

HDV IgM

**“Capture” Enzyme ImmunoAssay
(ELISA) for the determination of IgM
antibodies to Hepatitis Delta Virus
in human plasma and sera**

- for “in vitro” diagnostic use only -



DIA.PRO

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HDV IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM class antibodies to Hepatitis Delta Virus or HDV in human plasma and sera with the "capture" system. The kit is intended for the classification of the viral infective agent and the follow-up of HDV infected patients.

For "in vitro" diagnostic use only.

B. INTRODUCTION

The Hepatitis Delta Virus or HDV is a RNA defective virus composed of a core presenting the delta-specific antigen, encapsulated by HBsAg, that requires the helper function of HBV to support its replication.

Infection by HDV occurs in the presence of acute or chronic HBV infection. When acute delta and acute HBV simultaneously occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone. In contrast, a patient with chronic HBV infection can support HDV replication indefinitely, usually with a less severe illness appearing as a clinical exacerbation.

The determination of HDV specific serological markers (HDV Ag, HDV IgM and HDV IgG) represents in these cases an important tool to the clinician for the classification of the etiological agent, for the follow up of infected patients and their treatment.

The detection of HDV IgM and IgG antibodies allows the classification of the illness and the monitoring of the seroconversion event.

C. PRINCIPLE OF THE TEST

Microplates are coated with a monoclonal anti-hIgM antibody that in the 1st incubation "captures" specifically this class of antibodies.

After washing out all the other components of the sample, in the 2nd incubation bound anti HDV IgM are detected by the addition of recombinant HDV antigen immunocomplexed with a specific antibody, labeled with peroxidase (HRP).

After washing, the enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of IgM antibodies present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to carry out 96 tests.

1. Microplate: MICROPLATE

12 strips of 8 breakwells coated with purified anti human IgM specific mouse monoclonal antibody 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. Negative Control: CONTROL -

1x2.0 ml/vial. Ready to use. It contains, human antibodies negative to HDV, 3% skimmed milk, 0.2M Tris buffer pH 6.0+/-0.1, 0.2% Tween 20, 0.09% Na azide and 0.045% ProClin 300 as preservatives.

The Negative Control is pale yellow color coded.

3. Positive Control: CONTROL +

1x2.0 ml/vial. Ready to use. It contains, human IgM antibodies positive to HDV, 3% skimmed milk, 0.2M Tris buffer pH 6.0+/-0.1, 0.2% Tween 20, 0.09% Na azide and 0.045% ProClin 300 as preservatives.

The Positive Control is green yellow color coded.

Important Note: Even if this material has been chemically inactivated, handle as potentially infectious.

4. Calibrator: CAL ...

n° 1 vial. Lyophilized reagent to be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, human IgM antibodies to HDV, 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. Even if this material has been chemically inactivated, handle as potentially infectious.

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 20X: CONJ 20X

1x0.8 ml/vial. 20X concentrated solution. It contains peroxidase labeled polyclonal antibody to HDV. The reagent is dissolved into a buffer solution 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

7. HDV Antigen: Ag HDV

1x6 vials. Lyophilized reagent to be dissolved with 1.9 ml proper diluent. It contains non infective recombinant HDV Antigen, 25 mM Tris buffer pH 7.8+/-0.1 and 5% human serum proteins.

8. HDV Antigen Diluent: Ag DIL

1x16 ml/vial. Buffered solution for the dissolution of the lyophilized HDV antigen. It contains 0.2 M Tris buffer pH 6.0+/-0.1, 0.045% ProClin 300 and 0.2% Triton X100. The component is red colour coded.

9. Specimen Diluent: DILSPE

2x60.0 ml/vial. Buffered solution for the dilution of samples; it contains 0.2M Tris buffer pH 6.0+/-0.1, 0.2% Tween 20, 3% Skimmed milk, 0.045% ProClin 300 and 0.09% sodium azide as preservatives. The component is blue clour coded.

10. Chromogen/Substrate: SUBS TMB

1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine or TMB, 4% dimethylsulphoxide and 0.02% hydrogen peroxide of H₂O₂.

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

11. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. Contains 0.3 M H₂SO₄ 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 in the range 10-1000 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.
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/H₂O₂) 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 labware 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 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 RECOMMENDATIONS

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 0.2-0.8µ 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. When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative and Positive Controls:

Ready to use. Mix well on vortex before use.

Calibrator:

Lyophilized reagent to be dissolved with EIA grade water as reported in the label.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at -20°C.

Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml 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.

Immunocomplex:

Dissolve the lyophilized HDV Antigen with 1.9 ml HDV Antigen Diluent and mix gently in order to dissolve completely the content of the vial.

When all the powder is dissolved, add 100 µl 20X concentrated Enzyme Conjugate and mix gently on vortex.

Important Notes:

1. The preparation of the Immunocomplex has to be carried out just after the dispensation of controls&calibrator and samples into the microplate.
2. The so prepared immunocomplex is not stable when liquid. Freeze what not used in aliquots at -20°C. Thaw only once and do not use this frozen material after the expiration date of the kit.

Specimen Diluent

Ready to use. Mix on vortex before use.

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 (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 $\pm 0.5^\circ\text{C}$) 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 $\leq 10\text{ nm}$; (b) absorbance range from 0 to 4; (c) linearity to 4; 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 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 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/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 performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

- Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples.
- Dilute samples 1:200 dispensing 1 ml Specimen Diluent into a disposable tube and then 5 µl sample; mix on vortex before use. Do not dilute controls & calibrator as they are ready-to-use.
- Leave the A1 well empty for blanking purposes.
- Pipette 100 µl of the Negative Control in triplicate, 100 µl of the Calibrator in duplicate and 100 µl of the Positive Control in single.
- Then pipette 100 µl of diluted samples in the proper wells.
- Finally incubate the microplate **for 60 min at +37°C**.

Important notes:

- 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.*
- Prepare the Immunocomplex as described.*

- When the first incubation is finished, wash the microwells as previously described (section I.3)
- In all the wells except A1, pipette 100 µl Immunocomplex and 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. Contamination might occur.

- When the second incubation is over, wash the microwells as previously described (section I.3)
- 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.

- Incubate the microplate protected from light at **room temperature (18-24°C) for 20 min.** Wells dispensed with positive samples, the Positive Control and the Calibrator as well will turn from clear to blue.
- Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 10. Addition of the stop solution will turn the Positive Control, the Calibrator and positive samples from blue to yellow.
- 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:

- Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
- 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.*
- 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	100 µl
Diluted samples (1:200)	100 µl
1st incubation	60 min
Temperature	+37°C
Washing steps	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Immunocomplex	100 µl
2nd incubation	60 min
Temperature	+37°C
Washing steps	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	100µl
2nd incubation	20 min
Temperature	room
Sulphuric Acid	100 µl
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported in the table 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 or S/Co values have been matched in the analysis.

Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm
Negative Control	< 0.200 OD450nm after blanking
Coefficient of variation	< 30%
Calibrator	S/Co > 2.5
Positive Control	> 0.900 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 > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control > 0.200 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study;
coefficient of variation > 30%	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 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 S/Co < 2.5	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.
Positive Control < 0.900 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the 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 13.

P. CALCULATION OF DATA

If the test turns out to be valid, results are calculated from the mean OD450nm / 620-630nm value of the Negative Control (NC) by means of a cut-off value (Co) determined with the following formula:

$$\text{Cut-Off} = \text{NC} + 0.250$$

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 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
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient is not infected by HDV (acute phase).

Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.

A positive result is indicative of an ongoing HDV infection and therefore the patient should be treated accordingly.

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. 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.

3. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
4. Diagnosis of viral hepatitis infection has to be taken 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 13).

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 0.200 – Accepted

Positive Control: 2.000 OD450nm
Higher than 0.900 – Accepted

$$\text{Cut-Off} = 0.100 + 0.250 = 0.350$$

Calibrator: 1.000 – 1.100 OD450nm
Mean value: 1.050 OD450nm S/Co = 3.0
S/Co higher than 2.5 – Accepted
Sample 1: 0.080 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCE CHARACTERISTICS

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

No international standard for HDV IgM Antibody detection has been defined by CTS.

In its absence, an Internal Gold Standard (or IGS), derived from a patient in the acute phase of the infection, 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 three lots by comparison with a commercial European kit.

A limiting dilution curve was prepared in negative plasma.

Results of Quality Control are given in the following table:

Internal Gold Standard (IGS)

IGS	Lot #	1102	Lot #	0103	Lot #	0403	DiaSorin
dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co	S/Co
1 X	0.728	2.5	0.783	2.6	0.837	2.7	2.6
2 X	0.443	1.5	0.461	1.5	0.471	1.5	1.4
4 X	0.286	1.0	0.281	0.9	0.305	1.0	1.0
8 X	0.154	0.5	0.160	0.5	0.185	0.6	0.5
Plasma -	0.039	0.1	0.054	0.2	0.065	0.2	0.2

2. Diagnostic Sensitivity and Specificity:

The diagnostic performances were evaluated in a performance evaluation conducted by the Department of Gastro-Hepatology, Prof. M.Rizzetto, S.Giovanni Battista hospital, Torino, Italy, on more than 400 samples against a reference European kit.

Positive samples were collected from patients undergoing acute HDV infection.

The diagnostic specificity has been determined on panels of more than 250 negative samples from normal individuals and blood donors, classified negative with the reference kit.

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 samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.

Samples derived from patients with different viral (HCV, HAV) and non viral pathologies of the liver that may interfere with the test were examined. No cross reaction were observed.

The Performance Evaluation study conducted in a qualified external reference center on more than 400 samples has provided the following values :

Sensitivity	> 98 %
Specificity	> 98 %

3. Reproducibility:

It has been calculated on three samples examined in replicates in different runs. The mean values obtained from a study conducted on three samples of different HDV IgM reactivity, examined in 16 replicates in three separate runs is reported below:

DIM.CE: lot # 1102

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.061	0.056	0.056	0.058
Std.Deviation	0.008	0.007	0.007	0.008
CV %	13.9	13.0	12.9	13.3

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.798	0.810	0.802	0.803
Std.Deviation	0.044	0.041	0.046	0.044
CV %	5.5	5.1	5.7	5.4
S/Co	2.6	2.6	2.6	2.6

Positive Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.133	2.143	2.134	2.137
Std.Deviation	0.081	0.081	0.095	0.086
CV %	3.8	3.8	4.4	4.0
S/Co	6.9	7.0	7.0	7.0

DIM.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.062	0.059	0.066	0.062
Std.Deviation	0.008	0.005	0.006	0.006
CV %	12.4	9.3	9.2	10.3

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.843	0.843	0.826	0.837
Std.Deviation	0.051	0.051	0.045	0.049
CV %	6.0	6.0	5.4	5.8
S/Co	2.7	2.7	2.7	2.7

Positive Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.299	2.278	2.227	2.268
Std.Deviation	0.115	0.102	0.112	0.110
CV %	5.0	4.5	5.0	4.8
S/Co	7.4	7.4	7.0	7.3

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Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.066	0.070	0.067	0.068
Std.Deviation	0.006	0.008	0.008	0.007
CV %	9.8	10.7	11.3	10.6

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.800	0.813	0.815	0.809
Std.Deviation	0.044	0.046	0.049	0.046
CV %	5.5	5.7	6.0	5.7
S/Co	2.5	2.5	2.6	2.5

Positive Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.352	2.328	2.339	2.340
Std.Deviation	0.093	0.098	0.105	0.099
CV %	3.9	4.2	4.5	4.2
S/Co	7.5	7.3	7.4	7.4

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 13.

S. LIMITATIONS

False positivity has been assessed as less than 2 % of the normal population, mostly due to high titers of Rheumatoid Factor.

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

T. CONFIRMATION TEST

The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of HDV 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 µl concentrated Enzymatic Conjugate are diluted in 500 µl Antigen Diluent and mixed gently on vortex. Do not use any lyophilized vial of HDV Antigen 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 1:201, 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.

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.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 0.9 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 1.1 and in position E1 shows a S/Co value still higher than 1.1 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of HDV Antigen and a crossreaction with the polyclonal antibody, labeled with HRP, has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.1 and in position E1 shows a S/Co value lower than 0.9 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of HDV Antigen and not due to any crossreaction.

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
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CE
0318

The following table is reported for the interpretation of results:

Well	S/Co		
D1	< 0.9	> 1.1	> 1.1
E1	< 0.9	> 1.1	< 0.9
Interpretation	Problem of contamination	False positive	True positive

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HDV IgM

**Ensayo inmunoenzimático (ELISA)
de “captura” para la determinación
de anticuerpos IgM frente al
virus de la hepatitis Delta
en plasma y suero humanos**

Uso exclusivo para diagnóstico “in vitro”



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REF DIM.CE
96 pruebas

IgM HDV

A. OBJETIVO DEL EQUIPO

Ensayo inmunoenzimático (ELISA) para la determinación de anticuerpos de la clase IgM frente al Virus de la Hepatitis Delta (HDV) en plasma y suero humanos, mediante un sistema de captura. El equipo ha sido desarrollado para la clasificación del agente vírico infeccioso y para el seguimiento de pacientes infectados con HDV.

Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.

El Virus de la Hepatitis Delta es un virus ARN defectivo. Se compone de un núcleo con los antígenos delta específicos, y está encapsulado por el HBsAg. Para su replicación necesita ayuda funcional de VHB.

La infección por HDV ocurre en presencia de una infección aguda o crónica por VHB. Cuando se presenta simultáneamente la infección aguda por los dos virus, la enfermedad es grave y el cuadro clínico, así como las características bioquímicas, son prácticamente indistinguibles de una infección por VHB sólo. Sin embargo, una persona infectada por VHB de forma crónica puede soportar indefinidamente la replicación del HDV, normalmente la enfermedad es menos grave y aparece como exacerbadón clínica.

La determinación de los marcadores serológicos específicos de HDV (Ac HDV, IgM HDV e IgG HDV) representa una herramienta importante para los clínicos en la clasificación del agente etiológico, en el seguimiento de los pacientes y en el tratamiento.

La detección de anticuerpos IgM e IgG frente al HDV permite la clasificación de la enfermedad y la monitorización de la seroconversión.

C. PRINCIPIOS DEL ENSAYO

Las microplacas están recubiertas con un anticuerpo monoclonal anti-IgM que, durante la primera incubación, captura específicamente esta clase de anticuerpos.

En la segunda incubación, después del lavado, se añade un inmunocomplejo (un antígeno recombinante del HDV y un anticuerpo específico) marcado con peroxidasa (HRP), a fin de detectar los anticuerpos anti-HDV clase IgM unidos a la fase sólida.

Después del lavado, la enzima capturada en la fase sólida se combina con la mezcla cromógeno/substrato, generando una señal óptica proporcional a la cantidad de anticuerpos IgM presentes en la muestra.

D. COMPONENTES

Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE

12 tiras intercambiables de 8 pocillos, recubiertos con un anticuerpo monoclonal de ratón anti-IgM humano 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 4°C.

2. Control negativo: CONTROL

1x2.0 ml/vial. Listo para el uso. Contiene anticuerpos humanos negativos al HDV, 3% de leche descremada, tampón Tris-0.2M a pH 6.0 +/-0.1, 0.2% de Tween 20, 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+

1x2.0 ml/vial. Listo para el uso. Contiene anticuerpos IgM humanos positivos al HDV, 3% de leche descremada, tampón Tris 0.2M a pH 6.0 +/-0.1, 0.2% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como conservantes.

El control positivo está codificado con el color verde-amarillo.

Nota importante: Aun cuando los anticuerpos han sido inactivados químicamente, manipule el material como potencialmente infeccioso.

4. Calibrador: CAL ...

n° 1 vial. Reactivo liofilizado para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero fetal bovino, anticuerpos humanos clase IgM frente a HDV, además de sulfato de gentamicina 0.2 mg/ml 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 indicado en la etiqueta.

2. Aún cuando los anticuerpos han sido inactivados químicamente, manipule el material como potencialmente infeccioso.

5. Solución de lavado concentrada: WASHBUF 20X

1x60ml/frasco. Solución concentrada 20x.

Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, 0.05% de Tween 20 y ProClin 300 0.045%.

6. Conjugado 20X: CONJ 20X

1x0.8 ml/vial. Solución concentrada 20X. Contiene anticuerpo policlonal anti-HDV conjugado con peroxidasa (HPR). El reactivo se disuelve en una solución tampón: tampón Tris 10mM a pH 6.8 +/- 0.1, 5% de albúmina de suero bovino, ProClin 300 0.045% y 0.02% de sulfato de gentamicina como conservantes..

7. Antígeno HDV: Ag HDV

1x6 viales. Reactivo liofilizado para disolver en 1.9 ml del diluyente adecuado. Contiene antígeno recombinante HDV, no infeccioso, tampón Tris 25 mM a pH 7.8 +/-0.1 y 5% de proteínas del suero humano.

8. Diluyente de antígeno HDV: Ag DIL

1x16 ml/vial. Solución tamponada para la disolución del antígeno HDV liofilizado. Contiene tampón Tris 0.2M a pH 6.0 +/- 0.1, ProClin 300 0.045% y 0.2% de Tritón 100X. El componente está codificado con el color rojo.

9. Diluyente de muestras: DILSPE

2x60.0 ml/vial. Solución tamponada para la disolución de las muestras. Contiene tampón Tris 0.2M a pH 6.0 +/- 0.1, 3% de leche descremada, 0.2% de Tween 20, azida sódica 0.09% y ProClin 300 0.045% como conservantes. El reactivo está codificado con el color azul.

10. Cromógeno/substrato SUBS TMB

1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM a pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H₂O₂) 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 H₂SO₄ 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, 100 y 10 µl) y puntas de plástico desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para eliminar oxidantes químicos 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) ajustado a +37°C.
6. Lector calibrado de micropocillos ELISA con filtros de 450nm (lectura) y de 620-630 nm (blanco).
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 indumentales 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 microbianos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del cromógeno/substrato (TMB/H₂O₂) y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 °C, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar componentes de diferentes lotes ni tampoco de diferentes equipos del mismo lote.
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 después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en la etiqueta externa (envase primario) y en las etiquetas internas (viales).
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 cruzadas.
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 procedentes del

procedimiento de lavado, de restos de controles y muestras deben ser tratados como material potencialmente infeccioso y deben ser inactivados. Se recomienda la inactivación con una concentración final de lejía al 10% durante 16 a 18 horas o la inactivación con calor mediante autoclave a 121 °C durante 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 el uso de conservantes, en particular azida sódica, ya que puede afectar la actividad enzimática del conjugado.
3. Las muestras deben ser identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Cuando el equipo se emplea para el cribado de unidades de sangre, se recomienda el uso del código de barras y la lectura electrónica.
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 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.
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

En un estudio realizado con un equipo abierto no se ha detectado pérdida de actividad relevante utilizándolo hasta 6 veces y 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 color verde oscuro, lo que indicaría un defecto de fabricación.

De ser así, llame al servicio de atención al cliente de Dia.Pro. Las tiras no utilizadas deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8°C. Cuando se abre por primera vez, las tiras sobrantes se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambia de amarillo a verde.

Controles negativo y positivo:

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

Calibrador:

Reactivo liofilizado para disolver con agua de calidad EIA, según lo indicado en la etiqueta.

Nota: El calibrador disuelto no es estable. Se recomienda almacenar congelado en alícuotas a -20°C .

Solución de lavado concentrada:

Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada hasta 1200 ml y mezclarse suavemente antes de usarse.

Durante la preparación hay que evitar la formación de espuma y burbujas, que podrían reducir la eficiencia de lavado.

Nota: Una vez diluida, la solución es estable por una semana a temperaturas entre $+2$ y 8°C .

Inmunocomplejo:

Disolver el antígeno HDV liofilizado en 1.9 ml de diluyente de antígeno HDV y mezclar suavemente hasta disolver completamente el contenido del vial.

Cuando el polvo se haya disuelto completamente, añadir 100 μl del Conjugado concentrado 20X y mezclar suavemente en un vórtex.

Notas importantes:

1. La preparación de inmunocomplejo debe efectuarse justo después de dispensar en la placa los controles, el calibrador y las muestras.
2. El inmunocomplejo preparado así no es estable en estado líquido. Separar en alícuotas y congelar a -20°C el inmunocomplejo no utilizado. Descongelar una sola vez y no usar este material congelado después de la fecha de caducidad del equipo.

Diluyente 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 la 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 (etanol 70%, lejía 10%, desinfectantes de calidad hospitalaria). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de $\pm 2\%$.
2. La incubadora ELISA debe ser ajustada a 37°C ($\pm 0.5^{\circ}\text{C}$ 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 $\pm 5\%$.
5. El lector de microplaca ELISA debe estar provisto de un filtro de lectura de 450nm 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 $\leq 10\text{nm}$ b) Rango de absorbancia de 0 a 4, c) Linealidad a 4.0, reproducibilidad $\geq 1\%$. 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 que se mide la densidad óptica correcta. Periódicamente debe procederse al mantenimiento según las instrucciones del fabricante.
6. En caso de usar un sistema automatizado 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 interno de calidad". 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 contaminación de pocillos adyacentes. Se recomienda el uso de sistemas automatizados de Elisa para el cribado en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por serie.
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. También se ofrece apoyo para la instalación de nuevos instrumentos a usar con el equipo.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO

1. Comprobar la fecha de caducidad indicada en la etiqueta externa (envase primario). No usar si ha caducado.

- Comprobar que los componentes líquidos no están contaminados con partículas ni agregados visibles. Comprobar que el cromógeno/substrato es incoloro o azul pálido, aspirando un pequeño volumen con una pipeta estéril de plástico. Comprobar que no han ocurrido roturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Comprobar que la bolsa de aluminio que contiene la microplaca no está perforada ni dañada.
- Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
- Disolver el calibrador como se ha descrito anteriormente y mezclar suavemente.
- Dejar los componentes restantes hasta alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
- Ajustar la incubadora de ELISA a 37°C 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.
- Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
- Si se utiliza un sistema automatizado, encenderlo y comprobar que los protocolos estén correctamente programados.
- Comprobar que las micropipetas estén fijadas en el volumen requerido.
- 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.

- Poner el número de tiras necesarias en el soporte de plástico e identificar cuidadosamente los pocillos para estándares y muestras.
- Diluir las muestras 1:200 dispensando en un tubo desechable 1 ml de diluyente de muestras y 5 µl de muestra, mezclar con vórtex antes de usar. No es necesario diluir los controles ni el calibrador, ya que están listos para el uso.
- Dejar el pocillo A1 vacío para el blanco.
- Dispensar 100 µl del control negativo, por triplicado, 100 µl de calibrador, por duplicado, y 100 µl del control positivo.
- Posteriormente, añadir 100 µl de muestras diluidas en sus respectivos pocillos.
- Incubar la microplaca **60 min a +37°C**.

Notas importantes:

- Las tiras se deben sellar con el adhesivo suministrado sólo cuando se hace el ensayo manualmente. No sellar cuando se emplean equipos automatizados de ELISA.
- Preparar el inmunocomplejo según se ha descrito.

- Tras la primera incubación, lavar los pocillos como se ha descrito previamente (sección I.3).
- Dispensar 100 µl del inmunocomplejo en todos los pocillos, excepto el A1. Incubar la microplaca **60 min a +37°C**.

Nota importante: Hay que tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta. Podría producirse contaminación.

- Tras la segunda incubación, lavar los pocillos según lo descrito previamente (sección I.3).
- Dispensar 100 µl de cromógeno/substrato en todos los pocillos, incluido el A1.

Nota importante: No exponer directamente a fuerte iluminación, de lo contrario se puede generar un fondo excesivo.

- Incubar la microplaca protegida de la luz a **temperatura ambiente (18-24°C) durante 20 minutos**. Los pocillos con muestras positivas, el control positivo y el calibrador pasarán de un tono claro a azul.
- Dispensar 100 µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática usando la misma secuencia que en el paso 10. La adición de la solución de parada cambia el color del control positivo, el calibrador y las muestras positivas de azul a amarillo.
- Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450nm (lectura) y otro de 620-630nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas importantes:

- Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.
- La lectura debería hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos más de 20 minutos de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.
- 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 y calibrador	100 µl
Muestras diluidas (1:200)	100 µl
1ª incubación	60 min
Temperatura	+37°C
Pasos de lavado	5 ciclos con 20" de remojo o 6 ciclos sin remojo
Inmunocomplejo	100 µl
2da incubación	60 min
Temperatura	+37°C
Pasos de lavado	5 ciclos con 20" de remojo o 6 ciclos sin remojo
Cromógeno/substrato	100 µl
3ª incubación	20 min
Temperatura	t.a.*
Ácido sulfúrico	100 µl
Lectura D.O.	450 nm / 620-630 nm

*t.a.: temperatura ambiente

En la tabla siguiente 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 una comprobación en los controles/calibrador cada vez que se usa el equipo para verificar si los valores DO450nm/620-630nm o M/Co son los esperados en el análisis. Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Requerimientos
Pocillo blanco	Valor < 0.100 DO450nm
Control negativo (CN)	Valor < 0.200 DO450nm después de leer el blanco
Coeficiente de variación	< 30%
Calibrador	M/Co > 2.5
Control positivo (CP)	> 0.900 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	Comprobar que
Pocillo blanco > 0.100 DO a 450nm	1. la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control negativo (CN) > 0.200 DO a 450nm después de leer el blanco	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.
Coeficiente de variación > 30%	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, a derrames o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas ni con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Calibrador M/Co < 2.5	1. el procedimiento ha sido ejecutado correctamente. 2. no se han cometido errores en 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 estándar.
Control positivo < 0.900 DO450nm	1. el procedimiento ha sido ejecutado correctamente. 2. no se han cometido errores durante la distribución del control. 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 se ha producido 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 13.

P. CÁLCULO DE DATOS

Si la prueba resulta válida, los resultados se calculan a partir del valor medio de DO450nm/620-630nm del Control Negativo (CN) por medio de un Valor de Corte (Co) determinado con la siguiente fórmula:

$$\text{Valor de corte} = \text{CN} + 0.250$$

Nota importante: Cuando el cálculo de los resultados se realiza mediante el sistema operativo de un equipo automatizado de ELISA, es necesario asegurarse de que se utiliza la formulación adecuada para generar la correcta interpretación de los resultados.

Q. INTERPRETACIÓN DE LOS RESULTADOS

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

M/Co	Interpretación
< 0.9	Negativo
0.9 – 1.1	Equívoco
> 1.1	Positivo

Un resultado negativo indica que el paciente no está infectado por HDV (fase aguda).

Cualquier paciente cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre extraída 1 ó 2 semanas después de la inicial.

Un resultado positivo es indicativo de infección en curso por HDV y por consiguiente el paciente debe ser tratado adecuadamente.

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. Todas las muestras positivas deben someterse a la prueba de confirmación descrito en la sección T antes de emitir el resultado de positividad. Mediante la realización de dicha prueba pueden detectarse —y descartarse— reacciones falsas, causantes de interpretaciones erróneas del resultado analítico.
3. Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
4. El diagnóstico de infección con un virus de la hepatitis debe ser realizado y comunicado al paciente por un médico cualificado.

A continuación se incluye un ejemplo de los cálculos (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 13).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos por el usuario:

Control negativo: 0.100 – 0.120 – 0.080 DO450nm
 Valor medio: 0.100 DO450nm
 Menor de: 0.200 – Válido

Control positivo: 2.000 DO450nm
 Mayor de 0.900 – Válido

Valor de corte = 0.100+0.250 = 0.350

Calibrador: 1.000 – 1.100 DO450nm
 Valor medio: 1.050 DO450nm M/Co = 3.0
 M/Co mayor de 2.5 – Válido

Muestra 1: 0.080 DO450nm
 Muestra 2: 1.800 DO450nm
 Muestra 1 M/Co < 0.9 = negativa
 Muestra 2 M/Co > 1.1 = positiva

R. CARACTERÍSTICAS DE RENDIMIENTO

La evaluación del rendimiento ha sido realizada según lo establecido en las Especificaciones Técnicas Comunes (ETC) (Art. 5, Capítulo 3 de la Directiva IVD 98/79/CE).

1. Límite de detección.

Ningún estándar internacional para la detección de anticuerpos IgM anti-HDV ha sido definido en las ETC.

Con el objetivo de garantizar una excelente y constante sensibilidad del dispositivo, fue definido un estándar de oro interno (IGS), a partir de un paciente en fase aguda de la infección.

El límite de detección del ensayo ha sido calculado en tres lotes, usando un equipo comercial europeo como referencia.

Se preparó una curva de dilución límite en plasma negativo.

Los resultados del control de calidad se muestran en la siguiente tabla:

Estándar de oro interno (IGS)

IGS	Lote n.º 1102	Lote n.º 0103	Lote n.º 0403	DiaSorin			
dilución	DO450nm	M/Co	DO450nm	M/Co	M/Co		
1 X	0.728	2.5	0.783	2.6	0.837	2.7	2.6
2 X	0.443	1.5	0.461	1.5	0.471	1.5	1.4
4 X	0.286	1.0	0.281	0.9	0.305	1.0	1.0
8 X	0.154	0.5	0.160	0.5	0.185	0.6	0.5
Plasma -	0.039	0.1	0.054	0.2	0.065	0.2	0.2

2. Especificidad y sensibilidad diagnóstica

La evaluación del rendimiento diagnóstico se realizó mediante un ensayo con más de 400 muestras frente a un equipo de referencia europeo realizado por el Prof. M. Rizzetto, Departamento de Gastro-Hepatología del hospital S. Giovanni Battista de Turín, Italia.

Se recogieron muestras positivas de pacientes en etapa aguda de la infección por HDV.

La especificidad clínica ha sido determinada utilizando paneles de más de 250 muestras, provenientes de individuos sanos y donantes de sangre, clasificadas como negativas mediante un equipo de referencia.

Se emplearon, además, plasma sometido a métodos de tratamiento estándar (citratado, EDTA y heparina) y suero humano para determinar la especificidad. No se ha observado falsa reactividad debida a los métodos de preparación de muestras.

Las muestras congeladas también se han probado para comprobar si la congelación interfiere con el rendimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de partículas.

Se examinaron muestras procedentes de pacientes afectados por hepatitis víricas (HCV, HVA) y patologías no víricas del hígado, que pudieran provocar interferencia en el ensayo, sin embargo no se observó reacción cruzada.

El estudio del rendimiento se realizó mediante un ensayo con más de 400 muestras, en un centro de referencia externo cualificado. Se obtuvieron los siguientes valores:

Sensibilidad	> 98 %
Especificidad	> 98 %

3. Reproducibilidad.

Ha sido calculada con tres muestras, examinadas en réplicas en series diferentes.

Se realizó un estudio con 3 muestras de diferente reactividad IgM HDV, examinadas en 16 réplicas, en tres series separadas. Los valores medios obtenidos se facilitan a continuación:

DIM.CE:lote n.º 1102

Control negativo (N = 16)

Valores medios	1ª serie	2ª serie	3ª serie	Valor promedio
DO450nm	0.061	0.056	0.056	0.058
Desviación estándar	0.008	0.007	0.007	0.008
CV %	13.9	13.0	12.9	13.3

Calibrador (N = 16)

Valores medios	1ª serie	2ª serie	3ª serie	Valor promedio
DO 450nm	0.798	0.810	0.802	0.803
Desviación estándar	0.044	0.041	0.046	0.044
CV %	5.5	5.1	5.7	5.4
M/Co	2.6	2.6	2.6	2.6

Control positivo (N = 16)

Valores medios	1ª serie	2ª serie	3ª serie	Valor promedio
DO 450nm	2.133	2.143	2.134	2.137
Desviación estándar	0.081	0.081	0.095	0.086
CV %	3.8	3.8	4.4	4.0
M/Co	6.9	7.0	7.0	7.0

DIM.CE:lote n.º 0103

Control negativo (N = 16)

Valores medios	1ª serie	2ª serie	3ª serie	Valor promedio
DO 450nm	0.062	0.059	0.066	0.062
Desviación estándar	0.008	0.005	0.006	0.006
CV %	12.4	9.3	9.2	10.3

Calibrador (N = 16)

Valores medios	1ª serie	2ª serie	3ª serie	Valor promedio
DO 450nm	0.843	0.843	0.826	0.837
Desviación estándar	0.051	0.051	0.045	0.049
CV %	6.0	6.0	5.4	5.8
M/Co	2.7	2.7	2.7	2.7

Control positivo (N = 16)

Valores medios	1ª serie	2ª serie	3ª serie	Valor promedio
DO 450nm	2.299	2.278	2.227	2.268
Desviación estándar	0.115	0.102	0.112	0.110
CV %	5.0	4.5	5.0	4.8
M/Co	7.4	7.4	7.0	7.3

DIM.CE:lote n.º 0403

Control negativo (N = 16)

Valores medios	1ª serie	2ª serie	3ª serie	Valor promedio
DO 450nm	0.066	0.070	0.067	0.068
Desviación estándar	0.006	0.008	0.008	0.007
CV %	9.8	10.7	11.3	10.6

Calibrador (N = 16)

Valores medios	1ª serie	2ª serie	3ª serie	Valor promedio
DO 450nm	0.800	0.813	0.815	0.809
Desviación estándar	0.044	0.046	0.049	0.046
CV %	5.5	5.7	6.0	5.7
M/Co	2.5	2.5	2.6	2.5

Control positivo (N = 16)

Valores medios	1ª serie	2ª serie	3ª serie	Valor promedio
DO 450nm	2.352	2.328	2.339	2.340
Desviación estándar	0.093	0.098	0.105	0.099
CV %	3.9	4.2	4.5	4.2
M/Co	7.5	7.3	7.4	7.4

La variabilidad mostrada en las tablas no dio 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 13.

S. LIMITACIONES

Los falsos positivos fueron estimados como menos del 2% de la población normal, debido principalmente a altos títulos de Factor Reumatoide.

Las muestras que tras ser descongeladas presentan partículas de fibrina o agregados pueden generar algunos resultados falsos positivos.

T. PRUEBA DE CONFIRMACIÓN

La prueba de confirmación debe realizarse en todas las muestras positivas antes de remitir el diagnóstico de infección primaria por HDV al médico.

Proceder del modo siguiente para la confirmación:

1. Preparar el complejo antígeno/conjugado tal como se describe en la sección correspondiente. Este reactivo se denomina Solución A.
2. A continuación, se diluyen 25 µl de conjugado enzimático concentrado en 500 µl de diluyente para antígeno y mezclar suavemente usando un vórtex. ¡No utilizar ningún vial de antígeno HDV liofilizado para este procedimiento! Esta solución se denomina Solución B.
3. Dejar el pocillo A1 de la tira vacío para el blanco.
4. El Control negativo se dispensa en las posiciones B1+C1 de la tira. Esto se utiliza para el cálculo de los valores de corte y M/Co.
5. La muestra positiva a confirmar se dispensa diluida al 1:201 en las posiciones D1+E1 de la tira.
6. La tira se incuba durante 60 minutos a +37°C.
7. Tras el lavado, el pocillo blanco A1 se deja vacío.
8. Se dispensan 100 µl de Solución A en los pocillos B1+C1+D1.
9. A continuación, se añaden 100 µl de Solución B al pocillo E1.
10. La tira se incuba durante 60 minutos a +37°C.
11. Tras el lavado, se añaden 100 µl de cromógeno/sustrato a todos los pocillos y la tira se incuba durante 20 minutos a temperatura ambiente.
12. Se añaden 100 µl de ácido sulfúrico a todos los pocillos y entonces se mide la intensidad del color de éstos a 450nm (filtro de lectura) y a 620-630nm (substracción del fondo), calibrando el instrumento con el pocillo A1 (blanco).

La interpretación de los resultados se realiza del modo siguiente:

1. Si la muestra del pocillo D1 presenta un valor de M/Co inferior a 0.9, es probable que haya ocurrido un problema de dispensación o de contaminación en la primera prueba. Debe repetirse el procedimiento de ensayo descrito en la sección M, como doble comprobación del análisis.
2. Si la muestra de la posición D1 presenta un valor de M/Co superior a 1.1 y la muestra de la posición E1 presenta un valor de M/Co también superior a 1.1, la muestra se considera como un **falso positivo**. La reactividad de la muestra no es dependiente de la presencia específica de antígeno HDV y se ha producido una reacción cruzada con el anticuerpo policlonal marcado con HRP.
3. Si la muestra de la posición D1 presenta un valor de M/Co superior a 1.1 y la muestra de la posición E1 presenta un valor de M/Co inferior a 0.9, la muestra se considera como un **verdadero positivo**. La reactividad de la muestra es dependiente de la presencia específica de antígeno HDV y no es debida a ninguna reacción cruzada.

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

Pocillo	M/Co		
D1	< 0.9	> 1.1	> 1.1
E1	< 0.9	> 1.1	< 0.9
Interpretación	Problema de contaminación	Falso positivo	Verdadero 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:

Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (Mi) – Italia



0318

HDV Ab

**Competitive Enzyme Immunoassay
for the qualitative determination of
antibodies to Hepatitis Delta Virus
in human serum and plasma**

- for “in vitro” diagnostic use only -



DIA.PRO

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HDV Ab

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the qualitative determination of antibodies to Hepatitis Delta Virus or HDV in human plasma and sera with a "two-steps" methodology.

The kit is used for the follow-up of patients infected by HDV.

For "in vitro" diagnostic use only.

B. INTRODUCTION

The Hepatitis Delta Virus or HDV is a RNA defective virus composed of a core presenting the delta-specific antigen, encapsulated by HBsAg, that requires the helper function of HBV to support its replication.

Infection by HDV occurs in the presence of acute or chronic HBV infection. When acute delta and acute HBV simultaneously occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone. In contrast, a patient with chronic HBV infection can support HDV replication indefinitely, usually with a less severe illness appearing as a clinical exacerbation.

The determination of HDV specific serological markers (HDV Ag, HDV Ab, HDV IgM and HDV IgG) represents in these cases an important tool to the clinician for the classification of the etiological agent, for the follow up of infected patients and their treatment. The detection of HDV total antibodies allows the classification of the illness and the monitoring of the seroconversion event.

C. PRINCIPLE OF THE TEST

Anti-HDV antibodies, if present in the sample, compete with a virus-specific polyclonal IgG, labeled with peroxidase (HRP), for a fixed amount of rec-HDV coated on the microplate. The test is carried out with a two steps incubation competitive system. First the sample is added to the plate and specific anti HDV antibodies bind to the adsorbed antigen. After washing, an enzyme conjugated antibody to HDV is added and binds to the free portion of the antigen coated. After washing a chromogen/substrate mixture is dispensed. The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti-HDV antibodies in the sample and its activity is detected by the added chromogen/substrate. The concentration of HDV-specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti-HDV antibodies.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE

8x12 microwell strips coated with recombinant HDV-specific antigen 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. Negative Control: CONTROL -

1x2.0ml/vial. Ready to use. Contains goat serum proteins, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives. The negative control is colour coded pale yellow.

3. Positive Control: CONTROL +

1x2.0ml/vial. Ready to use. Contains goat serum proteins, high titer anti HDV antibodies, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives. The positive control is colour coded green.

4. Calibrator: CAL

n° 1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, low titer human antibodies to HDV, 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. 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 antibody to HDV in presence of 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives. The component is colour coded red.

7. Chromogen/Substrate: SUBS TMB

1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% DMSO, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H₂O₂.

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 H₂SO₄ solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Plate sealers n° 2

Instructions for Use n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes in the range 10-1000 ul and disposable plastic tips.
2. EIA grade water (double distilled or deionized, 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-borne microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB/H₂O₂) 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 labware 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 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 RECOMMENDATIONS

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 3 months.

1. Antigen coated microwells:

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.

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:

Low positive control. Add precisely the volume of EIA grade water, reported on its label, to the lyophilized 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. When thawed do not freeze again; discard it.

5. Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml 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. 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 +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 350µl/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 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 ≤ 10 nm; (b) absorbance range from 0 to 4; (c) linearity to 4; repeatability ≥ 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/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.

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. Pipette 100 µl of Negative Control in triplicate, 100 µl Positive Control in single and then 100 µl of samples. Check that controls 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. Then incubate the microplate at **+37°C for 60 min.**

Important note: Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.

5. Wash the microplate as described.

6. Pipette 100 µl TMB/H₂O₂ 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 min**.

Important note: Do not expose to strong direct light as a high background might be generated.

7. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step n° 6 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.

8. 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 use of the Calibrator, a low positive control, is not mandatory for the assay as the CAL does not enter into the cut-off calculation. The CAL may be used as a low titer positive control when a laboratory internal quality verification is required by the management. When used for such purpose, dispense 100 µl of it, possibly in duplicate.

N. ASSAY SCHEME

Controls/Calibrator	100 µl
Samples	100 µl
1st incubation	60 min
Temperature	+37°C
Washing 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
Washing step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H ₂ O ₂ mix	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
Reading OD	450nm / 620-630nm

An example of dispensation scheme (including CAL) is reported in the table 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 negative and positive controls any time, and on the Calibrator in addition when the kit is used for the first time, 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	< 0.100 OD450nm value
Negative Control (NC)	> 1.000 OD450nm after blanking If lower carefully control the washing procedure and decrease the number of cycles or the soaking time coefficient of variation < 30%
Positive Control (PC)	OD450 nm < NC/10
Calibrator (CAL)	PC ≤ OD450nm < (NC+PC)/5

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

If they don't, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 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 OD450nm Outside the range	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of 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.
Positive Control OD450nm > NC/10	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 8.

P. RESULTS

The results are calculated by means of a cut-off value determined with the following formula:

$$\text{Cut-Off} = (\text{NC} + \text{PC}) / 5$$

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 HDV.

Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample.

A positive result is indicative of HDV infection and therefore the patient should be treated accordingly.

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 8).

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

Negative Control: 2.100 – 2.200 – 2.000 OD450nm

Mean Value: 2.100 OD450nm

Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm

Lower than NC/10 – Accepted

$$\text{Cut-Off} = (2.100 + 0.100) / 5 = 0.440$$

Calibrator: 0.300-0.260 OD450nm

Mean value: 0.280 OD450nm

Within the range $\text{PC} \leq \text{OD450nm} < (\text{NC} + \text{PC})/5$ – Accepted

Sample 1: 0.020 OD450nm

Sample 2: 1.900 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:

In absence of an international standard, the sensitivity of the assay has been calculated by means of the product named Accurun n° 127 supplied by Boston Biomedica Inc. – USA . The table below reports the OD450nm shown by this preparation when diluted in Fetal Calf Serum to prepare a limiting dilution curve, in three different lots.

Co/S values

	DAB.CE	Lot # 1102	DAB.CE	Lot # 0103	DAB.CE	Lot # 0403
Accurun # 127	OD450 nm	Co/S value	OD450 nm	Co/S value	OD450 nm	Co/S value
1x	0.171	3.0	0.163	2.9	0.156	2.8
2x	0.187	2.7	0.176	2.6	0.179	2.5
4x	0.230	2.2	0.220	2.1	0.202	2.2
8x	0.298	1.7	0.285	1.6	0.271	1.6
16x	0.417	1.2	0.405	1.1	0.402	1.1
32x	0.514	1.0	0.490	0.9	0.482	0.9
64x	0.717	0.7	0.700	0.7	0.705	0.6
128x	1.063	0.5	1.006	0.5	1.015	0.4
CTRL (-)	2.484	////////	2.261	////////	2.114	////////

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The diagnostic performances were evaluated in a clinical trial conducted by the Department of Gastro-Hepatology, Prof. M.Rizzetto, S.Giovanni Battista hospital, Torino, Italy, on more than 400 samples against a reference kit.

Negative, positive and potentially interfering samples were examined in the trial.

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.

Results are briefly reported in the tables below:

Sensitivity	> 98 %
Specificity	> 98 %

3. PRECISION

The mean values obtained from a study conducted on two samples of different anti-HDV antibody reactivity, examined in 16 replicates in three separate runs for three lots of product, is reported below:

DAB.CE: lot #1102

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.342	2.428	2.433	2.401
Std.Deviation	0.113	0.106	0.122	0.114
CV %	4.8	4.4	5.0	4.7

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.298	0.289	0.286	0.291
Std.Deviation	0.023	0.027	0.026	0.025
CV %	7.7	9.3	9.1	8.7
Co/S	1.6	1.7	1.7	1.7

DAB.CE: lot #0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.208	2.237	2.246	2.230
Std.Deviation	0.105	0.108	0.108	0.107
CV %	4.7	4.8	4.8	4.8

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.269	0.277	0.266	0.271
Std.Deviation	0.026	0.024	0.025	0.025
CV %	9.8	8.5	9.5	9.3
Co/ S	1.7	1.7	1.7	1.7

DAB.CE: lot # 0403

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.246	2.221	2.182	2.216
Std.Deviation	0.097	0.103	0.118	0.106
CV %	4.3	4.6	5.4	4.8

Calibrator (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.286	0.273	0.280	0.280
Std.Deviation	0.027	0.023	0.026	0.025
CV %	9.3	8.5	9.1	9.0
Co/S	1.6	1.7	1.6	1.6

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 8.

S. LIMITATIONS

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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CE
0318

HDV Ab

**Ensayo inmunoenzimático competitivo
para la determinación cualitativa de
anticuerpos frente al Virus de la
Hepatitis Delta
en plasma y suero humanos**

Uso exclusivo para diagnóstico "in vitro"



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REF DAB.CE
96 pruebas

HDV Ab

A. OBJETIVO DEL EQUIPO.

Ensayo inmunoenzimático competitivo (ELISA) para la determinación cualitativa de anticuerpos frente al Virus de la Hepatitis Delta (HDV) en plasma y suero humanos con una metodología de "dos pasos".

El equipo ha sido desarrollado para el seguimiento de pacientes infectados con HDV.

Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.

El Virus de la Hepatitis Delta es un virus ARN defectivo. Se compone de un núcleo con los antígenos delta específicos, y está encapsulado por el HBsAg. Para su replicación necesita ayuda funcional de HBV.

La infección por HDV ocurre en presencia de una infección aguda o crónica por HBV. Cuando se presenta simultáneamente la infección aguda por los dos virus, la enfermedad es grave y el cuadro clínico, así como las características bioquímicas son prácticamente indistinguibles de una infección por HBV. Sin embargo, una persona infectada por HBV de forma crónica puede soportar indefinidamente la replicación por HDV, normalmente la enfermedad es menos severa y aparece como exacerbadión clínica.

La determinación de los marcadores serológicos específicos de HDV (HDV Ag, HDV Ab, HDV IgM y HDV IgG) representa una herramienta importante para los clínicos en la clasificación del agente etiológico, en el seguimiento de los pacientes así como en el tratamiento.

La detección de anticuerpos totales permite la clasificación de la enfermedad y el seguimiento de la seroconversión.

C. PRINCIPIOS DEL ENSAYO.

El ensayo es de tipo competitivo, donde los anticuerpos anti-HDV de la muestra compiten con un anticuerpo policlonal (IgG) específico para el virus y conjugado con peroxidasa (HRP), por el antígeno recombinante-HDV de la fase sólida.

El ensayo se realiza mediante un sistema de dos pasos con incubación competitiva. La muestra se añade a la placa y los anticuerpos específicos anti-HDV se combinan con el antígeno de la fase sólida. Después del lavado, se añade un anticuerpo conjugado con peroxidasa (HRP) que se une al antígeno libre en la placa. Previo lavado, se añade el substrato cromogénico.

La concentración de la enzima conjugada, unida a la fase sólida es inversamente proporcional a la cantidad de anticuerpos al HDV presentes en la muestra y su actividad se detecta por la adición del substrato cromogénico.

La concentración de anticuerpos específicos al HDV en la muestra se determina de manera semicuantitativa a través del cálculo de un valor de corte.

D. COMPONENTES.

Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE

12 tiras de 8 pocillos recubiertos con antígeno recombinante específico de HDV, 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 4°C.

2. Control Negativo: CONTROL -

1x2.0ml/vial. Listo para el uso. Contiene proteínas del suero de cabra, tampón Tris-HCl 100 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 +

1x2.0ml/vial. Listo para el uso. Contiene proteínas del suero de cabra, alto título de anticuerpos anti-HDV, tampón Tris-HCl 100 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 ...

n° 1 vial. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero bovino fetal, bajo título de anticuerpos humanos al HDV, además de sulfato de gentamicina 0.02 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. 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%

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 anti-HDV conjugado con peroxidasa (HRP) en presencia de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservante. El conjugado está codificado con el color rojo.

7. 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 (H₂O₂) 0.02% así como dimetilsulfóxido 4%.

Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

8. Ácido Sulfúrico: H₂SO₄ 0.3M

1x15ml/vial. Contiene solución de H₂SO₄ 0.3M

Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Sellador adhesivo, n° 2

Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (10-1000 µl) 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 37°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 sustrato (TMB/H₂O₂) a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 °C, 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. 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 pesquaje 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 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.
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 3 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 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 2-8°C. 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:

Control positivo bajo. Añadir de manera precisa al polvo liofilizado el volumen de agua de calidad EIA indicado en la etiqueta. Dejar disolver totalmente y mezclar suavemente en el vórtex.

Note: Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a -20°C. Cuando se descongele, descartar el agua en lugar de congelarla nuevamente.

5. Solución de Lavado Concentrada:

Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada hasta 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 8°C.

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. 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 o los reactivos (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 37°C (+/- 0.5°C) 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 microplaca ELISA debe estar provisto de un filtro de lectura de 450nm 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 $\leq 10\text{nm}$ b) Rango de absorbancia de 0 a 4, c) Linealidad a 4, reproducibilidad $\geq 1\%$. 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 que se mide la densidad óptica correcta. Periódicamente debe procederse al mantenimiento según las instrucciones 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 pesquaje 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 20x concentrada, 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 37°C 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. 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 100µl del Control Negativo, por triplicado, 100µl del Control Positivo una vez y, posteriormente, añadir 100µl de muestras. Comprobar que los controles y muestras se han añadido correctamente.

Después incubar la microplaca durante **60 minutos a +37°C**.

3. Lavar la microplaca según lo descrito previamente (sección I.3).

4. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1; comprobar que los reactivos se han añadido correctamente. 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.

5. Lavar la microplaca según lo descrito previamente (sección I.3).

6. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1.

Incubar la microplaca protegida de la luz a **temperatura ambiente (18-24°C) durante 20 minutos**.

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. La adición de la solución de parada cambia el color del Control Negativo y las muestras negativas de azul a amarillo.

8. 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:

- Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.
- 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.
- El uso del calibrador (CAL), un control negativo bajo, no es obligatorio para el ensayo ya que el calibrador (CAL) no afecta al cálculo del valor de corte. El calibrador (CAL) puede usarse como un control negativo bajo si la gestión requiere un control interno de calidad del laboratorio. Dispensar 100µl del calibrador (CAL), posiblemente por duplicado, cuando se utilice para este propósito.

N. ESQUEMA DEL ENSAYO.

Controles/Calibrador	100 µl
Muestras	100 µl
1ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Mezcla TMB/H2O2	100 µl
3ª 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 (incluido el calibrador (CAL):

		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 negativo y positivo cada vez que se usa el equipo, y con el calibrador la primera vez que se usa el equipo, para verificar si los valores DO450nm o Co/M son los esperados.

Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	valor < 0.100 DO450nm
Control Negativo (CN)	> 1.000 DO450nm después de leer el blanco Si es menor, controle cuidadosamente el proceso de lavado y disminuya los ciclos o el tiempo entre los mismos. Coeficiente de variación < 30%
Control Positivo (CP)	DO450 nm < CN/10
Calibrador (CAL)	CP ≤ DO450nm < (CN+CP)/5

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.
Control	1. el proceso de lavado y los parámetros

Negativo (CN) < 1.000 DO450nm después de leer el blanco Coeficiente de variación > 20%	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 DO450nm Fuera de rango	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 DO450nm > CN/10	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, informe al responsable para tomar las medidas pertinentes.

Nota importante:

El análisis debe seguir el paso de lectura descrito en la sección M, punto 8.

P. RESULTADOS.

Los resultados se calculan por medio de un valor de corte (cut-off) hallado con la siguiente fórmula:

$$\text{Valor de corte} = (\text{CN} + \text{CP}) / 5$$

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 las DO a 450nm / 620-630nm de las muestras y el Valor de corte 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 no está infectado por HDV.

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 HDV y por consiguiente el paciente debe ser tratado adecuadamente.

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 se incluye un ejemplo de los cálculos (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 8).

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

Control Negativo: 2.100 – 2.200 – 2.000 DO450nm

Valor medio: 2.100 DO450nm

Mayor de 1.000 – Válido

Control Positivo: 0.100 DO450nm

Menor de CN/10 – Válido

$$\text{Valor de corte} = (2.100 + 0.100) / 5 = 0.440$$

Calibrador: 0.300-0.260 DO450nm

Valor medio: 0.280 DO450nm

Dentro del rango $CP \leq DO450nm < (CN+CP)/5$ – Válido

Muestra 1: 0.020 DO450nm

Muestra 2: 1.900 DO450nm

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.

En ausencia de un estándar internacional, la sensibilidad del ensayo ha sido calculada por medio de un producto denominado Accurun n° 127 suministrado por Boston Biomedical Inc., Estados Unidos.

La siguiente tabla muestra los valores de DO450nm para esta preparación, diluido en suero bovino fetal (SFB), para construir la curva de dilución límite en tres lotes diferentes:

Valores Co/M						
	DAB.CE	Lote #	DAB.CE	Lote #	DAB.CE	Lote #
	DO450	Co/M	DO450	Co/M	DO450	Co/M
Accurun	nm	valor	nm	valor	nm	valor
# 127						
1x	0.171	3.0	0.163	2.9	0.156	2.8
2x	0.187	2.7	0.176	2.6	0.179	2.5
4x	0.230	2.2	0.220	2.1	0.202	2.2
8x	0.298	1.7	0.285	1.6	0.271	1.6
16x	0.417	1.2	0.405	1.1	0.402	1.1
32x	0.514	1.0	0.490	0.9	0.482	0.9
64x	0.717	0.7	0.700	0.7	0.705	0.6
128x	1.063	0.5	1.006	0.5	1.015	0.4
CTRL (-)	2.484	////////	2.261	////////	2.114	////////

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICA.

La evaluación del procedimiento diagnóstica se realizó mediante un ensayo con más de 400 muestras frente a un equipo de referencia. Este ensayo clínico fue conducido por el Prof. M. Rizzetto, Departamento de Gastro-Hepatología del hospital S. Giovanni Battista de Turín, Italia.

Se examinaron muestras negativas, positivas y otras que pudieran provocar interferencia.

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.

A continuación se muestran brevemente los resultados obtenidos:

Sensibilidad	> 98 %
Especificidad	> 98 %

3. PRECISIÓN.

Se realizó un estudio con 3 lotes y dos muestras de diferente reactividad anti-HDV, examinadas en 16 réplicas, en tres tandas separadas. Los valores medios obtenidos se reportan a continuación:

DAB.CE: lote #1102

Control Negativo (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	2.342	2.428	2.433	2.401
Desviación estándar	0.113	0.106	0.122	0.114
CV %	4.8	4.4	5.0	4.7

Calibrador (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	0.298	0.289	0.286	0.291
Desviación estándar	0.023	0.027	0.026	0.025
CV %	7.7	9.3	9.1	8.7
Co/M	1.6	1.7	1.7	1.7

DAB.CE: lote #0103

Control Negativo (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	2.208	2.237	2.246	2.230
Desviación estándar	0.105	0.108	0.108	0.107
CV %	4.7	4.8	4.8	4.8

Calibrador (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	0.269	0.277	0.266	0.271
Desviación estándar	0.026	0.024	0.025	0.025
CV %	9.8	8.5	9.5	9.3
Co/M	1.7	1.7	1.7	1.7

DAB.CE: lote # 0403

Control Negativo (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	2.246	2.221	2.182	2.216
Desviación estándar	0.097	0.103	0.118	0.106
CV %	4.3	4.6	5.4	4.8

Calibrador (N = 16)

Valores medios	1ª tanda	2ª tanda	3ª tanda	Valor Promedio
DO 450nm	0.286	0.273	0.280	0.280
Desviación estándar	0.027	0.023	0.026	0.025
CV %	9.3	8.5	9.1	9.0
Co/M	1.6	1.7	1.6	1.6

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 8.

S. LIMITACIONES.

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 (Mi) – Italia



0318

HP IgA

**Enzyme ImmunoAssay (ELISA) for
the quantitative/qualitative
determination of IgA antibodies to
Helicobacter pylori
in human serum and plasma**

- for “in vitro” diagnostic use only -



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HP IgA

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgA antibodies to *Helicobacter pylori* in human plasma and sera. The product is intended for the follow-up of patients showing gastrointestinal pathologies referable to *H.pylori* infection.

For "in vitro" diagnostic use only.

B. INTRODUCTION

Helicobacter pylori (HP) is a Gram negative bacterium, firstly isolated in gastric mucosa by Marshall and Warren in 1983.

Hp has been recognized to be the agent responsible of most of cases of gastric mucosal damage and to play a role in the evolution of gastric diseases to carcinoma.

Hp causes an immunological response during infection and specific antibodies of the different classes of IgG, IgA and IgM are produced by the patient.

ELISA are currently used to screen patients affected by gastritis or peptic ulcers for acute active infection due to some *Helicobacter pylori* virulent strains.

In particular the presence of IgA and IgM antibodies is reported to be correlated to the acute phase of illness, while IgG antibodies become present at different titers shortly after primary infections and last in blood for many years.

Quantitative ELISA are also used in the follow-up of patients undergoing antibiotic therapy, useful in monitoring IgG titer variations during and after the pharmaceutical treatment

C. PRINCIPLE OF THE TEST

Microplates are coated with *H.pylori* immunodominant antigens derived from tissue culture of a virulent strain.

In the 1st incubation, the solid phase is treated with diluted samples and anti-HP IgA are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound anti-HP IgA are detected by the addition of anti hIgA antibody, labeled 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-HP IgA antibodies present in the sample.

IgA in the sample may therefore be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (arbU/ml) as no international standard is available.

Neutralization of IgG anti-HP, carried out directly in the well, is performed in the assay in order to block interferences due to this class in the determination of IgA.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE

12 strips x 8 microwells coated with HP specific immunodominant antigens derived from tissue culture of a virulent strain. 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 4°C.

2. Calibration Curve: CAL N° ...

Ready to use and color coded standard curve ranging:

4 ml CAL1 = 0 arbU/ml

4 ml CAL2 = 5 arbU/ml

2 ml CAL3 = 10 arbU/ml

2 ml CAL4 = 20 arbU/ml

2 ml CAL 5 = 50 arbU/ml

4 ml CAL6 = 100 arbU/ml.

Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.

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 color coded.

3. Control Serum: CONTROL ...ml

1 vial. Lyophilized. It contains fetal bovine serum proteins, human IgA antibodies to HP at about 20 arbU/ml±20%, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

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

1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgA, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

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 H₂O₂).

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 H₂SO₄ 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. Neutralizing Reagent: SOLN NEUT

1x8ml/vial. Ready-to-use Reagent. It contains goat anti hIgG, 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.

10. Plate sealing foils n°2

11. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled 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-borne 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 freezed/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

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storage.

In this case call Dia.Pro's customer service.

Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°..8°C. When opened the first time, residual strips are stable till the indicator of humidity 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.

Neutralizing Reagent

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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 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 Control Serum as reported.
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:

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 50 µl of the Neutralizing Reagent (SOLN NTR) in all the wells of the samples. Do not add it in the wells used for the Calibrators and the Control Serum !

Important note: The Neutralizing Reagent is able to block false positive reactions due to RF. Positive samples in internal QC panels might be detected negative if such samples were tested positive with an IVD that does not carry out any RF blocking reaction.

4. Then dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
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 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.

8. Incubate the microplate for **60 min at +37°C**.
9. Wash microwells as in step 5.
10. 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.

11. 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.
12. 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:

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 50 µl of the Neutralizing Reagent (SOLN NTR) in all the wells of the samples. Do not add it in the wells used for the Calibrators !

Important note: The Neutralizing Reagent is able to block false positive reactions due to RF. Positive samples in internal QC panels might be detected negative if such samples were tested positive with an IVD that does not carry out any RF blocking reaction.

4. 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.
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 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.

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 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
12. 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
Neutralizing Reagent (only for samples)	50 µl
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/H ₂ O ₂	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

(*) Important Notes:

- The Control Serum (CS) 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	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 = 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	S3	S11									
B	CAL1	S4	S12									
C	CAL1	S5	S13									
D	CAL2	S6	S14									
E	CAL2	S7	S15									
F	CAL6	S8	S16									
G	S1	S9	S17									
H	S2	S10	S18									

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:

Check	Requirements
Blank well	< 0.100 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 > 0.100 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 (e.g.: 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 CAL4 ± 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 has occurred.

Anyway, if all other parameters (Blank, CAL1, CAL2, CAL 6), match the established requirements, the test may be considered valid.

Important note:

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

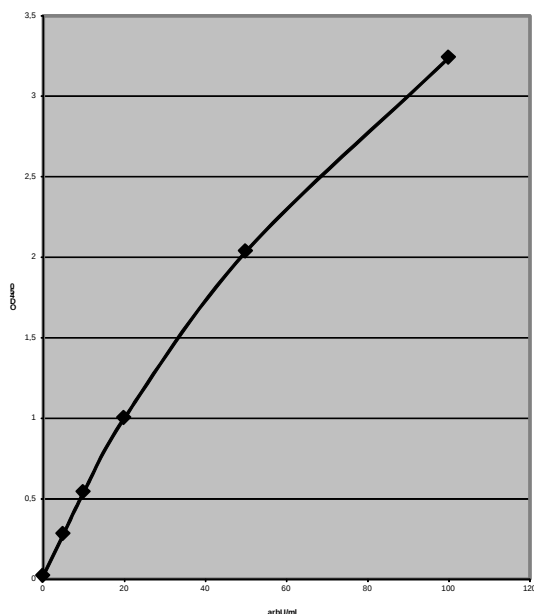
P. RESULTS

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 H.pylori IgA antibody in samples.

An example of Calibration curve is reported below.



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 (data obtained proceeding as the the reading step described in the section M, point 12):

Note: The following data must not be used instead of 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: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 100 arbU/ml: 2.045 OD450nm
Higher than 1.000 – Accepted

The OD450nm/620-630nm of the Calibrator 5 arbU/ml is considered the cut-off (or Co) of the system.

The ratio between the OD450nm/620-630nm value of the sample and the OD450nm/620-630nm of the Calibrator 5 arbU/ml (or S/Co) can provide a semi-quantitative estimation of the content of specific IgG in the sample.

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 5 arbU/ml are considered negative for anti H.pylori IgA antibody.

Samples with a concentration higher than 5 arbU/ml are considered positive for anti H.pylori IgA antibody.

Important notes:

1. *H.pylori IgA results alone are not enough to provide a clear diagnosis of Helicobacter pylori infection. Other tests for Helicobacter pylori (supplied by Dia.Pro Diagnostic BioProbes s.r.l. at code n° HPAG.CE, HPG.CE and HPM.CE), should be carried out.*
2. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted on panels of positive and negative samples in an external clinical laboratory with reference to a FDA approved reference kit.

1. Limit of detection

No international standard for HP IgA Antibody detection has been defined so far by the European Community.

In its absence, an Internal Gold Standard (or IGS), derived from a patient with an history of past HP infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:

The diagnostic performances were evaluated in a performance evaluation study conducted in an external center, with excellent experience in the diagnosis of infectious diseases.

The **diagnostic sensitivity** was studied in an external study on more than 50 samples, pre-tested positive with the reference kit of European origin in use at the laboratory. Positive samples were collected from patients with a clinical history of H.pylori infection.

The **diagnostic specificity** was determined in an external study on panels of more than 100 negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.

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.

In addition 35 cross-reacting specimens were studied internally to verify absence of interference on the assay results.

No interference was observed (100% specificity).

Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.

The external Performance Evaluation provided the following values :

Sensitivity	> 98 %
Specificity	> 98 %

3. Reproducibility:

A study conducted on three samples of different HP IgA reactivity, examined in 16 replicates in three separate runs has

shown CV% values ranging 7-18% depending on the OD450nm/620-630nm readings.
The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS

False positivity has been assessed as less than 2% of the normal population.
Frozen samples containing fibrin particles or aggregates may generate false positive results.

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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



HP IgA

**Ensayo Inmunoenzimático (ELISA) para
la determinación cuantitativa/cualitativa
de anticuerpos IgA frente a
Helicobacter pylori
en suero y plasma humano**

Uso exclusivo para diagnóstico *in vitro*



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HP IgA

A. OBJETIVO DEL ESTUCHE

Ensayo inmunoenzimático (ELISA) para la determinación cuantitativa/cualitativa de anticuerpos IgA frente a *Helicobacter pylori* en suero y plasma humano. El producto ha sido diseñado para el estudio de pacientes que muestran patología gastrointestinal producida por infección de *H. pylori*.

Uso exclusivo para diagnóstico *in vitro*.

B. INTRODUCCIÓN

Helicobacter pylori (HP) es una bacteria Gram negative que fue aislada en mucosa gástrica por Marshall y Warren en 1983.

Hp ha sido descrita como el agente causante de la mayoría de casos de daño en la mucosa gástrica, jugando un papel importante en la evolución de enfermedad gástrica a carcinoma. La infección por HP produce una respuesta inmune del paciente con la producción de anticuerpos específicos IgA, IgA e IgM.

Ensayos inmunoenzimáticos son usados en la determinación de pacientes afectados con gastritis o úlceras pépticas producidas por infecciones agudas de cepas virulentas de *Helicobacter pylori*.

Se ha descrito que la presencia de anticuerpos IgA e IgM están correlacionados con fases agudas de la enfermedad, mientras que la presencia de anticuerpos IgG se encuentran a diferentes niveles poco tiempo después de la primera infección, manteniéndose en sangre durante algunos años.

ELISA cuantitativos también son usados en el estudio de pacientes con tratamiento de antibióticos, muy útil en el seguimiento de niveles de IgG durante y después del tratamiento farmacéutico.

C. PRINCIPIO DEL ENSAYO

Las microplacas están recubiertas con antígenos inmunodominantes de *H. pylori* procedentes de cultivos titulares de una cepa virulenta.

En la 1ª incubación, la fase sólida es tratada con muestras diluidas y los anticuerpos anti-HP IgA quedan unidos a los antígenos de la fase sólida.

Después de lavar, los componentes de la muestra que no se hayan unido son eliminados. En la 2ª incubación, los anticuerpos anti-HP IgA unidos son detectados por la adición de anticuerpos anti-IgA humana marcados con peroxidasa (HRP).

La enzima queda capturada en la fase sólida y actúa sobre el cromógeno/substrato, la actividad de la enzima genera una señal óptica proporcional a la cantidad de anticuerpo anti-HP IgA presente en la muestra.

La cantidad de IgA presente en la muestra es cuantificada usando una curva estándar calibrada en unidades arbitrarias por mililitro (Uarb/ml).

La neutralización de anticuerpos anti-HP IgG es necesaria para eliminar interferencias en la determinación de IgA. La neutralización se realiza directamente en los pocillos.

D. COMPONENTES

Cada estuche posee los reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE

12 tiras x 8 pocillos unidos a antígenos inmunodominantes de HP procedentes de cultivos en tejidos de una cepa virulenta de HP. Las placas están selladas en bolsas que contienen desecantes.

Permitir que las microplacas alcancen la temperatura ambiente antes de abrir; sellar las tiras no usadas en la bolsa con desecantes y conservar a 4°C

2. Curva de Calibración: CAL N° ...

Listo para el uso y codificados con color estándar rangos de curva:

4 ml CAL1 = 0 arbU/ml
4 ml CAL2 = 5 arbU/ml
2 ml CAL3 = 10 arbU/ml
2 ml CAL4 = 20 arbU/ml
2 ml CAL 5 = 50 arbU/ml
4 ml CAL6 = 100 arbU/ml.

Los estándares están calibrados según un interno Gold Standard or IGS ya que no se ha definido uno internacional.

Contiene proteínas de suero humano, 2% caseína, 10mM tampón citrato sódico pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Azida sódica y 0.045% ProClin 300 como conservantes. Los estándares están codificados con color azul.

3. Suero Control: CONTROL ...ml

1 vial. Liofilizado.

Contiene proteínas de suero bovino fetal, anticuerpos IgA humanos frente a HP a 20 Uarb/ml±20%, 0.3 mg/ml sulfato de gentamicina y 0.045% ProClin 300 como conservantes.

4. Tampón de lavado concentrado: WASHBUF 20X

1x60ml/botella 20x solución concentrada.

Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM pH 7.0±0.2, 0.05% Tween 20 y 0.045% ProClin 300.

5. Enzima conjugada: CONJ

1x16ml/vial. Listo para usar y codificada con el color rojo. Contiene anticuerpos policlonales que reconocen IgA humana y están marcados con peroxidasa de rábano, 5% BSA, 10 mM de tampón Tris pH 6.8±0.1, 0.045% ProClin 300 y 0.02% de sulfato de gentamicina como conservantes.

6. Cromógeno/Substrato: SUBS TMB

1x16ml/vial.

Contiene 50 mM tampon citrato-fostato pH 3.5-3.8, 4% dimetilsulfóxido, 0.03% tetra-methyl-benzidina (TMB) y 0.02% peróxido de hidrógeno (H₂O₂).

Nota: Evitar la exposición a la luz, es fotosensible.

7. Ácido sulfúrico: H₂SO₄ 0.3 M

1x15ml/vial, contiene solución 0.3 M H₂SO₄.

Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Diluyente de muestras: DILSPE

2x60ml/vial. Contiene 2% caseína, 10 mM tampon citrato sódico pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% azida sódica y 0.045% ProClin 300 como conservantes. Usar para diluir las muestras.

9. Reactivo Neutralizante: SOLN NEUT

1x8ml/vial. Listo para usar. Contiene anticuerpos de cabra anti IgG humana, 2% caseína, 10 mM tampon citrate sódico pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% azida sódica y 0.045% ProClin 300 como conservantes.

10. Sellador adhesivo n°2

11. Libro de instrucciones n°1

E. MATERIALES NECESARIOS PERO NO SUMINISTRADOS

1. Micropipetas calibradas (1000ul, 100 y 10ul) y puntas de plástico desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para eliminar agentes químicos oxidantes usados como desinfectantes).
3. Reloj con un rango de 60 minutos o más.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA (seco o húmedo) capaz de alcanzar una temperatura de 37°C (+/- 0.5°C).
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de filtros de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Agitador 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 entrenado 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 cromógeno/ substrato a la luz y también las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el estuche a temperaturas entre 2-8 °C, 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 son transparentes y no contienen precipitados ni agregados en el momento del uso. En caso contrario, informar al supervisor del laboratorio para realizar el procedimiento pertinente y reemplazar el estuche.
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 estuche 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 estuche e internamente en los reactivos. Un estudio dirigido a comprobar la actividad de estuches abiertos, no mostró pérdida de actividad en estuches usados hasta 6 veces en un periodo de 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 antes de tirar. 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. Cuando el estuche se emplea para el pesquiasaje 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, 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 COMPONENTES Y PRECAUCIONES

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 2-8°C. 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 calibración:

Componentes listos para usar. Mezclar cuidadosamente con agitador antes de usar.

Suero control:

Añadir el volumen de agua de ELISA, indicada en la marca, dejar disolver y mezclar con agitador.

Nota: El control después de la disolución no es estable. Almacenar a -20°C en alícuotas.

Tampón de lavado concentrado:

Todo el contenido del tampón concentrado 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 8°C.

Enzima conjugada:

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.

Cromogeno/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.

Diluyente de muestras:

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

Reactivo Neutralizante:

Listo 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 HERRAMIENTAS USADAS 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 (alcohol, lejía 10%, desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periódicamente los residuos de los componentes del estuche.
2. El incubador de ELISA debe ser ajustada a 37°C (+/- 0.5°C) 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 (620-630nm, obligatorio) para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda $\leq 10\text{nm}$ b) Rango de absorbancia de 0 a ≥ 2.0 , c) Linealidad ≥ 2.0 , reproducibilidad $\geq 1\%$. 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 la sección "Control interno de calidad". 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 de ELISA cuando el número de muestras para analizar 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. CONTROLES Y OPERACIONES PREVIAS 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.
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 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.
5. Disolver totalmente el contenido del Suero Control, como se ha descrito anteriormente.
6. Diluir totalmente el tampón de lavado concentrada 20X, como se ha descrito anteriormente.
7. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
8. Ajustar la incubadora de ELISA a 37°C 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.
9. Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
10. En caso de trabajar automáticamente, conectar 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 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.

El estuche puede ser usado para determinaciones cuantitativas y cualitativas.

M1. DETERMINACIÓN CUANTITATIVA:

1. Diluir las muestras 1:101 dentro de un apropiado tubo (ejemplo: 1000 µl Diluyente de muestras + 10 µl muestra). No diluir el grupo de Calibración ya que están listos para usarse. Mezclar todos los reactivos líquidos en un agitador y continua como se describe a continuación.
2. Poner el número de tiras necesarias en el soporte de plástico. Dejar el primer pocillo A1 y B1 vacío para el blanco.
3. Dispensar 50 µl del Reactivo Neutralizante (SOLN NTR) en todos los pocillos de las muestras. ¡No añadirlo dentro de los pocillos usados para los Calibradores y el Suero Control!

Nota importante: El reactivo neutralizante puede bloquear falsas reacciones positivas debido a RF. Las muestras positivas en paneles de control de calidad internos podrían ser detectadas como negativas si estas muestras se analizaron como positivas con un IVD que no realiza ninguna reacción de bloqueo de RF.

4. Dispensar 100 µl de Calibrador y 100 µl de Suero Control por duplicado. Una vez hecho esto, añadir 100 µl de las muestras diluidas en cada uno de los pocillos marcados específicamente.
5. Incubar la microplaca durante **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 con el lavador automático dispensando según se indica (sección I.3).
7. Dispensar 100 µL de la Enzima Conjugada en todos los pocillos, excepto en el A1 y B1, 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 conjugado. Podría producirse contaminación.

8. Incubar la microplaca **60 min a +37°C.**
9. Lavar los pocillos como en el paso 6.
10. Dispensar 100 µl del Cromógeno/Substrato en todos los pocillos, incluido el A1 y B1. 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 100 µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición de la solución de parada cambia el color del calibrador positivo, el suero control y las muestras positivas 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 o B1 o ambos (blanco).

M2. DETERMINACIÓN CUALITATIVA

Para realizar una determinación cualitativa, proceder como se describe a continuación:

1. Diluir las muestras 1:101 dentro de un apropiado tubo (ejemplo: 1000 µl Diluyente de muestras + 10 µl muestra). No diluir el Set Calibrador ya que están listos para usarse. Mezclar todos los reactivos líquidos en un agitador y continua como se describe a continuación.
2. Poner el número de tiras necesarias en el soporte de plástico. Dejar el primer pocillo (A1) vacío para el blanco.
3. Dispensar 50 µl del Reactivo Neutralizante (SOLN NTR) en todos los pocillos de las muestras. ¡No añadirlo dentro de los pocillos usados para los calibradores!

Nota importante: El reactivo neutralizante puede bloquear falsas reacciones positivas debido a RF. Las muestras positivas en paneles de control de calidad internos podrían ser detectadas como negativas si estas muestras se analizaron como positivas con un IVD que no realiza ninguna reacción de bloqueo de RF.

4. Dispensar 100 µl de Calibrador 0Uarb/ml y Calibrador 5Uarb/ml por duplicado y Calibrador 100Uarb/ml en un solo pocillo. Una vez hecho esto, añadir 100 µl de las muestras diluidas en cada uno de los pocillos marcados específicamente.
5. Incubar la microplaca durante **60min 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 con el lavador automático como se indicó previamente (Sección I.3).
7. Dispensar 100 µL de la Enzima Conjugada 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 conjugado. Podría producirse contaminación.

8. Incubar la microplaca durante **60 min a +37°C.**
9. Lavar los pocillos como en el paso 6.
10. Dispensar 100 µl del Cromógeno/Substrato en todos los pocillos, incluido el blanco. 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 100 µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición de la solución de parada cambia el color de los calibradores positivos, el suero control y las muestras positivas 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 ni polvo 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:

Método	Operaciones
Reactivo Neutralizante (sólo para muestras)	50 µl
Calibradores y Control(*)	100 µl
Muestras diluidas 1:101	100 µl
1ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Enzima conjugada	100 µl
2ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
TMB/H ₂ O ₂	100 µl
3ª incubación	20 min
Temperatura	18°-24°C
Ácido sulfúrico	100 ul
Lectura DO	450nm/620-630 nm

(*) Notas importantes:

- 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

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	CAL4	M1									
B	BL	CAL4	M2									
C	CAL1	CAL5	M3									
D	CAL1	CAL5	M4									
E	CAL2	CAL6	M5									
F	CAL2	CAL6	M6									
G	CAL3	SC(*)	M7									
H	CAL3	SC(*)	M8									

Leyenda: BL= Blanco CAL= Calibrador
SC(*)= Suero Control - No obligatorio M= Muestra

A continuación se describe un ejemplo del esquema en un ensayo cualitativo:

Microplaca												
	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M3	M11									
B	CAL1	M4	M12									
C	CAL1	M5	M13									
D	CAL2	M6	M14									
E	CAL2	M7	M15									
F	CAL6	M8	M16									
G	M1	M9	M17									
H	M2	M10	M18									

Leyenda: BL= Blanco CAL = Calibradores
M= Muestra

O. CONTROL INTERNO DE CALIDAD

Se realiza un grupo de pruebas con los controles cada vez que se usa el estuche para verificar si los valores DO 450nm/620-630nm son los esperados.

Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	Valor < 0.100 DO 450nm
CAL 1 0 Uarb/ml	Media valor < 0.150 DO 450nm después del blanco Coeficiente de variación < 30%
CAL 2 5 Uarb/ml	DO 450nm > DO 450nm CAL1 + 0.100
CAL 6 100 Uarb/ml	DO 450nm > 1.000

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 DO 450nm	1. La solución Cromogeno/Substrato no se ha contaminado durante el ensayo
CAL 1 0 Uarb/ml > 0.150 DO 450nm después del 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 (dispensar el calibrador positivo en lugar del negativo). 4. no ha existido contaminación del calibrador 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.
CAL 2 5 Uarb/ml DO 450nm < DO 450nm CAL1 + 0.100	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en la distribución (ej. dispensar un calibrador incorrecto). 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.
CAL 6 100 Uarb/ml < 1.000 DO 450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en la distribución (ej. dispensar un calibrador incorrecto). 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 la comprobación, informe al supervisor para tomar las medidas pertinentes.

** Notas

Si se ha usado suero de control, comprobar los siguientes datos:

Parámetro	Exigencia
Suero Control	valor medio de DO450nm CAL4 +/-20%

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

Problema	Compruebe que
Suero Control	1. el procedimiento ha sido realizado correctamente.
Diferente de los valores esperados	2. no se han cometido errores en la distribución (ej. dispensar un calibrador incorrecto).
	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.

En cualquier caso, si todos los demás parámetros (blanco, CAL1, CAL2, CAL6) se corresponden con los requisitos establecidos, la prueba puede considerarse válida.

Nota importante:

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

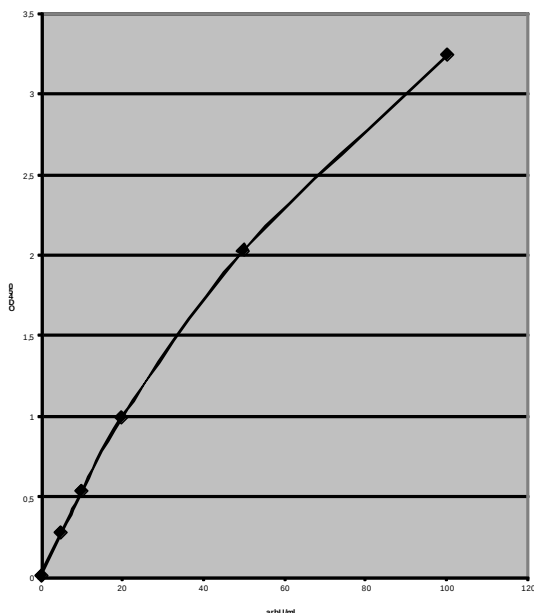
P. RESULTADOS

P.1 Método Cuantitativo

Para la determinación cuantitativa se hace necesario usar un programa adecuado para dibujar una curva de calibrado con los valores obtenidos de la lectura a 450nm/620-630nm.

La concentración de anticuerpos anti-*H. Pylori* presente en las muestras se calculará utilizando la curva de calibración.

Un ejemplo de Curva de Calibración se muestra a continuación:



Nota Importante: No usar esta curva de calibración para hacer los cálculos.

P.2 Método Cualitativo

En el método cualitativo, calcula la media de los valores DO 450nm/620-630nm del calibrador 0 y 5 Uarb/ml y comprueba que el ensayo es válido.

Ejemplo de cálculos (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12).

Nota: Los siguientes datos no deben ser usados en lugar de los datos reales obtenidos.

Calibrador 0 Uarb/ml: 0.020 – 0.024 DO 450nm
Media Valor: 0.022 DO 450nm
Menor que 0.150 – Aceptado

Calibrador 5 Uarb/ml: 0.250 – 0.270 DO 450nm
Media Valor: 0.260 DO 450nm
Mayor que Cal 0 + 0.100 – Aceptado

Calibrador 100 Uarb/ml: 2.045 DO 450nm
Mayor que 1.000 – Aceptado

La DO 450nm/620-630nm del Calibrador 5 Uarb/ml es considerado el valor del corte (cut-off o Co).

La relación entre el valor de DO 450nm/620-630nm de la muestra y la DO450nm/620-630nm del Calibrador 5 Uarb/ml (o M/Co) puede dar información semi-cuantitativa de la cantidad de IgG de la muestra.

Q. INTERPRETACIÓN DE LOS RESULTADOS

Muestras con una concentración menor de 5 Uarb/ml son consideradas negativas para la presencia de anticuerpos anti-*H.pylori* IgA.-4

Muestras con una concentración mayor de 5 Uarb/ml son consideradas positivas para la presencia de anticuerpos anti-*H.pylori* IgA.

Notas importantes:

1. La presencia de anticuerpos anti-*H.pylori* IgA no son suficientes para diagnosticar infección por *Helicobacter pylori*. Otros estudios para for *Helicobacter pylori* (suministrados por Dia.Pro Diagnostic BioProbes s.r.l. con código n° HPAG.CE, HPG.CE y HPM.CE), pueden ser realizados.
2. 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.
3. Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
4. El diagnóstico debe ser evaluado y comunicado al paciente por un médico calificado.

R. REALIZACIONES CARACTERÍSTICAS

La evaluación de las realizaciones deben ser dirigidas sobre paneles de muestras positivas y negativas en un laboratorio clínico externos con referencia a FDA.

1. Límite de detección

La comunidad Europea no ha definido estándares internacionales para la detección de anticuerpos anti-HP IgA.

En su ausencia, un Internal Gold Standard (o IGS), derivado de pacientes con historia de infección por HP, ha sido definido con la finalidad de proporcionar un procedimiento con alta sensibilidad.

2. Especificidad y Sensibilidad Diagnósticas:

Las realizaciones diagnósticas fueron evaluadas en un centro externo de amplia experiencia en el diagnóstico de enfermedades infecciosas.

La **sensibilidad diagnóstica** fue estudiada en un estudio externo en más de 50 muestras, pre-probadas como positivas con la referencia de kits de origen Europeo utilizados en los laboratorios. Muestras positivas fueron tomadas de pacientes con historial clínico de infección por *H. pylori*.

La especificidad diagnóstica fue determinada en un estudio externo en paneles de más de 100 muestras negativas de individuos normales y donantes de sangre, clasificados como negativos con la referencia del kit, incluidas las muestras potencialmente interferentes.

Tanto el plasma, derivado con diferentes técnicas de preparación estándar (citrato, EDTA y heparina) y los sueros se han utilizado para determinar la especificidad.

No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

Además fueron estudiadas 35 muestras de reacciones cruzadas para verificar la ausencia de interferencias en el ensayo de los resultados. No se observó interferencia (100% de especificidad).

También se han analizado muestras congeladas, para verificar si las muestras congeladas interfieren con la realización de la prueba. No se observaron interferencias en el lavado y en las partículas de muestras gratuitas.

La Evaluación externa nos ofreció los siguientes valores:

Sensibilidad	> 98 %
Especificidad	> 98 %

3. Reproducibilidad:

Un estudio realizado en tres muestras de diferentes anticuerpos anti-HP IgA reactividad, examinado en 16 replicaciones in 3 tiras separadas ha mostrado valores CV% entre 7-18% dependiendo de la lectura de la DO 450nm/620-630nm.

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

S. LIMITACIONES

Falsos positivos han sido estudiados con menos del 2% de la población normal.

Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.

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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 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 Giovanni
(Milán) – Italia



HP IgG

**Enzyme ImmunoAssay (ELISA)
for the quantitative/qualitative
determination of IgG antibodies to
Helicobacter pylori
in human serum and plasma**

- for “in vitro” diagnostic use only -



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HP IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to *Helicobacter pylori* in human plasma and sera. The product is intended for the follow-up of patients showing gastrointestinal pathologies potentially correlated to HP infection.

For "in vitro" diagnostic use only.

B. INTRODUCTION

Helicobacter pylori (HP) is a Gram negative bacterium, firstly isolated in gastric mucosa by Marshall and Warren in 1983.

HP has been recognized to be the agent responsible of most of cases of gastric mucosal damage and to play a role in the evolution of gastric diseases to carcinoma.

HP causes an immunological response during infection and specific antibodies of the different classes of IgG, IgA and IgM are produced by the patient.

ELISA are currently used to screen patients affected by gastritis or peptic ulcers for acute active infection due to some *Helicobacter pylori* virulent strains.

In particular the presence of IgA and IgM antibodies is reported to be correlated to the acute phase of illness, while IgG antibodies become present at different titers shortly after primary infections and last in blood for many years.

Quantitative ELISA are also used in the follow-up of patients undergoing antibiotic therapy, useful in monitoring IgG titer variations during and after the pharmaceutical treatment

C. PRINCIPLE OF THE TEST

Microplates are coated with H.pylori immunodominant antigens derived from tissue culture of a virulent strain.

In the 1st incubation, the solid phase is treated with diluted samples and anti-HP IgG are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound anti-HP IgG are detected by the addition of anti hlgG antibody, labeled 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-HP IgG antibodies present in the sample.

IgG in the sample may therefore be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (arbU/ml) as no international standard is available.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE

12 strips x 8 microwells coated with HP specific immunodominant antigens derived from tissue culture of a virulent strain. 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 4°C.

2. Calibration Curve: CAL N° ...

Ready to use and color coded standard curve ranging:

4 ml CAL1 = 0 arbU/ml
4 ml CAL2 = 5 arbU/ml
2 ml CAL3 = 10 arbU/ml
2 ml CAL4 = 20 arbU/ml
2 ml CAL 5 = 50 arbU/ml
4 ml CAL6 = 100 arbU/ml.

Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.

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 HP at about 20 arbU/ml±20%, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

4. 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.

5. Enzyme conjugate : CONJ

1x16ml/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 and 0.02% gentamicine sulphate as preservatives.

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 H₂O₂).

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

7. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial contains 0.3 M H₂SO₄ 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, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled 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, 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-borne 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

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of conservation.

In this case call Dia.Pro's customer service.

Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°..8°C. When opened the first time, residual strips are stable till the indicator of humidity 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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 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 Control Serum as reported.
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:

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.

- Wash the microplate with an automatic as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 5.
- 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.

- 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.
- 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), blanking the instrument on A1 or B1 or both (mandatory).

M2. QUALITATIVE DETERMINATION

If only a qualitative determination is required, proceed as described below:

- 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.
- Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
- 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.
- 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.

- Wash the microplate with an automatic washer as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 5.
- 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.

- 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.
- 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), blanking the instrument on A1 or B1 or both (mandatory).

General Important notes:

- Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
- 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/H ₂ O ₂	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
S = Sample
CAL = Calibrators

O. INTERNAL QUALITY CONTROL

A validation 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
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 > 0.100 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 CAL4 +/-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 has occurred.

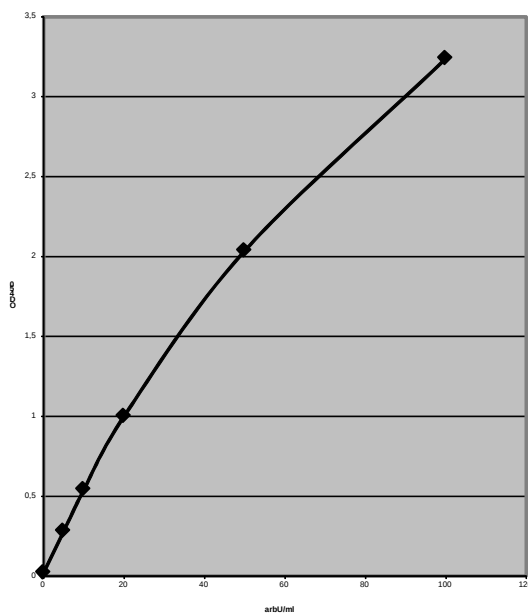
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL 6), match the established requirements, the test may be considered valid.

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 H.pylori IgG antibody in samples.

An example of Calibration curve is reported below.



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:

Note: The following data must not be used instead of 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: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 100 arbU/ml: 2.045 OD450nm
Higher than 1.000 – Accepted

The OD450nm of the Calibrator 5 arbU/ml is considered the cut-off (or Co) of the system.

The ratio between the OD450nm value of the sample and the OD450nm of the Calibrator 5 arbU/ml (or S/Co) can provide a semi-quantitative estimation of the content of specific IgG in the sample.

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 5 arbU/ml are considered negative for anti H.pylori IgG antibody. Samples with a concentration higher than 5 arbU/ml are considered positive for anti H.pylori IgG antibody.

Important notes:

1. H.pylori IgG results alone are not enough to provide a clear diagnosis of Helicobacter pylori infection. Other tests for Helicobacter pylori (supplied by Dia.Pro Diagnostic BioProbes s.r.l. at code n° HPAG.CE, HPA.CE and HPM.CE), should be carried out.

2. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.
3. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
4. Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted on panels of positive and negative samples in an external clinical laboratory with reference to a FDA approved reference kit.

1. Limit of detection

No international standard for HP IgG Antibody detection has been defined so far by the European Community. In its absence, an Internal Gold Standard (or IGS), derived from a patient with an history of past mononucleosis infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:

The diagnostic performances were evaluated in a performance evaluation study conducted in an external center, with excellent experience in the diagnosis of infectious diseases.

The diagnostic sensitivity was studied on more than 50 samples, pre-tested positive with the reference kit of European origin in use at the laboratory. Positive samples were collected from patients with a clinical history of H.pylori infection.

The diagnostic specificity was determined on panels of more than 100 negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.

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 samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.

The Performance Evaluation provided the following values :

Sensitivity	> 98 %
Specificity	> 98 %

3. Reproducibility:

A study conducted on three samples of different HP IgG reactivity, examined in 16 replicates in three separate runs has shown CV% values ranging 2-18% depending on the OD450nm readings.

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

S. LIMITATIONS

False positivity has been assessed as less than 2% of the normal population.

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

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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



HP IgM

Enzyme ImmunoAssay (ELISA) for
the determination of IgM antibodies
to *Helicobacter pylori*
in human serum and plasma

- for “in vitro” diagnostic use only -



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HP IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to *Helicobacter pylori* in human plasma and sera. For "in vitro" diagnostic use only.

B. INTRODUCTION

Helicobacter pylori (HP) is a Gram negative bacterium, firstly isolated in gastric mucosa by Marshall and Warren in 1983. Hp has been recognized to be the agent responsible of most of cases of gastric mucosal damage and to play a role in the evolution of gastric diseases to carcinoma.

Hp causes an immunological response during infection and specific antibodies of the different classes of IgG, IgA and IgM are produced by the patient.

ELISA are currently used to screen patients affected by gastritis or peptic ulcers for acute active infection due to some *Helicobacter pylori* virulent strains.

In particular the presence of IgA and IgM antibodies is reported to be correlated to the acute phase of illness, while IgG antibodies become present at different titers shortly after primary infections and last in blood for many years.

Quantitative ELISA are also used in the follow-up of patients undergoing antibiotic therapy, useful in monitoring IgG titer variations during and after the pharmaceutical treatment

C. PRINCIPLE OF THE TEST

Microplates are coated with *H.pylori* immunodominant antigens derived from tissue culture of a virulent strain.

In the 1st incubation, the solid phase is treated with diluted samples and anti-HP IgM are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound anti-HP IgM are detected by the addition of anti hlgM antibody, labeled 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-HP IgM antibodies present in the sample.

The presence of IgM in the sample may therefore be determined by means of a cut-off value able to discriminate between negative and positive samples.

Neutralization of IgG anti-HP, carried out directly in the well, is performed in the assay in order to block interferences due to this class of antibodies in the determination of IgM.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE

12 strips x 8 microwells coated with HP specific immunodominant antigens derived from tissue culture of a virulent strain. 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 4°C.

2. Negative Control: CONTROL -

1x4.0 ml/vial. Ready to use. It contains, human IgM antibodies negative to HP, 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.

The Negative Control is pale yellow color coded.

3. Positive Control: CONTROL +

1x4.0 ml/vial. Ready to use. It contains high titer human IgM antibodies positive to HP, 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.

The Positive Control is green yellow color coded.

4. Calibrator: CAL ...

n° 1 vial. Lyophilized reagent to be dissolved with EIA grade water as reported in the label. It contains bovine serum proteins, low titer human IgM antibodies to HP, 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. 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.

6. Enzyme conjugate : CONJ

1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

7. 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 H₂O₂).

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

8. Sulphuric Acid: H2SO4 0.3 M

1x15ml/vialIt contains 0.3 M H₂SO₄ solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. 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.

10. Neutralizing Reagent: SOLN NEUT

1x8ml/vial. It contains goat anti hlgG, 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.

11. Plate sealing foils n°2

12. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled 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 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 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

Microplate:

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

Negative Control

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

Positive Control

Ready to use components. Mix carefully 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.

Note: *The dissolved calibrator is not stable. Store it 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.

Neutralizing Reagent

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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 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 "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 Calibrator as reported.
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.

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Controls/Calibrator as they 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 50 µl Neutralizing Reagent in all the wells, except A1 used for blanking operations and in the wells used for the Controls and the Calibrator.

Important note: The Neutralizing Reagent is able to block false positive reactions due to RF. Positive samples in internal QC panels might be detected negative if such samples were tested positive with an IVD that does not carry out any RF blocking reaction.

4. Dispense 100 µl of Negative Control in triplicate, 100 µl of Positive Control in single, 100 µl of Calibrator in duplicate and 100 µl of diluted samples in each properly identified well.
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 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.

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 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
12. 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
Neutralizing Reagent (only for samples)	50 µl
Calibrator(*) & Controls	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 µl
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 in the table 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
PC = Positive Control S = Sample
CAL(*) = Calibrator – Not Mandatory

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 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
Negative Control	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
Positive Control	OD450nm > 0.500

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
Negative Control > 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 control instead of the negative one); 4. that no contamination of the negative control 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.
Positive Control < 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 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.

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

** 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 executed; 2. that no mistake has been done in its distribution (dispensation of a wrong 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. RESULTS

If the test turns out to be valid, results are calculated from the mean OD450nm/620-630nm value of the Negative Control (NC) by means of a cut-off value (Co) determined with the following formula:

$$\text{Cut-Off} = \text{NC} + 0.250$$

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 generate the correct interpretation of results.

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm/620-630nm value (S) and the cut-off value (Co), 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 has not developed IgM antibodies to H.pylori.

Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.

A positive result is indicative of an ongoing H.pylori infection and therefore the patient should be treated accordingly.

Important notes:

1. *H.pylori IgM results alone are not enough to provide a clear diagnosis of Helicobacter pylori infection. Other tests for Helicobacter pylori (supplied by Dia.Pro Diagnostic BioProbes s.r.l. at code n° HPAG.CE, HPA.CE and HPG.CE), should be carried out.*
2. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below.

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: 1.000 OD450nm
Higher than 0.500 – Accepted

$$\text{Cut-Off} = 0.100 + 0.250 = 0.350$$

Calibrator: 0.500 – 0.540 OD450nm
Mean value: 0.520 OD450nm
S/Co higher than 1.0 – Accepted

Sample 1: 0.080 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 1.0 = negative
Sample 2 S/Co > 1.2 = positive

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in accordance to what reported in the Essential Requirements of the Directive 98/79/EC.

1. Limit of detection

No international standard for HP IgM Antibody detection has been defined so far by the European Community. In its absence, an Internal Gold Standard (or IGS), derived from a patient with an history of past mononucleosis infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:

The diagnostic performances were evaluated in a performance evaluation study conducted in an external center, with excellent experience in the diagnosis of infectious diseases.

The diagnostic sensitivity was studied on more than 50 samples, pre-tested positive with the reference kit of European origin in use at the laboratory. Positive samples were collected from patients with a clinical history of H.pylori acute infection.

The diagnostic specificity was determined on panels of more than 100 negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.

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 samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.

The Performance Evaluation provided the following values:

Sensitivity	> 98 %
Specificity	> 98 %

3. Reproducibility:

It has been calculated on three samples examined in replicates in different runs. CV% values obtained from a study conducted on three samples of different HP IgM reactivity, examined in 16 replicates in three separate runs ranged between 4-15%, depending on the OD450nm/620-630nm reading.

The variability observed did not result in sample misclassification.

S. LIMITATIONS

False positivity has been assessed as less than 2% of the normal population.

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

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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.

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HP IgM

Ensayo Inmunoenzimático (ELISA) para la
determinación de anticuerpos IgM frente a
Helicobacter pylori
en suero y plasma humano

Uso exclusivo para diagnóstico *in vitro*



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HP IgM

A. OBJETIVO DEL ESTUCHE

Ensayo inmunoenzimático (ELISA) para la determinación cuantitativa/cualitativa de anticuerpos IgM frente a *Helicobacter pylori* en suero y plasma humano.

Uso exclusivo para diagnóstico *in vitro*.

B. INTRODUCCIÓN

Helicobacter pylori (HP) es una bacteria Gram negative que fue aislada en mucosa gástrica por Marshall y Warren en 1983.

Hp ha sido descrita como el agente causante de la mayoría de casos de daño en la mucosa gástrica, jugando un papel importante en la evolución de enfermedad gástrica a carcinoma. La infección por HP produce una respuesta inmune del paciente con la producción de anticuerpos específicos IgA, IgA e IgM.

Ensayos inmunoenzimáticos son usados en la determinación de pacientes afectados con gastritis o úlceras pépticas producidas por infecciones agudas de cepas virulentas de *Helicobacter pylori*.

Se ha descrito que la presencia de anticuerpos IgA e IgM están correlacionados con fases agudas de la enfermedad, mientras que la presencia de anticuerpos IgG se encuentran a diferentes niveles poco tiempo después de la primera infección, manteniéndose en sangre durante algunos años.

ELISA cuantitativos también son usados en el estudio de pacientes con tratamiento de antibióticos, muy útil en el seguimiento de niveles de IgG durante y después del tratamiento farmacéutico.

C. PRINCIPIO DEL ENSAYO

Las microplacas están recubiertas con antígenos inmunodominantes de *H. pylori* procedentes de cultivos titulares de una cepa virulenta.

En la 1ª incubación, la fase sólida es tratada con muestras diluidas y los anticuerpos anti-HP IgG quedan unidos a los antígenos de la fase sólida.

Después de lavar, los componentes de la muestra que no se hayan unido son eliminados. En la 2ª incubación, los anticuerpos anti-HP IgM unidos son detectados por la adición de anticuerpos anti-IgM humana marcados con peroxidasa (HRP).

La enzima queda capturada en la fase sólida y actúa sobre el cromógeno/substrato, la actividad de la enzima genera una señal óptica proporcional a la cantidad de anticuerpo anti-HP IgM presente en la muestra.

La cantidad de IgM presente en la muestra es determinada usando la media del valor de corte (cut-off o co) que permite discriminar entre muestras positivas y negativas.

La neutralización de anticuerpos anti-HP IgG es necesaria para eliminar interferencias en la determinación de IgM. La neutralización se realiza directamente en los pocillos.

D. COMPONENTES

Cada estuche posee los reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE

12 tiras x 8 pocillos unidos a antígenos inmunodominantes de HP procedentes de cultivos en tejidos de una cepa virulenta de HP. Las placas están selladas en bolsas que contienen desecantes.

Permitir que las microplacas alcancen la temperatura ambiente antes de abrir; sellar las tiras no usadas en la bolsa con desecantes y conservar a 4°C

2. Control Negativo: CONTROL -

1x4.0ml/vial.

Listo para el uso. Contiene, anticuerpos humanos IgM negativos para HP, 2% de caseína, 10mM de tampón Citrato Sódico pH

6.0 +/-0.1, 0.1% Tween 20, 0.09% Azida sódica y 0.045% ProClin 300, como conservantes.

El control negativo está codificado con el color amarillo.

3. Control Positivo: CONTROL +

1x4.0ml/vial.

Listo para el uso. Contiene, altos niveles de anticuerpos humanos IgM positivos para HP, 2% caseína, 10mM de tampón Citrato Sódico pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Azida sódica y 0.045% ProClin 300, como conservantes.

El control positivo está codificado en el color verde-amarillo oscuro.

4. Calibrador: CAL ...

Vial nº1. 1 vial. Liofilizado para ser disuelto en agua EIA, como queda marcado en el recipiente. Contiene proteínas de suero bovino, bajos niveles de anticuerpos humanos IgM frente a HP, 0,2mg/ml sulfato de gentamicina y 0.045% ProClin 300, como conservantes.

Nota: El volumen necesario para disolver el contenido del vial puede variar de un lote a otro. Usar el volumen correcto marcado.

5. Tampón de lavado concentrado: WASHBUF 20X

1x60ml/botella 20x solución concentrada.

Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM pH 7.0+/-0.2, 0.05% Tween 20 y 0.045% ProClin 300.

6. Enzima conjugada : CONJ

1x16ml/vial. Listo para usar y codificada con el color rojo. Contiene anticuerpos policlonales que reconocen IgM humana y están marcados con peroxidasa de rábano, 5% BSA, 10 mM de tampón Tris pH 6.8+/-0.1, 0.045% ProClin 300 y 0.02% de sulfato de gentamicina como conservantes.

7. Cromogeno/Substrato: SUBS TMB

1x16ml/vial.

Contiene 50 mM tampón citrato-fosfato pH 3.5-3.8, 4% dimetilsulfóxido, 0.03% tetra-metil-benzidina (TMB) y 0.02% peróxido de hidrógeno (H₂O₂).

Nota: Evitar la exposición a la luz, es fotosensible.

8. Ácido sulfúrico: H2SO4 0.3 M

1x15ml/vial, contiene solución 0.3 M H₂SO₄.

Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Diluyente de muestras: DILSPE

2x60ml/vial. Contiene 2% caseína, 10 mM tampón citrato sódico pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% azida sódica y 0.045% ProClin 300 como conservantes. Usar para diluir las muestras.

10. Reactivo Neutralizante: SOLN NEUT

1x8ml/vial. Contiene anticuerpos de cabra anti IgG humana, 2% caseína, 10 mM tampón citrato sódico pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% azida sódica y 0.045% ProClin 300 como conservantes.

11. Sellador adhesivo nº2

12. Libro de instrucciones nº1

E. MATERIALES NECESARIOS PERO NO SUMINISTRADOS

1. Micropipetas calibradas (1000ul, 100 y 10ul) y puntas de plástico desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para eliminar agentes químicos oxidantes usados como desinfectantes).
3. Reloj con un rango de 60 minutos o más.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA (seco o húmedo) capaz de alcanzar una temperatura de 37°C (+/- 0.5°C).

6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de filtros de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Agitador 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 entrenado 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 cromógeno/ substrato a la luz y también las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el estuche a temperaturas entre 2-8 °C, 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 son transparentes y no contienen precipitados ni agregados en el momento del uso. En caso contrario, informar al supervisor del laboratorio para realizar el procedimiento pertinente y reemplazar el estuche.
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 estuche 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 estuche e internamente en los reactivos. Un estudio dirigido a comprobar la actividad de estuches abiertos, no mostró pérdida de actividad en estuches usados hasta 6 veces en un periodo de 6 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 antes de tirar. 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. Cuando el estuche se emplea para el pesquiasaje 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, 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 COMPONENTES Y PRECAUCIONES

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 2-8°C. 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 usar. Mezclar cuidadosamente con agitador antes de usar.

Control Positivo

Listo para usar. Mezclar cuidadosamente con agitador antes de usar.

Calibrador

Añadir el volumen de agua de ELISA, indicada en la marca, dejar disolver y mezclar con agitador.

Nota: El calibrador disuelto no es estable. Almacenar a -20°C en alícuotas.

Tampón de lavado concentrado:

Todo el contenido del tampón concentrado 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 8°C.

Enzima conjugada:

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.

Cromogeno/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.

Diluyente de muestras:

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

Reactivo Neutralizante:

Listo 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 HERRAMIENTAS USADAS 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 (alcohol, lejía 10%, desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periódicamente los residuos de los componentes del estuche.
2. El incubador de ELISA debe ser ajustada a 37°C (+/- 0.5°C) 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 (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 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 interno de calidad". 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 de ELISA cuando el número de muestras para analizar 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. CONTROLES Y OPERACIONES PREVIAS 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.
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 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.
5. Disolver totalmente el contenido del Calibrador, como se ha descrito anteriormente.
6. Diluir totalmente el tampón de lavado concentrada 20X, como se ha descrito anteriormente.
7. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
8. Ajustar la incubadora de ELISA a 37°C y cebear 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.

- Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
- En caso de trabajar automáticamente, conectar el equipo y comprobar que los protocolos estén correctamente programados.
- Comprobar que las micropipetas estén fijadas en el volumen requerido.
- 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 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.

- Diluir las muestras 1:101 dentro de un apropiado tubo (ejemplo: 1000 µl Diluyente de muestras + 10 µl muestra). No diluir el Control/Calibración ya que están listos para usarse. Mezclar todos los reactivos líquidos en un agitador y continua como se describe a continuación.
- Poner el número de tiras necesarias en el soporte de plástico. Dejar el primer pocillo A1 vacío para el blanco.
- Dispensar 50 µl del Reactivo Neutralizante (SOLN NTR) en todos los pocillos de las muestras, excepto en A1. ¡No añadirlo dentro de los pocillos usados para los Calibradores y los Controles!

Nota importante: El reactivo neutralizante puede bloquear falsas reacciones positivas debido a RF. Las muestras positivas en paneles de control de calidad internos podrían ser detectadas como negativas si estas muestras se analizaron como positivas con un IVD que no realiza ninguna reacción de bloqueo de RF.

- Dispensar 100 µl de Control Negativo por triplicado, Calibrador por duplicado y 100 µl de Control Positivo en un solo pocillo y 100 µl de muestras diluidas en cada uno de los pocillos marcados específicamente.
- Incubar la microplaca durante **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.

- Lavar la microplaca con el lavador automático dispensando según se indica (section I.3).
- Dispensar 100 µl de la Enzima Conjugada 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 conjugado. Podría producirse contaminación.

- Incubar la microplaca **60 min a +37°C**.
- Lavar los pocillos como en el paso 6.
- Dispensar 100 µl 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.

- Dispensar 100 µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición de la solución de parada cambia el color del calibrador positivo, el suero control y las muestras positivas de amarillo a azul.
- 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:

- Asegurarse de que no hay impresiones digitales ni polvo en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.
- 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:

Método	Operaciones
Reactivo Neutralizante (sólo para muestras)	50 µl
Calibrador(*) y Controles	100 µl
Muestras diluidas 1:101	100 µl
1ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Enzime conjugada	100 µl
2ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
TMB/H2O2	100 µl
3ª incubación	20 min
Temperatura	18-24°C
Ácido sulfúrico	100 ul
Lectura DO	450nm/620-630nm

(*) 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	M2										
B	CN	M3										
C	CN	M4										
D	CN	M5										
E	CAL(*)	M6										
F	CAL(*)	M7										
G	CP	M8										
H	M1	M9										

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

O. CONTROL INTERNO DE CALIDAD

Se realiza un grupo de pruebas con los controles cada vez que se usa el estuche para verificar si los valores DO 450nm son los esperados.

Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo blanco	Valor < 0.100 DO 450nm
Control Negativo	Valor Medio < 0.150 DO 450nm después del blanco Coeficiente de variación < 30%
Control Positivo	DO 450nm > 0.500

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 DO 450nm	1. La solución Cromogeno/Substrato no se ha contaminado durante el ensayo
Control Negativo > 0.150 DO 450nm después del 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 (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 < 1.000 OD450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en la distribución (ej. dispensar un control 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 control positivo.

Si ocurre alguno de los problemas anteriores, después de la comprobación, informe al supervisor para tomar las medidas pertinentes.

** Nota:

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

Parámetro	Exigencia
Calibrador	S/Co > 1.0

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

Problema	Compruebe que
Calibrador S/Co < 1.0	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en la distribución (ej. dispensar un control 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.

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. RESULTADOS

Los resultados se calculan con la media de la DO 450nm/650-630nm del control Negativo y por medio de un valor de corte (cut-off) hallado con la siguiente fórmula:

$$\text{Cut-Off} = \text{CN} + 0.250$$

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 y el Valor de corte (M/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 ha desarrollado anticuerpos IgM frente a *H.pylori*.

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 *H.pylori*. y por consiguiente el paciente debe ser tratado adecuadamente.

Notas importantes:

- La presencia de anticuerpos anti-*H.pylori* IgA no son suficientes para diagnosticar infección por *Helicobacter pylori*. Otros estudios para *for Helicobacter pylori* (suministrados por Dia.Pro Diagnostic BioProbes s.r.l. con código n° HPAG.CE, HPA.CE y HPG.CE), pueden ser realizados.
- 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.
- Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
- El diagnóstico debe ser evaluado y comunicado al paciente por un médico calificado.

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: 0.100 – 0.120 – 0.080 DO 450nm
Media del Valor: 0.100 DO 450nm
Menor que 0.150 – Aceptado

Control Positivo: 1.000 DO 450nm
Mayor que 0.500 – Aceptado

Valor de Corte o Cut-Off = 0.100+0.250 = 0.350

Calibrador: 0.500 – 0.540 DO 450nm
Meida del Valor: 0.520 DO 450nm
S/Co mayor que 1.0 – Aceptado

Muestra 1: 0.080 DO 450nm
Muestra 2: 1.800 DO 450nm
Muestra 1 S/Co < 1.0 = Negativo
Muestra 2 S/Co > 1.2 =Positivo

R. REALIZACIONES CARATERÍSTICAS

La evaluación de las realizaciones debe ser dirigida de acuerdo a lo establecido en Essential Requirements of the Directive 98/79/EC.

1. Límite de detección

La comunidad Europea no ha definido estándares internacionales para la detección de anticuerpos anti-HP IgM. En su ausencia, un Internal Gold Standard (o IGS), derivado de pacientes con historia de infección por mononucleosis infecciosa, ha sido definido con la finalidad de proporcionar un procedimiento con alta sensibilidad.

2. Especificidad y Sensibilidad Diagnósticas:

Las realizaciones diagnósticas fueron evaluadas en un centro externo de amplia experiencia en el diagnóstico de enfermedades infecciosas.

La sensibilidad diagnóstica fue estudiada en más de 50 muestras, pre-probadas como positivas. Muestras positivas fueron tomadas de pacientes con historial clínico de infección por *H. pylori*.

La especificidad diagnóstica fue determinada en paneles de más de 100 muestras negativas de donantes normales, clasificados como negativos incluyendo especímenes que pudieran interferir potencialmente.

Se emplearon, 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.

Por último se analizaron muestras congeladas, para determinar posibles interferencias debidas a la toma de muestra y al almacenamiento. No se observaron interferencias.

La Evaluación de las Realizaciones nos ofreció los siguientes valores:

Sensibilidad	> 98 %
Especificidad	> 98 %

3. Reproducibilidad:

Ha sido calculada en tres muestras determinadas en diferentes filas. Valores de CV% de un estudio sobre tres muestras de diferentes reactividad IgM anti-HP, realizadas en tres filas separadas, muestra resultados entre 4-15%, dependiendo de la lectura de la DO 450nm/620-630nm.

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

S. LIMITACIONES

Falsos positivos han sido estudiados con menos del 2% de la población normal.

Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.

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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 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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(Milán) – Italia

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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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
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 HSV1 at about 20 arbU/ml $\pm$ 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 H₂O₂).

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

7. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. It contains 0.3 M H₂SO₄ 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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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 μ l Sample Diluent and then 10 μ l 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 (*)	100 µl
Samples diluted 1:101	100 µl
1 st 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
2 nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H ₂ O ₂	100 µl
3 rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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 $\pm$ 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

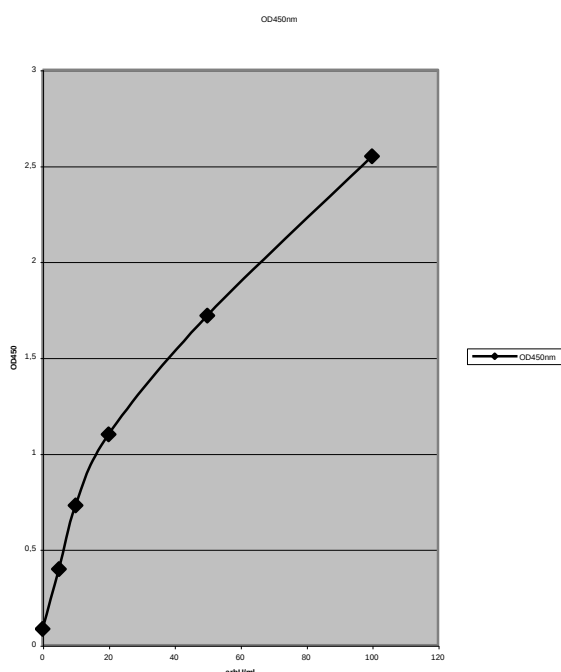
P.1 Quantitative method

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 of 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	3 rd 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	3 rd 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	3 rd 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



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 (H₂O₂) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. It contains 0.3 M H₂SO₄ 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 Immucplex 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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 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.

- Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
- 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.
- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- 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.

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:

- Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
- Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
- 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 !
- 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.
- 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.

- Wash the microplate with an automatic washer as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 6.
- 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.

- 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.
- 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:

- Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
- 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(*)	100 ul
Samples diluted 1:101	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:

$$\text{Cut-Off} = \text{NC} + 0.250$$

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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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 µl concentrated Enzymatic Conjugate are diluted in 500 µl 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



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 proteínas 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 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 sustrato cromogénico. En presencia del conjugado el sustrato es hidrolizado generando 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 proteínas 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 8°C.

2. Control Negativo: **CONTROL-**

1x4.0 ml/vial. Listo para el uso. Contiene 1% de proteínas del suero humano, 2% de caseína, tampón Tris 10 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 Tris 10 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 proteínas 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 Diluyente 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 (HRP) diluido 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. Diluyente 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. Diluyente 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 (H₂O₂) 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 H₂SO₄ 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 37°C (+/-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 sustrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el estuche a temperaturas entre 2-8 °C, 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 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.

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 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 2-8°C. 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 cuidadosamente con el vórtex antes de usar.

Nota: Para preservar la reactividad se recomienda mantenerla congelada en alícuotas a -20°C . 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 8°C .

Inmunocomplejo Ag/Ab:

Proceder cuidadosamente según se indica:

1. Disolver el contenido de un vial liofilizado utilizando 1.9 ml de Diluyente 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.

Diluyente 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 (alcohol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de $\pm 2\%$. Deben descontaminarse periódicamente los residuos de los componentes del estuche.
2. La incubadora de ELISA debe ser ajustada a 37°C ($\pm 0.5^{\circ}\text{C}$ 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 $\pm 5\%$.
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 $\leq 10\text{nm}$ b) Rango de absorbancia de 0 a ≥ 2.0 , c) Linealidad ≥ 2.0 , reproducibilidad $\geq 1\%$. 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 pesquiasaje 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 37°C 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 Diluyente 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 diluidas, 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 Diluyente de Muestras + 10 µl de muestra) en una segunda plataforma de dilución. Programar el equipo para aspirar primeramente 100 µl de Diluyente 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 Diluyente 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 diluidas 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 (*)	100 ul
Muestras diluidas 1:101	100 ul
1^{ra} incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Inmunocomplejo	100 ul
2^{da} incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Mezcla TMB/H ₂ O ₂	100 ul
3^{ra} 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 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 (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:

$$\text{Valor de corte} = \text{CN} + 0.250$$

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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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 precisión 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 Diluyente de Antígeno (ej: 25 ul de Conjugado concentrado en 500 ul de Diluyente 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 Giovanni (Milán) –
Italia



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 -



DIA.PRO

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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 H₂O₂).

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

7. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. It contains 0.3 M H₂SO₄ 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-borne 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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 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 (*)	100 µl
Samples diluted 1:101	100 µl
1 st 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
2 nd 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
3 rd 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 $\pm$ 20%

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

Problem	Check
Control Serum	1. that the procedure has been correctly executed;
Different from expected value	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

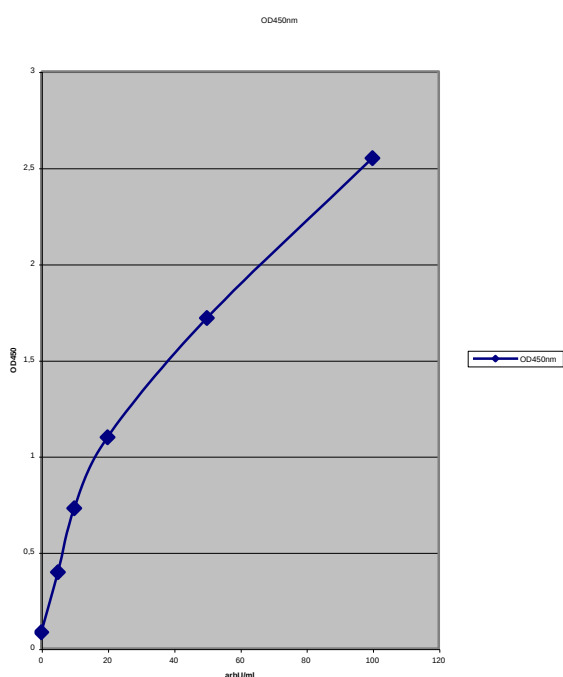
P.1 Quantitative method

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 of 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 $\geq 98\%$.

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	3 rd 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	3 rd 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	3 rd 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



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 -



DIA.PRO

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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 device 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 (H₂O₂) and 4% dimethylsulphoxide.

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

11. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. It contains 0.3 M H₂SO₄ 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-borne 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 Immunocomplex 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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 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.

- Dissolve the Calibrator as described above and gently mix.
- Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
- 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.
- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- 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.

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:

- Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
- Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
- 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 !
- 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.
- 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.

- Wash the microplate with an automatic as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 6.
- 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.

- 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.
- 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:

- Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
- 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 (*)	100 ul
Samples diluted 1:101	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:

$$\text{Cut-Off} = \text{NC} + 0.250$$

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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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 µl concentrated Enzymatic Conjugate are diluted in 500 µl 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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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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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 proteínas 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 sustrato cromogénico. En presencia del conjugado, el sustrato 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 proteínas 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 8°C.

2. Control Negativo: CONTROL -

1x4.0 ml/vial. Listo para el uso. Contiene 1% de proteínas del suero humano, 2% de caseína, tampón Tris 10 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 Tris 10 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 proteínas 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 Diluyente 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 (HRP) diluido 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. Diluyente 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. Diluyente 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 (H₂O₂) 0.02% así como dimetilsulfóxido al 4%.

Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

11. Ácido Sulfúrico: H₂SO₄ 0.3 M

1x15ml/vial. Contiene solución de H₂SO₄ 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 37°C (+/-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 sustrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el estuche a temperaturas entre 2-8 °C, 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 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.

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 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 2-8°C. 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 cuidadosamente con el vórtex antes de usar.

Nota: Para preservar la reactividad se recomienda mantenerla congelada en alícuotas a -20°C . 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 8°C .

Inmunocomplejo Ag/Ab:

Proceder cuidadosamente según se indica:

1. Disolver el contenido de un vial liofilizado utilizando 1.9 ml de Diluyente 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.

Diluyente 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 $\pm 2\%$. Deben descontaminarse periódicamente los residuos de los componentes del estuche.
2. La incubadora de ELISA debe ser ajustada a 37°C ($\pm 0.5^{\circ}\text{C}$ 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 $\pm 5\%$.
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 $\leq 10\text{nm}$ b) Rango de absorbancia de 0 a ≥ 2.0 , c) Linealidad ≥ 2.0 , reproducibilidad $\geq 1\%$. 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 pesquiasaje 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 37°C 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 Diluyente 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 diluidas, 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 Diluyente de Muestras + 10 µl de muestra) en una segunda plataforma de dilución. Programar el equipo para aspirar primeramente 100 µl de Diluyente 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 Diluyente 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 diluidas 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 sección 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 (*)	100 ul
Muestras diluidas 1:101	100 ul
1^{ra} incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Inmunocomplejo	100 ul
2^{da} incubación	60 min

Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Mezcla TMB/H ₂ O ₂	100 ul
3 ^{ra} 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 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 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 (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:

$$\text{Valor de corte} = \text{CN} + 0.250$$

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:

- 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.
- 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.
- 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.
- Antes de emitir un resultado positivo, cada muestra reactiva debe someterse al examen de confirmación reportado en la sección T, lo cual permite una correcta interpretación de los resultados ya que descarta los falsos positivos.
- Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
- 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 aprobado 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 (citrate, EDTA y heparina) y suero humano 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 débilmente 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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 precisión 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 Diluyente de Antígeno (ej: 25 µl de Conjugado concentrado en 500 µl de Diluyente 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.
Via G. Carducci n° 27 – Sesto San Giovanni (Milán) – Italia



ORGENTEC Diagnostika GmbH

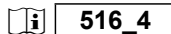
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ORG 516 AMA-M2

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



In vitro diagnostic medical device



Manufacturer



Catalogue number



Sufficient for 96 determinations



Batch code



Use by



Temperature limitation



Keep away from sunlight



Do not reuse



Date of manufacture



CE marked according to 98/79/EC



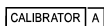
Consult instructions for use



Electronic Instruction For Use: version



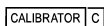
Microplate



Calibrator



Calibrator



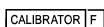
Calibrator



Calibrator



Calibrator



Calibrator



Control positive



Control negative



Sample Buffer P



Enzyme Conjugate



TMB Substrate



Stop solution



Wash Buffer



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. Subsequently 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 stops 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, classification 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		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 unopened 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, perform 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.

- 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.
- Dispense **100 µl** 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.
- Dispense **100 µl** of TMB substrate solution into each well.
Incubate for **15 minutes** at room temperature
- 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

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 establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

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3. Berg, P.A. and Klein, R. Immunology of primary biliary cirrhosis. Ballière's Clin.Gastroenterol. 1987; 1: 675 - 706.
4. Baum, H. and Palmer, C. The PBC specific antigen. Mol. Aspects Med. 1985; 8: 201 - 234.
5. Fussey, S.P.M., Guest, J.R., James, O.F W. et al. Identification and analysis of the major M2 autoantigens in primary biliary cirrhosis. PNAS, USA 1988; 85: 8654 - 8658.

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**