8x12 microwell strips coated with recombinant HBcAg and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control CONTROL -
1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is pale yellow color coded.

3. Positive Control CONTROL +
1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, anti HBcAg antibodies at a concentration of about 10 PEI U/ml, (calibrated on PEI HBc Reference Material 82), 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The positive control is green color coded.

4. Calibrator CAL ….
n° 1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains fetal bovine serum, human antibodies to HBcAg at a concentration of 2 PEI U/ml +/-10% (calibrated on PEI HBc Reference Material 82) and 0.045% ProClin 300 as preservative.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme Conjugate CONJ
1x16ml/vial. Ready-to-use solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated mouse monoclonal antibody to HBcAg in presence of 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300. as preservatives. The component is red colour coded.

7. Chromogen/Substrate SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.6 +/-0.1, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide
Note: To be stored protected from light as sensitive to strong illumination.

8. Specimen Diluent DILSPE
4x3ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.045% ProClin 300 for the pre-treatment of samples and controls in the plate, blocking interference. The component is blue colour coded.

Note: Use all the content of one vial before opening a second one. The reagent is sensitive to oxidation.

9. Sulphuric Acid H_2SO_4 O.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foil n° 2

11. Instruction manual n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
11. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
15. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water.

Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
   Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 6 months.

1. Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storage.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use.

4. Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.*

5. Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

8. Specimen Diluent
Ready to use solution. Mix gently on vortex before use. Use all the content of one vial before opening a second one. The reagent is sensitive to oxidation.

9. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm$0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right

dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.

4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of (620-630nm, mandatory) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Dispense 50 ul Specimen Diluent into all the control and sample wells.
4. Pipette 50 µl of the Negative Control in triplicate, 50 ul of the Calibrator in duplicate and then 50 ul of the Positive Control in single. Then dispense 50 ul of each of the samples.
5. Incubate the microplate for **60 min at +37°C.**

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

6. When the first incubation is finished, wash the microwells as previously described (section I.3)
7. Pipette 100 µl Enzyme Conjugate in all the wells, except A1; incubate the microplate for **60 min at +37°C.**

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

8. When the second incubation is finished, wash the microwells as previously described (section I.3)
9. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light. as a high background might be generated.*

10. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with negative control and negative samples will turn from clear to blue (competitive method).
11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.
12. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator*

may be used only when a laboratory internal quality control is required by the management.

N. ASSAY SCHEME

Specimen Diluent	50 ul
Controls&calibrator and samples	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm/620-630nm or Co/S values have been matched in the analysis.
Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.050 OD450nm value
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 20%
Calibrator (about 2 PEI U/ml)	Co/S > 1
Positive Control	< 0.200 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.050 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 20%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator Co/S < 1	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control > 0.200 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 12.

P. RESULTS

The results are calculated by means of a cut-off value determined with the following formula:

Cut-Off = (NC + PC) / 5

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Results are interpreted as ratio between the cut-off value and the sample OD450nm/620-630nm or Co/S.

Results are interpreted according to the following table:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HBV.
Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample.
The blood unit should not be transfused.
A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 12):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 2.000 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than 0.200 – Accepted

Cut-Off = (2.100 + 0.100) / 5 = 0.440

Calibrator: 0.400-0.360 OD450nm
Mean value: 0.380 OD450nm
Co/S>1 – Accepted

Sample 1: 0.028 OD450nm
Sample 2: 1.890 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION:

The sensitivity of the assay has been calculated by means of the reference preparation for HBcAb supplied by Paul Erlich Institute (PEI HBc Reference Material 82). The assay shows a sensitivity of about 1.25 PEI U/ml.
The table below reports the Co/S values shown by the PEI standard diluted as suggested by the manufacturer to prepare a limiting dilution curve in Fetal Calf Serum (FCS).

PEI U/ml	Lot 1001	Lot 0702	Lot 0702/2	Lot 1202
5	**22.6**	**18.0**	**19.0**	**17.7**
2.5	**8.0**	**5.5**	**5.4**	**5.0**
1.25	**1.1**	**1.3**	**1.0**	**1.0**
0.625	0.4	0.4	0.4	0.4

In addition Accurun 1 – series 3000 – supplied by Boston Biomedica Inc., USA, was tested to determine its Co/S value. Results are reported in the table below:

Accurun 1 – series 3000

Value	Lot 1001	Lot 0702	Lot 1202
Co/S	2.9	2.3	2.2

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The Performance Evaluation of the device was carried out in a trial conducted on more than total 6000 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where a total of 5179 unselected donors, including 1st time donors, 206 samples from hospitalized patients and 164 potentially interfering specimen were examined, the diagnostic specificity was recently assessed by testing a total of 1498 negative samples on seven different lots. A value of specificity of 100% was observed. In addition to the above population, 189 potentially interfering samples (other liver diseases,pregnant women, hemolized, lipemic, RF positives) have been tested and found negative, confirming a 100% of specificity of the device.
Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
In addition to the first Performance Evaluation Study, in order to further evaluate the diagnostic sensitivity of the device, a total of 262 positive samples were recently evaluated. The respective results, collected from seven different lots of the device show a diagnostic sensitivity of 100%.

3. PRECISION

The mean values obtained from a study conducted on three lots and on two samples of different anti-HBcAg reactivity, examined in 16 replicates in three separate runs is reported below:

BCAB.CE: lot # 1202

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.943	1.939	1.924	1.935
Std.Deviation	0.081	0.078	0.103	0.087
CV %	4.2	4.0	5.3	4.5

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.143	0.147	0.148	0.146
Std.Deviation	0.014	0.017	0.018	0.016
CV %	9.8	11.4	12.1	11.1
Co/S	2.8	2.7	2.6	2.7

BCAB.CE: lot # 0702

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.163	2.110	2.106	2.126
Std.Deviation	0.105	0.088	0.139	0.111
CV %	4.9	4.2	6.6	5.2

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.182	0.193	0.195	0.190
Std.Deviation	0.018	0.023	0.019	0.020
CV %	10.0	12.0	9.9	10.6
Co/S	2.5	2.2	2.3	2.3

BCAB.CE: lot # 0702/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.278	2.098	2.130	2.169
Std.Deviation	0.135	0.126	0.159	0.140
CV %	5.9	6.0	7.5	6.5

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.193	0.190	0.199	0.134
Std.Deviation	0.023	0.023	0.027	0.025
CV %	12.1	12.3	13.5	12.6
Co/S	2.4	2.2	2.2	2.3

The variability shown in the tables did not result in sample misclassification.

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 12.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte. This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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HBcAb

Ensayo inmunoenzimático competitivo (ELISA) para la determinación de anticuerpos frente al Antígeno *core* del virus de la Hepatitis B en plasma y suero humanos

Uso exclusivo para diagnóstico “in vitro”

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REF. BCAB.CE
96 pruebas

HBcAb

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático competitivo (ELISA) para la determinación de anticuerpos frente al antígeno *core* del virus de la Hepatitis B en plasma y suero.
El equipo está diseñado para el cribado en unidades de sangre y para el seguimiento de los pacientes infectados con HBV.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
La Organización Mundial de la Salud (OMS) define la infección por el virus de la Hepatitis B como:

"La Hepatitis B es una de las enfermedades más importantes que aquejan a la humanidad y constituye un problema de salud pública global. El término hepatitis significa inflamación del hígado, y la causa más común es la infección por uno de los cinco virus, denominados A, B, C, D y E. Estos virus pueden causar una enfermedad aguda cuyos síntomas persisten por varias semanas, se caracterizan por el color amarillo de la piel y los ojos (ictericia); orina oscura; fatiga extrema; náuseas; vómitos y dolor abdominal. La recuperación puede tardar de varios meses a un año. Los virus de la Hepatitis son causantes de infecciones crónicas en las que el paciente nunca se libera del virus e incluso, años más tarde, desarrolla cirrosis hepática o cáncer de hígado.
El tipo más serio de hepatitis viral es la causada por el HBV, siendo el único tipo, de los que provocan infección crónica, para el cual existe una vacuna disponible. El virus de la Hepatitis B se transmite por contacto con sangre o fluidos corporales de personas infectadas, de la misma forma que el virus de la inmunodeficiencia humana (HIV), agente causal del SIDA. Sin embargo, el HBV es entre 50 y 100 veces más infeccioso que el HIV. Las principales vías de transmisión del HBV son: (a) vía perinatal (transmisión de madre a hijo durante el parto); (b) de niño a niño; (c) mediante inyecciones y transfusiones inseguras (d) por contacto sexual.
A nivel mundial, la mayor parte de las infecciones ocurre de madre infectada a hijo, de niño a niño en hogares infantiles y por la reutilización de agujas y jeringuillas sin previa esterilización. En muchos países desarrollados (Europa Occidental y Norteamérica), el patrón de transmisión es diferente. En estos países, la transmisión de madre a hijo y de niño a niño representaban cerca de un tercio de las infecciones crónicas antes de que se implantara el programa de vacunación infantil. Sin embargo, la mayoría de las infecciones en estos países se adquiere por la actividad sexual durante la adolescencia, y por el consumo de drogas inyectables. Por otra parte, el virus de la hepatitis B constituye el principal riesgo en el trabajo, dentro del colectivo de los profesionales de la salud, motivo por el cual se ha aplicado la vacunación para la protección de los mismos.
El virus de la hepatitis B no se trasmite por la comida o agua contaminadas, ni por contactos casuales en el ámbito laboral. En zonas del Este y Centro de Europa se han encontrado tasas elevadas de infección crónica por HBV. En el Asia Central y en regiones de la India, aproximadamente el 5% de la población está infectada de forma crónica, mientras que en Europa Occidental y Norteamérica, los índices son menores del 1%.
Los niños infectados con HBV, constituyen el grupo más susceptible a la infección crónica. Aproximadamente el 90% de los niños infectados durante el primer año de vida y entre el 30 y el 50% de los niños infectados entre 1 y 4 años, desarrollan este tipo de infección. La mortalidad por cáncer de hígado o cirrosis asociados al HBV es cerca del 25%, entre las personas que han adquirido la infección crónica en la niñez. En determinado grupo de pacientes, la hepatitis B crónica es tratada con interferones o lamivudinas, lo cual puede ayudar en ocasiones. En algunos casos de cirrosis se han realizado trasplantes de hígado, pero el resultado ha sido variable.
La prevención de esta enfermedad a través de la vacunación, constituye la mejor opción. La vacuna contra la Hepatitis B tiene índices de seguridad y eficacia demostrados. A partir de 1982, han sido administradas mundialmente, alrededor de un billón de dosis. Se aplica por vía intramuscular en series de tres dosis. Los estudios realizados demuestran un 95% de eficacia en la prevención de la infección crónica en niños y adultos sin infección previa. En muchos países donde el índice de infección crónica en niños oscila entre 8% y 15%, se ha observado una reducción a menos del 1% en grupos de niños inmunizados. Desde 1991, la OMS ha hecho un llamamiento para la introducción de la vacuna contra la hepatitis B en todos los programas nacionales de vacunación."
El antígeno core *del virus de la Hepatitis B (HBcAg) es el elemento principal del núcleo viral. Está compuesto por un polipéptido simple de 17 kD, el cual es liberado en el proceso de disgregación de la partícula viral. Este antígeno contiene al menos un determinante inmunogénico. Durante la infección primaria, los anticuerpos anti- HbcAg, son unos de los primeros marcadores del HBV que aparecen en el suero, poco antes de que aparezca el antígeno de superficie (HBsAg). Los títulos de anticuerpos producidos contra HBcAg son altos y pueden ser detectados incluso varios años después de la infección. Debido a su presencia en bolsas de sangre de donantes se ha implementado esta técnica para el cribado en las unidades de sangre.*

La determinación de HBcAb es de gran importancia para la clasificación del agente viral en suero y plasma, conjuntamente con la detección del resto de los marcadores de la infección por HBV.

C. PRINCIPIOS DEL ENSAYO.
El ensayo es de tipo competitivo, donde los anticuerpos de la muestra compiten con un anticuerpo monoclonal por el antígeno de la fase sólida.
Los pocillos de la placa están recubiertos con el antígeno *core* del virus de la hepatitis B, obtenido por vía recombinante y purificado.
El suero/plasma de los pacientes se añade a los pocillos conjuntamente a una solución capaz de bloquear interferencias que puedan deberse a la naturaleza de la muestra.
A continuación, previo lavado que elimina los componentes no fijados de la muestra, se adiciona un anticuerpo monoclonal anti-HBcAg conjugado con Peroxidasa (HRP), el cual se une a cualquier traza de antígeno remanente en la placa.
Después de una segunda incubación, los pocillos son lavados para eliminar el conjugado en exceso, luego se añade el substrato cromogénico, que en presencia de la peroxidasa es hidrolizado a un producto final con color.
La intensidad del color es inversamente proporcional a la presencia de anticuerpos al HBcAg presentes en la muestra.

D. COMPONENTES.
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca MICROPLATE
12 tiras de 8 pocillos recubiertos con HBcAg recombinante, en bolsas selladas con desecante. Se deben poner las placas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar a 2-8ºC.

2. Control Negativo CONTROL -
1x1.0ml/vial. Listo para el uso. Contiene 5% de albúmina de suero bovino, tampón fosfato 10 mM pH 7.4 +/- 0.1, además de azida sódica 0.09% y ProClin 300 0.045% como conservantes. El control negativo está codificado con el color amarillo pálido.

3. Control Positivo CONTROL +
1x1.0ml/vial. Listo para el uso. Contiene 5% de albúmina de suero bovino, anticuerpos anti HBcAg a una concentración aproximada de 10 PEI U/ml, (Calibrado según PEI HBc Reference Material 82), tampón fosfato 10 mM pH 7.4 +/- 0.1, además de azida sódica 0.09% y ProClin 300 0.045% como conservantes. El control positivo está codificado con el color verde.

4. Calibrador CAL ….
vial nº 1. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero fetal bovino, anticuerpos humanos al HBcAg en una concentración de 2 PEI U/ml +/- 10% (Calibrado según PEI HBc Reference Material 82) y ProClin 300 0.045% como conservante.
Nota: El volumen necesario para disolver el contenido del frasco, varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Tampón de Lavado Concentrado WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y ProClin 300 0.045%.

6. Conjugado CONJ
1x16ml/vial. Solución lista para el uso. Contiene 5% de albúmina de suero bovino, tampón Tris 10mM a pH 6.8 +/- 0.1, anticuerpo monoclonal de ratón anti-HBcAg conjugado con peroxidasa (HPR) en presencia de 0.3 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservantes. El conjugado está codificado con el color rojo.

7. Cromógeno/Substrato SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM pH 3.6 +/- 0.1, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02% así como dimetilsulfóxido 4%.
Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

8. Diluente de muestras DILSPE
4x3ml/vial. Contiene una solución tamponada Tris 10 mM pH 8.0 +/- 0.1 y ProClin 300 0.045% para el pretratamiento de las muestras y controles en la placa, así como para bloquear inespecificidades. El componente está codificado con el color azul.

Note: Use todo el contenido del vial antes de abrir un segundo. El reactivo es sensible a oxidación.

9. Ácido Sulfúrico: H_2SO_4 O.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+ P351+P338, P337+P313, P362+P363).

10. Sellador adhesivo, n° 2

11. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.
1. Micropipetas calibradas (100µl y 50µl) y puntas plásticas desechables.
2. Agua de calidad EIA (Bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC.
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.
1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Cuando el equipo es usado para cribado en unidades de sangre, el laboratorio debe estar certificado y calificado para realizar este tipo de análisis (Ministerio de Salud o entidad similar).
3. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
4. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
5. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
6. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
7. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
8. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente.
9. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
11. No usar el producto después de la fecha de caducidad indicada en el equipo e internamente en los reactivos.
12. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
13. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
14. Los desechos producidos durante el uso del equipo deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
15. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.

16. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
17. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Evitar el uso de conservantes, en particular azida sódica, ya que pudiera afectar la actividad enzimática del conjugado.
3. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Cuando el equipo se emplea para el cribado en unidades de sangre, se recomienda el uso del código de barras.
4. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
5. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante almenos 12 meses, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
6. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 6 meses.

1. Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de conservación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

2. Control Negativo:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

3. Control Positivo:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

4. Calibrador:

Añadir al polvo liofilizado el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver totalmente y mezclar suavemente en el vórtex.

Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

5. Solución de Lavado Concentrada:

Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.

Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

6. Conjugado:

Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

7. Cromógeno/ Substrato:

Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

8. Diluente de muestras :

Solución lista para el uso. Mezclar bien con un vórtex antes de usar. Use todo el contenido del vial antes de abrir un segundo. El reactivo es sensible a oxidación.

9. Ácido Sulfúrico:

Listo para el uso. Mezclar bien con un vórtex antes de usar.

Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+ P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra (etanol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser

regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10 b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y las de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el cribado en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
4. Disolver el Calibrador como se ha descrito anteriormente y mezclar suavemente usando un vórtex.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, conectar el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
11. En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

1. Poner el número necesario de tiras en el soporte plástico e identificar cuidadosamente los pocillos para los controles, calibrador y muestras.
2. Dejar el pocillo A1 vacío para el blanco.
3. Dispensar 50µl de Diluente de Muestras en todos los pocillos para muestras y controles & calibrador.
4. Dispensar 50µl del Control Negativo, por triplicado, 50µl de Calibrador, por duplicado, y 50µl del Control Positivo. Posteriormente, añadir 50µl de cada muestra.
5. Incubar la microplaca durante **60 minutos a +37ºC.**

***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace la prueba manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Después de la primera incubación, lavar los pocillos según lo descrito previamente (sección I.3).
7. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1; incubar la microplaca durante **60 minutos a +37°C.**

***Nota importante**: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.*

8. Después de la segunda incubación, lavar los pocillos según lo descrito previamente (sección I.3).
9. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1.

***Nota importante**: No exponer directamente a fuerte iluminació, de lo contrario se generan interferencias.*

10. Incubar la microplaca protegida de la luz a **temperatura ambiente (18-24°C) durante 20 minutos**. Los pocillos con Control Negativo y muestras negativas deben pasar de un tono claro a azul, (método competitivo).

11. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición de la solución de parada cambia el color del Control Negativo y las muestras negativas de azul a amarillo.
12. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas importantes:

1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*
3. *El calibrador (CAL) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba. El calibrador (CAL) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

N. ESQUEMA DEL ENSAYO.

Diluente de Muestras	50 ul
Controles&Calibrador y muestras	50 ul
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 ul
2da incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H_2O_2	100 ul
3ra incubación	**20 min**
Temperatura	t.a.*
Acido Sulfúrico	100 ul
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M2										
B	CN	M3										
C	CN	M4										
D	CN	M5										
E	CAL	M6										
F	CAL	M7										
G	CP	M8										
H	M1	M9										

Leyenda: BL = Blanco CN = Control Negativo
CAL = Calibrador CP = Control Positivo M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un grupo de pruebas con los controles/calibrador cada vez que se usa el equipo para verificar si los valores DO450nm / 620-630 nm o Co/M son los esperados.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	Valor < 0.050 DO450nm
Control Negativo (CN)	Valor > 1.000 DO450nm después de leer el blanco Coeficiente de variación < 20%
Calibrador (aprox. 2 PEI U/ml)	Co/M > 1
Control Positivo	Valor< 0.200 DO450nm

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.
En caso contrario, no siga adelante y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.050 DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control Negativo (CN) < 1.000DO450nm después de leer el blanco Coeficiente de variación > 20%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Calibrador Co/M < 1	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Control Positivo > 0.200DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, informe al responsable para tomar las medidas pertinentes.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 12.

P. RESULTADOS.
Los resultados se calculan por medio de un valor de corte (cut-off) hallado con la siguiente fórmula:

Valor de corte = (CN + CP) / 5

Nota Importante*: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS.
La interpretación de los resultados se realiza mediante la razón entre el Valor de corte y las DO a 450nm / 620-630nm de las muestras (Co/M).
Los resultados se interpretan según la siguiente tabla:

Co/M	Interpretación
< 0.9	**Negativo**
0.9 - 1.1	**Equívoco**
> 1.1	**Positivo**

Un resultado negativo indica que el paciente noestáinfectado por HBV.
Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial. En este caso la unidad de la sangre no debe ser transfundida.
Un resultado positivo es indicativo de infección por HBV y por consiguiente el paciente debe ser tratado adecuadamente. La unidad de la sangre debe ser descartada.

Notas importantes:
1. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
2. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
3. *El diagnóstico de infección con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.*

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Control Negativo: 2.000 – 2.200 – 2.000 DO 450nm
Valor medio: 2.100 DO 450nm
Mayor de 1.000 – Válido

Control Positivo: 0.100 DO 450nm
Menor de 0.200 – Válido

Valor de corte = (2.100 + 0.100) / 5 = 0.440

Calibrador: 0.400-0.360 DO 450nm
Valor medio: 0.380 DO 450nm
Co/M>1 – Válido
Muestra 1: 0.028 DO 450nm
Muestra 2: 1.890 DO 450nm
Muestra 1 Co/M > 1.1 positiva
Muestra 2 Co/M < 0.9 negativa

R. FUNCIONAMIENTO.
La evaluación del funcionamiento ha sido realizada según lo reportado en las Especificaciones Técnicas Comunes (ETC) (art. 5, Capítulo 3 de las Directivas IVD 98/79/EC).

1. Límite de detección.
La sensibilidad del ensayo ha sido calculada por medio de una preparación estándar de referencia para HBcAb suministrada por el Instituto Paul Erlich (PEI HBc Reference Material 82). El ensayo muestra una sensibilidad de aproximadamente 1.25 PEI U/ml.

La siguiente tabla muestra los valores de Co/M para PEI estándar diluido, como se sugiere por el fabricante, para construir la curva de dilución límite en suero fetal bovino (SFB).

PEI U/ml	Lote 1001	Lote 0702	Lote 0702/2	Lote 1202
5	**22.6**	**18.0**	**19.0**	**17.7**
2.5	**8.0**	**5.5**	**5.4**	**5.0**
1.25	**1.1**	**1.3**	**1.0**	**1.0**
0.625	0.4	0.4	0.4	0.4

Se evaluaron además, paneles Accurun 1 – series 3000 – suministrados por Boston Biomedical Inc., Estados Unidos, a fin de determinar sus valores Co/M. Los resultados obtenidos se muestran a continuación:

Accurun 1 – series 3000

Valor	Lote 1001	Lote 0702	Lote 1202
Co/M	2.9	2.3	2.2

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICAS.
La evaluación del procedimiento diagnóstico se realizó mediante un ensayo con más de 6000 muestras.

2.1 Especificidad Diagnóstica.
Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico.
Además del primer estudio, donde se examinaron en total 5179 muestras de donantes no seleccionados, incluyendo donantes por 1ª vez, 206 muestras de pacientes hospitalizados y 164 muestras que pudieran provocar interferencia, la especificidad diagnóstica se evaluó recientemente examinando un total de 1498 muestras negativas en siete lotes distintos. Se observó un valor de especificidad de 100%. Además de la población anterior, se examinaron 189 muestras que pudieran provocar interferencia (pacientes con otras enfermedades hepáticas, mujeres embarazadas, hemolizadas, lipémicas, RF positivas) y se encontraron negativas, confirmando un 100% de especificidad del dispositivo.
Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

2.2 Sensibilidad Diagnóstica.
Se define como la probabilidad del ensayo de detectar positivos en presencia del analito específico.
Además del primer estudio de evaluación del rendimiento, para evaluar adicionalmente la sensibilidad diagnóstica del producto, se examinaron recientemente un total de 262 muestras positivas. Los resultados correspondientes, obtenidos de siete lotes distintos del dispositivo, muestran una sensibilidad diagnóstica de 100%.

3. Precisión.
Se realizó un estudio con 3 lotes y dos muestras de diferente reactividad anti-HBcAg, en 16 réplicas, en tres tandas separadas. Los valores medios obtenidos se expresan a continuación :

BCAB.CE: lote # 1202

Control Negativo (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	1.943	1.939	1.924	1.935
Desviación estándar	0.081	0.078	0.103	0.087
CV %	4.2	4.0	5.3	4.5

Calibrador (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.143	0.147	0.148	0.146
Desviación estándar	0.014	0.017	0.018	0.016
CV %	9.8	11.4	12.1	11.1
Co/M	2.8	2.7	2.6	2.7

BCAB.CE: lote # 0702

Control Negativo (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.163	2.110	2.106	2.126
Desviación estándar	0.105	0.088	0.139	0.111
CV %	4.9	4.2	6.6	5.2

Calibrador (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.182	0.193	0.195	0.190
Desviación estándar	0.018	0.023	0.019	0.020
CV %	10.0	12.0	9.9	10.6
Co/M	2.5	2.2	2.3	2.3

BCAB.CE: lote# 0702/2

Control Negativo (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.278	2.098	2.130	2.169
Desviación estándar	0.135	0.126	0.159	0.140
CV %	5.9	6.0	7.5	6.5

Calibrador (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.193	0.190	0.199	0.134
Desviación estándar	0.023	0.023	0.027	0.025
CV %	12.1	12.3	13.5	12.6
Co/M	2.4	2.2	2.2	2.3

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

Nota importante:
Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 12.

S. LIMITACIONES DEL PROCEDIMIENTO.
La contaminación bacteriana de las muestras o la inactivaciòn por calor pueden modificar los valores de absorbancia con la consiguiente alteración de los niveles del analito. Este ensayo es adecuado solo para el análisis de muestras individuales y no para mezclas.
El diagnóstico de una enfermedad infecciosa no se debe formular en base al resultado de un solo ensayo, sino que es necesario tomar en consideración la historia clínica y la sintomatología del paciente así como otros datos diagnósticos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (Mi) – Italia

CE
0318

HBsAb

Enzyme Immunoassay for qualitative/quantitative determination of antibodies to Hepatitis B surface Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

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REF SAB.CE
96 Tests

HBs Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for both the quantitative and qualitative determination of antibodies to the Surface Antigen of Hepatitis B Virus in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B surface Antigen (HBsAg) is the major structural polypeptide of the envelope of the Hepatitis B Virus (HBV).
This antigen is composed mainly of the type common determinant "a" and the type specific determinants "d" and "y", present only on the specific serotypes.
Upon infection, a strong immunological response develops firstly against the type specific determinants and in a second time against the "a" determinant.
Anti "a" antibodies are however recognised to be most effective in the neutralisation of the virus, protecting the patient from other infections and leading it to convalescence.
The detection of HBsAb has become important for the follow up of patients infected by HBV and the monitoring of recipients upon vaccination with synthetic and natural HBsAg.

C. PRINCIPLE OF THE TEST

Microplates are coated with a preparation of highly purified HBsAg that in the first incubation with sample specifically captures anti HBsAg antibodies to the solid phase.
After washing, captured antibodies are detected by an HBsAg, labelled with peroxidase (HRP), that specifically binds the second available binding site of these antibodies.
The enzyme specifically bound to wells, by acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and can be detected by an ELISA reader.
The amount of antibodies may be quantitated by means of a standard curve calibrated against the W.H.O reference preparation.
Samples are pre treated in the well with an specimen diluent able to block interference present in vaccinated individuals.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with purified heat-inactivated HBsAg of both subtypes (ad and ay) from human origin and sealed into a bag with desiccant.
Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
5x2.0 ml/vial. Ready to use and colour coded standard curve, derived from HBsAb positive plasma titrated on WHO standard for anti HBsAg (1st reference preparation 1977, lot 17-2-77), ranging: CAL1 = 0 mIU/ml // CAL2 = 10 mIU/ml // CAL3 = 50 mIU/ml // CAL4 = 100 mIU/ml // CAL 5 = 250 mIU/ml.
Contains human serum proteins, 5% BSA, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. Standards are blue coloured.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

4. Enzyme conjugate : CONJ
1x16.0 ml/vial. Ready-to-use solution and red color coded.
It contains inactivated purified HBsAg of both subtypes ad and ay, labelled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

5. Chromogen/Substrate: SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
1x8ml. 10 mM Tris Buffered solution ph 7.4 +/-0.1,suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide as preservatives.

8. Control Serum: CONTROL …ml
1 vial. Lyophilized.
Contains fetal bovine serum proteins, human anti HBsAg antibodies calibrated at 50 ± 10% WHO mIU/ml. 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

9. Plate sealing foil n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-1°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking, strongly recommended) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-HBsAg antibody concentration is expected to be higher than 250 mIU/ml should be diluted before use either 1:10 or 1:100 in the Calibrator 0 mIU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex when preparing the diluted samples.

H. PREPARATION OF COMPONENTS AND WARNINGS

1. Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Calibration Curve
Ready to use. Mix well on vortex before use.

3. Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

4. Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

5. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

6. Specimen Diluent:
Ready to use. Mix well on vortex before use.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, air-driven dust or microbes. Do not expose to strong light, oxidising agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

8. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm$1°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Control Serum as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipments are available and ready to use.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two procedures can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 and B1 wells empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
Then Dispense in all the wells to be used for the test, except for A1 and B1, 50µl of the Specimen Diluent.

Important note: *This additive is added before distributing samples and controls into specific wells and is particularly intended for blocking some substances present in people undergoing vaccination and capable to mask antibodies.*

2. Pipette 100µl of all the Calibrators, 100µl of Control Serum in duplicate and then 100ul of samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators, Control Serum and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

Important note: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

3. Wash the microplate as reported in section I.3.

4. In all the wells except A1 and B1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important notes:

1) *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*
2) *Mix thoroughly the Enzyme Conjugate on vortex before use.*

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, mandatory), blanking the instrument on A1 and B1 wells.

M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
2. Dispense 50 ul Specimen Diluent in all the wells, except for the blank A1. Then pipette 100µl of the Calibrator 0 mIU/ml in duplicate, 100µl of the Calibrator 10 mIU/ml in duplicate, 100µl of the Calibrator 250 mIU/ml in single, and then 100ul of samples. Check that Calibrators and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.
3. Wash the microplate as reported in section I.3.
4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important notes:

1) *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*
2) *Mix thoroughly the Enzyme Conjugate on vortex before use.*

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, mandatory), blanking the instrument on A1 and B1 wells.

Important general notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME (standard procedure)

Specimen Diluent Calibrators Control Serum Samples	50 ul 100 ul 100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme in quantitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3									
B	BLK	CAL4	S4									
C	CAL1	CAL5	S5									
D	CAL1	CAL5	S6									
E	CAL2	CS	S7									
F	CAL2	CS	S8									
G	CAL3	S1	S9									
H	CAL3	S2	S10									

Legenda: BLK = Blank // CAL = Calibrators // CS = Control Serum // S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL5	S 8	S 16									
G	S1	S 9	S 17									
H	S2	S 10	S 18									

Legenda: BLK = Blank // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameters	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 WHO mIU/ml	< 0.200 OD450nm after blanking
Calibrator 10 WHO mIU/ml	OD450nm higher than the OD450nm of the Calibrator 0 mIU/ml + 0.100
Calibrator 250 WHO mIU/ml	> 1.500 OD450nm
Control Serum	OD450nm = OD450nm CAL 50 mIU/ml ± 10%
Coefficient of variation	< 30% for the Calibrator 0 mIU/ml

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 mIU/ml > 0.200 coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure when the dispensation of standards is carried out; 4. that no contamination of the Cal 0 mIU/ml or of the wells where it was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

Calibrator 10 mIU/ml OD450nm < Cal 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Calibrator 250 mIU/ml < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (e.g.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

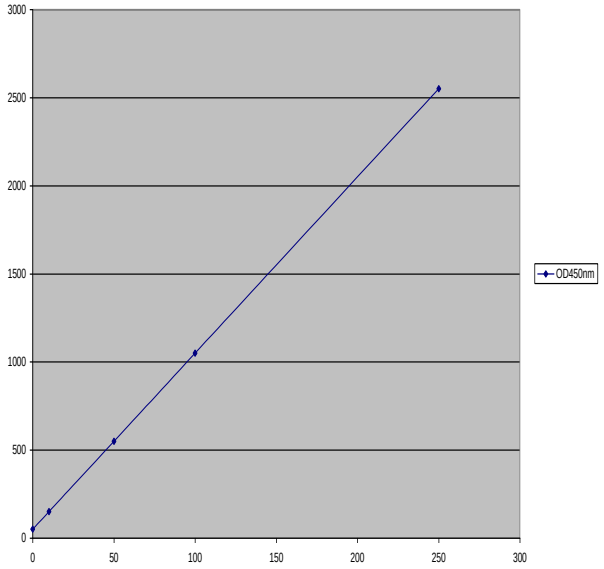
Important note:
The analysis must be done proceeding as the reading step described in the section M, point 7.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti HBsAg antibody in samples.
An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm/620-630nm values for the Calibrators 0 and 10 mIU/ml and then check that the assay is valid.

Example of calculation (data obtained proceeding as the reading step described in the section M, point 7).

The following data must not be used instead of real figures obtained by the user.

Calibrator 0 mIU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.200 – Accepted

Calibrator 10 mIU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 250 mIU/ml: 2.845 OD450nm
Higher than 1.500 – Accepted

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 10 WHO mIU/ml are considered negative for anti HBsAg antibody by most of the international medical literature.
Samples with a concentration higher than 10 WHO mIU/ml are considered positive for anti HBsAg antibody.
In the follow up of vaccination recipients, however, the value of 20 WHO mIU/ml is usually accepted by the medical literature as the minimum concentration at which the patient is considered clinically protected against HBV infection.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*

3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION:

The limit of detection of the assay has been calculated by means of the HBsAb international preparation supplied by CLB on behalf of WHO (1st reference preparation 1977, lot 17-2-77), on which Calibration Curve has been calibrated. HBV negative serum was used as diluent, as recommended by the supplier.
Results of Quality Control are given in the following table:

WHO mIU/ml	SAB.CE Lot # 1002	SAB.CE Lot # 1001	SAB.CE Lot # 1002/2
50	0.933	0.812	0.846
10	0.219	0.192	0.194
5	0.110	0.096	0.104
2.5	0.057	0.058	0.067
Std 0	0.021	0.015	0.023

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

A Performance Evaluation has been conducted on a total number of more than 700 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.
More than 500 negative specimens were tested, internally and externally, against a European company.
A diagnostic specificity of 98.8% was assessed. .
Moreover, diagnostic specificity was assessed by testing 113 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) against the European company. A value of specificity of 100% was assessed.
Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
106 vaccinated patients were evaluated providing a diagnostic sensitivity of 100%.
More than 100 HBV naturally infected patients were tested, internally and externally, against the European company; a diagnostic sensitivity of 100% was found.

3. PRECISION:

The mean values obtained from a study conducted on three samples of different anti-HBsAg reactivity, examined in 16 replicates in three separate runs is reported below:

SAB.CE: lot # 1202

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.038	0.038	0.039	0.039
Std.Deviation	0.003	0.004	0.005	0.004
CV %	8.8	9.5	11.8	10.0

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.250	0.243	0.244	0.246
Std.Deviation	0.020	0.023	0.017	0.020
CV %	8.0	9.3	7.0	8.1

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.998	3.000	3.259	3.085
Std.Deviation	0.152	0.151	0.158	0.153
CV %	5.1	5.0	4.8	5.0

SAB.CE: lot # 1002

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.048	0.048	0.050	0.049
Std.Deviation	0.005	0.004	0.006	0.005
CV %	9.4	8.4	11.5	9.8

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.249	0.252	0.242	0.248
Std.Deviation	0.021	0.020	0.023	0.021
CV %	8.3	7.9	9.6	8.6

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.544	3.653	3.612	3.603
Std.Deviation	0.153	0.176	0.138	0.156
CV %	4.3	4.8	3.8	4.3

SAB.CE: lot # 1002/2

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.050	0.051	0.050	0.050
Std.Deviation	0.005	0.006	0.006	0.005
CV %	10.0	10.9	11.9	10.9

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.226	0.238	0.239	0.234
Std.Deviation	0.015	0.017	0.018	0.016
CV %	6.5	7.0	7.5	7.0

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.526	3.457	3.499	3.494
Std.Deviation	0.137	0.143	0.162	0.147
CV %	3.9	4.1	4.6	4.2

The variability shown in the tables did not result in sample misclassification.

4. ACCURACY

The assay accuracy has been checked by the dilution and recovery tests. Any “hook effect”, underestimation likely to happen at high doses of analyte, was ruled out up to 10.000 mIU/ml.

Important note:

The performance data have been obtained proceeding as the reading step described in the section M, point 7.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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Manufacturer:
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CE
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HBsAb

Ensayo inmunoenzimático (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos frente al Antígeno de superficie del virus de la Hepatitis B en plasma y suero humanos

Uso exclusivo para diagnóstico “in vitro”

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REF SAB.CE
96 pruebas

HBs Ab

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos frente al antígeno de superficie del virus de la Hepatitis B, en plasma y suero humanos.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
La Organización Mundial de la Salud (OMS) define la infección por el virus de la Hepatitis B como:

"La Hepatitis B es una de las enfermedades más importantes que aquejan a la humanidad y constituye un problema de salud pública global. El término hepatitis significa inflamación del hígado, y la causa más común es la infección por uno de los cinco virus, denominados A, B, C, D y E. Estos virus pueden causar una enfermedad aguda cuyos síntomas persisten por varias semanas, se caracterizan por el color amarillo de la piel y los ojos (ictericia); orina oscura; fatiga extrema; náuseas; vómitos y dolor abdominal. La recuperación puede tardar de varios meses a un año. Los virus de la Hepatitis son causantes de infecciones crónicas en las que el paciente nunca se libera del virus e incluso, años más tarde, desarrolla cirrosis hepática o cáncer de hígado.
El tipo más serio de hepatitis viral es la causada por el HBV, siendo el único tipo, de los que provocan infección crónica, para el cual existe una vacuna disponible. El virus de la Hepatitis B se transmite por contacto con sangre o fluidos corporales de personas infectadas, de la misma forma que el virus de la inmunodeficiencia humana (HIV), agente causal del SIDA. Sin embargo, el HBV es entre 50 y 100 veces más infeccioso que el HIV. Las principales vías de transmisión del HBV son: (a) vía perinatal (transmisión de madre a hijo durante el parto); (b) de niño a niño; (c) mediante inyecciones y transfusiones inseguras (d) por contacto sexual.
A nivel mundial, la mayor parte de las infecciones ocurre de madre infectada a hijo, de niño a niño en hogares infantiles y por la reutilización de agujas y jeringuillas sin previa esterilización. En muchos países desarrollados (Europa Occidental y Norteamérica), el patrón de transmisión es diferente. En estos casos, la transmisión de madre a hijo y de niño a niño representaban cerca de un tercio de las infecciones crónicas antes de que se implementara el programa de vacunación infantil. Sin embargo, la mayoría de las infecciones en estos países se adquiere por la actividad sexual durante la adolescencia, y por el consumo de drogas inyectables. Por otra parte, el virus de la Hepatitis B constituye el principal riesgo en el trabajo, dentro del colectivo de los profesionales de la salud, motivo por el cual se ha aplicado la vacunación para la protección de los mismos.
El virus de la Hepatitis B no se propaga por la comida o agua contaminadas, ni por contactos casuales en el ámbito laboral. En zonas del Este y Centro de Europa se han reportado tasas elevadas de infección crónica por HBV. En el Asia Central y en regiones de la India, aproximadamente el 5% de la población está infectada de forma crónica, mientras que en Europa Occidental y Norteamérica, los índices son menores del 1%.
Los niños infectados con HBV, constituyen el grupo más susceptible a la infección crónica. Aproximadamente el 90% de los niños infectados durante el primer año de vida y entre el 30 y el 50% de los niños infectados entre 1 y 4 años, desarrollan este tipo de infección. La mortalidad por cáncer de hígado o cirrosis asociados al HBV es cerca del 25%, entre las personas que han presentado infección crónica en la niñez. En determinado grupo de pacientes, la Hepatitis B crónica es tratada con interferon, lamivudina, etc., lo cual puede ayudar en ocasiones. En algunos casos de cirrosis se han realizado trasplantes de hígado, pero el resultado ha sido variable.
La prevención de esta enfermedad a través de la vacunación, constituye la mejor opción. La vacuna contra la Hepatitis B tiene índices de seguridad y eficacia demostrados. A partir de 1982, han sido administradas mundialmente, alrededor de un billón de dosis. Se aplica por vía intramuscular en series de tres dosis. Los estudios realizados demuestran un 95% de eficacia en la prevención de la infección crónica en niños y adultos sin infección previa. En muchos países donde el índice de infección crónica en niños oscila entre 8% y 15%, se ha observado una reducción a menos del 1% en grupos de niños inmunizados. Desde 1991, la OMS ha hecho un llamado para la introducción de la vacuna contra la hepatitis B en todos los programas nacionales de vacunación."

El antígeno de superficie del virus de la Hepatitis B (HBsAg) es el elemento principal de la envoltura viral. Está compuesto fundamentalmente por el determinante común de tipo "a" y los específicos "d" e "y" específicos de serotipo.
Después de la infección se produce una respuesta inmunológica potente, primero contra los determinantes antigénicos específicos y después contra el determinante común "a". Los anticuerpos anti "a" son considerados los más eficaces en la neutralización del virus, contribuyen a la protección del paciente de otras infecciones y lo guian a la convalescencia.
La detección del HBsAb es importante para el seguimiento de los pacientes infectados por HBV y para el monitoreo de los receptores de vacunas elaboradas con el antígeno natural o sintético.

C. PRINCIPIOS DEL ENSAYO.
Los pocillos de la placa están recubiertos con una preparación del antígeno de superficie del virus de la hepatitis B, altamente purificado, que durante una primera incubación con la muestra, captura de forma específica anticuerpos anti HBsAg en la fase sólida. A continuación, previo lavado, se adiciona un HBsAg conjugado con Peroxidasa (HRP), el cual se combina de forma específica a un segundo sitio de unión disponible en estos anticuerpos. Después de la adición del substrato cromogénico y producto de la combinación del mismo con la enzima conjugada, se genera una señal coloreada proporcional a la presencia de anticuerpos al HBsAg en la muestra y puede detectarse mediante el lector ELISA. La cantidad de anticuerpos debe ser cuantificada utilizando una curva estándar calibrada, contra la referencia preparada por la O.M.S.
Las muestras son pretratadas en los pocillos con un diluente de muestras capaz de bloquear la interferencia presente en individuos vacunados.

D. COMPONENTES.
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
8x12 pocillos recubiertos con HBsAg humano correspondiente a los subtipos "ad" y "ay", inactivado por calor y purificado, en bolsas selladas con desecante. Se deben poner las placas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar a 4ºC.

2. Curva de Calibración: CAL N° …
5x2.0 ml/vial. Listo para el uso y curva con código estándar de color, elaborada a partir de plasma positivo a HBsAb, titulada según estándar de O.M.S. para anti-HBsAg (1ra preparación de referencia 1977, lote 17-2-77), con rangos: CAL1 = 0 mIU/ml // CAL2 = 10 mIU/ml // CAL3 = 50 mIU/ml // CAL4 = 100 mIU/ml // CAL 5 = 250 mIU/ml.

Contiene proteínas séricas, BSA 5%, tampón fosfato 10 mM pH 7.4+/-0.1, así como azida sódica 0.09% y 0.045% ProClin 300 como conservantes. Los estándar son de color azul.

3. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y 0.045% ProClin 300.

4. Conjugado: CONJ
1x16ml/vial. Solución lista para el uso. Codificado con el color rojo. Contiene HBsAg humano correspondiente a los subtipos "ad" y "ay", el cual ha sido inactivado por calor, purificado y marcado con HRP; BSA 5%; tampón Tris 10 mM pH 6.8+/-0.1; además de sulfato de gentamicina 0.3 mg/ml y 0.045% ProClin 300 como conservantes.

5. Cromógeno/Substrato. SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM pH 3.5-3.8, dimetilsulfóxido 4%, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02%.
Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

6. Ácido Sulfúrico: H_2SO_4 O.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Diluente de muestras : DILSPE
1x8ml. Contiene una solución tamponada Tris 10 mM pH 7.4 +/- 0.1, se recomienda en el seguimiento de vacunaciones. Contiene azida sódica 0.09% como preservativo.

8. Suero Control: CONTROL …ml
1 vial. Liofilizado.
Contiene proteínas del suero bovino fetal, anticuerpos humanos anti-HBsAg a una concentración aproximada de 50 ±10% mIU/ml (O.M.S.), además de sulfato de gentamicina 0.3 mg/ml y 0.045% ProClin 300 como conservantes.

9. Sellador adhesivo, n° 2

10. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (100µl y 50µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (tolerancia+/-1°C).
6. Lector calibrado de microplacas de ELISA con filtros de 450 nm (lectura) y filtros de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el equipo e internamente en los reactivos.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben de ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Cuando el equipo se emplea para

el pesquisaje en unidades de sangre, se recomienda el uso del código de barras.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante al menos 12 meses. Evitar congelar/descongelar cada muestra más de una vez, ya que pueden generarse partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.
6. Aquellas muestras cuya concentración de anticuerpos se espera sea mayor de 250 mIU/ml deben diluirse previamente a 1:10 o 1:100 con el Calibrador 0 mIU/ml. Las diluciones deben hacerse en tubos limpios desechables añadiendo 50 µl de muestra y 450 µl de Cal 0 (1:10). Mezclar después con ayuda del vórtex.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

1. Microplacas:
Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde, lo que indicaría un defecto de conservazión. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.
Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

2. Curva de Calibración:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

3. Suero Control:
Añadir al polvo liofilizado el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver totalmente y mezclar suavemente en el vórtex.
Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

4. Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

5. Conjugado:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

6. Diluente de muestras :
Solución lista para el uso. Mezclar bien con un vórtex antes de usar.

7. Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

8. Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra (etanol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de $\pm$5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450 nm y de un segundo filtro de 620-630 nm, obligatorio para reducir interferencias en la lectura. El

procedimiento estándar debe contemplar: a) Ancho de banda <= 10 b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección "Procedimiento del ensayo". El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.

6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones "Control interno de calidad" y "Procedimiento del ensayo". El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y las de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
4. Disolver el Suero Control como se ha descrito anteriormente.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar después suavemente en el vórtex todos los reactivos líquidos.
1. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
6. Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
7. En caso de trabajar automáticamente, conectar el equipo y comprobar que los protocolos estén correctamente programados.
8. Comprobar que las micropipetas estén fijadas en el volumen requerido.
9. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.

En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.
Pueden realizarse dos procedimientos acorde a los requerimientos del clínico.

M.1 Análisis Cuantitativo

1. Poner el número necesario de tiras en el soporte plástico. Dejar los pocillos A1 y B1 vacíos para el blanco.
Almacenar las tiras restantes en la bolsa con el desecante a temperaturas entre 2 y 8°C.
Dispensar 50µl de Diluente de Muestras en todos los pocillos, con excepción de A1 y B1.

Nota importante: *Este reactivo se adiciona antes de la distribución de las muestras y controles en los pocillos con el fin de bloquear cualquier elemento presente en el suero de personas vacunadas, lo cual pudiera enmascarar los anticuerpos.*

2. Dispensar 100µl de los Calibradores, 100µl del Suero Control por duplicado y después 100µl de las muestras. El Suero Control se emplea para verificar que el sistema analítico funciona como es debido. Comprobar que el Suero Control, los Calibradores y las muestras han sido añadidos adecuadamente. Incubar la microplaca durante **60 minutos a +37ºC.**
Nota importante*: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace la prueba manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

3. Lavar los pocillos según lo descrito previamente (sección I.3).
4. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1 y B1, controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **60 minutos a +37°C.**

Nota importante*:*
1) Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.
2) Mezclar el Conjugado con ayuda del vórtex antes de usar.

5. Lavar los pocillos según lo descrito previamente.
6. Dispensar 100µl de TMB/H_2O_2 en todos los pocillos, incluidos los del blanco. Controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **20 minutos a temperatura ambiente.**

Nota importante*: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

7. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 6. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, recomendado), calibrando el instrumento con el pocillo A1 (blanco) y B1 (blanco).

M.2 Análisis Cualitativo

1. Poner el número necesario de tiras en el soporte plástico. Dejar el pocillo A1 vacío para el blanco.
Almacenar las tiras restantes en la bolsa con el desecante a temperaturas entre 2 y 8°C.
2. Dispensar 50µl de Diluente de Muestras en todos los pocillos con excepción de A1. Dispensar 100µl del Calibrator 0 mIU/ml por duplicado, 100µl del Calibrator 10 mIU/ml por duplicado, 100µl del Calibrator 250 mIU/ml,añadir después 100µl de cada muestra. Comprobar que los Calibradores y las muestras han sido añadidos adecuadamente.
Incube la microplaca durante **60 minutos a +37ºC.**
3**.** Lavar los pocillos según lo descrito previamente (sección I.3).

4. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1, controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **60 minutos a +37°C.**

***Nota importante**:*
1. *Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.*
2. *Mezclar el Conjugado con ayuda del vórtex antes de usar.*

5. Lavar los pocillos según lo descrito previamente.
6. Dispensar 100µl de TMB/H_2O_2 en todos los pocillos, incluido el del blanco. Controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **20 minutos a temperatura ambiente.**

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

7. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 6. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas generales importantes:
1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*
3. *El suero de control (CS) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba. El suero de control (CS) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

N. ESQUEMA DEL ENSAYO (procedimiento estándar).

Diluente de Muestras Calibradores Suero Control Muestras	50 µl 100 µl 100 µl 100 µl
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2da incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H2O2	100 µl
3ra incubación	**20 min**
Temperatura	t.a.*
Acido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado en el análisis cuantitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	CAL4	M 3									
B	BL	CAL4	M 4									
C	CAL1	CAL5	M 5									
D	CAL1	CAL5	M 6									
E	CAL2	SC	M 7									
F	CAL2	SC	M 8									
G	CAL3	M1	M 9									
H	CAL3	M2	M10									

Leyenda: BL = Blanco // CAL = Calibradores // SC= Suero Control // M = Muestra

A continuación se describe un ejemplo del esquema de dispensado en el análisis cualitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 3	M 11									
B	CAL1	M 4	M 12									
C	CAL1	M 5	M 13									
D	CAL2	M 6	M 14									
E	CAL2	M 7	M 15									
F	CAL5	M 8	M 16									
G	M1	M 9	M 17									
H	M2	M 10	M 18									

Leyenda: BL = Blanco // CAL = Calibradores // M = Muestra

O. CONTROL DE CALIDAD INTERNO.
Se realiza un grupo de pruebas con los controles cada vez que se usa el equipo para verificar si el procedimiento durante el ensayo se ha realizado correctamente.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	< 0.100 DO450nm
Calibrador 0 O.M.S. mIU/ml	< 0.200 DO450nm después de leer el blanco
Calibrador 10 O.M.S. mIU/ml	DO450nm mayor que la DO450nm del Calibrador 0 mIU/ml + 0.100
Calibrador 250 O.M.S. mIU/ml	> 1.500 DO450nm
Suero Control	DO450nm = DO450nm CAL 50 mIU/ml +/-10%
Coeficiente de variación	< 30% para el Calibrador 0 mIU/ml

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.
En caso contrario, no siga adelante y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.100DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Calibrador 0 mIU/ml > 0.200 Coeficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento durante el dispensado del estándar 4. no ha existido contaminación del Cal 0 o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.

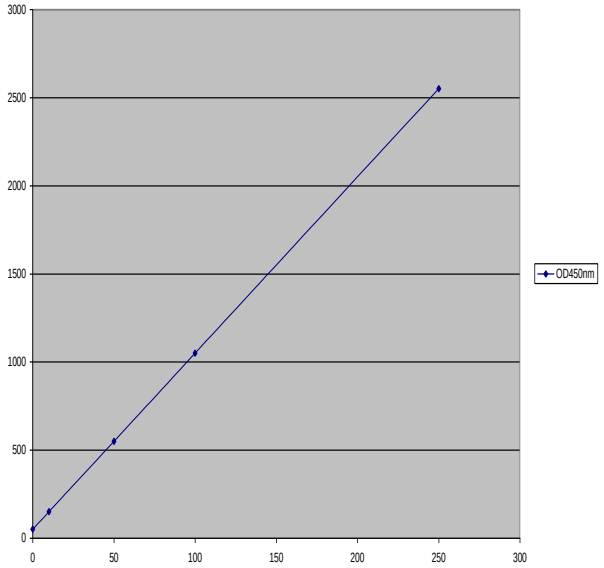
Calibrador 10 mIU/ml DO450nm < Cal 0 + 0.100	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Calibrador 250 mIU/ml < 1.500 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución. 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Suero Control Valor distinto al esperado	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar una muestra equivocada). 3. el proceso de lavado y los parámetros del lavador son correctos. 4. no ha ocurrido contaminación externa de los controles. 5. el Suero Control ha sido disuelto con el volumen correcto indicado en la etiqueta Si se indica un error, el ensayo debe repetirse tras eliminar la causa del mismo. En caso de no encontrar un error, procédase como sigue: a) si se obtiene un valor hasta +/-20%: la precisión global del laboratorio podría no permitir alcanzar +/-10% del valor esperado. Comunicar el problema al responsable para aceptar ó rechazar este resultado. b) si se obtiene un valor superior a +/-20%: en este caso el test es inválido y hay que avisar al servicio de atención al cliente de DiaPro

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 7.

P. RESULTADOS.

P.1 Método cuantitativo.
Si el ensayo resulta válido, usar para el método cuantitativo un programa de ajuste de curva para diseñar la curva de calibración con los valores obtenidos en la lectura a 450nm / 620-630nm (se sugiere interpolar 4 parámetros). Después calcular sobre la curva de calibración la concentración de anticuerpos anti-HBsAg presentes en la muestra.
A continuación, un ejemplo de curva de calibración:

Ejemplo de curva de calibración:



Nota Importante:
No usar la curva anterior para formular los cálculos.

P.2 Método cualitativo.
En el método cualitativo, calcular los valores medios de DO450nm para los Calibradores 0 y 10 mIU/ml, después comprobar que el ensayo es válido.

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 7):

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Calibrador 0 mIU/ml: 0.020 – 0.024 DO450nm
Valor medio: 0.022 DO450nm
Menor de 0.200 – Válido

Calibrador 10 mIU/ml: 0.250 – 0.270 DO450nm
Valor medio: 0.260 DO450nm
Mayor de Cal 0 + 0.100 – Válido

Calibrador 250 mIU/ml: 2.845 DO450nm
Mayor de 1.500 – *Válido*

Q. INTERPRETACION DE LOS RESULTADOS.
Las muestras con una concentración menor de 10 O.M.S. mIU/ml se consideran negativas a anti- HBsAg en la mayoría de la literatura médica internacional.
Las muestras con una concentración mayor de 10 O.M.S. mIU/ml se consideran positivas a anti- HBsAg.
En el seguimiento de receptores de vacunas, sin embargo, se aceptan por la literatura médica valores de 20 O.M.S. mIU/m como la concentración mínima a la que un paciente es considerado clínicamente protegido contra la infección por HBV.

Notas importantes:
1. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*

2. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
3. *El diagnóstico de infección con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.*

R. FUNCIONAMIENTO.
La evaluación del funcionamiento ha sido realizada según lo reportado en las Especificaciones Técnicas Comunes (ETC) (art. 5, Capítulo 3 de las Directivas IVD 98/79/EC).

1. LÍMITE DE DETECCIÓN.
El límite de detección del ensayo ha sido calculado por medio de una preparación estándar de referencia para HBsAb suministrada por CLB respaldado por O.M.S. (1ra preparación de referencia 1977, lote 17-2-77). Como diluente se empleó suero negativo a HBV, según lo recomendado por el fabricante.

La siguiente tabla muestra los resultados del Control de Calidad:

O.M.S. mIU/ml	SAB.CE Lote # 1002	SAB.CE Lote # 1001	SAB.CE Lote # 1002/2
50	0.933	0.812	0.846
10	0.219	0.192	0.194
5	0.110	0.096	0.104
2.5	0.057	0.058	0.067
Std 0	0.021	0.015	0.023

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICAS.
La evaluación del procedimiento diagnóstico se realizó mediante un ensayo con más de 700 muestras.

2.1 Especificidad Diagnóstica.
Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico. Se examinaron más de 500 muestras negativas de origen interno y externo, contra la referencia de una compañía europea. Se obtuvo una especificidad diagnóstica del 98.8%.
También contra esta referencia se analizaron 113 muestras que pudieran provocar interferencia (por ejemplo: otras enfermedades infecciosas, pacientes afectados por hepatitis no virales, pacientes sometidos a diálisis, mujeres embarazadas, hemofílicos, lipémicos, etc.). La especificidad obtenida fue del 100%.
Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

2.2 Sensibilidad Diagnóstica.
Se define como la probabilidad del ensayo de detectar positivos en presencia del analito específico.
Se evaluaron 106 pacientes vacunados, la sensibilidad diagnóstica fue del 100%.
Se probaron (interna y externamente) contra la referencia de la compañía europea, muestras de más de 100 pacientes infectados de manera natural con HBV. La sensibilidad diagnóstica fue del 100%.

3. PRECISIÓN.
Se realizó un estudio con 3 muestras de diferente reactividad anti-HBsAg, examinadas en 16 réplicas, en tres corridas separadas.
Los valores medios obtenidos se reportan a continuación :

SAB.CE: lote # 1202

Calibrador 0 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.038	0.038	0.039	0.039
Desviación estándar	0.003	0.004	0.005	0.004
CV %	8.8	9.5	11.8	10.0

Calibrador 10 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.250	0.243	0.244	0.246
Desviación estándar	0.020	0.023	0.017	0.020
CV %	8.0	9.3	7.0	8.1

Calibrador 250 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.998	3.000	3.259	3.085
Desviación estándar	0.152	0.151	0.158	0.153
CV %	5.1	5.0	4.8	5.0

SAB.CE: lote # 1002

Calibrador 0 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.048	0.048	0.050	0.049
Desviación estándar	0.005	0.004	0.006	0.005
CV %	9.4	8.4	11.5	9.8

Calibrador 10 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.249	0.252	0.242	0.248
Desviación estándar	0.021	0.020	0.023	0.021
CV %	8.3	7.9	9.6	8.6

Calibrador 250 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	3.544	3.653	3.612	3.603
Desviación estándar	0.153	0.176	0.138	0.156
CV %	4.3	4.8	3.8	4.3

SAB.CE: lote # 1002/2

Calibrador 0 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.050	0.051	0.050	0.050
Desviación estándar	0.005	0.006	0.006	0.005
CV %	10.0	10.9	11.9	10.9

Calibrador 10 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.226	0.238	0.239	0.234
Desviación estándar	0.015	0.017	0.018	0.016
CV %	6.5	7.0	7.5	7.0

Calibrador 250 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	3.526	3.457	3.499	3.494
Desviación estándar	0.137	0.143	0.162	0.147
CV %	3.9	4.1	4.6	4.2

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

4. EXACTITUD.

La exactitud del ensayo ha sido comprobada mediante diluciones y pruebas de recuperación. Cualquier "efecto gancho", estimación errónea que puede presentarse a elevadas dosis del analito, no se manifiesta hasta 10.000 mIU/ml.

Nota importante:

Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 7.

S. LIMITACIONES DEL PROCEDIMIENTO.

La contaminación bacteriana de las muestras o la inactivación por calor pueden modificar los valores de absorbancia con la consiguiente alteración de los niveles del analito. Este ensayo es adecuado solo para el análisis de muestras individuales y no para mezclas.

El diagnóstico de una enfermedad infecciosa no se debe formular en base al resultado de un solo ensayo, sino que es necesario tomar en consideración la historia clínica y la sintomatología del paciente así como otros datos diagnósticos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
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(Milán) – Italia

CE
0318

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Anti-Thyroid Peroxidase (Anti-TPO) Test System

Product Code: 1125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroid Peroxidase (TPO) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric.
Measurements of TPO autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Antibodies to thyroid peroxidase have been shown to be characteristically present from patients with Hashimoto thyroiditis (95%), idiopathic myedema (90%) and Graves Disease (80%)[1]. In fact 72% of patients positive for anti-TPO exhibit some degree of thyroid dysfunction.[2] This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction.

Measurements of antibodies to TPO have been done in the past by Passive Hemaglutination (PHA). PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the detectability of subclinical levels of antibodies to TPO. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation.

Monobind's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated Thyroid Peroxidase Antigen (TPO) is added, and then the reactants are mixed. Reaction results between the autoantibodies to TPO and the biotinylated TPO to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce color.

The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with autoimmune antibody level.

3.0 PRINCIPLE

A Sequential ELISA Method (TYPE 1)
The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated thyroid peroxidase antigen.

Upon mixing the biotinylated antigen and a serum containing the autoantibody, a reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$h\text{-}Ab_{(X\text{-}TPO)} + {}^{Btn}Ag_{(TPO)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} h\text{-}Ab_{(X\text{-}TPO)} - {}^{Btn}Ag_{(TPO)}$$

$^{Btn}Ag_{(TPO)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(X\text{-}TPO)}$ = Human Auto-Antibody (Variable Quantity)
$Ab_{(X\text{-}TPO)} - {}^{Btn}Ag_{(TPO)}$ = Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:

$h\text{-}Ab_{(X\text{-}TPO)} - {}^{Btn}Ag_{(TPO)} + \underline{Streptavidin}_{C.W.} \Rightarrow$ Immobilized complex (IC)

$\underline{Streptavidin}_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex (IC) = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.

$I.C._{(h\text{-}IgG)} + {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} \Rightarrow {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$

$I.C._{(h\text{-}IgG)}$ = Immobilized Immune complex (Variable Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG}$ = Enzyme-antibody Conjugate (Constant Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$ = Ag-Ab Complex (Variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained

4.0 REAGENTS

Materials Provided

A. Anti-TPO Calibrators – 1ml/vial Icons A-F
Six (6) vials of references for anti-TPO at levels of 0(A), 25(B), 50(C), 100(D), 250(E) and 500(F) IU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the Medical Research Council (MRC) International Standard 66/387 for anti thyroid microsome.

B. TPO Biotin Reagent – 13ml/vial – Icon ▽
One (1) vial of biotinylated thyroid peroxidase antigen stabilized in a buffering matrix. A preservative has been added. Store at 2-8°C

C. Anti-TPO Enzyme Reagent – 13ml/vial - Icon
One (1) vial of anti-human IgG-horseradish peroxidase (HRP) conjugate stabilized in a bufferred matrix. A preservative has been added. Store at 2-8°C

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Serum Diluent – 20ml/vial
One (1) vial of serum diluent concentrate that containing buffer salts and a dye. Store at 2-8°C.

F. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

G. Substrate A – 7ml/vial - Icon S[A]
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

H. Substrate B – 7ml/vial - Icon S[B]
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

I. Stop Solution – 8ml/vial - Icon
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

J. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

Required But Not Provided:
1. Pipette capable of delivering 0.010ml (10µl), 0.025ml (25µl), and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Test tube(s) for patient dilution.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8ºC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the samples(s) may be stored at temperatures of -20ºC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Serum Diluent**
   Dilute the serum diluent to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer**
   Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
3. **Working Substrate Solution** – Stable for one (1) year.
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.
4. **Patient Sample Dilution (1/100)**
   Dispense 0.010ml (10µl) of each patient specimen into 1ml (1000µl) of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note1 : Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well.
3. Add 0.100 ml (100µl) of the TPO Biotin Reagent
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of the x-TPO Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Incubate for thirty (30) minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 350µl of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
    DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 500 IU/ml, dilute the sample an additional 1:5 or 1:10 using the original diluted material. Multiply by the dilution factor to obtain the concentration of the specimen.

10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-TPO in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-TPO activity in IU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the level of anti-TPO activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.323) intersects the dose response curve at 200 IU/ml anti-TPO concentration (See Figure 1).

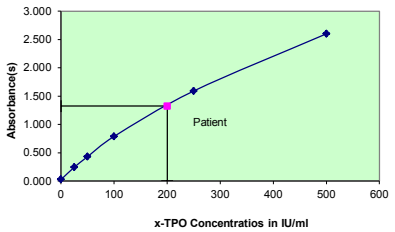
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (IU/ml)
Cal A	A1	0.022	0.026	0
	B1	0.030		
Cal B	C1	0.240	0.244	25
	D1	0.247		
Cal C	E1	0.437	0.430	50
	F1	0.422		
Cal D	G1	0.795	0.788	100
	H1	0.782		
Cal E	A2	1.610	1.590	250
	B2	1.572		
Cal F	C2	2.659	2.600	500
	D2	2.533		
Patient	E2	1.294	1.323	**200**
	F2	1.351		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-TPO in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

The presence of autoantibodies to TPO is confirmed when the serum level exceeds 40 IU/ml. The clinical significance of the result, coupled with anti-thyroglobulin activity, should be used in evaluating the thyroid condition. However, clinical inferences should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests.

13.0 EXPECTED RANGES OF VALUES

A study of normal population was undertaken to determine expected values for the anti-TPO AccuBind® ELISA test system. The number (n), mean (x) and standard deviation (σ) are given in Table 1. Values in excess of 40IU/ml are considered positive for the presence of anti-TPO autoantibodies.

TABLE I
Expected Values for the Anti-TPO ELISA Test System (In IU/ml)

Number	**100**
Mean	17.6
Standard deviation	10.8
Upper 95% (+2σ) level	39.2

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the anti-TPO AccuBind® ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V) for each of these control sera are presented in Tables 2 and 3.

TABLE 2
Within Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	20	25.5	1.5	5.7%
Pool 2	20	120.5	4.6	3.8%
Pool 3	20	352.4	14.8	4.2%

TABLE 3*
Between Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	10	26.5	1.8	6.8%
Pool 2	10	118.5	5.3	4.5%
Pool 3	10	365.4	22.5	6.2%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The anti-TPO AccuBind® ELISA test system has a sensitivity of 0.92 IU/ml. The sensitivity (detection limit) was ascertained by determining the variablility of the '0 IU/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The anti-TPO AccuBind® ELISA test system was compared with a reference anti-TPO ELISA microplate. Biological specimens from normal and disease states populations were used. The disease states included; Hashimoto's thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 82. The least square regression equation and the correlation coefficient were computed for the anti-TPO AccuBind® ELISA test system in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	122.9	y = 1.02 (x) – 5.1	0.989
Reference	127.0		

14.4 Specificity

Interferences from ANA, DNA, thyroglobulin (TPO) and rheumatoid antibodies were found to be insignificant

15.0 REFERENCES

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9. Ekholm R, "Biosysnthesis of thyroid hormones", *Int Rev Cytol,* 120, 243-288 (1990).
10. Degroot LJ, "Heterogeneity of human antibodies to TPO Thyroperoxidase", *Thyroid Autoimmunity,* **207,**177-182 (1990).

Revision: 4 Date: 2019-JUL-16 DCO: 1353
MP1125 Product Code: *1125-300*

Size		96(A)
Reagent (fill)	A)	1ml set
	B)	1 (13ml)
	C)	1 (13ml)
	D)	1 plate
	E)	1 (20ml)
	F)	1 (20ml)
	G)	1 (7ml)
	H)	1 (7ml)
	I)	1(8ml)

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IVD 2° 8° CE
EC REP CEpartner4U, Esdoornlaan 13 3951 DBMaarn, The Neatherlands www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device
2° 8° Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF Catalogue Number
Σ Contains Sufficient Test for Σ
LOT Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP Authorized Rep in European Country
CE European Conformity

NovaTec Immundiagnostica GmbH

NovaLisa®

Trichinella spiralis IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: TRIG0480 (96 Determinations)

ENGLISH

1. INTRODUCTION

Trichinosis (also called trichinellosis) is caused by nematodes (roundworms) of the genus Trichinella. In addition to the classical agent Trichinella spiralis, which is found worldwide in many carnivorous and omnivorous animals, four other species (T. pseudospiralis, T. nativa, T. nelsoni, and T. britovi) are recognized. Trichinosis is acquired by ingesting meat containing cysts of Trichinella. After exposure to gastric acid and pepsin, the larvae are released from the cysts and invade the small bowel mucosa where they develop into adult worms (female 2.2 mm in length, males 1.2 mm). After 1 week, the females release larvae that migrate to the striated muscles where they encyst. Encystment is completed in 4 to 5 weeks and the encysted larvae may remain viable for several years. Ingestion of the encysted larvae perpetuates the cycle.

Trichinosis infection occurs worldwide, but is most common in parts of Europe and the United States. Light infections may be asymptomatic. For mild to moderate infections, most symptoms subside within a few months whereas fatigue, weakness, and diarrhoea may last for months. In severe cases, death can occur.

Species	Disease	Symptoms (e.g.)	Transmission route
Trichinella spiralis	Trichinosis	Nausea, diarrhea, vomiting, fatigue, fever and abdominal discomfort. Larval migration into muscle tissue can cause edema, conjunctivitis, myalgias, splinter hemorrhages, rashes and blood eosinophilia.	Infection can occur by eating raw or undercooked pork and wild game products infected with the larvae of Trichinella worms

Infection or presence of pathogen may be identified by:

- Microscopy: muscle biopsy
- Serology: Detection of antibodies by ELISA

2. INTENDED USE

The Trichinella spiralis IgG ELISA is intended for the qualitative determination of IgG class antibodies against Trichinella spiralis in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Trichinella spiralis antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled Protein A in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplate
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Trichinella spiralis antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20…25°C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.748	6.37
#2	24	1.225	4.21
#3	24	1.570	5.13

Interassay	n	Mean (NTU)	CV (%)
#1	12	17.50	8.38
#2	12	21.33	13.08
#3	12	3.69	10.38

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 94.81% (95% confidence interval: 87.23% - 98.57%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 79.41% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray.
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: TRIG0480 Trichinella spiralis IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Trichinella spiralis ist der Erreger der Trichinose, einer weltweit verbreiteten Erkrankung des Menschen und zahlreicher Tiere. Trichinellen gehören zu den Fadenwürmern (Nematoden). Sie sind Gewebeparasiten primär von karnivoren (Fleischfresser) und omnivoren (Allesfresser) Säugetieren, einschließlich des Menschen. Nebst Trichinella spiralis sind verschiedene Arten und Unterarten von Trichinellen bekannt: T. britovi parasitiert in Luchs und Fuchs, T. nativa in Polarbär und Meeressäugern, T. nelsoni in Hyäne und Löwe und T. pseudospiralis in Vögeln und Nagern.

Adulte, geschlechtsreife Darmtrichinellen sind wenige mm lang und leben bevorzugt in der Schleimhaut des Dünndarmes. Das Weibchen setzt nach der Befruchtung laufend Larven frei, die sich durch die Darmwand bohren und über Blut- und Lymphweg in der Skelettmuskulatur des Wirtes einnisten. Diese Muskeltrichinellen stellen die infektiöse Form im Zyklus dar und bleiben in den wirtseigenen Fibrinkapseln der Muskulatur jahrelang infektiös. Mit einer Fleischmahlzeit gelangen die Muskeltrichinellen in neue Wirte, wo sie sich wiederum im Dünndarm einnisten und so den Zyklus als adulte, geschlechtsreife Stadien schließen.

Der klinische Verlauf der Erkrankung ist abhängig von der Anzahl der inkorporierten Trichinenlarven. Ab einer Aufnahme von ca. 50-70 Larven können Symptome auftreten, ab 3000 Muskeltrichinellen werden die Auswirkungen bereits bedrohlich. Als erste Symptome treten Nausea, Erbrechen, gastrointestinale Störungen mit Durchfall und leichtem Fieber auf, später Myositis mit Muskelschmerzen und -steifheit, Atem- und Schluckbeschwerden, Fieber, Lid- und Gesichtsödem und Hautexanthem. Gefürchtete Komplikationen sind Myokarditis und Meningoenzephalitis. Sobald die eingekapselten Trichinellen ihren Stoffwechsel reduzieren, klingen die Symptome ab. Eine gewisse Leistungsbeeinträchtigung der Muskulatur sowie rheumatoide Beschwerden können jedoch dauernd bestehen bleiben.

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
Trichinella spiralis	Trichinose (Trichinellose)	Übelkeit, Durchfall, Erbrechen, Müdigkeit, Fieber und abdominal Beschwerden. Larvale Migration in Muskelgewebe kann Ödem, Konjunktivitis, Myalgien, Splitterblutung, Hautausschläge und Blut Eosinophilie verursachen	Infektion kann auftreten durch den Verzehr von rohem oder nicht durchgebratenem Schweinefleisch und Wildprodukten, die mit den Larven von Trichinella Würmern infiziert sind.

Nachweis des Erregers bzw. der Infektion durch:

- Mikroskopie, Muskelbiopsie
- Serologie: Nachweis spezifischer Antikörper mittels ELISA

2. VERWENDUNGSZWECK

Der Trichinella spiralis IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Trichinella spiralis in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

4. MATERIALIEN

4.1. Mitgelieferte Reagenzien

- **Mikrotiterplatte:** 12 teilbare 8er-Streifen, beschichtet mit Trichinella spiralis Antigenen; in wieder verschließbarem Aluminiumbeutel.
- **IgG-Probenverdünnungspuffer:** 1 Flasche mit 100 mL Phosphatpuffer (10 mM) zur Probenverdünnung; pH 7,2 ± 0,2; gelb gefärbt; gebrauchsfertig; weiße Verschlusskappe; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Stopplösung:** 1 Flasche mit 15 mL Schwefelsäure, 0,2 mol/L; gebrauchsfertig; rote Verschlusskappe.
- **Waschpuffer (20x konz.):** 1 Flasche mit 50 mL eines 20-fach konzentrierten Phosphatpuffers (0,2 M), zum Waschen der Kavitäten; pH 7,2 ± 0,2; weiße Verschlusskappe.
- **Konjugat:** 1 Flasche mit 20 mL Peroxidase-konjugiertem Protein A in Phosphatpuffer (10 mM); blau gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **TMB-Substratlösung:** 1 Flasche mit 15 mL 3,3`,5,5`-Tetramethylbenzidin (TMB), < 0,1 %; gebrauchsfertig; gelbe Verschlusskappe.
- **Positivkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; rote Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Cut-off Kontrolle:** 1 Fläschchen mit 3 mL Kontrolle; gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Negativkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Für Gefahren- und Sicherheitshinweise siehe 12.1.
Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Arbeitsanleitung
- 1 Plattenlayout

4.3. Erforderliche Materialien und Geräte

- Mikrotiterplatten-Photometer mit Filtern 450/620 nm
- Inkubator 37 °C
- Manuelle oder automatische Waschvorrichtung für Mikrotiterplatten
- Mikropipetten (10 - 1000 µL)
- Vortex-Mischer
- Destilliertes Wasser
- Plastikröhrchen für den einmaligen Gebrauch

5. STABILITÄT UND LAGERUNG

Testkit bei 2...8 °C lagern. Die geöffneten Reagenzien sind bis zu den auf den Etiketten angegebenen Verfallsdaten verwendbar, wenn sie bei 2...8 °C gelagert werden.

6. VORBEREITUNG DER REAGENZIEN

Es ist sehr wichtig, alle Reagenzien und Proben vor ihrer Verwendung auf Raumtemperatur (20...25 °C) zu bringen und zu mischen!

6.1. Mikrotiterplatte

Die abbrechbaren Streifen sind mit Trichinella spiralis Antigenen beschichtet. Nicht verbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8 °C lagern.

6.2. Waschpuffer (20x konz.)

Der Waschpuffer ist im Verhältnis 1 + 19 zu verdünnen; z.B. 10 mL Waschpuffer + 190 mL destilliertes Wasser.
Der verdünnte Puffer ist bei Raumtemperatur (20…25°C) 5 Tage haltbar. Sollten Kristalle im Konzentrat auftreten, die Lösung z.B. in einem Wasserbad auf 37 °C erwärmen und vor dem Verdünnen gut mischen.

6.3. TMB-Substratlösung

Die gebrauchsfertige Lösung ist bei 2...8 °C vor Licht geschützt aufzubewahren. Die Lösung ist farblos, kann aber auch leicht hellblau sein. Sollte die TMB-Substratlösung blau sein, ist sie kontaminiert und kann nicht im Test verwendet werden.

7. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum- oder Plasmaproben (Citrat, Heparin) verwendet werden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8 °C aufbewahrt werden, sonst aliquotieren und tiefgefrieren (-70…-20 °C). Wieder aufgetaute Proben vor dem Verdünnen gut schütteln. Wiederholtes Tiefgefrieren und Auftauen vermeiden!
Hitzeinaktivierung der Proben wird nicht empfohlen.

7.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1 + 100 mit IgG-Probenverdünnungspuffer verdünnen, z. B. 10 µL Probe und 1 mL IgG-Probenverdünnungspuffer in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1 + 100 zu erhalten; gut mischen (Vortex).

8. TESTDURCHFÜHRUNG

Arbeitsanleitung **vor** Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Arbeitsanleitung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschritte von drei bis fünf und das Volumen des Waschpuffers von 300 µL auf 350 µL zu erhöhen. Kapitel 12 beachten. Vor Testbeginn auf dem mitgelieferten Plattenlayout die Verteilung bzw. Position der Proben und der Standards/Kontrollen (Doppelbestimmung empfohlen) genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Standards/Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Inkubator auf 37 ± 1 °C einstellen.

1. Je 100 µL Standards/Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h ± 5 min bei 37 ± 1 °C inkubieren.**
4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300 µL Waschpuffer waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Das Intervall zwischen Waschen und Absaugen sollte > 5 sec betragen. Nach dem Waschen die Teststreifen auf Fließpapier ausklopfen, um die restliche Flüssigkeit zu entfernen.

   Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falschen Messergebnissen führt!
5. 100 µL Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes A1 vorgesehenen, pipettieren.
6. **30 min bei Raumtemperatur (20...25 °C) inkubieren.** Nicht dem direkten Sonnenlicht aussetzen.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100 µL TMB-Substratlösung in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25 °C) inkubieren.** Bei enzymatischer Reaktion findet eine Blaufärbung statt.
10. In alle Vertiefungen 100 µL Stopplösung in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei Zugabe der TMB-Substratlösung pipettieren, dadurch erfolgt ein Farbwechsel von blau nach gelb.
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe der Stopplösung messen.

8.1. Messung

Mit Hilfe des Substratleerwertes den **Nullabgleich** des Mikrotiterplatten-Photometers vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert des Substratleerwertes von allen anderen Extinktionswerten subtrahiert werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Standards/Kontrollen und Proben in das Plattenlayout eintragen.

Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

9. BERECHNUNG DER ERGEBNISSE

9.1. Testgültigkeitskriterien

Damit ein Testlauf als valide betrachtet werden kann, muss diese Gebrauchsanweisung strikt befolgt werden, und die folgenden Kriterien müssen erfüllt sein:

- **Substrat-Leerwert**: Extinktionswert **< 0,100**
- **Negativkontrolle**: Extinktionswert **< 0,200 und < Cut-off**
- **Cut-off Kontrolle**: Extinktionswert **0,150 – 1,300**
- **Positivkontrolle**: Extinktionswert **> Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

9.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der Cut-off Kontrolle.

Beispiel: 0,44 OD Cut-off Kontrolle + 0,42 OD Cut-off Kontrolle = 0,86 : 2 = 0,43

Cut-off = 0,43

9.2.1. Ergebnisse in Einheiten [NTU]

$$\frac{\text{Mittlere Extinktion der Probe} \times 10}{\text{Cut-off}} = \text{[NovaTec Einheiten = NTU]}$$

Beispiel: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretation der Ergebnisse

Cut-off	10 NTU	-
Positiv	> 11 NTU	Es liegen Antikörper gegen den Erreger vor. Ein Kontakt mit dem Antigen (Erreger bzw. Impfstoff) hat stattgefunden.
Grenzwertig	9 – 11 NTU	Antikörper gegen den Erreger können nicht eindeutig nachgewiesen werden. Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen. Finden sich die Ergebnisse erneut im grenzwertigen Bereich, gilt die Probe als **negativ**.
Negativ	< 9 NTU	Es liegen keine Antikörper gegen den Erreger vor. Ein vorausgegangener Kontakt mit dem Antigen (Erreger bzw. Impfstoff) ist unwahrscheinlich.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden. Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse serologischer Tests nur einen begrenzten Wert.		

10. TESTMERKMALE

Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen.

Für weitere Informationen zu den Testmerkmalen kontaktieren Sie bitte NovaTec Immundiagnostica GmbH.

10.1. Präzision

Intraassay	n	Mittelwert (E)	Vk (%)
#1	24	0,748	6,37
#2	24	1,225	4,21
#3	24	1,570	5,13

Interassay	n	Mittelwert (NTU)	Vk (%)
#1	12	17,50	8,38
#2	12	21,33	13,08
#3	12	3,69	10,38

10.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 94,81% (95% Konfidenzintervall: 87,23% - 98,57%).

10.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie ist 100% (95% Konfidenzintervall: 79,41% - 100%).

10.4. Interferenzen

Hämolytische, lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/mL Hämoglobin, 5 mg/mL Triglyceride und 0,5 mg/mL Bilirubin keine Interferenzen im vorliegenden ELISA.

10.5. Kreuzreaktivität

Die Untersuchung eines Probenpanels mit Antikörperaktivitäten gegen potenziell kreuzreagierende Parameter ließ keine signifikanten Anzeichen von falsch-positiven Ergebnissen aufgrund von Kreuzreaktivitäten erkennen.

11. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.

12. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Arbeitsanleitung sind strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle Materialien menschlichen oder tierischen Ursprungs sind als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAg nicht-reaktiv getestet.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen, um Kreuzkontaminationen zu vermeiden.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat und Standards/Kontrollen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten, Reagenzien sorgfältig in die Kavitäten pipettieren.
- Der ELISA ist nur für qualifiziertes Personal bestimmt, das den Standards der Guten Laborpraxis (GLP) folgt.
- Zur weiteren internen Qualitätskontrolle sollte jedes Labor zusätzlich bekannte Proben verwenden.

12.1. Sicherheitshinweis für Reagenzien, die Gefahrstoffe enthalten

Die Reagenzien können CMIT/MIT (3:1) oder MIT enthalten (siehe 4.1)
Daher gelten die folgenden Gefahren- und Sicherheitshinweise.

Achtung

H317	Kann allergische Hautreaktionen verursachen.
P261	Einatmen von Aerosol vermeiden.
P280	Schutzhandschuhe/ Schutzkleidung tragen.
P302+P352	BEI BERÜHRUNG MIT DER HAUT: Mit viel Seife und Wasser waschen.
P333+P313	Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ ärztliche Hilfe hinzuziehen.
P362+P364	Kontaminierte Kleidung ausziehen und vor erneutem Tragen waschen.

Weitere Informationen können dem Sicherheitsdatenblatt entnommen werden.

12.2. Entsorgungshinweise

Chemikalien und Zubereitungen sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den nationalen abfallrechtlichen Gesetzen und Verordnungen. Die zuständige Behörde informiert über die Entsorgung von Sonderabfällen.

13. BESTELLINFORMATIONEN

Produktnummer: TRIG0480 Trichinella spiralis IgG ELISA (96 Bestimmungen)

FRANÇAIS

1. INTRODUCTION

La trichinose (également appelée trichinellose) est causée par des nématodes (ascarides lombricoïdes) du genre Trichinella. En plus de l'agent classique Trichinella spiralis, qu'on trouve dans le monde entier dans beaucoup d'animaux carnivores et omnivores, quatre autres espèces (T. pseudospiralis, T. nativa, T. nelsoni, et T. britovi) sont connues. La trichinose est transmise par ingestion de viande qui contient des kystes de Trichinella. Après avoir été exposées à l'acide gastrique et à la pepsine, les kystes libèrent des larves qui envahissent la muqueuse de l'intestin grêle où elles se développent en vers adultes (femelle: 2.2 millimètres en longueur; mâles: 1.2 millimètre). Après une semaine, les femelles libèrent des larves qui migrent aux muscles striés où elles s'enkystent. L'enkystement est accompli en 4 à 5 semaines et les larves enkystées peuvent rester vivantes pendant plusieurs années. L'ingestion des larves enkystées perpétue le cycle.

L'infection de trichinose se produit dans le monde entier, mais surtout dans certaines régions d'Europe et des Etats-Unis. Les infections légères peuvent être asymptomatiques.

La plupart des symptômes d'une infection modérée passe dans quelques mois, tandis que la fatigue, la faiblesse, et la diarrhée peuvent durer plus longtemps. Des cas graves, peuvent même être mortels.

Espèce	La maladie	Symptômes (p.ex.)	Modes de transmission
Trichinella spiralis	Trichinose	Nausée, diarrhée, vomissement, fatigue, fièvre et malaise abdominale. La migration larvaire dans les muscles peut causer des œdèmes, la conjonctivite, les myalgies, des hémorragies, des éruptions et l'éosinophilie.	L'infection peut se produire en mangeant du porc infecté cru ou pas assez cuit et du gibier infecté des larves de Trichinella

L'infection ou la présence d'un agent pathogène peut être identifiée par:

- Microscopie, biopsie de muscles
- Sérologie: Détection des anticorps par ELISA

2. INDICATION D'UTILISATION

La trousse Trichinella spiralis IgG ELISA est prévue pour la détection qualitative des anticorps IgG anti-Trichinella spiralis dans le sérum humain ou plasma (citrate, héparine).

3. PRINCIPE DU TEST

La détermination immunoenzymatique qualitative des anticorps spécifiques est basée sur le la technique ELISA (du anglais, Enzyme-Linked Immunosorbent Assay).
Plaques de Microtitrage sont recouvertes d'antigènes spécifiques pour lier les anticorps correspondants de l'échantillon. Après le lavage des puits pour éliminer l'échantillon détaché, le conjugué peroxydase de raifort (HRP) est ajouté. Ce conjugué se lie aux anticorps capturés. Dans une deuxième étape de lavage, le conjugué non lié est éliminé. Le complexe immun formé par le conjugué lié est visualisé par l'addition tétraméthylbenzidine (TMB) qui donne un produit de réaction bleu.
L'intensité de ce produit est proportionnelle à la quantité d'anticorps spécifiques dans l'échantillon. L'acide sulfurique est ajouté pour arrêter la réaction. Cela produit un changement du bleu au jaune. L'absorbance à 450/620 nm est lue en utilisant un photomètre de Plaques de Microtitrage ELISA.

4. MATERIEL

4.1. Réactifs fournis

- **Plaque de Microtitrage:** 12 barrettes de 8 puits sécables revêtus d'antigène d'Trichinella spiralis; en sachets d'aluminium refermables.
- **Tampon de Dilution d'Échantillon IgG:** 1 flacon contenant 100 mL de tampon phosphaté (10 mM) pour la dilution de l'échantillon; pH 7,2 ± 0,2; prêt à l'emploi; couleur jaune; bouchon blanc; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solution d'Arrêt:** 1 flacon contenant 15 mL d'acide sulfurique, 0,2 mol/L; prêt à l'emploi; bouchon rouge.
- **Tampon de Lavage (concentré x 20):** 1 flacon contenant 50 mL d'un tampon phosphaté (0,2 M) concentré 20 fois (pH 7,2 ± 0,2) pour laver les puits; bouchon blanc.
- **Conjugué:** 1 flacon contenant 20 mL de Protein A conjuguées à de la peroxydase du raifort dans le tampon phosphaté (10 mM); prêt à l'emploi; couleur bleue, bouchon noir.
- **Solution de Substrat TMB:** 1 flacon contenant 15 mL de 3,3',5,5'-tétraméthylbenzidine (TMB), < 0,1%; prêt à l'emploi; bouchon jaune.
- **Contrôle Positif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon rouge; ≤ 0,02% (v/v) MIT.
- **Contrôle Cut-off:** 1 flacon contenant 3 mL contrôle; prêt à l'emploi; couleur jaune; bouchon vert; ≤ 0,02% (v/v) MIT.
- **Contrôle Négatif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon bleu; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Pour les mentions de danger et les conseils de prudence voir chapitre 12.1
Pour les substances potentiellement dangereuses s'il vous plaît vérifiez la fiche de données de sécurité.

4.2. Matériel fourni

- 1 couvercle autocollante
- 1 instructions d'utilisation
- 1 présentation de la plaque

4.3. Matériel et équipement requis

- Photomètre de Plaque de Microtitrage ELISA, pour mesurer l'absorbance à 450/620 nm
- Incubateur 37 °C
- Laveur manuel ou automatique pour le lavage des Plaque de Microtitrage
- Pipettes pour utilisation entre 10 et 1000 µL
- Mélangeur Vortex
- Eau distillée
- Tubes jetables

5. STABILITE ET CONSERVATION

Conserver le kit à 2...8 °C. Les réactifs ouverts sont stables jusqu'à la date de péremption indiquée sur l'étiquette lorsqu'il est conservé à 2...8°C.

6. PREPARATION DES REACTIFS

Il est très important porter tous les réactifs et échantillons à température ambiante (20...25 °C) et les mélanger avant de commencer le test!

6.1. Plaque de Microtitrage

Les barrettes sécables sont revêtues d'antigène d'Trichinella spiralis. Immédiatement après avoir prélevé les barrettes nécessaires, les barrette restantes doivent être scellés le vide dans de feuille d'aluminium avec le sac de silicium (le déshydratant) fourni et emmagasiner à 2...8 °C.

6.2. Tampon de Lavage (conc. x 20)

Diluer le Tampon de Lavage 1+19; par exemple 10 mL du Tampon de Lavage + 190 mL d'eau distillée. Le Tampon de Lavage diluée est stable pendant 5 jours à la température ambiante (20...25 °C). Cas apparaissent des cristaux dans le concentré, chauffer la solution à 37 °C par exemple dans un bain-marie mélangez bien avant dilution.

6.3. Solution de Substrat TMB

La solution est prête à utiliser et doit être emmagasiné à 2...8 °C, à l'abri de la lumière. La solution doit être incolore ou pourrait avoir une légère couleur bleu clair. Si le substrat devient bleu, il peut avoir été contaminé et ne peut pas être utilisé dans le test.

7. PRELEVEMENT ET PREPARATION DES ECHANTILLONS

Utiliser des échantillons humains de sérum ou plasma (citrate, héparine) pour ce test. Si le test est réalisé dans les 5 jours après le prélèvement, les échantillons doivent être conservés à 2...8 °C; autrement ils doivent être aliquotés et conservés surgelés (-70…-20 °C). Si les échantillons sont conservés congelés, bien mélanger les échantillons décongelés avant le test. Éviter les cycles répétés de congélation et décongélation.
L'inactivation par la chaleur des échantillons n'est pas recommandée.

7.1. Dilution de l'échantillon

Avant du test, tous les échantillons doivent être dilués 1 + 100 avec Tampon de Dilution d'Échantillon IgG. Diluer 10 µL d'échantillon avec 1 mL I Tampon de Dilution d'Échantillon IgG dans des tubes pour obtenir une dilution 1 + 100 et mélanger soigneusement sur un Vortex.

8. PROCEDE DE TEST

Lire attentivement les instructions d'utilisation **avant de** réaliser le test. La fiabilité des résultats dépend du suivi strict d'utilisation comme décrit. La technique de test suivante a été validée uniquement pour une procédure manuelle. Si le test doit être effectué sur un système automatique pour ELISA, nous conseillons d'augmenter le nombre d'étapes de lavage de trois à cinq et le volume du Tampon de Lavage de 300 à 350 µL. Faites attention au chapitre 12. Avant de commencer le test, le plan de distribution et d'identification de tous les échantillons et les étalons/contrôles (il est recommandé déterminer en double) doivent être soigneusement établi sur la feuille présentation de la plaque prévue dans le conseil de kit. Sélectionner le nombre de barrettes ou de puits nécessaires et les placer sur le support.

Réaliser toutes les étapes du test dans l'ordre donné et sans délai.

Un embout de pipette propre et jetable doit être utilisé pour distribuer chaque étalon/contrôle et échantillon.

Régler l'incubateur à 37 ± 1 °C.

1. Pipeter 100 µL de étalons/contrôles et d'échantillons dilués dans leurs puits respectifs. Garder le puits A1 pour le blanc substrat.
2. Couvrir les puits avec le couvercle, fourni dans le kit.
3. **Incuber pendant 1 heure ± 5 minutes à 37 ± 1 °C.**
4. A la fin de l'incubation, enlever le couvercle, aspirer le contenu des puits et laver chaque puits trois fois avec 300 µL du Tampon de Lavage. Éviter les débordements des puits de réaction. L'intervalle entre le cycle de lavage et l'aspiration doit être > 5 sec. À la fin, enlever soigneusement le liquide restant en tapotant les barrettes sur du papier absorbant avant la prochaine étape.

   Note: L'étape de lavage est très importante! Un lavage insuffisant peut conduire à une précision faible et de faux résultats!
5. Pipeter 100 µL du conjugué dans tous les puits sauf le puits Blanc A1.
6. **Incuber pendant 30 minutes à température ambiante (20…25°C).** N'exposer pas à la lumière directe du soleil.
7. Répéter l'étape numéro 4.
8. Pipeter 100 µL de la Solution de Substrat TMB dans tous les puits.
9. **Incuber pendant exactement 15 minutes à température ambiante (20…25 °C) dans l'obscurité.** Une couleur bleue se produit en raison d'une réaction enzymatique.
10. Pipeter 100 µL de la Solution d'Arrêt dans tous les puits dans le même ordre et à la même vitesse que pour la Solution de Substrat TMB, ainsi, il y a un changement du bleu au jaune.
11. Mesurer l'absorbance à 450/620 nm dans les 30 minutes après l'addition de la Solution d'Arrêt.

8.1. Mesure

Réglez le Photomètre de Plaque de Microtitrage ELISA **à zéro** en utilisant **le Blanc substrat.**
Si - pour des raisons techniques - le Photomètre de Plaque de Microtitrage ELISA ne peut pas être ajusté à zéro en utilisant le Blanc substrat, la valeur d'absorbance de cette doit être soustraire la valeur d'absorbance de toutes les autres valeurs d'absorbance mesurées afin d'obtenir des résultats fiables!

Mesurer l'absorbance de tous les puits **à 450 nm** et enregistrer les valeurs d'absorbance pour chaque étalon/contrôle et échantillon dans la présentation de la plaque.

Il est recommandé d'effectuer la mesure **dichromatique** utilisant 620 nm comme longueur d'onde de référence.
Si doubles déterminations ont été effectuées, calculer **les valeurs moyennes d'absorbance.**

9. RESULTATS

9.1. Critères de validation

Pour qu'une série d'analyses soit considérée comme valide, ces instructions d'utilisation doivent être strictement suivies, et les critères suivants doivent être respectés:

- **Blanc Substrat**: Valeur d'absorbance **< 0,100**
- **Contrôle Négatif**: Valeur d'absorbance **< 0,200 et < Cut-off**
- **Contrôle Cut-off**: Valeur d'absorbance **0,150 – 1,300**
- **Contrôle Positif**: Valeur d'absorbance **> Contrôle Cut-off**

Lorsque ces critères ne sont pas remplis, le test n'est pas valide et doit être recommencé.

9.2. Calcul des résultats

La valeur seuil correspond à la moyenne des valeurs d'absorbance du Contrôle Cut-off.

Exemple: 0,44 DO Contrôle Cut-off + 0,42 DO Contrôle Cut-off = 0,86 : 2 = 0,43
Cut-off = 0,43

9.2.1. Résultats en unités [NTU]

$$\frac{\text{Valeur (moyenne) d'absorbance de l' échantillon} \times 10}{\text{Cut-off}} = \text{[unités NovaTec = NTU]}$$

Exemple: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interprétation des résultats

Cut-off	10 NTU	-
Positif	> 11 NTU	Les anticorps dirigés contre l'agent pathogène sont présents. Il ya eu un contact avec l'antigène (pathogène resp. vaccin).
Zone grise	9 – 11 NTU	Les anticorps dirigés contre l'agent pathogène ne pouvaient pas être détectés clairement. Il est recommandé de répéter le test avec un échantillon frais dans 2 à 4 semaines. Si le résultat est encore dans la zone grise l'échantillon est jugé **négatif**.
Negatif	< 9 NTU	L'échantillon ne contient pas d'anticorps contre l'agent pathogène. Un contact préalable avec l'antigène (pathogène resp. vaccin) est peu probable.
Le diagnostic d'une maladie infectieuse ne devrait pas être établi sur la base du résultat d'une seule analyse. Un diagnostic précis devrait prendre en considération l'histoire clinique, la symptomatologie ainsi que les données sérologiques. Les données sérologiques sont de valeur limité dans le cas des patients immunodéprimés et des nouveaux-nés.		

10. PERFORMANCES DU TEST

Ces résultats s'appuient sur les groupes d'échantillons étudiés; il n'agit pas de caractéristiques techniques garanties.

Pour plus d'informations sur les performances du test s'il vous plaît contactez NovaTec Immundiagnostica GmbH.

10.1. Précision

Intra-essai	n	moyenne (E)	CV (%)
#1	24	0,748	6,37
#2	24	1,225	4,21
#3	24	1,570	5,13

Inter-essai	n	moyenne (NTU)	CV (%)
#1	12	17,50	8,38
#2	12	21,33	13,08
#3	12	3,69	10,38

10.2. Spécificité diagnostique

La spécificité diagnostique est définie comme la probabilité d'obtenir un résultat négatif en l'absence d'un analyte spécifique. Elle est 94,81% (95% Intervalle de confiance: 87,23% - 98,57%).

10.3. Sensibilité diagnostique

La sensibilité diagnostique est définie comme la probabilité d'obtenir un résultat positif en présence d'un analyte spécifique. Elle est 100% (95% Intervalle de confiance: 79,41% - 100%).

10.4. Interférences

Des échantillons hémolytiques ou lipémiques ou ictériques n'ont pas montré d'interférences, avec des concentrations jusqu'à 10 mg/mL de hémoglobine, 5 mg/mL de triglycérides et 0,5 mg/mL de bilirubine.

10.5. Réaction croisée

L'étude d'un panel d'échantillons avec des anticorps dirigés contre différents paramètres interférents n'a pas révélé de preuves significatives de résultats faussement positifs dus à des réactions croisées.

11. LIMITES DE LA TECHNIQUE

Une contamination bactérienne ou des cycles de congélation/décongélation répétés de l´échantillon peuvent affecter les valeurs d'absorption.

12. PRECAUTIONS ET AVERTISSEMENTS

- La procédure de test, l'information, les précautions et mises en garde de la notice d'emploi, doivent être suivies de façon stricte. L'utilisation de ces trousses avec des automates ou dispositifs similaires doit être validée. Aucun changement de la conception, composition et procédure de test, ainsi que l'utilisation avec d'autres produits non approuvés par le fabricant, ne sont pas autorisés; seul l'utilisateur est responsable de tels changements. Le fabricant n'est pas responsable des faux résultats et des incidents dus à ces motifs. Le fabricant n'est pas responsable des résultats fournis par analyse visuelle des échantillons des patients.
- Uniquement pour diagnostic in vitro.
- Tous les matériaux d'origine humaine ou animale doivent être considérés et traités comme étant potentiellement infectieux.
- Tous les composants d'origine humaine utilisés pour la fabrication de ces réactifs ont été analysés et ont été trouvés non réactifs en Ag HBs, en anticorps anti-VHI 1 et 2 et en anticorps anti-VHC.
- Ne pas échanger les réactifs ou les Plaque de Microtitrage provenant de différents lots de production.
- Ne pas utiliser de réactifs provenant d'autres fabricants avec les réactifs de cette trousse.
- Ne pas utiliser les réactifs après la date de péremption indiquée sur l'étiquette.
- Utiliser seulement des embouts de pipette, des distributeurs et du matériel de laboratoire propres.
- Ne pas échanger les bouchons des flacons, pour éviter la contamination croisée.
- Fermer soigneusement les flacons après utilisation pour éviter l'évaporation et la contamination microbienne.
- Avant une nouvelle utilisation, vérifier les flacons de conjugué et de étalon/contrôle, déjà utilisés, pour exclure une contamination microbienne.
- Pour éviter la contamination croisée et des résultats faussement élevés, introduire les échantillons de patients et les réactifs exactement au fond des puits sans éclabousser.
- L'ELISA est uniquement conçu pour le personnel qualifié suivant les normes de bonnes pratiques de laboratoire (Good Laboratory Practice, GLP).
- Pour un contrôle de qualité interne plus poussé, chaque laboratoire doit en outre utiliser des échantillons connus.

12.1. Note de sécurité pour les réactifs contenant des substances dangereuses

Les réactifs peuvent contenir du CMIT/MIT (3 :1) ou du MIT (voir chapitre 4.1)
Par conséquent, les mentions de danger et les conseils de prudence suivants s'appliquent.

Attenttion	H317	Peut provoquer une allergie cutanée.
	P261	Éviter de respirer les aérosols.
	P280	Porter des gants de protection/ des vêtements de protection.
	P302+P352	EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment savon à l'eau.
	P333+P313	En cas d'irritation ou d'éruption cutanée: consulter un médecin.
	P362+P364	Enlever les vêtements contaminés et les laver avant réutilisation.

De plus amples informations peuvent être trouvées dans la fiche de données de sécurité.

12.2. Elimination des déchets

Les résidus des produits chimiques et des préparations sont considérés en général comme des déchets dangereux. L'élimination de ce type de déchet est réglementée par des lois et réglementations nationales et régionales. Contacter les autorités compétentes ou les sociétés de gestion des déchets pour obtenir des renseignements sur l'élimination des déchets dangereux.

13. INFORMATION POUR LES COMMANDES

Référence: TRIG0480 Trichinella spiralis IgG ELISA (96 déterminations)

ITALIANO

1. INTRODUZIONE

La trichinella spiralis é l'agente eziologico della trichinosi, una malattia molto diffusa tra gli essere umani ed anche numerosi animali. La trichinella appartiene alla famiglia dei vermi nematodi. Sono parassiti di carnivori e onnivori che si dividono in vari sottogruppi, per esempio T. britovi nelle volpi e linci, T. nativa negli orsi polare e nei mammiferi marini, T. nelsoni nei leoni e nelle iene e T. pseudospiralis negli ucelli e nei roditori.

Le trichinelle adulte e fertili vivono preferibilmente nella mucosa dell'intestino tenue. La femmina rilascia continuamente una grande quantità di larve. Queste perforano la parete intestinale e si diffondono per via ematica o linfatica. Infine si insediano in cisti localizzate nei muscoli dove possono vivere per anni e rappresentano la forma contagiosa. L'uomo contrae la trichinosi mangiando carne malcotta infestata dalle cisti larvali del parassita. Il quadro clinico dipende dal numero delle larvi incorporati. I sintomi in genere iniziano da un numero di 50-70 larve circa. Con più di 3000 larve la malattia può diventare pericolosa. I primi sintomi in genere sono nausea, vomito, diarrea e febbre non molto alta. Questi sintomi possono essere seguiti da dolore e irrigidimento muscolare, miosite, edemi nel viso e esantema della pelle. Complicazioni pericolose sono la miocardite e la meningoencefalite. I sintomi diminuiscono nel momento in cui le trichinelle rallentano il loro metabolismo.

Specie	Malattia	Sintomi (p.es.)	Via di trasmissione
Trichinella spiralis	Trichinosi	Nausea, diarrea, vomito, affaticamento, febbre e dolori addominali. Migrazione larvale nel tessuto muscolare può causare edema, congiuntiviti, mialgie, emorragie scheggia, eruzioni cutanee ed eosinofilia del sangue.	L'infezione accade quando se mangia carne di maiale infetta cruda o poco cotta e prodotti di selvaggina infettati con le larve di vermi Trichinella

L'infezione o la presenza di un agente patogeno può essere identificata da:
- Microscopia: Biopsia muscolare
- Sierologia: ELISA

2. USO PREVISTO

Il Trichinella spiralis IgG ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgG per Trichinella spiralis nel siero o plasma (citrato, eparina) umano.

3. PRINCIPIO DEL TEST

La determinazione immunoenzimatico qualitativa degli anticorpi specifici si basa sulla tecnica ELISA (d'inglese Enzyme-linked immunosorbent assay).
Piastre di Microtitolazione sono rivestiti con antigeni specifici che si legano agli anticorpi presenti nel campione. Dopo aver lavato i pozzetti per rimuovere tutto il materiale campione non legato, il coniugato di perossidasi di rafano (HRP) è aggiunto. Questo coniugato si lega agli anticorpi catturati. In una seconda fase di lavaggio coniugato, non legato è rimosso. Il complesso immunitario formato dal coniugato legato sarà evidenziato aggiungendo tetrametilbenzidina (TMB) substrato che dà una colorazione blu.
L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici presenti nel campione. Acido solforico è aggiunto per bloccare la reazione. Questo produce un cambiamento di colore dal blu al giallo. Assorbanza a 450/620 nm viene letto utilizzando un fotometro di Piastre di Microtitolazione ELISA.

4. MATERIALI

4.1. Reagenti forniti

- **Piastre di Microtitolazione:** 12 strisce divisibili in 8 pozzetti, con adesi antigeni della Trichinella spiralis; dentro una busta d'alluminio richiudibile.
- **Tampone Diluizione del Campione IgG:** 1 flacone contenente 100 mL di tampone fosfato (10 mM) per diluire i campioni; pH 7,2 ± 0,2; colore giallo; pronto all'uso; tappo bianco; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Soluzione Bloccante:** 1 flacone contenente 15 mL di acido solforico, 0,2 mol/L, pronto all'uso; tappo rosso.
- **Tampone di Lavaggio (20x conc.):** 1 flacone contenente 50 mL di un tampone fosfato concentrato 20 volte (0,2 M) per il lavaggio dei pozzetti; pH 7,2 ± 0,2; tappo bianco.
- **Coniugato:** 1 flacone contenente 20 mL di Proteina A, coniugati a perossidasi in tampone fosfato (10 mM); colore azzurro; pronto all'uso; tappo nero.
- **Soluzione Substrato TMB:** 1 flacone contenente 15 mL di 3,3`,5,5`-Tetrametilbenzidina (TMB), < 0,1 %; pronto all'uso; tappo giallo.
- **Controllo Positivo:** 1 flacone da 2 mL controllo; colore giallo; tappo rosso; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Cut-off:** 1 flacone da 3 mL controllo; colore giallo; tappo verde; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Negativo:** 1 flacone da 2 mL controllo; colore giallo; tappo blu; pronto all'uso; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Le indicazioni di pericolo e consigli di prudenza vedi capitolo 12.1.

Per le sostanze potenziali pericolose si prega di leggere la scheda di dati di sicurezza.

4.2. Accessori forniti

- 1 pellicola adesiva
- 1 istruzione per l'uso
- 1 schema della piastra

4.3. Materiali e attrezzature necessari

- Fotometro per Piastre di Microtitolazione con filtri da 450/620 nm
- Incubatrice 37 °C
- Lavatore, manuale o automatico, di Piastre di Microtitolazione
- Micropipette per l'uso tra 10-1000 µL
- Vortex-Mixer
- Acqua distillata
- Provette monouso

5. MODALITÀ DI CONSERVAZIONE

Conservare il kit a 2...8 °C. I reagenti aperti sono stabili fino alla data di scadenza indicata sull'etichetta quando sono conservati a 2...8 °C.

6. PREPARAZIONE DEI REAGENTI

È molto importante, portare tutti i reagenti e campioni a temperatura ambiente (20…25 °C) e mescolare prima di iniziare il test.

6.1. Piastre di Microtitolazione

Le strisce divisibili sono rivestite con l'antigeni della Trichinella spiralis. Immediatamente dopo la rimozione degli strisce necessari, le strisce rimanenti devono essere sigillare nuovamente in un foglio di alluminio insieme con il sacchetto di gel di silice conservati a 2...8 °C.

6.2. Tampone di Lavaggio (20x conc.)

Diluire il Tampone di Lavaggio 1+19; per esempio: 10 mL del Tampone di Lavaggio + 190 mL di acqua distillata. Il Tampone di Lavaggio diluito è stabile per 5 giorni a temperatura ambiente (20...25 °C). Se cristalli appaiono nel concentrato, riscaldare la soluzione a 37 °C per esempio in un bagnomaria. Mescolare bene prima della diluizione.

6.3. Soluzione Substrato TMB

La soluzione sta pronta all'uso e deve essere conservata a 2...8 °C, al riparo dalla luce. La soluzione deve essere incolore o potrebbe avere un leggero colore blu chiaro. Se il substrato diventa blu, potrebbe essere stato contaminato e non può essere utilizzato nel test.

7. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Per questo test si prega di usare campioni di siero o plasma (citrato, eparina) umano. Se il test è fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2...8 °C. Altrimenti devono essere aliquotati e congelati tra (-70…-20 °C). Se i campioni sono conservati congelati, mescolare bene i campioni scongelati prima del test. Evitare cicli ripetuti di congelamento/scongelamento.
L'inattivazione dei campioni per mezzo del calore non è raccomandata.

7.1. Diluizione dei campioni

Prima del test, diluire i campioni 1+100 con Tampone Diluizione del Campione IgG. Per esempio, pipettare nelle provette 10 µL di campione + 1 mL di Tampone Diluizione del Campione IgG e mescolare bene (Vortex).

8. PROCEDIMENTO

Leggere bene le istruzioni per l'uso **prima** di iniziare il teste. L'affidabilità dei risultati dipende dalla stretta aderenza le istruzioni per l'uso di prova come descritto. La seguente procedura è stata validata per l'esecuzione manuale. Per un'esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi de 3 a 5 volte e il volume del Tampone di Lavaggio da 300 a 350 µL per evitare effetti di lavaggio. Prestare attenzione al capitolo 12. Stabilire innanzitutto il piano di distribuzione e identificazione dei campioni e standards/controlli (è raccomandato determinare in duplicato) sullo schema della piastra fornito con il kit. Inserire i pozzetti necessari nel supporto.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza ritardi.

Sul pipettaggio utilizzare puntali nuovi e puliti per ogni campione e standard/controllo.

Regolare l'incubatore a 37 ± 1 °C.

1. Pipettare 100 µL di standard/controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il Bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva, fornita nel kit.
3. **Incubare 1 ora ± 5 min a 37 ± 1°C.**
4. Al termine dell'incubazione, togliere la pellicola e aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µL di Tampone di Lavaggio. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere > 5 sec. Dopo il lavaggio picchiettare delicatamente i pozzetti su una carta assorbente per togliere completamente il liquido, prima del passo successivo.

   Attenzione: Il lavaggio è una fase molto importante. Da lavaggio insufficiente risulta una bassa precisione e risultati falsi!
5. Pipettare 100 µL di Coniugato in tutti i pozzetti, escludendo quello con il Bianco-substrato (Blank) A1.
6. **Incubare per 30 min a temperatura ambiente (20...25 °C).** Non esporre a fonti di luce diretta.
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100 µL di Soluzione Substrato TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente (20...25 °C) al buio.** Un colore blu verifica a causa della reazione enzimatica.
10. Pipettare 100 µL di Soluzione Bloccante in tutti i pozzetti, nello stesso ordine della Soluzione Substrato TMB, in tal modo un cambiamento di colore dal blu al giallo si verifica.
11. Misurare l'assorbanza a 450/620 nm entro 30 min dopo l'aggiunta della Soluzione Bloccante.

8.1. Misurazione

Regolare il fotometro per le Piastre di Microtitolazione ELISA **a zero** usando il substrato-Bianco (Blank).

Se, per motivi tecnici, non è possibile regolare il fotometro per le Piastre di Microtitolazione a zero usando il Bianco-substrato, il valore de assorbanza de questo deve essere sottratto dai valori dell'assorbanza da tutti i valori delle altre assorbanze per ottenere risultati affidabili!

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e inserire tutti i valori misurati nello schema della piastra.

È raccomandato fare le misurazioni delle onde **bichrome** (due colori). Utilizzando la lunghezza d'onda de 620 nm come misura di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze**.

9. RISULTATI

9.1. Validazione del test

Affinché un test possa essere considerato valido, le presenti Istruzioni per l'uso devono essere rigorosamente seguite e devono essere soddisfatti i seguenti criteri:

- **Substrato Bianco (Blank)**: Valore di assorbanza **< 0,100**
- **Controllo Negativo**: Valore di assorbanza **< 0,200 e < Cut-off**
- **Controllo Cut-off**: Valore di assorbanza **0,150 – 1,300**
- **Controllo Positivo**: Valore di assorbanza **> Cut-off**

Se non sono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

9.2. Calcolo dei risultati

Il Cut-off è la media dei valori di assorbanza dei Controlli Cut-off.

Esempio: Valore di assorbanza del Controllo Cut-off 0,44 + valore di assorbanza del Controllo Cut-off 0,42 = 0,86/2 = 0,43
Cut-off = 0,43

9.2.1. Risultati in unità [NTU]

$$\frac{\text{Assorbanza media del campione} \times 10}{\text{Cut-off}} = \text{[unità NovaTec = NTU]}$$

Esempio: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretazione dei risultati

Cut-off	10 NTU	-
Positivo	> 11 NTU	Anticorpi contro il patogeno sono presenti. C'è stato un contatto con l'antigene (patogeno resp. vaccino).
Zona grigia	9 – 11 NTU	Anticorpi contro il patogeno non è stato possibile rilevare chiaramente. Si consiglia di ripetere il test con un nuovo campione in 2-4 settimane. Se il risultato è nuovamente nella zona grigia, il campione viene giudicato come **negativo**.
Negativo	< 9 NTU	Il campione non contiene anticorpi contro il patogeno. Un precedente contatto con l'antigene (patogeno resp. vaccino) è improbabile.
La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato.		

10. CARATTERISTICHE DEL TEST

I risultati si riferiscono al gruppo di campioni investigato; questi non sono specifiche garantite.

Per ulteriori informazioni su caratteristiche del test, si prega, di contattare NovaTec Immundiagnostica GmbH.

10.1. Precisione

Intradosaggio	**n**	**Media (E)**	**CV (%)**
#1	24	0,748	6,37
#2	24	1,225	4,21
#3	24	1,570	5,13
Interdosaggio	**n**	**Media (NTU)**	**CV (%)**
#1	12	17,50	8,38
#2	12	21,33	13,08
#3	12	3,69	10,38

10.2. Specificità diagnostica

La specificità diagnostica è la probabilità del test di fornire un risultato negativo in assenza di analita specifici.
La specificità diagnostica è 94,81 % (95% intervallo di confidenza: 87,23% - 98,57%).

10.3. Sensibilità diagnostica

La sensibilità diagnostica è la probabilità del test di fornire un risultato positivo alla presenza di analita specifici.
La sensibilità diagnostica è 100% (95% intervallo di confidenza: 79,41% - 100%).

10.4. Possibili interferenze

Campioni emolitici, lipidici et itterici contenenti fino a 10 mg/mL di emoglobina, 5 mg/mL di trigliceridi e 0,5 mg/mL di bilirubina non hanno presentato fenomeni d'interferenza nel presente test.

10.5. Reattività crociata

L'investigazione di un gruppo di campioni con attività di anticorpi contro parametri potenzialmente interferenti non ha rivelato alcuna evidenza rilevante di risultati falsamente positivi dovuto a reattività crociata.

11. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelamento possono alterare i valori delle assorbanze.

12. PRECAUZIONI E AVVERTENZE

- La procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature similari deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i materiali di origine umana o animale devono essere considerati potenzialmente contagiosi e infettivi.
- Tutti gli elementi di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg.
- Non scambiare reagenti e Piastre di Microtitolazione di lotti diversi.
- Non utilizzare reagenti d'altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto punte per pipette, distributori, e articoli da laboratorio puliti.
- Non scambiare i tappi dei flaconi, per evitare contaminazione crociata.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti senza spruzzi.
- L'ELISA è progettato solo per il personale qualificato che segue le norme di buona pratica di laboratorio (Good Laboratory Practice, GLP).
- Per un ulteriore controllo di qualità interno ogni laboratorio dovrebbe inoltre utilizzare campioni noti.

12.1. Nota di sicurezza per i reagenti contenenti sostanze pericolose

I reagenti possono contenere CMIT/MIT (3:1) o MIT (vedi capitolo 4.1)
Pertanto, si applicano le seguenti indicazioni di pericolo e le consigli di prudenza.

Attenzione

H317	Può provocare una reazione allergica cutanea.
P261	Evitare di respirare gli aerosol.
P280	Indossare guanti/ indumenti protettivi.
P302+P352	IN CASO DI CONTATTO CON LA PELLE: lavare abbondantemente con sapone acqua.
P333+P313	In caso di irritazione o eruzione della pelle: consultare un medico.
P362+P364	Togliere tutti gli indumenti contaminati e lavarli prima di indossarli nuovamente.

Ulteriori informazioni sono disponibili nella scheda di dati di sicurezza.

12.2. Smaltimento

In genere tutte le sostanze chimiche sono considerati rifiuti pericolosi. Lo smaltimento è regolato da leggi nazionali. Per ulteriori informazioni contattare l'autorità locale.

13. INFORMAZIONI PER GLI ORDINI

Numero del prodotto: TRIG0480 Trichinella spiralis IgG-ELISA (96 determinazioni)

ESPAÑOL

1. INTRODUCCIÓN

La Trichinella spirals es el agente patógeno de la triquinosis, un nemátodo de manifiestación mundial en el humano y varios animales. Son párasitos del tejido de mamíferos carnívoros y omnivoros incluyendo al hombre. Se conocen varios subtipos: T. britovi que parasita en el zorro y el lince, T. nativa en el oso polar y los mamíferos marinos, T. nelsoni en el léon y la hiena y T. pseudospiralis en las aves y los roedores.

Las Trichinellas adultas sólo mieden unos mm y vieven preferentemente en la mucosa del intestino delgado. La hembra segrega a menudo larvas después de su fecundación que migran a través de la pared intestinal y el torrente sanguíneo al tejido muscular, donde forman quistes. Estos quistes presentan la forma infecciosa del ciclo de vida y son infeciosos por varios años dentro de las propias cápsulas de fibrina del huéspued. La enfermedad se transmite al comer la carne infectada donde se anidan de nuevo en la mucosa intestinal para cerrar el ciclo como estados adultos y maduros.

El curso clínico de la enfermedad depende del numero de larvas incorporadas. Con un numero de aprox. 50 a 70 larvas se pueden mostrar síntomas como náusea, vómito, dificultades respiratorias y dificultad de deglución, fiebre, edemas de la cara y exantemas de la piel. Complicaciónes temidas son miocarditis y meningoencefalitis. A menudo cuando las Trichinellas reducen su metabolismo, desaparecen los síntomas. Sin embargo pueden persistir una deficiencia muscular y molestias reumáticas..

Especies	**Enfermedad**	**Síntomas (p.ej.)**	**Vía de transmisión**
Trichinella spiralis	Triquinosis (triquinelosis)	Náuseas, diarrea, vómitos, fatiga, fiebre y malestar abdominal. La migración larvaria en el tejido muscular puede causar edema, conjuntivitis, mialgias, hemorragias en astillas, erupciones cutáneas y eosinofilia en sangre.	La infección se produce cuando la ingesta de carne infectada (porcino, salvaje) cruda o mal cocida y productos de caza silvestres infectados con larvas de gusanos de Trichinella

La infección o la presencia de un patógeno puede identificarse mediante:

- Microscopia, biopsia muscular
- Serología: Detección de anticuerpos a través de ELISA

2. USO PREVISTO

El enzimoinmunoensayo Trichinella spiralis IgG ELISA se utiliza para la determinación cualitativa de anticuerpos IgG específicos contra Trichinella spiralis en suero o plasma (citrato, heparina) humano.

3. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimática cualitativa de anticuerpos específicos se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay).
Las Placas de Microtitulación están recubiertas con antígenos específicos unen a los anticuerpos de la muestra. Después de lavar los pocillos para eliminar todo el material de muestra no unida, el conjugado de peroxidasa de rábano (HRP) se añade. Este conjugado se une a los anticuerpos capturados. En una segunda etapa de lavado se retira el conjugado no unido. El complejo inmune formado por el conjugado unido se visualiza añadiendo substrato tetrametilbencidina (TMB), que da un producto de reacción azul.
La intensidad de este producto es proporcional a la cantidad de anticuerpos específicos en la muestra. se añade ácido sulfúrico para detener la reacción. Esto produce un cambio de color de azul a amarillo. La extinción a 450/620 nm se mide con un fotómetro de Placa de Microtitulación ELISA.

4. MATERIALES

4.1. Reactivos suministrados

- **Placa de Microtitulación:** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos de Trichinella spiralis, en bolsa de aluminio.
- **Tampón de Dilución de Muestras IgG:** 1 botella de 100 mL de solución de tampón de fosfato (10 mM) para diluir la muestra; pH 7,2 ± 0,2; color amarillo; listo para ser utilizado; tapa blanca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solución de Parada:** 1 botella de 15 mL de ácido sulfúrico, 0,2 mol/L, listo para ser utilizado; tapa roja.
- **Tampón de Lavado (20x conc.):** 1 botella de 50 mL de una solución de tampón de fosfato 20x concentrado (0,2 M) para lavar los pocillos; pH 7,2 ± 0,2; tapa blanca.
- **Conjugado:** 1 botella de 20 mL de conjugado de proteína A con peroxidasa en tampón de fosfato (10 mM); color azul; tapa negra; listo para ser utilizado.
- **Solución de Sustrato de TMB:** 1 botella de 15 mL 3,3',5,5'-tetrametilbenzindina (TMB), < 0,1 %; listo para ser utilizado; tapa amarilla.
- **Control Positivo:** 1 botella de 2 mL control; color amarillo; tapa roja; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Cut-off:** 1 botella de 3 mL control; color amarillo; tapa verde; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Negativo:** 1 botella de 2 mL control; color amarillo; tapa azul; listo para ser utilizado; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para indicaciones de peligro y consejos de prudencia consulte el cap. 12.1.
Para sustancias potencialmente peligrosas por favor revise la ficha de datos de seguridad.

4.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 instrucciones de uso
- 1 esquema de la placa

4.3. Materiales e instrumentos necesarios

- Fotómetro de Placa de Microtitulación con filtros de 450/620 nm
- Incubadora 37 °C
- Dispositivo de lavado manual o automático de Placa de Microtitulación
- Micropipetas para uso de (10-1000 µL)
- Mezcladora Vortex
- Agua destilada
- Tubos de plástico desechables

5. ESTABILIDAD Y ALMACENAJE

Almacene el kit a 2...8 °C. Los reactivos abiertos son estables hasta la fecha de caducidad indicada en la etiqueta cuando se almacena a 2...8 °C.

6. PREPARACIÓN DE LOS REACTIVOS

Es muy importante llevar todos los reactivos y las muestras a temperatura ambiente (20...25 °C) y mezclarlos antes de ser utilizados!

6.1. Placa de Microtitulación

Las tiras rompibles están recubiertas con antígeno de Trichinella spiralis. Inmediatamente después de la eliminación de las tiras, las tiras restantes deben sellarse de nuevo en el papel de aluminio junto con la bolsita di dióxio de silicio y almacenar a 2...8 °C.

6.2. Tampón de Lavado (20x conc.)

Diluir el Tampón de Lavado 1+19; por ejemplo 10 mL del Tampón de Lavado + 190 mL de agua destilada. El Tampón de Lavado diluido es estable durante 5 días a temperatura ambiente (20…25 °C). En caso de aparecer cristales en el concentrado, calentar la solución a 37 °C, por ejemplo, en un baño María. Mezclar bien antes de la dilución.

6.3. Solución de Sustrato de TMB

La solución está lista para su uso y debe almacenarse a 2...8 °C, protegida de la luz. La solución debe ser incolora o podría tener un color ligeramente azul claro. Si el sustrato se convierte en azul, es posible que haya sido contaminado y no puede ser utilizado en el ensayo.

7. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero o plasma (citrato, heparina) humano. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas a 2...8 °C, en caso contrario deben ser alicuotadas y almacenadas congeladas (-70...-20 °C). Agitar bien las muestras descongeladas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas.
No se recomienda la inactivación por calor de las muestras.

7.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1 + 100 con el Tampón de Dilución de Muestras IgG, p. e. 10 µL de la muestra con 1 mL de Tampón de Dilución de Muestras IgG, mezclar bien con la mezcladora Vortex.

8. PROCEDIMIENTO

Por favor, leer cuidadosamente las instrucciones de uso del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido solamente para el método manual. Si se realiza el ensayo en los sistemas automáticos de ELISA es aconsejable elevar el número de lavados de tres hasta cinco veces y el volumen de Tampón de Lavado de 300 µL a 350 µL para excluir efectos de lavado. Preste atención al capítulo 12. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los estándares/controles (se recomienda determinar en duplicado) en el esquema de la placa suministrada. Usar la cantidad necesaria de tiras o pocillos e insertarlos en el soporte.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los estándares/controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a 37 ± 1°C.

1. Pipetear 100 µL de estándares/controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. **Incubar 1 h ± 5 min a 37 ± 1°C.**
4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300 µL del Tampón de Lavado. Evitar el rebosamiento de los pocillos. El intervalo entre lavado y aspiración debe ser > 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.

   Nota: El lavado es muy importante! Un mal lavado insuficiente provoca una baja precisión y resultados falsamente elevados!
5. Pipetear 100 µL de conjugado en cada pocillo con excepción del blanco substrato A1.
6. **Incubar 30 min a la temperatura ambiente (20…25 °C).** Evitar la luz solar directa.
7. Repetir el lavado como en el paso numero 4.
8. Pipetear 100 µL de Solución de Sustrato de TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente (20...25 °C).** Un color azul se produce en las muestras positivas debido a la reacción enzimática.
10. Pipetear en todos los pocillos 100 µL de la Solución de Parada en el mismo orden y mismo intervalo de tiempo como con el Solución de Sustrato de TMB, por lo tanto un cambio de color de azul a amarillo se produce.
11. Medir la extinción con 450/620 nm en un periodo de 30 min después de añadir la Solución de Parada.

8.1. Medición

Ajustar el fotómetro de Placa de Microtitulación ELISA al cero utilizando el Blanco.

Si por razones técnicas el fotómetro de Placa de Microtitulación de ELISA no se puede ajustar a cero utilizando el Blanco, el valor de la absorbancia de este debe ser sustraído de los demás valores de absorbancia medidos con el fin de obtener resultados fiables!

Medir la **extinción** de todos los pocillos con **450 nm** y anotar los resultados de los estándares/controles y de las muestras en el esquema de la placa.

Es aconsejable realizar la medición **bicromática** a una longitud de onda de referencia de 620 nm.

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

9. CÁLCULO DE LOS RESULTADOS

9.1. Criterios de validez del ensayo

Para que un ensayo se considere válido, deben seguirse estrictamente las presentes instrucciones de uso y deben cumplirse los siguientes criterios:

- **Blanco:** valor de la extinción **< 0,100**
- **Control Negativo:** valor de la extinción **< 0,200 y < Cut-off**
- **Control Cut-off:** valor de la extinción **0,150 – 1,300**
- **Control Positivo:** valor de la extinción **> Cut-off**

Si estos criterios no se cumplen, la prueba no es váida y deberá repetirse.

9.2. Cálculo del valor de la medición

El Cut-off se obtiene de los valores de la extinción de los dos controles Cut-off.

Ejemplo: 0,42 OD Control Cut-off + 0,44 OD Control Cut-off = 0,86:2 = 0,43

Cut-off = 0,43

9.2.1. Resultados en unidades [NTU]

$$\frac{\text{Promedio valor de la extinción de la muestra x 10}}{\text{Cut-off}} = \text{[NovaTec-unidades = NTU]}$$

Ejemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretación de los resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Los anticuerpos contra el patógeno están presentes. Ha producido un contacto con el antígeno (patógeno resp. vacuna).
Zona intermedia	9 – 11 NTU	Los anticuerpos contra el patógeno no se pudieron detectar claramente. Se recomienda repetir la prueba con una muestra fresca en 2 a 4 semanas. Si el resultado es de nuevo en la zona intermedia, la muestra se considera como **negativa**.
Negativo	< 9 NTU	La muestra no contiene anticuerpos contra el patógeno. Un contacto previo con el antígeno (patógeno resp. vacuna) es poco probable.
El diagnóstico de una infección no solamente se debe basar en el resultado del ensayo. Es necesario considerar la anamnésis y la sintomatología del paciente junto al resultado serológico. Estos resultados sólo tienen valor restringido en pacientes inmunodeprimidos o en neonatos.		

10. CARACTERÍSTICAS DEL ENSAYO

Los resultados están basados en el grupo de pruebas investigado; no se trata de especificaciones garantizadas.

Para obtener más información sobre las características del ensayo, por favor, entre en contacto NovaTec Immundiagnostica GmbH.

10.1. Precisión

Intra ensayo	**n**	**Promedio (E)**	**CV (%)**
#1	24	0,748	6,37
#2	24	1,225	4,21
#3	24	1,570	5,13
Inter ensayo	**n**	**Promedio (NTU)**	**CV (%)**
#1	12	17,50	8,38
#2	12	21,33	13,08
#3	12	3,69	10,38

10.2. Especificad diagnóstica

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia del analítico específico. Es 94,81% (95% intervallo di confidenza: 87,23% - 98,57%).

10.3. Sensibilidad de diagnóstico

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico. Es 100% (95% Intervalo de confianza: 79,41% - 100%).

10.4. Interferencias

Las muestras lipémicas, ictéricas e hemolíticas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/mL para triglicéridos, de 0,5 mg/mL para bilirrubina y de 10 mg/mL hemoglobina.

10.5. Reactividad cruzada

La investigación del panel de muestras con actividad de los anticuerpos en los parámetros con potencial de reacción cruzada no reveló ninguna evidencia significativa de resultados positivos falsos debido a reacciones cruzadas.

11. LIMITACIONES DEL ENSAYO

Una contaminación de las muestras con bacterias, o una congelación y descongelación repetida pueden producir cambios en los valores de la extinción.

12. PRECAUCIONES Y ADVERTENCIAS

- El procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnostico in vitro.
- Todos los materiales de origen humano o animal deberán ser considerados y tratados como potencialmente infecciosos.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG.
- No intercambiar reactivos y Placa de Microtitulación de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.
- No intercambiar las tapas de los diferentes reactivos, para evitar la contaminación cruzada.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Para evitar contaminaciones cruzadas y resultados erróneamente aumentados, Pipetear cuidadosamente las muestras y los reactivos en los pocillos sin salpicar.
- El ELISA sólo está diseñado para personal cualificado siguiendo las normas de buenas prácticas de laboratorio (Good Laboratory Practice,GLP).
- Para un mayor control de calidad interno, cada laboratorio deberá utilizar además muestras conocidas.

12.1. Nota de seguridad para los reactivos que contienen sustancias peligrosas

Los reactivos pueden contener CMIT/MIT (3:1) o MIT (consulte el cap. 4.1)
Por lo tanto, se aplican las indicaciones de peligro y consejos de prudencia.

Atención

H317	Puede provocar una reacción alérgica en la piel.
P261	Evitar respirar el aerosol.
P280	Llevar guantes/ prendas de protección.
P302+P352	EN CASO DE CONTACTO CON LA PIEL: Lavar con abundante jabón agua.
P333+P313	En caso de irritación o erupción cutánea: Consultar a un médico.
P362+P364	Quitar las prendas contaminadas y lavarlas antes de volver a usarlas.

Se puede encontrar más información en la ficha de datos de seguridad.

12.2. Indicaciones para la eliminación de residuos

Por regla general, los productos químicos y las preparaciones son residuos peligrosos. Su eliminación esta sometida a las leyes y los decretos nacionales sobre la eliminación de residuos. Las autoridades informan sobre la eliminación de residuos peligroso.

13. INFORMACIONES PARA PEDIDOS

N° del producto:	TRIG0480	Trichinella spiralis IgG ELISA (96 determinaciones)

PORTUGUÊS

1. INTRODUÇÃO

A triquinose (também designada por triquinelose) é causada por nemátodos (vermes redondos) do género Trichinella. Além do agente clássico Trichinella spiralis, que é encontrado a nível mundial em muitos animais carnívoros e omnívoros, são identificadas quatro outras espécies (T. pseudospiralis, T. nativa, T. nelsoni e T. britovi). A triquinose resulta da ingestão de carne que contém larvas sob a forma de quistos de Trichinella. Após a exposição ao ácido gástrico e pepsina, as larvas são libertadas dos quistos e invadem a mucosa do intestino delgado onde se desenvolvem em vermes adultos (as fêmeas têm 2,2 mm e os machos 1,2 mm de comprimento). Após 1 semana, as fêmeas libertam larvas que migram para os músculos estriados onde vão enquistar. O enquistamento fica concluído ao fim de 4 a 5 semanas e as larvas enquistadas poderão conservar-se viáveis durante vários anos. A ingestão de larvas enquistadas vai perpetuar o ciclo.

A infecção por triquinose ocorre em todo o mundo, embora seja mais comum em algumas partes da Europa e nos Estados Unidos. As infecções leves poderão ser assintomáticas. No caso das infecções ligeiras a moderadas, a maioria dos sintomas diminui no espaço de alguns meses, enquanto a fadiga, fraqueza e diarreia poderão durar vários meses. Nos casos graves, a infecção pode resultar em morte.

Espécies	Doença	Sintomas (p.ex.)	Via de transmissão
Trichinella spiralis	Triquinose	Náuseas, diarreia, vómitos, fadiga, febre e desconforto abdominal. A migração larvar para o tecido muscular pode originar edema, conjuntivite, mialgias, hemorragias em lasca, erupções cutâneas e eosinofilia sanguínea.	A infecção ocorre quando há ingestão de carne de porco infectada crua ou mal cozinhada e produtos de caça selvagem infectados com as larvas de vermes de Trichinella

Infecção ou presença de patógeno pode ser identificada por:

- Microscopia, biopsia muscular
- Serologia: Detecção de anticorpos pelo método ELISA

2. UTILIZAÇÃO PRETENDIDA

O kit Trichinella spiralis IgG ELISA destina-se à determinação qualitativa de anticorpos da classe IgG contra Trichinella spiralis no soro ou plasma (citrato, heparina) humanos.

3. PRINCÍPIO DO ENSAIO

A determinação imunoenzimática qualitativa de anticorpos específicos é baseado na técnica de ELISA (do inglês Enzyme-linked Immunosorbent Assay).
As Placas de Microtitulação são revestidas com antigénios específicos que se ligam os anticorpos correspondentes da amostra. Após lavagem dos poços, para remover todo o material de amostra não ligada, um conjugado de peroxidase de rábano (HRP) é adicionado. Este conjugado se liga aos anticorpos capturados. Num segundo passo de lavagem o conjugado não ligado é removido. O complexo imune formado pelo conjugado ligado é visualizado por adição de substrato de tetrametilbenzidina (TMB), o que dá um produto de reacção azul.
A intensidade deste produto é proporcional à quantidade de anticorpos específicos da amostra. O ácido sulfúrico é adicionado para parar a reacção. Isso produz uma mudança de cor de azul para amarelo. Absorvância a 450/620 nm é lida utilizando um fotômetro de Placa de Microtitulação ELISA.

4. MATERIAIS

4.1. Reagentes fornecidos

- **Placa de Microtitulação:** 12 tiras de 8 poços, destacáveis e quebráveis, revestidas com antigénio de Trichinella spiralis, em bolsas de folha de alumínio com fecho.
- **Tampão de Diluição de Amostra IgG:** 1 frasco contendo 100 mL de tampão fosfato (10 mM) para diluição da amostra, pH 7,2 ± 0,2; de cor amarela; pronto a usar; tampa branca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solução de Bloqueio:** 1 frasco contendo 15 mL ácido sulfúrico; 0,2 mol/L; pronto a usar; tampa vermelha.
- **Tampão de Lavagem (conc. 20x):** 1 frasco contendo 50 mL de um tampão fosfato (0,2 M); concentrado 20 vezes (pH 7,2 ± 0,2) para a lavagem dos poços; tampa branca.
- **Conjugado:** 1 frasco contendo 20 mL de Protein A marcados com peroxidase no tampão fosfato (10 mM); de cor azul, pronto a usar; tampa preta.
- **Solução Substrato TMB:** 1 frasco contendo 15 mL de 3,3',5,5'-tetrametilbenzidina (TMB), < 0,1 %; pronto a usar; tampa amarela.
- **Control Positivo:** 1 botella de 2 mL control; color amarillo; tapa roja; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Cut-off:** 1 botella de 3 mL control; color amarillo; tapa verde; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Negativo:** 1 botella de 2 mL control; color amarillo; tapa azul; listo para ser utilizado; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para indicaciones de peligro y consejos de prudencia consulte el cap. 12.1.
Para substâncias potencialmente perigosas verifique a ficha de dados de segurança.

4.2. Materiais fornecidos

- 1 Película de cobertura
- 1 Instruções de uso
- 1 Layout da placa

4.3. Materiais e Equipamento necessários

- Fotômetro de Placa de Microtitulaçãos ELISA, equipado para a medição da absorvância a 450/620 nm
- Incubadora 37 °C
- Equipamento manual ou automático para a lavagem de Placa de Microtitulação
- Pipetas para dispensar volumes entre 10 e 1000 µL
- Agitador de tubos tipo Vortex
- Água destilada
- Tubos descartáveis

5. ESTABILIDADE E ARMAZENAMENTO

Armazene o kit a 2...8 °C. Os reagentes abertos são estáveis até o prazo de validade impresso no rótulo quando armazenado a 2...8 °C.

6. PREPARAÇÃO DOS REAGENTES

É muito importante deixar todos os reagentes e amostras estabilizar à temperatura ambiente (20...25 °C) misturá-los antes de iniciar o teste!

6.1. Placa de Microtitulação

As tiras quebraveis são revestidas com antigénio de Trichinella spiralis. Imediatamente após a remoção das tiras necessarias, as tiras restantes devem ser lacradas de novo na folha de alumínio juntamente com o saquinho de silicio fornecido e armazenadar a 2...8 °C.

6.2. Tampão de Lavagem (conc. 20x)

Diluir o Tampão de Lavagem 1+19; por exemplo: 10 mL do Tampão de Lavagem + 190 mL de água destilada. O Tampão de Lavagem diluído é estavel durante 5 dias à temperatura ambiente (20...25 °C). Caso apareça cristais no concentrado, aquecer a solução a 37 °C por exemplo, em banho Maria. Misture bem antes da diluição.

6.3. Solução Substrato TMB

A solução está pronta para uso e tem de ser armazenada à 2...8 °C, protegida da luz. A solução deve ser incolor ou poderia ter uma ligeira coloração azul claro. Se o substrato se transforma em azul, pode ter sido contaminado e não pode ser usado no teste.

7. COLHEITA E PREPARAÇÃO DAS AMOSTRAS

Usar com este ensaio amostras de soro ou plasma (citrato, heparina) humanos. Se o ensaio for realizado dentro de 5 dias após colheita da amostra, o espécime deve ser mantido a 2…8 °C; caso contrário devem ser alicotadas e armazenadas congeladas (-70...-20 °C). Se as amostras forem armazenadas congeladas, misturar bem as amostras descongeladas antes de testar. Evitar congelar e descongelar repetidamente.
Não é recomendada a inactivação por calor das amostras.

7.1. Diluição das amostras

Antes de testar todas as amostras devem ser diluídas 1 + 100 com Tampão de Diluição de Amostra IgG. Dispensar 10 µL de amostra e 1 mL de Tampão de Diluição de Amostra IgG em tubos para obter uma diluição 1 + 100 e misturar meticulosamente com um vortex.

8. PROCEDIMENTO DO ENSAIO

Por favor, ler atentamente as instruções de uso **antes** de realizar o teste. A fiabilidade dos resultados depende da adesão estrita ao as instruções de uso, conforme descritas. O procedimento de ensaio a seguir está validado apenas para o procedimento manual. Se o teste for realizado em sistemas automáticos para teste ELISA é recomendável aumentar os passos de lavagem de três até cinco e o volume da Tampão de Lavagem de 300 µL para 350 µL para evitar efeitos de lavagem. Preste atenção ao capítulo 12. Antes de iniciar o teste, o plano de distribuição e identificação de todas as amostras e calibradores/controles (é recomendado determinar em duplicidade) deve ser cuidadosamente estabelecido no Layout da placa fornecida no kit. Seleccionar o número necessário de tiras ou poços e inserir os mesmos no suporte.

Realizar todas as etapas do teste na ordem indicada e sem atrasos significativos.

Na pipetagem deve ser utilizada uma ponta limpa e descartável para dispensar cada controle e amostra.

Ajustar a incubadora para 37 ± 1 °C.

1. Dispensar 100 µL dos calibradores/controles e das amostras diluídas nos poços respectivos. Deixar o poço A1 vazio para o branco substrato.
2. Cobrir os poços com a película fornecida no kit.
3. **Incubar durante 1 hora ± 5 min a 37 ± 1 °C.**
4. Quando terminar a incubação, remover a película, aspirar o conteúdo dos poços e lavar cada poço três vezes com 300 µL de Tampão de Lavagem. Evitar que os poços de reacção transbordem. O intervalo entre a lavagem e a aspiração deve ser > 5 seg. No final, retirar cuidadosamente o fluido restante batendo delicadamente as tiras sobre papel absorvente, antes da próxima etapa!

   Nota: A lavagem é muito importante! Lavagem insuficiente resulta em baixa precisão e falsos resultados.
5. Dispensar 100 µL de Conjugado em todos os poços, excepto no poço do Branco substrato A1.
6. **Incubar durante 30 min à temperatura ambiente (20...25°C).** Não expor diretamente à luz solar.
7. Repetir a etapa 4.
8. Dispensar 100 µL de Solução Substrato TMB em todos os poços.
9. **Incubar durante exactamente 15 min à temperatura ambiente (20...25 °C) e no escuro.** A cor azul devido a uma reacção enzimática.
10. Dispensar 100 µL de Solução de Bloqueio em todos os poços, pela mesma ordem e com a mesma velocidade a que foi dispensada a Solução Substrato TMB,desse modo uma mudança de cor de azul para amarelo ocorre.
11. Medir a absorvância a 450/620 nm dentro de 30 min após a adição da Solução de Bloqueio.

8.1. Medição

Ajustar o fotômetro para Placa de Microtitulação ELISA **a zero** usando o **Branco substrato .**

Se - devido à razões técnicas – o fotômetro para Placa de Microtitulação ELISA não puder ser ajustado a zero usando o Branco substrato, valor da absorvância deste deve ser subtraido de todos os outros valores de absorvância medidos de forma a obter resultados fiáveis!

Medir a absorvância de todos os poços a **450 nm** e registar os valores da absorvância para cada calibrador/controle e amostra no Layout da placa.

É recomendado fazer a medição **dicromática** usando como referência um comprimento de onda de 620 nm.

Se determinações duplas foram realizadas, calcular **os valores médios de absorvância**.

9. RESULTADOS

9.1. Critérios de validação do ensaio

Para que um ensaio seja considerado válido, estas Instruções de Uso devem ser rigorosamente seguidas, e os seguintes critérios devem ser cumpridos:

- **Branco substrato**: Valor de Absorvância **< 0,100**
- **Controle Negativo**: Valor de Absorvância **< 0,200 e < Cut-off**
- **Controle Cut-off**: Valor de Absorvância **0,150 – 1,300**
- **Controle Positivo**: Valor de Absorvância **> Cut-off**

Se estes critérios não forem cumpridos, o teste não é válido e deve ser repetido.

9.2. Cálculo dos Resultados

O Cut-off é o valor médio da absorvância das determinações do Controle Cut-off.

Exemplo: Valor da absorvância do Controle Cut-off 0,42 + valor da absorvância do Controle Cut-off 0,44 = 0,86 : 2 = 0,43
Cut-off = 0,43

9.2.1. Resultados em Unidades [NTU]

$$\frac{\text{Valor da absorvância (média) da amostra x 10}}{\text{Cut-off}} = \text{[Unidades NovaTec = NTU]}$$

Exemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretação dos Resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Os anticorpos contra o agente patogênico estão presente. Houve um contacto com o antigénio (patógeno resp vacina).
Zona cinzenta	9 – 11 NTU	Os anticorpos contra o agente patogênico não puderam ser claramente detectados. Recomenda-se a repetir o teste com uma amostra fresca em 2 a 4 semanas. Se o resultado estiver novamente dentro da zona cinzenta, a amostra é julgada como **negativa**.
Negativo	< 9 NTU	A amostra não contém os anticorpos contra o agente patogênico. Um contato prévio com o antígeno (patógeno resp. vacina) é improvável.
O diagnóstico de uma doença infecciosa não deve ser estabelecido com base num único resultado do teste. Um diagnóstico preciso deve ter em consideração a história clínica, a sintomatologia bem como dados serológicos. Em pacientes imunossuprimidos e recém-nascidos os dados serológicos têm apenas valor restrito.		

10. CARACTERÍSTICAS DE DESEMPENHO ESPECÍFICAS

Os resultados referem-se aos grupos de amostras investigados; estas não são especificações garantidas.

Para mais informações sobre as características de desempenho específicas, por favor, entre em contato NovaTec Immundiagnostica GmbH.

10.1. Precisão

Intra ensaio	n	Média (E)	CV (%)
#1	24	0,748	6,37
#2	24	1,225	4,21
#3	24	1,570	5,13

Inter ensaio	n	Média (NTU)	CV (%)
#1	12	17,50	8,38
#2	12	21,33	13,08
#3	12	3,69	10,38

10.2. Especificidade Diagnóstica

A especificidade diagnóstica é definida como a probabilidade do ensaio ser negativo na ausência do analito específico.
É de 94,81% (95% Intervalo de confiança: 87,23% - 98,57%).

10.3. Sensibilidade Diagnóstica

A sensibilidade diagnóstica é definida como a probabilidade do ensaio ser positivo na presença do analito específico.
É de 100% (95% Intervalo de confiança: 79,41% - 100%).

10.4. Interferências

Não são observadas interferências com amostras hemolisadas, lipémicas ou ictéricas até uma concentração de hemoglobina de 10 mg/mL, de triglicerídeos de 5 mg/mL e de bilirrubina de 0,5 mg/mL.

10.5. Reacção cruzada

A investigação do painel de amostras com atividades de anticorpos em parâmetros com potencial de reação cruzada não revelou nenhuma significante evidencia de resultados falso-positivos devido a reações cruzadas.

11. LIMITAÇÕES DO PROCEDIMENTO

Contaminação bacteriana ou a repetição de ciclos de congelação-descongelação do espécime podem afectar os valores da absorvância.

12. PRECAUÇÕES E AVISOS

- O procedimento do teste, as informações, as precauções e avisos nas instruções para utilização têm de ser rigorosamente seguidas. O uso de kits de teste com analisadores e equipamento similar tem de ser validado. Qualquer alteração no desenho, composição e procedimento do teste bem como qualquer utilização em combinação com outros produtos não aprovados pelo fabricante não estão autorizados; o próprio utilizador é responsável por tais alterações. O fabricante não é legalmente responsável por resultados falsos e incidentes originados por estes motivos. O fabricante não é legalmente responsável por quaisquer resultados obtidos por análise visual das amostras dos pacientes.
- Apenas para uso no diagnóstico in-vitro.
- Todos os materiais de origem humana ou animal devem ser considerados e tratados como potencialmente infectantes.
- Todos os componentes de origem humana usados para a produção destes reagentes foram testados para anticorpos anti-HIV, anticorpos anti-HCV e HBsAg e foram considerados não-reactivos.
- Não trocar e/ou juntar reagentes ou Placa de Microtitulação de lotes de produção diferentes.
- Nenhuns reagentes de outros fabricantes devem ser usados juntamente com reagentes deste kit de teste.
- Não usar reagentes após a data de validade indicada no rótulo.
- Usar apenas pontas de pipeta, dispensadores e material de laboratório limpos.
- Não trocar as tampas dos frascos dos reagentes para evitar contaminação cruzada.
- Fechar firmemente os frascos dos reagentes imediatamente após a utilização para evitar evaporação e contaminação microbiana.
- Após a primeira abertura e armazenamento subsequente verificar se existe contaminação microbiana dos frascos do conjugado e dos calibradores/controles antes de utiliza-los novamente.
- Para evitar contaminação-cruzada e resultados falsamente elevados, pipetar as amostras dos pacientes e dispensar o reagentes precisamente nos poços sem salpicar.
- O ELISA é projetado apenas para pessoal qualificado seguindo os padrões de boas práticas de laboratório (Good Laboratory Practice, GLP).
- Para um controle de qualidade interno adicional cada laboratório deve utilizar amostras conhecidas.

12.1. Nota de segurança para reagentes que contenham substâncias perigosas

Os reagentes podem conter CMIT/MIT (3:1) ou MIT (ver capitulo 4.1)
Portanto, as seguintes advertências de perigo e recomendações de prudência aplicam-se.

Atenção

H317	Pode provocar uma reacção alérgica cutânea.
P261	Evitar respirar os aerossóis.
P280	Usar luvas de protecção/ vestuário de protecção.
P302+P352	SE ENTRAR EM CONTACTO COM A PELE: lavar abundantemente com sabão água
P333+P313	Em caso de irritação ou erupção cutânea: consulte um médico.
P362+P364	Retirar a roupa contaminada e lavá-la antes de a voltar a usar.

Mais informações podem ser encontradas na ficha de dados de segurança.

12.2. Considerações de Eliminação

Resíduos de químicos e preparações são geralmente considerados como resíduos perigosos. A eliminação deste tipo de resíduos está regulada por leis e normativas nacionais e regionais. Contactar as autoridades locais ou empresas de gestão de resíduos as quais podem aconselhar sobre como eliminar resíduos perigosos.

13. INFORMAÇÃO DE PEDIDO

Prod. No.: TRIG0480 Trichinella spiralis IgG ELISA (96 Determinações)

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ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIÓNES / ABREVIATURAS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
IVD	In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Dispositif médical de diagnostic in vitro / Diagnostico in vitro / Producto para diagnóstico In vitro / Dispositivo Médico para Diagnóstico In Vitro
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
CE	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / Marca CE / Marca CE
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso/ Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microtiterplate / Mikrotiterplatte / Plaque de Microtitrage / Piastre di Microtitolazione / Placa de Microtitulación / Placa de Microtitulação
CONJ	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CONTROL | -	Negative Control / Negativkontrolle / Contrôle Négatif / Controllo Negativo / Control Negativo / Controle Negativo
CONTROL | +	Positive Control / Positivkontrolle / Contrôle Positif / Controllo Positivo / Control Positivo / Controle Positivo
CUT OFF	Cut-off Control / Cut-off Kontrolle / Contrôle Cut-off / Controllo Cut-off / Control Cut-off / Controle Cut-off
DIL | G	IgG Sample Dilution Buffer / IgG-Probenverdünnungspuffer / Tampon de Dilution d'Échantillon IgG / Tampone Diluizione del Campione IgG / Tampón de Dilución de Muestras / Tampão de Diluição de Amostra IgG
SOLN | STOP	Stop Solution / Stopplösung / Solution d'Arrêt / Soluzione Bloccante /Solución de Parada/ Solução de Bloqueio
SUB | TMB	TMB Substrate Solution / TMB-Substratlösung / Solution de Substrat TMB / Soluzione Substrato TMB / Solución de Sustrato de TMB / Solução Substrato TMB
WASH | BUF | 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de Lavage concentré 20 x / Tampone di Lavaggio concentrazione x20 / Tampón de Lavado concentrado x20 / Tampão de Lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SUMMARY OF TEST PROCEDURE / KURZANLEITUNG TESTDURCHFÜHRUNG / RÉSUMÉ DE LA PROCEDURE DE TEST / SCHEMA DELLA PROCEDURA / RESUMEN DE LA TÉCNICA / RESUMO DO PROCEDIMENTO DE TESTE

SCHEME OF THE ASSAY

Trichinella spiralis IgG ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 ± 1 °C** Wash each well three times with 300 µL of Washing Buffer					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20…25°C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer					
TMB Substrate Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20…25°C) in the dark					
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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AccuBind
ELISA Microwells

Thyroglobulin Ab (Anti-Tg) Test System
Product Code: 1025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroglobulin (Tg) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric. Measurements of Tg autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Antibodies to thyroglobulin have been shown to be characteristically present from patients with thyroiditis and primary thyrotoxicosis.[1,2] This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction. Passive Hemaglutination (PHA) methods have been employed in the past for measurements of antibodies to Tg. PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the detectability of subclinical levels of antibodies to Tg. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation.

Monobind's microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated thyroglobulin (Tg) is added, and then the reactants are mixed. Reaction results between the autoantibodies to Tg and the biotinylated Tg to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin.

After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce color.

The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with autoimmune antibody level.

3.0 PRINCIPLE

A Sequential Sandwich ELISA Method (TYPE 1)
The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated thyroglobulin antigen.

Upon mixing biotinylated antigen and a serum containing the autoantibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$\text{h-Ab}_{(X\text{-}Tg)} + {}^{Btn}\text{Ag}_{(Tg)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} \text{h-Ab}_{(X\text{-}Tg)} - {}^{Btn}\text{Ag}_{(Tg)}$$

$^{Btn}Ag_{(Tg)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(X\text{-}Tg)}$ = Human Auto-Antibody (Variable Quantity)
$Ab_{(X\text{-}Tg)} - {}^{Btn}Ag_{(Tg)}$ = Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:
$h\text{-}Ab_{(X\text{-}Tg)} - {}^{Btn}Ag_{(Tg)} + \underline{\text{Streptavidin}}_{C.W.} \Rightarrow \underline{\text{immobilized complex}}$ (IC)
$\underline{\text{Streptavidin}}_{C.W.}$ = Streptavidin immobolized on well
Immobilized complex (IC) = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed.
$I.C._{(h\text{-}IgG)} + {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} \Rightarrow {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$
$I.C._{(h\text{-}IgG)}$ = Immobilized Immune complex (Variable Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG}$ = Enzyme-antibody Conjugate (Constant Quantity)
$^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$ = Ag-Ab Complex (Variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Anti-Tg Calibrators – 1ml/vial Icons A-F
Six (6) vials of references for anti-Tg at levels of 0(**A**), 50(**B**), 125(**C**), 500(**D**), 1000(**E**), and 2000(**F**) IU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using the 1st International Reference Preparation, which was assayed against the Medical Research Council (MRC) Research Standard A 65/93 for anti-thyroglobulin activity.

B. Tg Biotin Reagent – 13ml/vial – Icon ∇
One (1) vial of biotinylated thyroglobulin stabilized in a buffering matrix. A preservative has been added. Store at 2-8°C.

C. x-Tg Enzyme Reagent – 13ml/vial - Icon
One (1) vial of anti-human IgG-horseradish peroxidase (HRP) conjugate stabilized in a bufferred matrix. A preservative has been added. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Serum Diluent – 20ml/vial
One (1) vial of serum diluent concentrate that containing buffer salts and a dye. Store at 2-8°C.

F. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

G. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

H. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

I. Stop Solution – 8ml/vial - Icon
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

J. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.0101ml (10.1µl) and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Test tube(s) for patient dilution.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminate devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Serum Diluent**
Dilute the serum diluent to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer**
Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
3. **Working Substrate Solution** – Stable for one (1) year.
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.
4. **Patient Sample Dilution (1/100)**
Dispense 0.0101ml (10.1µl) of each patient specimen into 1ml (1000µl) of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well.
3. Add 0.100 ml (100µl) of Tg Biotin Reagent.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of x-Tg Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITON
9. Cover and incubate for thirty (30) minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (blot and tap) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of Working Substrate Solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITON
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 2000 IU/ml, dilute the sample an additional 1:5 or 1:10 using

the original diluted material. Multiply by the dilution factor to obtain the concentration of the specimen.

10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-Tg in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-Tg activity in IU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the level of anti-Tg activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.387) intersects the dose response curve at 790 IU/ml anti-Tg concentration (See Figure 1).

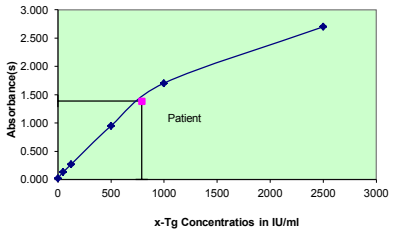
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (IU/ml)
Cal A	A1	0.022	0.025	0
	B1	0.028		
Cal B	C1	0.135	0.133	50
	D1	0.131		
Cal C	E1	0.280	0.270	125
	F1	0.261		
Cal D	G1	0.962	0.949	500
	H1	0.936		
Cal E	A2	1.709	1.703	1000
	B2	1.698		
Cal F	C2	2.730	2.698	2000
	D2	2.667		
Patient	E2	1.390	1.387	790
	F2	1.383		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 'F' should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-Tg in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. Samples, which are contaminated microbiologically, should not be used.
11. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
12. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
13. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
14. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. The presence of autoantibodies to Tg is confirmed when the serum level exceeds 125 IU/ml. The clinical significance of the result, coupled with anti-thyroid peroxidase activity, should be used in evaluating the thyroid condition. However, clinical inferences should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests.
8. The cost benefits should be considered in the use of thyroglobulin antibodies testing when performed in concert with anti- thyroid peroxidase (TPO). The widespread practice of performing both tests has been questioned.[4]

13.0 EXPECTED RANGES OF VALUES

A study of normal population was undertaken to determine expected values for the Anti-Tg AccuBind® test system. The number (n) mean (X) and standard deviation (σ) are given in Table 1. Values in excess of 125IU/ml are considered positive for the presence of anti-Tg autoantibodies.

TABLE I
Expected Values for Anti-Tg AccuBind® ELISA Test System (In IU/ml)

Number	**100**
Mean	74.3
Standard deviation	25.2
Upper 95% (+2σ) level	124.7

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the Anti-Tg AccuBind® ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value(X), standard deviation (σ) and coefficient of variation (C.V) for each of these control sera are presented in Tables 2 and 3.

TABLE 2
Within Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	20	65.5	3.3	5.0%
Pool 2	20	385.5	15.5	4.0%
Pool 3	20	1554.4	55.4	3.6%

TABLE 3*
Between Assay Precision (Values in IU/ml)

Sample	N	X	σ	C.V.
Pool 1	10	66.8	3.6	5.3%
Pool 2	10	374.2	18.5	4.9%
Pool 3	10	1625.5	65.2	4.0%

*As measured in ten experiments in duplicate.

12.2Sensitivity

The Anti-Tg AccuBind® ELISA has a sensitivity of 1.94 IU/ml. The sensitivity was ascertained by determining the variability of the '0 IU/ml' calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

12.3 Accuracy

The Anti-Tg AccuBind® ELISA test system was compared with a reference method. Biological specimens from normals, and disease states populations were used. The disease states included; Hashimoto's thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 181. The least square regression equation and the correlation coefficient were computed for the anti-Tg AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	415.6	y = 9.79 + 0.969 (x)	0.995
Reference	419.2		

Only slight amounts of bias between the anti-Tg AccuBind® ELISA method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

Interferences from ANA, DNA, thyroid peroxidase (TPO) and rheumatoid antibodies were found to be insignificant in the assay system.

15.0 REFERENCES

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Revision: 5 Date: 2019-JUL-16 DCO: 1353
MP1025 Product Code: *1025-300*

Size		96(A)
Reagent (fill)	A)	1ml set
	B)	1 (13ml)
	C)	1 (13ml)
	D)	1 plate
	E)	1 (20ml)
	F)	1 (20ml)
	G)	1 (7ml)
	H)	1 (7ml)
	I)	1(8ml)

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Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2° 8° Temperature Limitation Storage Condition (2-8°C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	

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AccuBind
ELISA Microwells

Cancer Antigen 125 (CA-125) Test System

Product Code: 3025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Cancer Antigen 125 (CA-125) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Cancer Antigen 125 (CA-125) is a glycoprotein that occurs in blood as high molecular weight entity (MB_{rB} > *200*,000). High concentrations of this antigen are associated with ovarian cancer and a range of benign and malignant diseases. Although the specificity and sensitivity of CA-125 assays are somewhat limited, especially in early diagnosis of ovarian cancer, the assay has found widespread use in the differential diagnosis of adnexal masses, in monitoring disease progression and response to therapy in ovarian cancer, and in the early detection of recurrence after surgery or chemotherapy for ovarian cancer. Published literature has shown that elevated serum CA-125 levels can be observed in patients with serious endometroid, clear cell and undifferentiated ovarian carcinoma. The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, and 6% of patients with non-neoplastic conditions (including, but not limited to, first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

In this method, CA-125 calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of CA-125) are added and the reactants mixed. Reaction between the various CA-125 antibodies and native CA-125 forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-CA-125 antibody bound conjugate is separated from the unbound enzyme-CA-125 conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of CA-125 levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CA-125 concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA-125 antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{CA\text{-}125} + {}^{Btn}Ab_{(m)} \underset{k\text{-}a}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{CA\text{-}125}$ = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)} + Streptavidin_{CW} \Rightarrow$ Immobilized complex
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. CA-125 Calibrators - 1ml/vial- Icons A-F
Six (6) vials of references CA-125 Antigen at levels of 0(**A**), 15(**B**), 50(**C**), 100(**D**), 200(**E**) and 400(**F**) U/ml. A preservative has been added. Store at 2-8°C.

Note: The human serum based standards were made using a >99% pure affinity purified preparation of CA-125. The preparation was calibrated against Centocor CA-125 IRMA test.

B. CA-125 Enzyme-Reagent – 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

F. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

G. Stop Solution – 8ml/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 & 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and elevated ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one (1) year
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the CA-125 Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution**.

10.0 CALCULATION OF RESULTS

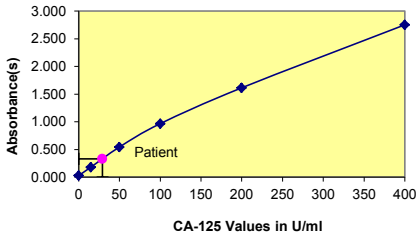
A dose response curve is used to ascertain the concentration of CA-125 in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA-125 concentration in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CA-125 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.331l) intersects the dose response curve at 29.3U/ml CA-125 concentration (See Figure 1).

Note: Computer data reduction software designed ELISA assays may also be used for the data reduction. *If such software is utilized, the validation of the software should be ascertained.*

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.035	0.029	0
	B1	0.022		
Cal B	C1	0.186	0.182	15
	D1	0.178		
Cal C	E1	0.536	0.545	50
	F1	0.554		
Cal D	G1	0.985	0.967	100
	H1	0.949		
Cal E	A2	1.615	1.615	200
	B2	1.616		
Cal F	C2	2.749	2.753	400
	D2	2.758		
Patient	A3	0.336	0.331	**29.3**
	B3	0.325		

Figure 1



*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CA-125 concentrations above 400 U/ml may be diluted (for example 1/10 or higher) with normal male serum (CA-125 < 5 U/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
2. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. CA-125 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CA-125 value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

13.0 EXPECTED RANGE OF VALUES

The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases and 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

TABLE I
Expected Values for CA-125 AccuBind® ELISA Test System

Healthy and non-pregnant subjects	U≤U 35 U/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the CA-125 AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	20	3.1	0.22	7.1%
Level 2	20	28.0	1.42	5.0%
Level 3	20	161.2	4.21	2.6%

TABLE 3
Between Assay Precision* (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	10	3.7	0.44	11.8%
Level 2	10	25.3	1.81	7.1%
Level 3	10	154.0	5.11	3.4%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CA-125 AccuBind® ELISA test system has a sensitivity of 1.0 U/ml. The sensitivity was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The CA-125 AccuBind® ELISA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 121. The least square regression equation and the correlation coefficient were computed for CA-125 in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (X)	5.67	y = -0.116 +1.032x	0.998
Reference (Y)	5.75		

14.4 Specificity

In order to test the specificity of the antibody pair used, massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. In addition some widely used, over-the-counter, drugs and some cytotoxic drugs (10 fold the normal dose) were tested in the assay. No cross reaction was found. Percent recoveries for some of these additions are listed below in Table 5.

TABLE 5

Analyte	Amount Added	% Recovery
Bilirubin	1 mMol/L	98 – 103%
Hemoglobin	1 mMol/L	100 – 106%
Triglycerides	10 mMol/L	96 – 110 %
RF	1000 kIU/L	97 – 107%
Biotin	25 µg/L	99 – 103%

15.0 REFERENCES

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Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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Glossary of Symbols
(EN 980/ISO 15223)