EBNA IgM

Enzyme ImmunoAssay for the qualitative determination of IgM antibodies to Epstein Barr Virus Nuclear Antigen (EBNA) in human plasma and sera

- for “in vitro” diagnostic use only -

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REF EBNM.CE
96 Tests

EBNA IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the qualitative determination of IgM class antibodies to Epstein Barr Virus Nuclear Antigen (EBNA) in human plasma and sera.
The kit is intended for the classification of the viral infective agent and the follow-up of EBV infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Epstein Barr Virus or EBV is the principal etiological agent of infectious mononucleosis, as well as a contributory factor in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma, or NPC.
A member of the family Herpesviridae, it has a worldwide distribution, such that 80 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life. While childhood infections are mostly asymptomatic, 50 to 70% of young adults undergoing primary EBV infections show mild to severe illness.
EBV may cause a persistent, latent infection which can be reactivated under immunosuppression or in AIDS affected patients. As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies raised in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection, had a past infection or may be having reactivated EBV infection.
The detection of EBV-specific IgG, IgM and IgA antibodies to its major immunodominant antigens has become therefore an important and useful determination for the monitoring and follow-up of EBV infected patients.

C. PRINCIPLE OF THE TEST

Microplates are coated with affinity purified native EBNA.
The solid phase is first treated with the diluted sample and anti-EBNA IgM are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-EBNA IgM are detected by the addition of anti hIgM antibody, 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-EBNA IgM antibodies present in the sample.
Interferences due to IgG and RF in samples are blocked directly into the well by a Neutralizing Reagent.

D. COMPONENTS

Each kit contains sufficient reagents to carry out 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 breakable wells coated with affinity-purified native EBNA 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 -
1x4ml. Human serum base not reactive for anti-EBNA IgM antibodies. It contains 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives. The Negative Control is pale yellow colour coded.

3. Positive Control: CONTROL +
1x4ml. Human serum base reactive for anti-EBNA IgM antibodies. It contains 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives. The Positive Control is green colour coded.

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 IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

6. Specimen Diluent: DILSPE
2x60.0 ml/vial. Buffered solution for the dilution of samples. It contains 2% casein, 0.2 M Tris buffer pH 6.0+/-0.1, 0.2% Tween 20, 0.045% ProClin 300 and 0.09% sodium azide as preservatives. The component is blue color coded.

7. Neutralizing Reagent: SOLN NEUT
1x8ml. Proteic solution for the neutralization of IgG and RF in samples. It contains a detergent, proteic stabilizers, 0.1% sodium azide and 0.045% ProClin 300 as preservatives.

8. 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 and 0.02% hydrogen peroxide of H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid: H_2SO_4 O.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Plate sealing foils n° 2

11. 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/H_2O_2) 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 RECOMMANDATIONS

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

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 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 inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
***Important Note**: After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Controls
Ready to use. Mix well on vortex before use.

Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 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.*

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

Neutralizing Reagent
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 ±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 and the right temperature of +37°C is assured to the microplate.
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, 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.
3. Check that the Chromogen/Substrate is colorless 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).
5. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
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 dispensing 1 ml Specimen Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the Controls as they are ready-to-use.
2. 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.
3. Dispense 50 µl Neutralizing Reagent in all the sample wells; do not dispense in A1 and in the Controls wells.

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. Pipette 100 µl Negative Control in triplicate and 100 µl Positive Control in single into appropriate wells. Then dispense 100 µl of samples into the appropriate sample wells.
5. Incubate the microplate at **+37°C for 60 min**.

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

6. Wash the microplate as reported in section I.3.
7. In all the wells, except A1, pipette 100 µl Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

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

8. Wash the microplate as described in section I.3.
9. Pipette 100 µl Chromogen/Substrate in each well, the blank well A1 included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

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

10. Stop the enzymatic reaction by pipetting 100 µl Sulphuric Acid into each well and using the same pipetting sequence as in step 9.
11. Then measure the color intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, mandatory), blanking the instrument on A1.

Important general notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*

N. ASSAY SCHEME

Neutralizing Reagent (only for samples)	50 ul
Controls	100 ul
Samples diluted 1:101	100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H_2O_2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-6230nm

An example of dispensation scheme is reported below

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S4										
B	NC	S5										
C	NC	S6										
D	NC	S7										
E	PC	S8										
F	S1	S9										
G	S2	S10										
H	S3	S11										

Legenda: BLK = Blank // NC = Negative Control
PC = Positive Control // S = Sample

O. INTERNAL QUALITY CONTROL

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

Parameters	Requirements
Blank well	< 0.100 OD450nm
Negative Control (NC)	< 0.150 OD450nm after blanking
Positive Control (PC)	> 0.500 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 OD450nm > 0.150	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure when the dispensation of Controls is carried out; 4. that no contamination of the Control or of the wells where it was dispensed has occurred due to spills of positive samples or Conjugate; 5. that micropipettes have not become contaminated with positive samples or with the Conjugate; 6. that the washer needles are not blocked or partially obstructed.
Positive Control OD450nm < 0.500	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: 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 Control has occurred.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 11.

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:

Cut-Off = NC + 0.250

Important Note: *When the calculation of results is performed by the operating system of an ELISA automated workstation, ensure that the proper formulation is used to generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS
Test results are interpreted as the 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 EBV.
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 EBV infection and therefore the patient should be treated accordingly.

Important notes:

1. *EBNA IgM results alone are not enough to provide a clear diagnosis of EBV infection. Other tests for EBV (supplied by Dia.Pro Diagnostic BioProbes s.r.l. at code n° EBNG.CE, EAG.CE, EAM.CE, VCAG.CE, VCAM.CE and RealTime PCR assay for EBV), 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 (data obtained proceeding as the the reading step described in the section M, point 11):

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

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
Cut-Off = 0.100+0.250 = 0.350
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 an external clinical center on panels of negative and positive samples with reference to a commercial kit.

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

2. Diagnostic Sensitivity and Specificity:
The **diagnostic sensitivity** was studied on 88 positive samples, pre-tested with the reference kit of European origin in use at the laboratory. Positive samples were collected from patients undergoing acute mononucleosis infection.
The **diagnostic specificity** was determined on 352 negative samples from normal individuals classified negative with the reference kit.
Moreover a panel of potentially interfering samples (RF+, unrelated virus infections, et.c) were tested with no false results.
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:
Data obtained from a study conducted on three samples of different EBNA IgM reactivity, examined in 16 replicates in three separate runs showed in general CV% values lower than 15%, 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, mostly due to high titers of Rheumatoid Factor.
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.

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CE

EBNA IgM

Ensayo inmunoenzimático para la determinación cualitativa de anticuerpos IgM contra el virus de Epstein Barr Antígeno nuclear (EBNA) en plasma y suero humanos

- Uso exclusivo para diagnóstico “in vitro”-

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REF EBNM.CE
96 pruebas

EBNA IgM

A. OBJETIVO DEL EQUIPO

Ensayo inmunoenzimático (ELISA) para la determinación cualitativa de los anticuerpos de clase IgM contra el antígeno nuclear del virus de Epstein-Barr (EBNA) en plasma y suero humanos.

El equipo ha sido desarrollado para la clasificación del agente vírico infeccioso y para el seguimiento de pacientes infectados con EBV.

Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN

El virus de Epstein Barr o EBV es el principal agente etiológico de la mononucleosis infecciosa, así como uno de los factores que contribuyen a la etiología del linfoma de Burkitt y del carcinoma nasofaríngeo (CNF).

Perteneciente a la familia Herpesviridae y de distribución mundial, se estima que entre un 80 y un 90% de los adultos han sido infectados con este virus. Las infecciones primarias suelen producirse durante los primeros diez años de vida. Mientras que las infecciones infantiles suelen ser asintomáticas, entre el 50 y el 70% de los adultos jóvenes con infecciones primarias puede desarrollar de formas leves a severas de la enfermedad.

El EBV puede producir una infección latente persistente que puede reactivarse por inmunosupresión o en pacientes con SIDA. Debido a que la respuesta humoral a la infección primaria por EBV es bastante rápida, la clase y los niveles de anticuerpos presentes en la mayoría de los casos permiten determinar si un paciente todavía es vulnerable al virus, si la infección primaria es actual o reciente, si contrajo la infección en el pasado o si la infección por EBV se está reactivando.

La detección de anticuerpos IgG, IgM e IgA específicos contra los antígenos inmunodominantes del virus constituye un medio importante y útil de control y seguimiento de los pacientes infectados por EBV.

C. PRINCIPIOS DEL ENSAYO

Se han recubierto las microplacas con EBNA nativo purificado.

La fase sólida se trata primero con la muestra diluida y se capturan los anticuerpos IgM anti-EBNA, si existen, mediante los antígenos.

Después del lavado, que elimina el resto de los componentes de la muestra, en la 2ª incubación se detectan los anticuerpos IgM anti-EBNA unidos mediante la adición de anticuerpo anti hIgM, marcado con peroxidasa (HRP).

La enzima capturada en la fase sólida, combinada con la mezcla substrato/cromógeno, genera una señal óptica proporcional a la cantidad de anticuerpos IgM anti-EBNA presentes en la muestra.

Las interferencias debidas a la presencia de IgG y RF en las muestras se bloquean directamente en el pocillo mediante un reactivo neutralizante.

D. COMPONENTES

Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLACA

12 tiras x 8 pocillos rompibles recubiertos con EBNA nativo purificado por afinidad en una bolsa sellada con desecante. Dejar la microplaca a temperatura ambiente antes de abrirla, sellar las tiras sobrantes en la bolsa con el desecante y conservar a 4ºC.

2. Control negativo: CONTROL -

1x4 ml. Suero humano no reactivo para anticuerpos IgM anti-EBNA. Contiene 0,2 mg/ml de sulfato de gentamicina y ProClin 300 GC al 0,045% como conservantes. El control negativo está codificado con el color amarillo pálido.

3. Control positivo: CONTROL +

1x4 ml. Suero humano reactivo para anticuerpos IgM anti-EBNA. Contiene 0,2 mg/ml de sulfato de gentamicina y ProClin 300 al 0,045% como conservantes. El control positivo está codificado con el color verde.

4. Solución de lavado concentrada: WASHBUF 20X

1x60 ml/botella. Solución concentrada 20x.

Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7,0 +/- 0,2, Tween 20 al 0,05% y ProClin 300 al 0,045%.

5. Conjugado: CONJ

1x16 ml/vial. Listo para el uso y codificado con color rojo. Contiene anticuerpos policlonales anti IgM humana conjugados con peroxidasa (HRP), 5% de albúmina de suero bovino (BSA), tampón Tris 10 mM a pH 6,8+/-0,1, ProClin 300 al 0,045% y sulfato de gentamicina al 0,02% como conservantes.

6. Diluente de muestras: DILSPE

2x60,0 ml/vial. Solución tamponada para la dilución de las muestras. Contiene caseína al 2%, tampón Tris 0,2M a pH 6,0 +/-0,1, Tween 20 al 0,2%, ProClin 300 al 0,045% y azida sódica al 0,09% como conservantes. El reactivo está codificado con el color azul.

7. Reactivo neutralizante: SOLN NEUT

1x8 ml Solución proteica para la neutralización de IgG y RF en las muestras. Contiene un detergente, estabilizantes proteicos, azida sódica al 0,1% y ProClin 300 al 0,045% como conservantes.

8. Cromógeno/ Substrato: SUBS TMB

1x16 ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM a pH 3,5-3,8, tetra-metil-benzidina (TMB) al 0,03% o peróxido de hidrógeno (H_2O_2) al 0,02%.

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

9. Ácido sulfúrico: H_2SO_4 O,3 M

1x15 ml/vial. Contiene solución de H_2SO_4 0,3 M

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

10. Sellador adhesivo, n° 2

11. Manual de instrucciones, 1 ud.

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 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) ajustado a +37ºC.
6. Lector calibrado de micropocillos ELISA con filtros de 450 nm (lectura) y 620-630 nm (blanco).
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES

1. El equipo solo debe usarse por personal técnico adecuadamente instruido, bajo la supervisión de un médico responsable del laboratorio.

2. Todo el personal que participe en la realización de los ensayos deberá llevar la indumentaria protectora adecuada de laboratorio, guantes sin talco y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). Todo el personal involucrado debe tener formación en procedimientos de bioseguridad, como recomienda el Centro de Control de Enfermedades de Atlanta, EE. UU., y como ha publicado 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 VHB y VHA, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el entorno del laboratorio para evitar la contaminación por polvo o agentes microbianos en el aire al abrir los viales del equipo y las microplacas, así como durante la realización del ensayo. Evitar la exposición del cromógeno/substrato (TMB/H_2O_2) y las vibraciones de la mesa de trabajo durante el ensayo.
5. Tras la recepción, conservar el equipo a una temperatura entre +2 y 8 °C, en un refrigerador con temperatura regulada o en una cámara de refrigeración.
6. No intercambiar componentes de lotes distintos, ni tampoco de dos equipos del mismo lote.
7. Comprobar que los reactivos estén claros y no contengan partículas pesadas visibles ni agregados. 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 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 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 lejía al 10% de 16 a 18 horas o el uso de la 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, lavar la superficie con abundante agua.
16. Los demás materiales de desecho que se generan durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben manipularse como fuentes potenciales de infección de acuerdo con las directivas nacionales y las 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 del laboratorio de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte a 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 identificarse claramente mediante códigos de barras o nombres, a fin de evitar una interpretación errónea de los resultados. Cuando el equipo se emplea para el cribado de unidades de sangre, se recomienda el uso de código de barras y lectura electrónica.
4. Las muestras hemolizadas (color rojo) y visiblemente hiperlipémicas (aspecto lechoso) deben descartarse para evitar falsos resultados, al igual que aquellas que contengan restos de fibrina, partículas pesadas o filamentos y organismos 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, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
6. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2 y 0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES

Estudios de estabilidad realizados en equipos en uso no han arrojado pérdida de actividad significativa en un período de hasta 3 meses.

Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Comprobar que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de conservación.

De ser así, contactar con el servicio de atención al cliente de Dia.Pro.

Las tiras de pocillos no utilizadas deben depositarse en la bolsa de aluminio otra vez con el desecante; la bolsa debe guardarse herméticamente cerrada a 2-8ºC.

***Nota importante**: Una vez abierto el envase, las tiras sobrantes se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambia de amarillo a verde.*

Controles

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

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 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 durante una semana a temperaturas entre +2 y 8ºC.*

Diluente 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.

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 intensa, agentes oxidantes y superficies metálicas.
En caso de que deba transferirse este componente, usar solo 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). Además, deben revisarse regularmente para mantener una precisión del 1% y una exactitud de $\pm$2%.
2. La incubadora ELISA debe ser ajustada a 37ºC ($\pm$ 0,5ºC de tolerancia) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua, siempre que estén validadas para la incubación de pruebas de ELISA y se garantice una temperatura de +37°C en la microplaca.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de $\pm$5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450 nm y de un segundo filtro de 620-630 nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda $\leq$ 10 nm; b) Rango de absorbancia de 0 a $\geq$ 2,0; c) Linealidad $\geq$ 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 calibrarse 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 instalarse en el sistema operativo de la unidad y validarse tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe validarse y fijarse correctamente. Debe prestarse especial atención para evitar el arrastre por las agujas de dispensación de muestras y de lavado a fin de minimizar la posibilidad de obtener falsos positivos a causa de la contaminación de los pocillos adyacentes por muestras fuertemente reactivas. 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 parte externa del equipo (envase primario). No usar si ha caducado.
2. Comprobar que los componentes líquidos no están contaminados con partículas o agregados visibles.
3. Asegurarse de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico.
4. Comprobar que no han ocurrido roturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte.
5. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
6. Diluir totalmente la solución de lavado concentrada 20x, como se ha descrito anteriormente.
7. Dejar los componentes restantes hasta alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
8. Ajustar la incubadora de ELISA a 37 ºC y alimentar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
9. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
10. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
11. Comprobar que las micropipetas estén ajustadas al volumen requerido.
12. Asegurarse de que el resto del equipamiento esté disponible y listo para el uso.
13. En caso de que surja algún problema, detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO

El ensayo debe realizarse de acuerdo con las instrucciones que se indican a continuación, teniendo cuidado de mantener en todas las muestras el mismo tiempo de incubación.

1. Diluir las muestras 1:101 dispensando en un tubo desechable 1 ml de diluente de muestras y 10 µl de muestra; mezclar con vórtex antes de usar. No diluir los controles, ya que están listos para el uso.
2. Poner el número de tiras necesarias en el soporte de la microplaca. Dejar el pocillo A1 vacío para la operación de blanco. Guardar las tiras restantes en la bolsa con desecante a 2 - 8 °C, sellada.
3. Dispensar 50 µl de reactivo neutralizante en todos los pocillos de muestras; no dispensar en el pocillo A1 ni en los pocillos de 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.*

4. Dispensar 100 µl de control negativo por triplicado y 100 µl de control positivo una sola vez en los pocillos adecuados. A continuación, dispensar 100 µl de las muestras en los pocillos de muestras adecuados.
5. Incubar la microplaca **60 min. a +37°C**.

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

6. Lavar la microplaca según se indica en la sección I.3.
7. Dispensar 100 µl de conjugado en todos los pocillos, excepto el A1. Comprobar que se haya añadido correctamente el reactivo. Incubar la microplaca **60 min. a +37°C**.

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

8. Lavar la microplaca como se describe en la sección I.3.
9. Dispensar 100 µl de cromógeno/substrato en todos los pocillos, incluidos el pocillo de blanco A1. Comprobar que se haya añadido correctamente el reactivo. A continuación, incubar la microplaca a **temperatura ambiente por 20 minutos**.

***Nota importante:** No exponer a luz intensa directa, ya que se podría generar un fondo excesivo.*

10. Dispensar 100 µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática usando la misma secuencia que en el paso 9.
11. Medir la intensidad del color con el lector de microplacas con filtro de 450 nm (lectura) y filtro de 620-630 nm (blanco, obligatorio) y leer el blanco en el pocillo A1.

Notas generales importantes:

1. *~~Si no se puede utilizar el segundo filtro~~, asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer ~~a 450 nm~~. Podrían generarse falsos positivos en la lectura.*
2. *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.*

N. ESQUEMA DEL ENSAYO

Reactivo neutralizante (solo para muestras)	50 µl
Controles	100 µl
Muestras diluidas 1:101	100 µl
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2ra incubación	**60 min**
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Temperatura	+37°C
Mezcla TMB/H_2O_2	100 µl
3ra incubación	**20 min**
Temperatura	t.a.
Ácido sulfúrico	100 µl
Lectura DO	450 nm / 620-630 nm

A continuación se incluye un ejemplo del esquema de dispensado:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	S4										
B	NC	S5										
C	NC	S6										
D	NC	S7										
E	PC	S8										
F	S1	S9										
G	S2	S10										
H	S3	S11										

Leyenda: BL = Blanco // NC = Control Negativo
CP = Control positivo // M = Muestra

O. CONTROL DE CALIDAD INTERNO

Se realiza una comprobación para validar los controles y el suero de control siempre que se utiliza el equipo, para verificar si el rendimiento del ensayo es el homologado.
Controlar que los datos siguientes coinciden:

Parámetros	Requisitos
Pocillo blanco	< 0,100 DO 450 nm
Control negativo (NC)	< 0,150 DO 450 nm después de leer el blanco
Control positivo (PC)	> 0,500 DO450nm

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

En caso contrario, detener el ensayo y comprobar lo siguiente:

Problema	Comprobar
Pocillo blanco > 0,100 DO 450 nm	1. la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control negativo DO 450 nm > 0,150	1. el proceso de lavado y los parámetros del lavador coinciden con los validados en los estudios previos de calificación; 2. se ha usado la solución de lavado apropiada y se ha alimentado con esta el lavador antes del uso; 3. no se han cometido errores en el

	procedimiento del ensayo al dispensar los controles; 4. el control o los pocillos en los que se ha dispensado no se han contaminado debido a derrames de muestras positivas o conjugado; 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado; 6. las agujas del lavador no están parcial o totalmente obstruidas.
Control positivo DO 450 nm < 0,500	1. el procedimiento ha sido realizado correctamente; 2. no ha habido errores durante su distribución (ejemplo, dispensación de un control incorrecto); 3. el proceso de lavado y los parámetros del lavador coinciden con los validados en los estudios previos de calificación; 4. no ha ocurrido contaminación externa del control.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 11.

P. RESULTADOS
Si la prueba es válida, los resultados se calculan a partir del valor medio de DO 450 nm /620-630 nm del control negativo (NC), mediante un valor de corte (Co) determinado con la siguiente fórmula:

Valor de corte = CN + 0,250

Nota importante: *Cuando el cálculo de los resultados se realiza mediante el sistema operativo de un equipo ELISA automático, hay que asegurarse de que la formulación usada para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS
La interpretación de los resultados se realiza mediante la relación entre el valor de DO450 nm /620-630nm de la muestra (M) y el valor de corte (Co), o 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 EBV.
Los pacientes cuya muestra resulte no concluyente deben someterse a una nueva prueba con una segunda muestra tomada 1 o 2 semanas después de la inicial.
Un resultado positivo es indicativo de infección en curso por EBV y por consiguiente el paciente debe ser tratado adecuadamente.

Notas importantes:

1. *Los resultados de la prueba EBNA IgM por si solos no son suficientes para establecer un diagnóstico claro de infección por EBV. Deben realizarse otras pruebas de detección de EBV (suministradas por Dia.Pro Diagnostic BioProbes s.r.l. con código n° EBNG.CE, EAG.CE, EAM.CE, VCAG.CE, VCAM.CE y RealTime PCR assay for EBV).*
2. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
3. *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 realizado y comunicado al paciente por un médico calificado.*

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 11).

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

Control negativo: 0,100 – 0,120 – 0,080 OD 450 nm
Valor medio: 0,100 DO 450 nm
Menor de 0,150 – Válido
Control positivo: 1,000 DO 450 nm
Mayor de 0,500 – Válido
Valor de corte = 0,100+0,250 = 0,350
Muestra 1: 0,080 DO 450 nm
Muestra 2: 1,800 DO 450 nm
Muestra1 M/Co < 1,0 = negativa
Muestra 2 M/Co > 1,2 = positiva

R. CARACTERÍSTICAS DEL RENDIMIENTO
La evaluación del rendimiento se ha realizado en un centro clínico externo en paneles de muestras positivas y negativas con respecto a un equipo de referencia.

1. Límite de detección
La Comunidad Europea no ha definido ningún estándar internacional para la prueba detección de anticuerpos EBNA IgM hasta el momento.
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 mononucleosis infecciosa.

2. Sensibilidad y especificidad diagnóstica:
La **sensibilidad diagnóstica** se ha estudiado en más de 88 muestras positivas previamente analizadas con el equipo de referencia europeo que se utiliza en el laboratorio. Se recogieron muestras positivas de pacientes con mononucleosis infecciosa aguda.
La **especificidad diagnóstica** ha sido determinada en 352 muestras negativas de individuos sanos clasificadas como negativas mediante un equipo de referencia.
Asimismo se ha analizado un panel de muestras potencialmente interferentes (RF+, infecciones no relacionadas con el virus, etc.) sin que se obtuvieran resultados falsos.
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 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 agregados.
En la evaluación del rendimiento se obtuvieron los siguientes valores:

Sensibilidad	≥ 98 %
Especificidad	≥ 98 %

3. Reproducibilidad:
Los datos obtenidos en un estudio con tres muestras de diferente reactividad EBNA IgM, examinadas en 16 réplicas en tres tandas separadas, presentaron valores CV% inferiores al

15% en general, dependiendo las lecturas de DO 450 nm/620-630 nm.
La variabilidad mostrada en las tablas no dio como resultado una clasificación errónea de las muestras.

S. LIMITACIONES

Los falsos positivos fueron estimados como menos del 2% de la población normal, debido principalmente a altos títulos de Factor Reumatoide.
La contaminación bacterica o la inactivación por calor de la muestra pueden afectar los valores de DO y por tanto alterar los niveles del analito.

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Todos los productos IVD que fabrica la empresa están sujetos a control mediante un sistema de gestión de calidad certificado conforme con la norma ISO 13485. Cada lote se somete a control de calidad y se comercializa solamente si cumple las especificaciones técnicas y los criterios de aceptación de la CE.

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

CE

HBe Ag&Ab

Enzyme Immunoassay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

- for “in vitro” diagnostic use only -

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REF. HBE.CE
96 Tests

HBe Ag&Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

The kit is intended for the follow-up of acute infection and of chronic patients under therapy.

For “in vitro” diagnostic use only.

B. INTRODUCTION

Hepatitis B “e” Antigen or HBeAg is known to be intimately associated with Hepatitis B Virus or HBV replication and the presence of infectious Dane particles in the blood.

Recently, it has been found that HBeAg is a product of proteolytic degradation of Hepatitis B core Antigen or HBcAg, occurring in hepatocites, whose expression is under the control of the precore region of HBV genome.

If HBeAg is considered a specific marker of infectivity, the presence of anti HBeAg antibodies in blood is recognised to be a clinical sign of recovery from infection to convalescence.

The determination of these two analytes in samples from HBV patients has become important for the classification of the phase of illness and as a prognostic value in the follow up of infected patients.

C. PRINCIPLE OF THE TEST

HBeAg:

HBeAg, if present in the sample, is captured by a specific monoclonal antibody, in the 1st incubation.

In the 2nd incubation, after washing, a tracer, composed of a mix of two specific anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP), is added to the microplate and binds to the captured HBeAg.

The concentration of the bound enzyme on the solid phase is proportional to the amount of HBeAg in the sample and its activity is detected by adding the chromogen/substrate in the 3rd incubation.

The presence of HBeAg in the sample is determined by means of a cut-off value that allows for the semiquantitative detection of the antigen.

HBeAb

Anti HBeAg antibodies, if present in the sample, compete with a recombinant HBeAg preparation for a fixed amount of an anti HBeAg antibody, coated on the microplate wells.

The competitive assay is carried out in two incubations, the first with the sample and recHBeAg, and the second with a tracer, composed of two anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP).

The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti HBeAg antibodies in the sample and its activity is detected by adding the chromogen/substrate in the third incubation.

The concentration of HBeAg specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti HBeAg antibodies.

D. COMPONENTS

The kit contains reagents for total 96 tests.

1. Microplate: MICROPLATE

n° 1 coated microplate

12 strips of 8 breakable wells coated with anti HBeAg specific monoclonal antibody, postcoated with bovine serum proteins and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -

1x2.0ml/vial. Ready to use control. It contains bovine serum, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is colorless.

3. Antigen Positive Control: CONTROL + Ag

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, non infectious recombinant HBeAg, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.

The positive control is green color coded.

4. Antibody Positive Control: **:** CONTROL + Ab

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, human anti HbeAg positive plasma at about 10 PEI U/ml, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The label is red colored.

The positive control is yellow color coded.

5. Antigen Calibrator: CALAG …ml

n° 1 vial. Lyophilised calibrator for HBeAg. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, non infectious recombinant HBeAg at 1 PEI U/ml +/- 10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Important 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.

6. Antibody Calibrator: CALAB …ml

n° 1 vial. Lyophilized calibrator for anti HBeAg antibody. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, positive plasma at 0.25 PEI U/ml +/-10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The label is red colored.

Important 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.

7. 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.

8. Enzyme conjugate: CONJ

1x16ml/vial. Ready to use conjugate. It contains Horseradish peroxidase conjugated with a mix of monoclonal antibodies to HBeAg, 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

The reagent is red color coded.

9. HBe Antigen: Ag-HBe

1x10ml/vial. Ready to use reagent. It contains recombinant HBeAg, fetal bovine serum, buffered solution pH 8.0+/-0.1, 0.045% ProClin 300 and 0.09% sodium azide as preservatives.

The reagent is blue color coded.

10. Chromogen/Substrate: SUBS TMB

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

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

11. Sulphuric Acid: H_2SO_4 O.3 M

1x15ml/vial. It 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 (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

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

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

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

H. PREPARATION OF COMPONENTS AND WARNINGS

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

1. 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.

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

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

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

5. Antigen 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.

6. Antibody 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.

7. 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.

8. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

9. HBe Antigen:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

10. 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.

11. 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. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the 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. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation

and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the 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.

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.

A) HBe Antigen:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 100 µl of the Negative Control in triplicate, 100 µl of the Antigen Calibrator in duplicate and then 100 µl of the Antigen Positive Control in single.
4. Then dispense 100 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked colour difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Incubate the microplate for **60 min at +37°C**.

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

7. When the first incubation is finished, wash the microwells as previously described (section I.3)
8. Dispense 100 µl Enzyme Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

9. Check that the reagent has been dispensed properly and then incubate the microplate for **60 min at +37°C**.
10. When the second incubation is finished, wash the microwells as previously described (section I.3)
11. 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.*

12. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive control and positive samples will turn from clear to blue.
13. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 11. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
14. Measure the color 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.

B) HBe Antibody:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 50 µl of the Negative Control in triplicate, 50 µl of the Antibody Calibrator in duplicate and then 50 µl of the Antibody Positive Control in single.
4. Then dispense 50 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Dispense then 50 µl of HBe Antigen in all the wells, except for A1.
7. Incubate the microplate for **60 min at +37°C**.

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

8. When the first incubation is finished, wash the microwells as previously described (section I.3)
9. Finally proceed as described for the HBeAg assay from point 8 to the last one.

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 should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

HBe antigen test

Controls and calibrator Samples	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

HBe antibody test

Controls and calibrator Samples	50 ul 50 ul
Neutralising antigen	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzymatic conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mixture	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

An example of dispensation scheme is reported below:

Microplate

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

Legenda: BLK = Blank // NC = Negative Control
PC = Positive Control // 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:

HBe Antigen

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	< 0.150 OD450nm after blanking coefficient of variation < 30%
Antigen Calibrator	S/Co > 2.0
Positive Control (PC)	> 1.500 OD450nm

HBe Antibody

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 10%
Antibody Calibrator	OD450nm < NC/1.5
Positive Control (PC)	OD450nm < NC/10

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

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

HBeAg

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.500 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.

HBe antibody

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 10%	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 (e.g.: dispensation of positive control instead of negative control; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred; 5. that micropipettes have not become contaminated with positive samples; 6. that the washer needles are not blocked or partially obstructed.

Calibrator OD450nm > NC/1.5	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 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; 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 14.

P. CALCULATION OF THE CUT-OFF
The results are calculated by means of a cut-off value determined with the following formula:

HBeAg:

NC + 0.100 = Cut-Off (Co)

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

HBeAb:

(NC + PC) / 3 = Cut-Off (Co)

***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 follows:

HBeAg:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

HBeAb:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

Note:
S = OD450nm/620-630nm of the sample
Co = cut-off value

An example of calculation for HBeAg assay is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

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

Negative Control: 0.020 – 0.030 – 0.025 OD450nm
Mean Value: 0.025 OD450nm
Lower than 0.150 – Accepted

Positive Control: 2.489 OD450nm
Higher than 1.500 – Accepted
Cut-Off = 0.025+0.100 = 0.125
Calibrator: 0.520 - 0.540 OD450nm
Mean value: 0.530 OD450nm S/Co = 4.2
S/Co higher than 2.0 – Accepted

Sample 1: 0.030 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

An example of calculation for HBeAb is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

The following data must not be used instead or 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

Cut-Off = (2.100 + 0.100) / 3 = 0.733
Calibrator: 0.720-0.760 OD450nm
Mean value: 0.740 OD450nm
OD450nm < NC/1.5 – 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

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory director to reduce the risk of judgment errors and misinterpretations.*
2. *The Identification of the clinical status of a HBV patient (acute, chronic, asymptomatic hepatitis) has to be done on the basis also of the other markers of HBV infection (HBsAg, HBsAb, HBcAb, HBcIgM);*
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 by and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

A) HBeAg

1. Limit of detection
The limit of detection of the assay has been calculated by means of the International Standard for HBeAg, supplied by Paul Erlich Institute (PEI).
The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAg
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 51, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

BBI's Accurun 51 (S/Co)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	**4.1**	**1.6**	0.9	0.6	0.4
0103/2	**4.1**	**1.7**	0.9	0.6	0.4
0303	**4.0**	**1.6**	0.9	0.5	0.4

2. Diagnostic Sensitivity:
The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from different HBV pathologies (acute, chronic) bearing HBeAg reactivity.
An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.
Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.
Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE S/Co	Abbott EIA S/Co	Sorin EIA S/Co
21	5.4	4.5	6.3
22	3.7	4.3	5.4
23	1.9	3.2	3.1
24	1.1	2.4	1.5
25	1.0	2.1	1.2
26	0.6	1.7	0.7
27	0.2	0.8	0.3
28	0.2	0.6	0.2
29	0.2	0.4	0.2
30	0.2	0.3	0.2
31	0.1	0.3	0.2
32	0.1	0.3	0.2

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for an other commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	3	3.3	7.0
2	6	17.5	21.9
3	26	30.1	37.1
4	31	29.4	23.5
5	1	1.1	2.2
6	2	2.3	6.9
7	35	30.1	24.6
8	38	29.2	31.9
9	4	16.6	10.8
10	-	0.3	0.2
11	1	3.4	3.6
12	< 1	0.2	1.2
13	< 1	0.9	1.4
14	-	0.2	0.2
15	-	0.4	0.1
16	-	0.5	0.1
17	-	0.3	0.2
18	-	0.2	0.2
19	-	0.2	0.1
20	-	0.2	0.1
21	-	0.3	1.0
22	-	0.3	0.1
23	-	0.4	0.1
24	-	0.2	0.2
25	-	0.3	0.2

3. Diagnostic Specificity:
The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a FDA approved 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 this 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 and 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 500 samples has provided a value > 98% .

4. Precision
It has been calculated on two samples examined in 16 replicate in three different runs on three lots.
The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.030	0.027	0.032	0.029
Std.Deviation	0.002	0.002	0.003	0.002
CV %	7.4	8.2	7.9	7.8

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.569	0.559	0.575	0.568
Std.Deviation	0.027	0.029	0.028	0.028
CV %	4.7	5.3	4.9	4.9
S/Co	4.4	4.4	4.4	4.4

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.033	0.031	0.030	0.032
Std.Deviation	0.003	0.003	0.002	0.003
CV %	7.9	8.5	7.4	8.0

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.565	0.573	0.568	0.569
Std.Deviation	0.026	0.025	0.024	0.025
CV %	4.7	4.3	4.2	4.4
S/Co	4.2	4.4	4.4	4.3

HBE.CE: lot # 0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.029	0.034	0.038	0.034
Std.Deviation	0.003	0.003	0.004	0.003
CV %	9.7	9.8	9.2	9.6

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.579	0.573	0.564	0.572
Std.Deviation	0.023	0.028	0.025	0.025
CV %	4.1	4.8	4.5	4.5
S/Co	4.5	4.3	4.1	4.3

B) HBe Antibody

1. Limit of detection

The limit of detection of the assay has been calculated by means of the International Standard for HBeAb, supplied by Paul Erlich Institute (PEI).

The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAb
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 52, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

Accurun 52 (Co/S)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	1.0	0.8	0.6	0.4	0.4
0103/2	1.0	0.8	0.6	0.5	0.4
0303	1.0	0.8	0.6	0.4	0.4

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive for HBeAb by a US FDA approved kit.

Positive samples were collected from different HBV pathologies bearing anti HBeAg antibody reactivity.

An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.

Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.

Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE Co/S	Abbott EIA Co/S	Sorin EIA Co/S
21	0.4	0.4	0.5
22	0.4	0.5	0.6
23	0.4	0.6	0.5
24	0.4	0.5	0.6
25	0.4	0.6	0.5
26	0.5	0.6	0.6
27	0.6	0.8	0.7
28	0.7	0.9	0.7
29	0.6	0.9	0.7
30	0.8	1.0	0.9
31	1.0	1.3	1.1
32	1.0	1.2	1.0

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for another commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	-	0.3	0.5
2	-	0.2	0.5
3	-	0.2	0.5
4	-	0.2	0.5
5	-	0.3	0.6
6	-	0.3	0.6
7	-	0.2	0.4
8	-	0.2	0.4
9	-	0.2	0.5
10	-	1.9	0.6
11	-	0.3	0.5
12	-	0.4	0.9
13	2	4.4	9.1
14	1	3.8	2.9
15	< 1	1.0	1.5
16	> 50	4.3	120.9
17	< 1	1.0	1.0
18	5	5.6	21.8
19	1	2.7	6.4
20	11	5.0	47.3
21	2	1.9	10.0
22	26	28.1	90.7
23	-	0.3	0.5
24	< 1	0.8	1.3
25	50	28.1	167.4

3. Diagnostic specificity:

The clinical specificity has been determined as described before for HBeAg.

The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

4. Precision:

It has been calculated on two samples examined in 16 replicate in three different runs on three lots.

The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.484	2.420	2.471	2.458
Std.Deviation	0.129	0.160	0.142	0.144
CV %	5.2	6.6	5.7	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.867	0.800	0.878	0.848
Std.Deviation	0.043	0.060	0.050	0.051
CV %	5.0	7.5	5.7	6.1
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.316	2.361	2.413	2.363
Std.Deviation	0.127	0.144	0.146	0.139
CV %	5.5	6.1	6.0	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.767	0.793	0.785	0.781
Std.Deviation	0.041	0.050	0.046	0.046
CV %	5.4	6.3	5.8	5.8
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot #0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.334	2.415	2.437	2.395
Std.Deviation	0.146	0.155	0.158	0.153
CV %	6.3	6.4	6.5	6.4

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.850	0.867	0.876	0.864
Std.Deviation	0.052	0.051	0.048	0.050
CV %	6.1	5.9	5.5	5.8
Co/S	0.9	1.0	1.0	1.0

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 14.

S. LIMITATIONS

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

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

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

HSV1 IgG

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

- for “in vitro” diagnostic use only -

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REF HSV1G.CE
96 Tests

HSV1 IgG

A. INTENDED USE

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

B. INTRODUCTION

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

C. PRINCIPLE OF THE TEST

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

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

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

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

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

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

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

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

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

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

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

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

F. WARNINGS AND PRECAUTIONS

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

G. SPECIMEN: PREPARATION AND WARNINGS

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

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

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

H. PREPARATION OF COMPONENTS AND WARNINGS

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

Microplate:

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

Calibration Curve

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

Control Serum

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

Wash buffer concentrate:

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

Enzyme conjugate:

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

Chromogen/Substrate:

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

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

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

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

Legenda:

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

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

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

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

L. PRE ASSAY CONTROLS AND OPERATIONS

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

M. ASSAY PROCEDURE

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

M1. QUANTITATIVE DETERMINATION:

Automated assay:

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

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

Manual assay:

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

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

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

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

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

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

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

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

Automated assay:

Proceed as described in section M1.

Manual assay:

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

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

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

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

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

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

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

General Important notes:

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

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

N. ASSAY SCHEME

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

(*) Important Notes:

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

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

Microplate

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

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

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

Microplate

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

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

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

Control that the following data are matched:

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

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

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

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

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

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL 4 ± 20%

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

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

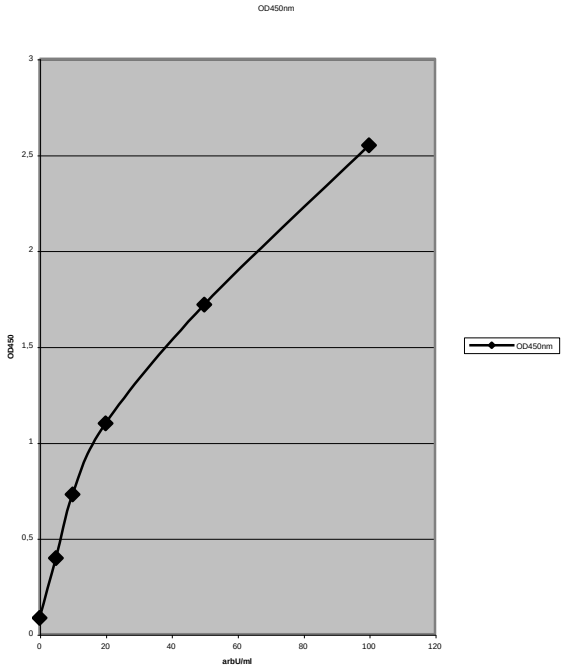
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL6), match the established requirements, the test may be considered valid.

P. RESULTS

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

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

Example of Calibration Curve :



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

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

Example of calculation:

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

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

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

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

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

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

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

R. PERFORMANCES

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

Mean OD450nm values (n = 2)

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

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

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

Mean OD450nm values (n = 2)

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

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

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

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

HSV1G.CE Lot # 1004

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

HSV1G.PU: lot 1203

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

HSV1G.PU: Lot 0204/2

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

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

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

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

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

CE

HSV1 IgM

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

- for "in vitro" diagnostic use only -

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REF HSV1M.CE
96 tests

HSV1 IgM

A. INTENDED USE

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

B. INTRODUCTION

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

C. PRINCIPLE OF THE TEST

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

D. COMPONENTS

The kit contains reagents for 96 tests.

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

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

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

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

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

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

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

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

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

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

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

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

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

F. WARNINGS AND PRECAUTIONS

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

G. SPECIMEN: PREPARATION AND WARNINGS

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

H. PREPARATION OF COMPONENTS AND WARNINGS

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

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

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

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

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

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

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

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

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

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

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

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

Legenda:

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

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

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

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

L. PRE ASSAY CONTROLS AND OPERATIONS

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

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

M. ASSAY PROCEDURE

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

M.1 Automated assay:

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

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

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

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

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

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

M. 2 Manual assay:

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

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

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

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

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

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

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

Important notes:

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

N. ASSAY SCHEME

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

(*) Important Notes:

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

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

An example of dispensation scheme is reported below:

Microplate

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

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

O. INTERNAL QUALITY CONTROL

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

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

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

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

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

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

If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.2

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

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

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

P. CALCULATION OF THE CUT-OFF

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

Cut-Off = NC + 0.250

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

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

Q. INTERPRETATION OF RESULTS

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

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

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

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

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

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

Cut-Off = 0.110+0.250 = 0.360

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

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

Important notes:

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

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

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

OD450nm values

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

2. Diagnostic sensitivity:

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

3. Diagnostic specificity:

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

4. Precision:

Results are reported as follows:

HSV1M.CE: lot # RD1

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

HSV1M.CE: lot # RD2

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

HSV1M.CE: lot # RD3

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

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

S. LIMITATIONS

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

T. CONFIRMATION TEST

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

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

Interpretation of results is carried out as follows:

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

The following table is reported for the interpretation of results

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

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

HSV1 IgM

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

- Uso exclusivo para diagnóstico "in vitro"-

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REF HSV1M.CE
96 pruebas

HSV1 IgM

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

12. Sellador adhesivo, n° 2

13. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

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

F. ADVERTENCIAS Y PRECAUCIONES.

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

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

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

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

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

Microplacas:

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

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

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

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

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

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

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

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

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

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

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

Leyenda:

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

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

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

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

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

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

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

M. PROCEDIMIENTO DEL ENSAYO.

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

M.1 Ensayo automatizado:

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

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

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

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

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

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

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

M.2 Ensayo Manual.

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

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

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

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

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

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

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

Notas generales importantes:

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

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

N. ESQUEMA DEL ENSAYO.

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

t.a.°temperatura ambiente

(*) Notas importantes:

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

A continuación se describe un ejemplo del esquema de dispensado:

Microplaca

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

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

O. CONTROL DE CALIDAD INTERNO.

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

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

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

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

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

***** Notas importantes:***

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

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

Parámetro	Exigencia
Calibrador	M/Co > 1.2

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

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

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

P. CÁLCULO DEL VALOR DE CORTE.

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

Valor de corte = CN + 0.250

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

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

Q. INTERPRETACIÓN DE LOS RESULTADOS.

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

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

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

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

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

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

Notas importantes:

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

R. PERFORMANCES.

1. Límite de detección.

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

Valores DO 450nm

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

2. Sensibilidad Diagnóstica:

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

3. Especificidad Diagnóstica :

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

4. Precisión:

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

HSV1M.CE: lote # RD1

Negativa (N = 16)

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

Débil reactiva (N = 16)

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

Altamente reactiva (N = 16)

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

HSV1M.CE: lote # RD2

Negativa (N = 16)

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

Débil reactiva (N = 16)

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

Altamente reactiva (N = 16)

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

HSV1M.CE: lote # RD3

Negativa (N = 16)

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

Débil reactiva (N = 16)

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

Altamente reactiva (N = 16)

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

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

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

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

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

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

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

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

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

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

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

CE

HSV2 IgG

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

- for “in vitro” diagnostic use only -

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REF HSV2G.CE
96 Tests

HSV2 IgG

A. INTENDED USE

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

B. INTRODUCTION

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

C. PRINCIPLE OF THE TEST

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

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

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

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

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

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

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

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

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

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

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

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

F. WARNINGS AND PRECAUTIONS

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

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

G. SPECIMEN: PREPARATION AND WARNINGS

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

H. PREPARATION OF COMPONENTS AND WARNINGS

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

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

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

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

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

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

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

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

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

Legenda:

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

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

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

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

L. PRE ASSAY CONTROLS AND OPERATIONS

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

M. ASSAY PROCEDURE

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

M1. QUANTITATIVE DETERMINATION:

Automated assay:

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

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

Manual assay:

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

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

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

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

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

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

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

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

Automated assay:

Proceed as described in section M1.

Manual assay:

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

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

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

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

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

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

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

General Important notes:

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

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

N. ASSAY SCHEME

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

(*) Important Notes:

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

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

Microplate

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

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

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

Microplate

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

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

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

Control that the following data are matched:

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

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

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

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

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

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL 4 ± 20%

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

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

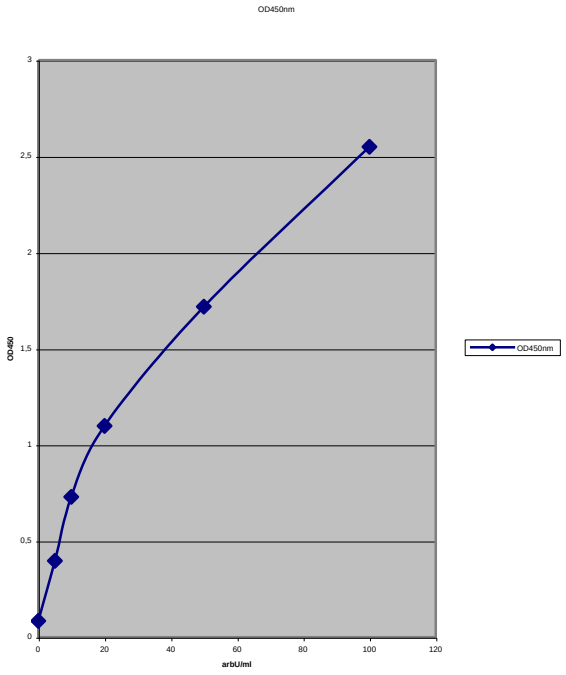
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL6), match the established requirements, the test may be considered valid.

P. RESULTS

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

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

Example of Calibration Curve :



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

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

Example of calculation:

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

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

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

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

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

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

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

R. PERFORMANCES

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

Mean OD450nm values (n = 2)

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

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

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

HSV2G.PU: lot 1103

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

HSV2G.PU: lot 1203

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

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

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

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

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

CE

HSV2 IgM

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

- for "in vitro" diagnostic use only -

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REF HSV2M.CE
96 tests

HSV2 IgM

A. INTENDED USE

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

B. INTRODUCTION

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

C. PRINCIPLE OF THE TEST

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

D. COMPONENTS

The kit contains reagents for 96 tests.

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

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

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

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

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

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

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

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

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

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

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

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

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

F. WARNINGS AND PRECAUTIONS

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

G. SPECIMEN: PREPARATION AND WARNINGS

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

H. PREPARATION OF COMPONENTS AND WARNINGS

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

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

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

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

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

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

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

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

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

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

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

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

Legenda:

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

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

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

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

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

M. ASSAY PROCEDURE

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

M.1 Automated assay:

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

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

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

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

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

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

M. 2 Manual assay:

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

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

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

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

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

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

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

Important notes:

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

N. ASSAY SCHEME

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

(*) Important Notes:

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

An example of dispensation scheme is reported below:

Microplate

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

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

O. INTERNAL QUALITY CONTROL

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

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

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

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

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

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

If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.2

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

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

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

P. CALCULATION OF THE CUT-OFF

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

Cut-Off = NC + 0.250

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

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

Q. INTERPRETATION OF RESULTS

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

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

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

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

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

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

Cut-Off = 0.100+0.250 = 0.350

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

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

Important notes:

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

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

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

OD450nm values

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

2. Diagnostic sensitivity:

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

3. Diagnostic specificity:

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

4. Precision:

Results are reported as follows:

HSV2M.CE: lot # RD1

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

HSV2M.CE: lot # RD2

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

HSV2M.CE: lot # RD3

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

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

S. LIMITATIONS

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

T. CONFIRMATION TEST

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

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

Interpretation of results is carried out as follows:

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

The following table is reported for the interpretation of results

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

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Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

HSV2 IgM

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

- Uso exclusivo para diagnóstico "in vitro"-

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REF HSV2M.CE
96 pruebas

HSV2 IgM

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

12. Sellador adhesivo, n° 2

13. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

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

F. ADVERTENCIAS Y PRECAUCIONES.

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

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

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

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

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

Microplacas:

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

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

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

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

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

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

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

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

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

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

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

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

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

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

M. PROCEDIMIENTO DEL ENSAYO.

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

M.1 Ensayo automatizado:

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

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

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

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

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

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

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

M.2 Ensayo Manual.

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

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

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

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

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

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

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

Notas generales importantes:

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

N. ESQUEMA DEL ENSAYO.

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

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

*t.a.*temperatura ambiente*

(*) Notas importantes:

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

A continuación se describe un ejemplo del esquema de dispensado:

Microplaca

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

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

O. CONTROL DE CALIDAD INTERNO.

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

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

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

En caso contrario, detenga el ensayo y compruebe:

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

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

***** Notas importantes:***

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

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

Parámetro	Exigencia
Calibrador	M/Co > 1.2

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

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

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

P. CÁLCULO DEL VALOR DE CORTE.

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

Valor de corte = CN + 0.250

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

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

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

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

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

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

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

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

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

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

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

R. PERFORMANCES.

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

Valores DO 450nm

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

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

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

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

HSV2M.CE: lote # RD1

Negativa (N = 16)

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

Débil reactiva (N = 16)

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

Altamente reactiva (N = 16)

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

HSV2M.CE: lote # RD2

Negativa (N = 16)

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

Débil reactiva (N = 16)

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

Altamente reactiva (N = 16)

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

HSV2M.CE: lote # RD3

Negativa (N = 16)

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

Débil reactiva (N = 16)

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

Altamente reactiva (N = 16)

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

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

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

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

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

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

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

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

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

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

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

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

HSV 1&2 IgG

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

- for “in vitro” diagnostic use only -

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REF HSVG.CE
96 Tests

HSV IgG

A. INTENDED USE

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

B. INTRODUCTION

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

C. PRINCIPLE OF THE TEST

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

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

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

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

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

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

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

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

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

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

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

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

F. WARNINGS AND PRECAUTIONS

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

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

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

G. SPECIMEN: PREPARATION AND WARNINGS

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

H. PREPARATION OF COMPONENTS AND WARNINGS

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

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

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

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

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

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

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

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

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

Legenda:

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

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

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

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

L. PRE ASSAY CONTROLS AND OPERATIONS

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

M. ASSAY PROCEDURE

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

M1. QUANTITATIVE DETERMINATION:

Automated assay:

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

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

Manual assay:

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

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

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

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

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

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

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

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

Automated assay:

Proceed as described in section M1.

Manual assay:

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

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

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

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

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

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

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

General Important notes:

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

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

N. ASSAY SCHEME

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

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

(*) Important Notes:

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

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

Microplate

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

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

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

Microplate

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

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

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

Control that the following data are matched:

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

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

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

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

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

**** Note:**

If Control Serum has used, verify the following data:

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

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

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

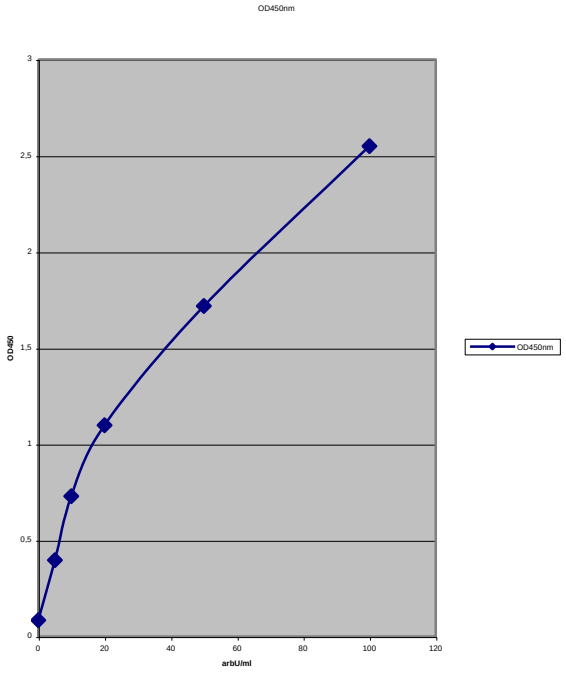
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL6), match the established requirements, the test may be considered valid.

P. RESULTS

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

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

Example of Calibration Curve :



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

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

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

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

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

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

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

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

R. PERFORMANCES

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

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

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

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

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

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

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

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

BBI Panel PTH 201 (Performance)

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

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

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

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

HSVG: lot 0603/2

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

HSVG.PU: lot 0603

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

HSVG: Lot 0403/M

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

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

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

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

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

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

CE

HSV 1&2 IgM

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

- for "in vitro" diagnostic use only -

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Fax +39 02 44386771
e-mail: info@diapro.it

REF HSVM.CE
96 tests

HSV1&2 IgM

A. INTENDED USE

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

B. INTRODUCTION

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

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

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

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

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

C. PRINCIPLE OF THE TEST

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

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

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

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

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

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE

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

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

2. Negative Control: CONTROL -

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

3. Positive Control: CONTROL +

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

The positive control is green colour coded.

4. Calibrator: CAL …ml

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

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

5. Lyophilized HSV1&2 Ag: AG HSV

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

6. Wash buffer concentrate: WASHBUF 20X

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

7. Enzyme conjugate: CONJ 20X

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

8. Antigen Diluent : AG DIL

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

9. Specimen Diluent : DILSPE

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

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

10. Chromogen/Substrate : SUBS TMB

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

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

11. Sulphuric Acid: H_2SO_4 0.3 M

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

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

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

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

F. WARNINGS AND PRECAUTIONS

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

G. SPECIMEN: PREPARATION AND WARNINGS

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

H. PREPARATION OF COMPONENTS AND WARNINGS

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

Microplate:

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

Negative Control:

Ready to use. Mix well on vortex before use.

Positive Control:

Ready to use. Mix well on vortex before use.

Calibrator:

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

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

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

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

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

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

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

Legenda:

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

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

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

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

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

M. ASSAY PROCEDURE

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

M.1 Automated assay:

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

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

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

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

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

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

M. 2 Manual assay:

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

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

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

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

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

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

9. Wash microwells as in section I.3.

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

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

Important notes:

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

N. ASSAY SCHEME

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

(*) Important Notes:

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

An example of dispensation scheme is reported below:

Microplate

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

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

O. INTERNAL QUALITY CONTROL

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

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

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

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

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

**** Important Note:**

If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co ≥ 1.0

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

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

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

P. CALCULATION OF THE CUT-OFF

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

Cut-Off = NC + 0.250

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

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

Q. INTERPRETATION OF RESULTS

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

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

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

An example of calculation is reported below:

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

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

Cut-Off = 0.090+0.250 = 0.340

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

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

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

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

OD450nm values

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

2. Diagnostic sensitivity:

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

3. Diagnostic specificity:

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

3. Precision:

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

Results are reported as follows:

HSVM.CE: lot # RD1

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

HSVM.CE: lot # RD2

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

HSVM.CE: lot # RD3

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.871	1.862	1.848	1.861
Std.Deviation	0.040	0.035	0.026	0.033
CV %	2.1	1.9	1.4	1.8

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of HSV is released to the doctor.

Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section. This reagent is called Solution A.
2. Then 25 ul concentrated Enzymatic Conjugate are diluted in 500 ul Antigen Diluent and mixed gently on vortex. Do not use any lyophilized antigen vial for this procedure ! This solution is called Solution B.
3. The well A1 of the strip is left empty for blanking.
4. The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and S/Co values.
5. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
6. The strip is incubated for 60 min at +37°C.
7. After washing, the blank well A1 is left empty.
8. 100 µl of Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl of Solution B are added to well E1.
10. The strip is incubated for 60 min at +37°C.
11. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
12. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 1.0 a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of HSV and a crossreaction with enzymatic conjugate has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value lower than 1.0 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of HSV and not due to any crossreaction.

The following table is reported for the interpretation of results

Well	**S/Co**		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.0
Interpretation	Problem of contam.	False positive	True positive

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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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CE

HBsAb

Enzyme Immunoassay for qualitative/quantitative determination of antibodies to Hepatitis B surface Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

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REF SAB.CE
96 Tests

HBs Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for both the quantitative and qualitative determination of antibodies to the Surface Antigen of Hepatitis B Virus in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B surface Antigen (HBsAg) is the major structural polypeptide of the envelope of the Hepatitis B Virus (HBV).
This antigen is composed mainly of the type common determinant "a" and the type specific determinants "d" and "y", present only on the specific serotypes.
Upon infection, a strong immunological response develops firstly against the type specific determinants and in a second time against the "a" determinant.
Anti "a" antibodies are however recognised to be most effective in the neutralisation of the virus, protecting the patient from other infections and leading it to convalescence.
The detection of HBsAb has become important for the follow up of patients infected by HBV and the monitoring of recipients upon vaccination with synthetic and natural HBsAg.

C. PRINCIPLE OF THE TEST

Microplates are coated with a preparation of highly purified HBsAg that in the first incubation with sample specifically captures anti HBsAg antibodies to the solid phase.
After washing, captured antibodies are detected by an HBsAg, labelled with peroxidase (HRP), that specifically binds the second available binding site of these antibodies.
The enzyme specifically bound to wells, by acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and can be detected by an ELISA reader.
The amount of antibodies may be quantitated by means of a standard curve calibrated against the W.H.O reference preparation.
Samples are pre treated in the well with an specimen diluent able to block interference present in vaccinated individuals.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with purified heat-inactivated HBsAg of both subtypes (ad and ay) from human origin and sealed into a bag with desiccant.
Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
5x2.0 ml/vial. Ready to use and colour coded standard curve, derived from HBsAb positive plasma titrated on WHO standard for anti HBsAg (1st reference preparation 1977, lot 17-2-77), ranging: CAL1 = 0 mIU/ml // CAL2 = 10 mIU/ml // CAL3 = 50 mIU/ml // CAL4 = 100 mIU/ml // CAL 5 = 250 mIU/ml.
Contains human serum proteins, 5% BSA, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. Standards are blue coloured.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

4. Enzyme conjugate : CONJ
1x16.0 ml/vial. Ready-to-use solution and red color coded.
It contains inactivated purified HBsAg of both subtypes ad and ay, labelled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

5. Chromogen/Substrate: SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
1x8ml. 10 mM Tris Buffered solution ph 7.4 +/-0.1,suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide as preservatives.

8. Control Serum: CONTROL …ml
1 vial. Lyophilized.
Contains fetal bovine serum proteins, human anti HBsAg antibodies calibrated at 50 ± 10% WHO mIU/ml. 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

9. Plate sealing foil n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-1°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking, strongly recommended) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-HBsAg antibody concentration is expected to be higher than 250 mIU/ml should be diluted before use either 1:10 or 1:100 in the Calibrator 0 mIU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex when preparing the diluted samples.

H. PREPARATION OF COMPONENTS AND WARNINGS

1. Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Calibration Curve
Ready to use. Mix well on vortex before use.

3. Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

4. Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

5. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

6. Specimen Diluent:
Ready to use. Mix well on vortex before use.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, air-driven dust or microbes. Do not expose to strong light, oxidising agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

8. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm$1°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Control Serum as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipments are available and ready to use.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two procedures can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 and B1 wells empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
Then Dispense in all the wells to be used for the test, except for A1 and B1, 50µl of the Specimen Diluent.

Important note: *This additive is added before distributing samples and controls into specific wells and is particularly intended for blocking some substances present in people undergoing vaccination and capable to mask antibodies.*

2. Pipette 100µl of all the Calibrators, 100µl of Control Serum in duplicate and then 100ul of samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators, Control Serum and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

Important note: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

3. Wash the microplate as reported in section I.3.

4. In all the wells except A1 and B1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important notes:

1) *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*
2) *Mix thoroughly the Enzyme Conjugate on vortex before use.*

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, mandatory), blanking the instrument on A1 and B1 wells.

M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
2. Dispense 50 ul Specimen Diluent in all the wells, except for the blank A1. Then pipette 100µl of the Calibrator 0 mIU/ml in duplicate, 100µl of the Calibrator 10 mIU/ml in duplicate, 100µl of the Calibrator 250 mIU/ml in single, and then 100ul of samples. Check that Calibrators and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.
3. Wash the microplate as reported in section I.3.
4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important notes:

1) *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*
2) *Mix thoroughly the Enzyme Conjugate on vortex before use.*

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, mandatory), blanking the instrument on A1 and B1 wells.

Important general notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME (standard procedure)

Specimen Diluent Calibrators Control Serum Samples	50 ul 100 ul 100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme in quantitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3									
B	BLK	CAL4	S4									
C	CAL1	CAL5	S5									
D	CAL1	CAL5	S6									
E	CAL2	CS	S7									
F	CAL2	CS	S8									
G	CAL3	S1	S9									
H	CAL3	S2	S10									

Legenda: BLK = Blank // CAL = Calibrators // CS = Control Serum // S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL5	S 8	S 16									
G	S1	S 9	S 17									
H	S2	S 10	S 18									

Legenda: BLK = Blank // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameters	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 WHO mIU/ml	< 0.200 OD450nm after blanking
Calibrator 10 WHO mIU/ml	OD450nm higher than the OD450nm of the Calibrator 0 mIU/ml + 0.100
Calibrator 250 WHO mIU/ml	> 1.500 OD450nm
Control Serum	OD450nm = OD450nm CAL 50 mIU/ml ± 10%
Coefficient of variation	< 30% for the Calibrator 0 mIU/ml

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 mIU/ml > 0.200 coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure when the dispensation of standards is carried out; 4. that no contamination of the Cal 0 mIU/ml or of the wells where it was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

Calibrator 10 mIU/ml OD450nm < Cal 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Calibrator 250 mIU/ml < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (e.g.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

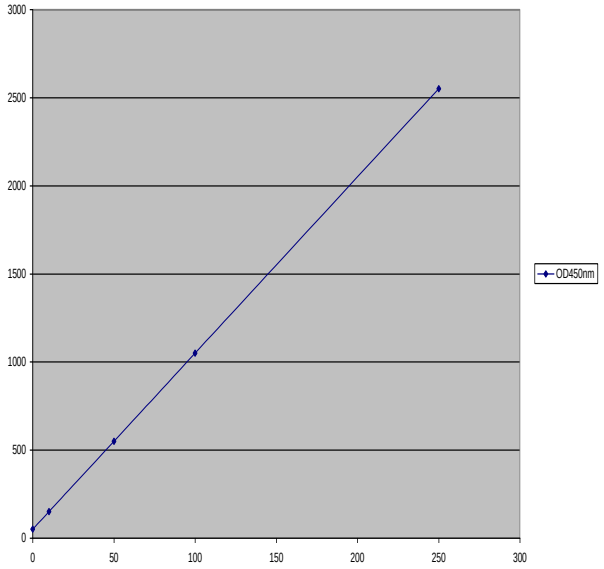
Important note:
The analysis must be done proceeding as the reading step described in the section M, point 7.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti HBsAg antibody in samples.
An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm/620-630nm values for the Calibrators 0 and 10 mIU/ml and then check that the assay is valid.

Example of calculation (data obtained proceeding as the reading step described in the section M, point 7).

The following data must not be used instead of real figures obtained by the user.

Calibrator 0 mIU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.200 – Accepted

Calibrator 10 mIU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 250 mIU/ml: 2.845 OD450nm
Higher than 1.500 – Accepted

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 10 WHO mIU/ml are considered negative for anti HBsAg antibody by most of the international medical literature.
Samples with a concentration higher than 10 WHO mIU/ml are considered positive for anti HBsAg antibody.
In the follow up of vaccination recipients, however, the value of 20 WHO mIU/ml is usually accepted by the medical literature as the minimum concentration at which the patient is considered clinically protected against HBV infection.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*

3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION:

The limit of detection of the assay has been calculated by means of the HBsAb international preparation supplied by CLB on behalf of WHO (1st reference preparation 1977, lot 17-2-77), on which Calibration Curve has been calibrated. HBV negative serum was used as diluent, as recommended by the supplier.
Results of Quality Control are given in the following table:

WHO mIU/ml	SAB.CE Lot # 1002	SAB.CE Lot # 1001	SAB.CE Lot # 1002/2
50	0.933	0.812	0.846
10	0.219	0.192	0.194
5	0.110	0.096	0.104
2.5	0.057	0.058	0.067
Std 0	0.021	0.015	0.023

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

A Performance Evaluation has been conducted on a total number of more than 700 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.
More than 500 negative specimens were tested, internally and externally, against a European company.
A diagnostic specificity of 98.8% was assessed. .
Moreover, diagnostic specificity was assessed by testing 113 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) against the European company. A value of specificity of 100% was assessed.
Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
106 vaccinated patients were evaluated providing a diagnostic sensitivity of 100%.
More than 100 HBV naturally infected patients were tested, internally and externally, against the European company; a diagnostic sensitivity of 100% was found.

3. PRECISION:

The mean values obtained from a study conducted on three samples of different anti-HBsAg reactivity, examined in 16 replicates in three separate runs is reported below:

SAB.CE: lot # 1202

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.038	0.038	0.039	0.039
Std.Deviation	0.003	0.004	0.005	0.004
CV %	8.8	9.5	11.8	10.0

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.250	0.243	0.244	0.246
Std.Deviation	0.020	0.023	0.017	0.020
CV %	8.0	9.3	7.0	8.1

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.998	3.000	3.259	3.085
Std.Deviation	0.152	0.151	0.158	0.153
CV %	5.1	5.0	4.8	5.0

SAB.CE: lot # 1002

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.048	0.048	0.050	0.049
Std.Deviation	0.005	0.004	0.006	0.005
CV %	9.4	8.4	11.5	9.8

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.249	0.252	0.242	0.248
Std.Deviation	0.021	0.020	0.023	0.021
CV %	8.3	7.9	9.6	8.6

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.544	3.653	3.612	3.603
Std.Deviation	0.153	0.176	0.138	0.156
CV %	4.3	4.8	3.8	4.3

SAB.CE: lot # 1002/2

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.050	0.051	0.050	0.050
Std.Deviation	0.005	0.006	0.006	0.005
CV %	10.0	10.9	11.9	10.9

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.226	0.238	0.239	0.234
Std.Deviation	0.015	0.017	0.018	0.016
CV %	6.5	7.0	7.5	7.0

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.526	3.457	3.499	3.494
Std.Deviation	0.137	0.143	0.162	0.147
CV %	3.9	4.1	4.6	4.2

The variability shown in the tables did not result in sample misclassification.

4. ACCURACY

The assay accuracy has been checked by the dilution and recovery tests. Any “hook effect”, underestimation likely to happen at high doses of analyte, was ruled out up to 10.000 mIU/ml.

Important note:

The performance data have been obtained proceeding as the reading step described in the section M, point 7.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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Manufacturer:
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HBsAb

Ensayo inmunoenzimático (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos frente al Antígeno de superficie del virus de la Hepatitis B en plasma y suero humanos

Uso exclusivo para diagnóstico “in vitro”

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REF SAB.CE
96 pruebas

HBs Ab

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos frente al antígeno de superficie del virus de la Hepatitis B, en plasma y suero humanos.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
La Organización Mundial de la Salud (OMS) define la infección por el virus de la Hepatitis B como:

"La Hepatitis B es una de las enfermedades más importantes que aquejan a la humanidad y constituye un problema de salud pública global. El término hepatitis significa inflamación del hígado, y la causa más común es la infección por uno de los cinco virus, denominados A, B, C, D y E. Estos virus pueden causar una enfermedad aguda cuyos síntomas persisten por varias semanas, se caracterizan por el color amarillo de la piel y los ojos (ictericia); orina oscura; fatiga extrema; náuseas; vómitos y dolor abdominal. La recuperación puede tardar de varios meses a un año. Los virus de la Hepatitis son causantes de infecciones crónicas en las que el paciente nunca se libera del virus e incluso, años más tarde, desarrolla cirrosis hepática o cáncer de hígado.
El tipo más serio de hepatitis viral es la causada por el HBV, siendo el único tipo, de los que provocan infección crónica, para el cual existe una vacuna disponible. El virus de la Hepatitis B se transmite por contacto con sangre o fluidos corporales de personas infectadas, de la misma forma que el virus de la inmunodeficiencia humana (HIV), agente causal del SIDA. Sin embargo, el HBV es entre 50 y 100 veces más infeccioso que el HIV. Las principales vías de transmisión del HBV son: (a) vía perinatal (transmisión de madre a hijo durante el parto); (b) de niño a niño; (c) mediante inyecciones y transfusiones inseguras (d) por contacto sexual.
A nivel mundial, la mayor parte de las infecciones ocurre de madre infectada a hijo, de niño a niño en hogares infantiles y por la reutilización de agujas y jeringuillas sin previa esterilización. En muchos países desarrollados (Europa Occidental y Norteamérica), el patrón de transmisión es diferente. En estos casos, la transmisión de madre a hijo y de niño a niño representaban cerca de un tercio de las infecciones crónicas antes de que se implementara el programa de vacunación infantil. Sin embargo, la mayoría de las infecciones en estos países se adquiere por la actividad sexual durante la adolescencia, y por el consumo de drogas inyectables. Por otra parte, el virus de la Hepatitis B constituye el principal riesgo en el trabajo, dentro del colectivo de los profesionales de la salud, motivo por el cual se ha aplicado la vacunación para la protección de los mismos.
El virus de la Hepatitis B no se propaga por la comida o agua contaminadas, ni por contactos casuales en el ámbito laboral. En zonas del Este y Centro de Europa se han reportado tasas elevadas de infección crónica por HBV. En el Asia Central y en regiones de la India, aproximadamente el 5% de la población está infectada de forma crónica, mientras que en Europa Occidental y Norteamérica, los índices son menores del 1%.
Los niños infectados con HBV, constituyen el grupo más susceptible a la infección crónica. Aproximadamente el 90% de los niños infectados durante el primer año de vida y entre el 30 y el 50% de los niños infectados entre 1 y 4 años, desarrollan este tipo de infección. La mortalidad por cáncer de hígado o cirrosis asociados al HBV es cerca del 25%, entre las personas que han presentado infección crónica en la niñez. En determinado grupo de pacientes, la Hepatitis B crónica es tratada con interferon, lamivudina, etc., lo cual puede ayudar en ocasiones. En algunos casos de cirrosis se han realizado trasplantes de hígado, pero el resultado ha sido variable.
La prevención de esta enfermedad a través de la vacunación, constituye la mejor opción. La vacuna contra la Hepatitis B tiene índices de seguridad y eficacia demostrados. A partir de 1982, han sido administradas mundialmente, alrededor de un billón de dosis. Se aplica por vía intramuscular en series de tres dosis. Los estudios realizados demuestran un 95% de eficacia en la prevención de la infección crónica en niños y adultos sin infección previa. En muchos países donde el índice de infección crónica en niños oscila entre 8% y 15%, se ha observado una reducción a menos del 1% en grupos de niños inmunizados. Desde 1991, la OMS ha hecho un llamado para la introducción de la vacuna contra la hepatitis B en todos los programas nacionales de vacunación."

El antígeno de superficie del virus de la Hepatitis B (HBsAg) es el elemento principal de la envoltura viral. Está compuesto fundamentalmente por el determinante común de tipo "a" y los específicos "d" e "y" específicos de serotipo.
Después de la infección se produce una respuesta inmunológica potente, primero contra los determinantes antigénicos específicos y después contra el determinante común "a". Los anticuerpos anti "a" son considerados los más eficaces en la neutralización del virus, contribuyen a la protección del paciente de otras infecciones y lo guian a la convalescencia.
La detección del HBsAb es importante para el seguimiento de los pacientes infectados por HBV y para el monitoreo de los receptores de vacunas elaboradas con el antígeno natural o sintético.

C. PRINCIPIOS DEL ENSAYO.
Los pocillos de la placa están recubiertos con una preparación del antígeno de superficie del virus de la hepatitis B, altamente purificado, que durante una primera incubación con la muestra, captura de forma específica anticuerpos anti HBsAg en la fase sólida. A continuación, previo lavado, se adiciona un HBsAg conjugado con Peroxidasa (HRP), el cual se combina de forma específica a un segundo sitio de unión disponible en estos anticuerpos. Después de la adición del substrato cromogénico y producto de la combinación del mismo con la enzima conjugada, se genera una señal coloreada proporcional a la presencia de anticuerpos al HBsAg en la muestra y puede detectarse mediante el lector ELISA. La cantidad de anticuerpos debe ser cuantificada utilizando una curva estándar calibrada, contra la referencia preparada por la O.M.S.
Las muestras son pretratadas en los pocillos con un diluente de muestras capaz de bloquear la interferencia presente en individuos vacunados.

D. COMPONENTES.
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
8x12 pocillos recubiertos con HBsAg humano correspondiente a los subtipos "ad" y "ay", inactivado por calor y purificado, en bolsas selladas con desecante. Se deben poner las placas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar a 4ºC.

2. Curva de Calibración: CAL N° …
5x2.0 ml/vial. Listo para el uso y curva con código estándar de color, elaborada a partir de plasma positivo a HBsAb, titulada según estándar de O.M.S. para anti-HBsAg (1ra preparación de referencia 1977, lote 17-2-77), con rangos: CAL1 = 0 mIU/ml // CAL2 = 10 mIU/ml // CAL3 = 50 mIU/ml // CAL4 = 100 mIU/ml // CAL 5 = 250 mIU/ml.

Contiene proteínas séricas, BSA 5%, tampón fosfato 10 mM pH 7.4+/-0.1, así como azida sódica 0.09% y 0.045% ProClin 300 como conservantes. Los estándar son de color azul.

3. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y 0.045% ProClin 300.

4. Conjugado: CONJ
1x16ml/vial. Solución lista para el uso. Codificado con el color rojo. Contiene HBsAg humano correspondiente a los subtipos "ad" y "ay", el cual ha sido inactivado por calor, purificado y marcado con HRP; BSA 5%; tampón Tris 10 mM pH 6.8+/-0.1; además de sulfato de gentamicina 0.3 mg/ml y 0.045% ProClin 300 como conservantes.

5. Cromógeno/Substrato. SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM pH 3.5-3.8, dimetilsulfóxido 4%, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02%.
Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

6. Ácido Sulfúrico: H_2SO_4 O.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Diluente de muestras : DILSPE
1x8ml. Contiene una solución tamponada Tris 10 mM pH 7.4 +/- 0.1, se recomienda en el seguimiento de vacunaciones. Contiene azida sódica 0.09% como preservativo.

8. Suero Control: CONTROL …ml
1 vial. Liofilizado.
Contiene proteínas del suero bovino fetal, anticuerpos humanos anti-HBsAg a una concentración aproximada de 50 ±10% mIU/ml (O.M.S.), además de sulfato de gentamicina 0.3 mg/ml y 0.045% ProClin 300 como conservantes.

9. Sellador adhesivo, n° 2

10. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (100µl y 50µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (tolerancia+/-1°C).
6. Lector calibrado de microplacas de ELISA con filtros de 450 nm (lectura) y filtros de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el equipo e internamente en los reactivos.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben de ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Cuando el equipo se emplea para

el pesquisaje en unidades de sangre, se recomienda el uso del código de barras.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante al menos 12 meses. Evitar congelar/descongelar cada muestra más de una vez, ya que pueden generarse partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.
6. Aquellas muestras cuya concentración de anticuerpos se espera sea mayor de 250 mIU/ml deben diluirse previamente a 1:10 o 1:100 con el Calibrador 0 mIU/ml. Las diluciones deben hacerse en tubos limpios desechables añadiendo 50 µl de muestra y 450 µl de Cal 0 (1:10). Mezclar después con ayuda del vórtex.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

1. Microplacas:
Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde, lo que indicaría un defecto de conservazión. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.
Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

2. Curva de Calibración:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

3. Suero Control:
Añadir al polvo liofilizado el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver totalmente y mezclar suavemente en el vórtex.
Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

4. Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

5. Conjugado:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

6. Diluente de muestras :
Solución lista para el uso. Mezclar bien con un vórtex antes de usar.

7. Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

8. Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra (etanol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de $\pm$5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450 nm y de un segundo filtro de 620-630 nm, obligatorio para reducir interferencias en la lectura. El

procedimiento estándar debe contemplar: a) Ancho de banda <= 10 b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección "Procedimiento del ensayo". El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.

6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones "Control interno de calidad" y "Procedimiento del ensayo". El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y las de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
4. Disolver el Suero Control como se ha descrito anteriormente.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar después suavemente en el vórtex todos los reactivos líquidos.
1. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
6. Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
7. En caso de trabajar automáticamente, conectar el equipo y comprobar que los protocolos estén correctamente programados.
8. Comprobar que las micropipetas estén fijadas en el volumen requerido.
9. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.

En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.
Pueden realizarse dos procedimientos acorde a los requerimientos del clínico.

M.1 Análisis Cuantitativo

1. Poner el número necesario de tiras en el soporte plástico. Dejar los pocillos A1 y B1 vacíos para el blanco.
Almacenar las tiras restantes en la bolsa con el desecante a temperaturas entre 2 y 8°C.
Dispensar 50µl de Diluente de Muestras en todos los pocillos, con excepción de A1 y B1.

Nota importante: *Este reactivo se adiciona antes de la distribución de las muestras y controles en los pocillos con el fin de bloquear cualquier elemento presente en el suero de personas vacunadas, lo cual pudiera enmascarar los anticuerpos.*

2. Dispensar 100µl de los Calibradores, 100µl del Suero Control por duplicado y después 100µl de las muestras. El Suero Control se emplea para verificar que el sistema analítico funciona como es debido. Comprobar que el Suero Control, los Calibradores y las muestras han sido añadidos adecuadamente. Incubar la microplaca durante **60 minutos a +37ºC.**
Nota importante*: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace la prueba manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

3. Lavar los pocillos según lo descrito previamente (sección I.3).
4. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1 y B1, controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **60 minutos a +37°C.**

Nota importante*:*
1) Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.
2) Mezclar el Conjugado con ayuda del vórtex antes de usar.

5. Lavar los pocillos según lo descrito previamente.
6. Dispensar 100µl de TMB/H_2O_2 en todos los pocillos, incluidos los del blanco. Controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **20 minutos a temperatura ambiente.**

Nota importante*: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

7. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 6. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, recomendado), calibrando el instrumento con el pocillo A1 (blanco) y B1 (blanco).

M.2 Análisis Cualitativo

1. Poner el número necesario de tiras en el soporte plástico. Dejar el pocillo A1 vacío para el blanco.
Almacenar las tiras restantes en la bolsa con el desecante a temperaturas entre 2 y 8°C.
2. Dispensar 50µl de Diluente de Muestras en todos los pocillos con excepción de A1. Dispensar 100µl del Calibrator 0 mIU/ml por duplicado, 100µl del Calibrator 10 mIU/ml por duplicado, 100µl del Calibrator 250 mIU/ml,añadir después 100µl de cada muestra. Comprobar que los Calibradores y las muestras han sido añadidos adecuadamente.
Incube la microplaca durante **60 minutos a +37ºC.**
3**.** Lavar los pocillos según lo descrito previamente (sección I.3).

4. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1, controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **60 minutos a +37°C.**

***Nota importante**:*
1. *Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.*
2. *Mezclar el Conjugado con ayuda del vórtex antes de usar.*

5. Lavar los pocillos según lo descrito previamente.
6. Dispensar 100µl de TMB/H_2O_2 en todos los pocillos, incluido el del blanco. Controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **20 minutos a temperatura ambiente.**

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

7. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 6. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas generales importantes:
1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*
3. *El suero de control (CS) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba. El suero de control (CS) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

N. ESQUEMA DEL ENSAYO (procedimiento estándar).

Diluente de Muestras Calibradores Suero Control Muestras	50 µl 100 µl 100 µl 100 µl
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2da incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H2O2	100 µl
3ra incubación	**20 min**
Temperatura	t.a.*
Acido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado en el análisis cuantitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	CAL4	M 3									
B	BL	CAL4	M 4									
C	CAL1	CAL5	M 5									
D	CAL1	CAL5	M 6									
E	CAL2	SC	M 7									
F	CAL2	SC	M 8									
G	CAL3	M1	M 9									
H	CAL3	M2	M10									

Leyenda: BL = Blanco // CAL = Calibradores // SC= Suero Control // M = Muestra

A continuación se describe un ejemplo del esquema de dispensado en el análisis cualitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 3	M 11									
B	CAL1	M 4	M 12									
C	CAL1	M 5	M 13									
D	CAL2	M 6	M 14									
E	CAL2	M 7	M 15									
F	CAL5	M 8	M 16									
G	M1	M 9	M 17									
H	M2	M 10	M 18									

Leyenda: BL = Blanco // CAL = Calibradores // M = Muestra

O. CONTROL DE CALIDAD INTERNO.
Se realiza un grupo de pruebas con los controles cada vez que se usa el equipo para verificar si el procedimiento durante el ensayo se ha realizado correctamente.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	< 0.100 DO450nm
Calibrador 0 O.M.S. mIU/ml	< 0.200 DO450nm después de leer el blanco
Calibrador 10 O.M.S. mIU/ml	DO450nm mayor que la DO450nm del Calibrador 0 mIU/ml + 0.100
Calibrador 250 O.M.S. mIU/ml	> 1.500 DO450nm
Suero Control	DO450nm = DO450nm CAL 50 mIU/ml +/-10%
Coeficiente de variación	< 30% para el Calibrador 0 mIU/ml

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.
En caso contrario, no siga adelante y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.100DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Calibrador 0 mIU/ml > 0.200 Coeficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento durante el dispensado del estándar 4. no ha existido contaminación del Cal 0 o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.

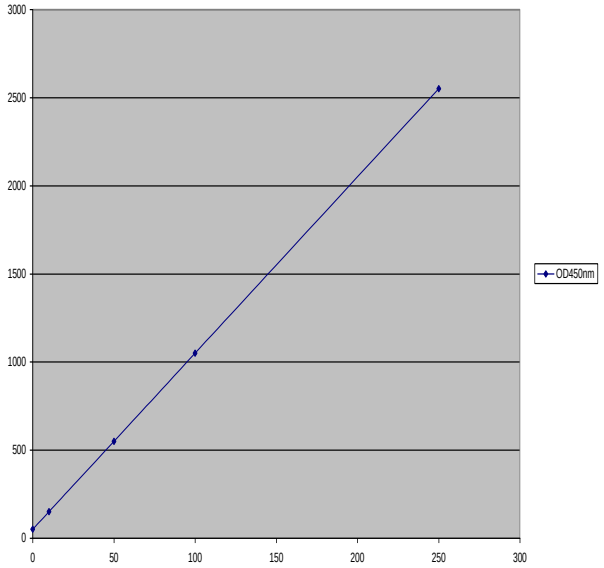
Calibrador 10 mIU/ml DO450nm < Cal 0 + 0.100	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Calibrador 250 mIU/ml < 1.500 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución. 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Suero Control Valor distinto al esperado	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar una muestra equivocada). 3. el proceso de lavado y los parámetros del lavador son correctos. 4. no ha ocurrido contaminación externa de los controles. 5. el Suero Control ha sido disuelto con el volumen correcto indicado en la etiqueta Si se indica un error, el ensayo debe repetirse tras eliminar la causa del mismo. En caso de no encontrar un error, procédase como sigue: a) si se obtiene un valor hasta +/-20%: la precisión global del laboratorio podría no permitir alcanzar +/-10% del valor esperado. Comunicar el problema al responsable para aceptar ó rechazar este resultado. b) si se obtiene un valor superior a +/-20%: en este caso el test es inválido y hay que avisar al servicio de atención al cliente de DiaPro

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 7.

P. RESULTADOS.

P.1 Método cuantitativo.
Si el ensayo resulta válido, usar para el método cuantitativo un programa de ajuste de curva para diseñar la curva de calibración con los valores obtenidos en la lectura a 450nm / 620-630nm (se sugiere interpolar 4 parámetros). Después calcular sobre la curva de calibración la concentración de anticuerpos anti-HBsAg presentes en la muestra.
A continuación, un ejemplo de curva de calibración:

Ejemplo de curva de calibración:



Nota Importante:
No usar la curva anterior para formular los cálculos.

P.2 Método cualitativo.
En el método cualitativo, calcular los valores medios de DO450nm para los Calibradores 0 y 10 mIU/ml, después comprobar que el ensayo es válido.

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 7):

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Calibrador 0 mIU/ml: 0.020 – 0.024 DO450nm
Valor medio: 0.022 DO450nm
Menor de 0.200 – Válido

Calibrador 10 mIU/ml: 0.250 – 0.270 DO450nm
Valor medio: 0.260 DO450nm
Mayor de Cal 0 + 0.100 – Válido

Calibrador 250 mIU/ml: 2.845 DO450nm
Mayor de 1.500 – *Válido*

Q. INTERPRETACION DE LOS RESULTADOS.
Las muestras con una concentración menor de 10 O.M.S. mIU/ml se consideran negativas a anti- HBsAg en la mayoría de la literatura médica internacional.
Las muestras con una concentración mayor de 10 O.M.S. mIU/ml se consideran positivas a anti- HBsAg.
En el seguimiento de receptores de vacunas, sin embargo, se aceptan por la literatura médica valores de 20 O.M.S. mIU/m como la concentración mínima a la que un paciente es considerado clínicamente protegido contra la infección por HBV.

Notas importantes:
1. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*

2. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
3. *El diagnóstico de infección con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.*

R. FUNCIONAMIENTO.
La evaluación del funcionamiento ha sido realizada según lo reportado en las Especificaciones Técnicas Comunes (ETC) (art. 5, Capítulo 3 de las Directivas IVD 98/79/EC).

1. LÍMITE DE DETECCIÓN.
El límite de detección del ensayo ha sido calculado por medio de una preparación estándar de referencia para HBsAb suministrada por CLB respaldado por O.M.S. (1ra preparación de referencia 1977, lote 17-2-77). Como diluente se empleó suero negativo a HBV, según lo recomendado por el fabricante.

La siguiente tabla muestra los resultados del Control de Calidad:

O.M.S. mIU/ml	SAB.CE Lote # 1002	SAB.CE Lote # 1001	SAB.CE Lote # 1002/2
50	0.933	0.812	0.846
10	0.219	0.192	0.194
5	0.110	0.096	0.104
2.5	0.057	0.058	0.067
Std 0	0.021	0.015	0.023

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICAS.
La evaluación del procedimiento diagnóstico se realizó mediante un ensayo con más de 700 muestras.

2.1 Especificidad Diagnóstica.
Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico. Se examinaron más de 500 muestras negativas de origen interno y externo, contra la referencia de una compañía europea. Se obtuvo una especificidad diagnóstica del 98.8%.
También contra esta referencia se analizaron 113 muestras que pudieran provocar interferencia (por ejemplo: otras enfermedades infecciosas, pacientes afectados por hepatitis no virales, pacientes sometidos a diálisis, mujeres embarazadas, hemofílicos, lipémicos, etc.). La especificidad obtenida fue del 100%.
Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

2.2 Sensibilidad Diagnóstica.
Se define como la probabilidad del ensayo de detectar positivos en presencia del analito específico.
Se evaluaron 106 pacientes vacunados, la sensibilidad diagnóstica fue del 100%.
Se probaron (interna y externamente) contra la referencia de la compañía europea, muestras de más de 100 pacientes infectados de manera natural con HBV. La sensibilidad diagnóstica fue del 100%.

3. PRECISIÓN.
Se realizó un estudio con 3 muestras de diferente reactividad anti-HBsAg, examinadas en 16 réplicas, en tres corridas separadas.
Los valores medios obtenidos se reportan a continuación :

SAB.CE: lote # 1202

Calibrador 0 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.038	0.038	0.039	0.039
Desviación estándar	0.003	0.004	0.005	0.004
CV %	8.8	9.5	11.8	10.0

Calibrador 10 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.250	0.243	0.244	0.246
Desviación estándar	0.020	0.023	0.017	0.020
CV %	8.0	9.3	7.0	8.1

Calibrador 250 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.998	3.000	3.259	3.085
Desviación estándar	0.152	0.151	0.158	0.153
CV %	5.1	5.0	4.8	5.0

SAB.CE: lote # 1002

Calibrador 0 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.048	0.048	0.050	0.049
Desviación estándar	0.005	0.004	0.006	0.005
CV %	9.4	8.4	11.5	9.8

Calibrador 10 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.249	0.252	0.242	0.248
Desviación estándar	0.021	0.020	0.023	0.021
CV %	8.3	7.9	9.6	8.6

Calibrador 250 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	3.544	3.653	3.612	3.603
Desviación estándar	0.153	0.176	0.138	0.156
CV %	4.3	4.8	3.8	4.3

SAB.CE: lote # 1002/2

Calibrador 0 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.050	0.051	0.050	0.050
Desviación estándar	0.005	0.006	0.006	0.005
CV %	10.0	10.9	11.9	10.9

Calibrador 10 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.226	0.238	0.239	0.234
Desviación estándar	0.015	0.017	0.018	0.016
CV %	6.5	7.0	7.5	7.0

Calibrador 250 mIU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	3.526	3.457	3.499	3.494
Desviación estándar	0.137	0.143	0.162	0.147
CV %	3.9	4.1	4.6	4.2

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

4. EXACTITUD.

La exactitud del ensayo ha sido comprobada mediante diluciones y pruebas de recuperación. Cualquier "efecto gancho", estimación errónea que puede presentarse a elevadas dosis del analito, no se manifiesta hasta 10.000 mIU/ml.

Nota importante:

Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 7.

S. LIMITACIONES DEL PROCEDIMIENTO.

La contaminación bacteriana de las muestras o la inactivación por calor pueden modificar los valores de absorbancia con la consiguiente alteración de los niveles del analito. Este ensayo es adecuado solo para el análisis de muestras individuales y no para mezclas.

El diagnóstico de una enfermedad infecciosa no se debe formular en base al resultado de un solo ensayo, sino que es necesario tomar en consideración la historia clínica y la sintomatología del paciente así como otros datos diagnósticos.

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CE
0318

HBsAg one

Version ULTRA

Fourth generation Enzyme Immunoassay (ELISA) for the determination of Hepatitis B surface Antigen or HBsAg in human serum and plasma

- for “in vitro” diagnostic use only -

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REF SAG1ULTRA.CE
96/192/480/960 Tests

HBsAg One version ULTRA

A. INTENDED USE

Fourth generation Enzyme Immunoassay (ELISA) for the one-step determination of Hepatitis B surface Antigen or HBsAg in human plasma and sera.
The kit is intended for the screening of blood units, is able to detect HBsAg mutants and finds application in the follow-up of HBV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood. Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programs."

Hepatitis B surface Antigen or HBsAg is the most important protein of the envelope of Hepatitis B Virus, responsible for acute and chronic viral hepatitis.
The surface antigen contains the determinant "a", common to all the known viral subtypes, immunologically distinguished by two distinct subgroups (ay and ad).
The ability to detect HBsAg with high sensitive immunoassays in the last years has led to an understanding of its distribution and epidemiology worldwide and to radically decrease the risk of infection in transfusion.

C. PRINCIPLE OF THE TEST

A mix of mouse monoclonal antibodies specific to the determinants "a", "d" and "y" of HBsAg is fixed to the surface of microwells. Patient's serum/plasma is added to the microwell together with a second mix of mouse monoclonal antibodies, conjugated with Horseradish Peroxidase (HRP) and directed against a different epitope of the determinant "a" and against "preS".
The specific immunocomplex, formed in the presence of HBsAg in the sample, is captured by the solid phase.
At the end of the one-step incubation, microwells are washed to remove unbound serum proteins and HRP conjugate.
The chromogen/substrate is then added and, in the presence of captured HBsAg immunocomplex, the colorless substrate is hydrolyzed by the bound HRP conjugate to a colored end-product. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.
The color intensity is proportional to the amount of HBsAg present in the sample.
The version ULTRA is particularly suitable for automated screenings and is able to detect "s" mutants.

D. COMPONENTS

The standard configuration contains reagents to perform 192 tests and is made of the following components:

1. Microplate MICROPLATE
n° 2. 12 strips of 8 breakable wells coated with anti HBsAg, affinity purified mouse monoclonal antibodies, specific to "a", "y" and "d" determinants, and sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains goat serum, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The negative control is pale yellow color coded.

3. Positive Control CONTROL +
1x4.0ml/vial. Ready to use control. It contains goat serum, non infectious recombinant HBsAg, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The positive control is color coded green.

4. Calibrator CAL ….
n° 2 vials. Lyophilized calibrator. To be dissolved with EIA grade water as reported in the label. Contains fetal bovine serum, non infectious recombinant HBsAg at 0.5 IU/ml (2nd WHO international standard for HBsAg, NIBSC code 00/588), 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% 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
2x60ml/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 Diluent CONJ DIL
2x16ml/vial. Ready to use and pink/red color coded regent. It contains 10 mM Tris buffer pH 6.8+/-0.1, 1% normal mouse serum, 5% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives. The solution is normally opalescent.

7. Enzyme Conjugate CONJ 20X
2x1ml/vial. 20X concentrated reagent. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibodies to HBsAg, determinant "a" and "preS", 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

8. Chromogen/Substrate SUBS TMB
2x25ml/bottle. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid H_2SO_4 O.3 M
1x25ml/bottle. It contains 0.3 M H2SO4 solution.
Note: Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foils n° 4

11. Package insert

Important note:
Only upon specific request , Dia.Pro can supply reagents for 96, 480, 960 tests , as reported below**:**

Microplates	N°1	N°5	N°10
Negative Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Positive Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Calibrator	N° 1 vial	N° 5 vials	N° 10 vials
Wash buffer concentrate	1x60ml/vial	5x60ml/vial	4x150ml/vial
Enzyme conjugate	1x0.8ml/vial	1x4ml/vial	2x4ml/vial
Conjugate Diluent	1x16ml/vial	2x40ml/vial	2x80ml/vial
Chromogen/Substrate	1x25ml/vial	3x42ml/vial	2x125ml/vial
Sulphuric Acid	1x15ml/vial	2x40ml/vial	2x80ml/vial
Plate sealing foils	N° 2	N° 10	N° 20
Package insert	N° 1	N° 1	N° 1
Number of tests	96	480	960
Code SAG1ULTRA.CE	96	480	960

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), capable to provide shaking at 1300 rpm+/-150, set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated 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..
15. 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.
16. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND 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. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and lipemic ("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 well as they could give rise to false positive results. Specimens with an altered pathway of coagulation, presenting particles after blood collection and preparation of serum/plasma as those coming from hemodialized patients, could give origin to false positive 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 sample should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If some turbidity is present or presence of microparticles is suspected after thawing, filter the sample on a disposable 0.2-0.8u filter to clean it up for testing or use the two-steps alternative method.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 6 months.

1. Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a decrease in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use. The positive control does not contain any infective HBV as it is composed of recombinant synthetic HBsAg.

4. 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. The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.

5. Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.

Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
The working solution is prepared by diluting the 20X concentrated reagent into the Conjugate
Mix well on vortex before use.
Avoid any contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic sterile disposable containers.
Important note*: The working solution is not stable. Prepare only the volume necessary for the work of the day. As an example when the kit is used in combination with other instruments or manually, dilute 0.1 ml 20X Conjugate with 1.9 ml Conjugate Diluent into a disposable plastic vial and mix carefully before use.*

7. Chromogen/Substrate:
Ready to use. Mix well by end-over-end mixing.
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 by end-over-end mixing.
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 ±1°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. In case of **shaking** during incubations, the instrument has to ensure 350 rpm ±150. Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.
4. 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.

5. **Incubation times** have a tolerance of $\pm$5%.
6. The **microplate reader** has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; (d) 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.
7. When using **ELISA automated workstations**, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) 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 by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing. The carry over effect 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.
8. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
9. **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 essential requirements of the assay. Support is also provided for the installation of new instruments to be used in combination with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dilute the 20X concentrated Enzyme Conjugate with its Diluent as reported.
5. Dissolve the Calibrator as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. 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.
8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. 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.

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense first 150 ul controls & calibrator, then all the samples and finally 100 ul diluted Enzyme Conjugate.
For the pre-washing step (point 1 of the assay procedure) and all 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. Place the required number of strips in the plastic holder and wash them once to hydrate wells. Carefully identify the wells for controls, calibrator and samples.

Important note: *Pre washing (1 cycle: dispensation of 350ul/well of washing solution+ aspiration) is fundamental to obtain reliable and specific results both in the manual and in the automatic procedures. Do not omit it !*

2. Leave the A1 well empty for blanking purposes.
3. Pipette 150µl of the Negative Control in triplicate, 150ul of the Calibrator in duplicate and then 150ul of the Positive Control in single followed by 150ul of each of the samples.
4. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).
5. Dispense 100ul diluted Enzymatic Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.*

6. Following addition of the conjugate, check that the color of the samples have changed from yellowish to pink/red and then incubate the microplate for **120 min at +37°C**.

Important notes:

a. 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.
b. If the procedure is carried out on shaking, be sure to deliver the rpm reported for in Section I.3 as otherwise intra-well contamination could occur.

7. When the first incubation is over, wash the microwells as previously described (section I.4)
8. Pipette 200 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.*

9. Incubate the microplate protected from light at **18-24°C for 30 min.** Wells dispensed with the positive control, the calibrator and positive samples will turn from clear to blue.
10. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 8. Addition of the acid solution will turn the positive control, the calibrator and positive samples from blue to yellow/brown.
11. Measure the color intensity of the solution in each well, as described in section I.6 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

Important general notes:

1. *Ensure that no fingerprints or dust are present on the external bottom of the microwell before reading. They could generate false positive results on reading.*
2. *Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.*
3. *When samples to be tested are not surely clean or have been stored frozen, the assay procedure reported below is recommended as long as it is far less sensitive to interferences due to hemolysis, hyperlipaemia, bacterial contamination and fibrin microparticles. The assay is carried out in two-steps at +37°C on shaking at* 350 rpm ±150 *as follows:*
   - *dispense 100 ul of controls, calibrator and samples*
   - *incubate 60 min at +37°C on shaking*
   - *wash according to instructions (section I.4)*
   - *dispense 100 ul diluted enzyme tracer*
   - *incubate 30 min at +37°C on shaking*
   - *wash*
   - *dispense 100 ul TMB&H2O2 mix*
   - *incubate 30 min at r.t. on shaking*
   - *stop and read*

   In this procedure the pre-wash can be omitted.
   This method shows performances similar to the standard one and therefore can be used in alternative.
4. *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

Operations	Procedure
Pre-Washing step	n° 1 cycle
Controls&Calibrator&samples Diluted Enzyme Conjugate	150 ul 100 ul
1st incubation	**120 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	200ul
2nd incubation	**30 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported in the following section:

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 results are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator 0.5 IU/ml	S/Co ≥ 2
Positive Control	> 1.000 OD450nm value

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 (NC) > 0.050 OD450nm after blanking	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 the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of 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	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 < 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. In this case, the negative control will have an OD450nm value > 0.050). 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 11.

P. CALCULATION OF THE CUT-OFF
The test results are calculated by means of a cut-off value determined on the mean OD450nm/620-630nm value of the negative control (NC) with the following formula:

NC + 0.050 = Cut-Off (Co)

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 (S) and the Cut-Off value (Co), mathematically 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 HBV and that the blood unit may be transfused.
Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample; the blood unit should not be transfused.
A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0.8 u filter to remove any microparticles interference. Then, if still positive, the sample has to be submitted to a confirmation test before a diagnosis of viral hepatitis is released.*
3. *When test results are transmitted from the laboratory to another department, 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 11):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.012 – 0.008 – 0.010 OD450nm
Mean Value: 0.010 OD450nm
Lower than 0.050 – Accepted
Positive Control: 2.489 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.010+0.050 = 0.060
Calibrator: 0.350 - 0.370 OD450nm
Mean value: 0.360 OD450nm S/Co = 6.0
S/Co higher than 2.0 – Accepted
Sample 1: 0.028 OD450nm
Sample 2: 1.690 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). Version ULTRA proved to be at least equivalent to the original design in a study conducted for the validation of the new version.

1. Analytical Sensitivity
The limit of detection of the assay has been calculated on the 2nd WHO international standard, NIBSC code 00/588.
In the following table, results are given for three lots (P1, P2 and P3) of the version ULTRA in comparison with the reference device (Ref.):

WHO IU/ml	Lot # P1 S/Co	Lot # P2 S/Co	Lot # P3 S/Co	Ref. S/Co
0.4	**4.6**	**4.8**	**4.6**	**4.6**
0.2	**2.3**	**2.4**	**2.4**	**2.4**
0.1	**1.4**	**1.4**	**1.5**	**1.2**
0.05	0.8	0.8	***1.0***	0.7
0.025	0.6	0.6	0.6	0.4
FCS (NC)	0.3	0.2	0.3	0.1

The assay shows an Analytical Sensitivity better than 0.1 WHO IU/ml of HBsAg.

In addition two panels of sensitivity supplied by EFS, France, and by SFTS, France, were tested and gave in the best conditions the following results:

Panel EFS Ag HBs HB1-HB6 lot n° 04

Sample ID	Characteristics	ng/ml	S/Co
HB1	diluent	/	0,2
HB2	adw2+ayw3	0.05	0,6
HB3	adw2+ayw3	0.1	**1,0**
HB4	adw2+ayw3	0.2	**1,8**
HB5	adw2+ayw3	0.3	**2,4**
HB6	adw2+ayw3	0.5	**4,2**

Sensitivity panel SFTS, France, Ag HBs 2005

Sample ID	Characteristics	ng/ml	S/Co
171	Adw2 + ayw3	2.21 ± 0.15	**15,4**
172	Adw2 + ayw3	1.18 ± 0.10	**8,7**
173	Adw2 + ayw3	1.02 ± 0.05	**6,1**
174	Adw2 + ayw3	0.64 ± 0.04	**4,0**
175	Adw2 + ayw3	0.49 ± 0.03	**3,4**
176	Adw2 + ayw3	0.39 ± 0.02	**2,6**
177	Adw2 + ayw3	0.25 ± 0.02	**2,0**
178	Adw2 + ayw3	0.11 ± 0.02	**1,3**
179	Adw2 + ayw3	0.06 ± 0.01	***0,9***
180	Adw2 + ayw3	0.03 ± 0.01	0,8
181	Adw2	0.5 – 1.0	**4,7**
182	Adw4	0.5 – 1.0	**3,6**
183	Adr	0.5 – 1.0	**4,5**
184	Ayw1	0.5 – 1.0	**5,1**
185	Ayw2	0.5 – 1.0	**6,4**
186	Ayw3	0.5 – 1.0	**7,3**
187	Ayw3	0.5 – 1.0	**5,8**
188	Ayw4	0.5 – 1.0	**6,9**
189	Ayr	0.5 – 1.0	**6,1**
190	diluent	/	0,6

The panel # 808, supplied by Boston Biomedical Inc., USA, was also tested to define the limit of sensitivity.
Results in the best conditions are as follows :

BBI panel PHA 808

Sample ID	Characteristics	ng/ml	S/Co
01	ad	2,49	**10,2**
02	ad	1,17	**4,8**
03	ad	1,02	**4,3**
04	ad	0,96	**3,8**
05	ad	0,69	**2,9**
06	ad	0,50	**2,2**
07	ad	0,41	**1,5**
08	ad	0,37	**1,3**
09	ad	0,30	**1,2**
10	ad	0,23	***1,0***
11	ay	2,51	**11,2**
12	ay	1,26	**5,9**
13	ay	0,97	**4,1**
14	ay	0,77	**3,7**
15	ay	0,63	**2,0**
16	ay	0,48	**2,4**
17	ay	0,42	**2,0**
18	ay	0,33	**1,8**
19	ay	0,23	**1,6**
20	ay	0,13	**1,1**
21	negative	/	0,6

2. Diagnostic Sensitivity:
The diagnostic sensitivity was tested according to what required by Common Technical Specifications (CTS) of the directive 98/79/EC on IVD for HBsAg testing.
Positive samples, including HBsAg subtypes and a panel of “s” mutants from most frequent mutations, were collected from different HBV pathologies (acute, a-symptomatic and chronic hepatitis B) or produced synthetically, and were detected positive in the assay.
All the HBsAg known subtypes, “ay” and “ad”, and isoforms “w” and “r”, supplied by CNTS, France, were tested in the assay and determined positive by the kit as expected.
An overall value of 100% has been found in a study conducted on a total number of more than 400 samples positive with the original reference IVD code SAG1.CE, CE marked.
A total of 30 sero-conversions were studied, most of them produced by Boston Biomedica Inc., USA.
Results obtained by examining eight panels supplied by Boston Biomedica Inc., USA, are reported below for the version ULTRA in comparison with the reference device code SAG1.CE.

Panel ID	1st sample positive	HBsAg subtype	HBsAg ng/ml	Version ULTRA S/Co	Ref. device S/Co
PHM 906	02	ad	0.5	**3.7**	**1.4**
PHM 907 (M)	06	ay	1.0	**4.4**	**2.9**
PHM 909	04	ad	0.3	**1.2**	0.8
PHM 914	04	ad	0.5	**1.1**	**1.1**
PHM 918	02	ad	0.1	**1.8**	0.5
PHM 923	03	ay	< 0.2	**2.2**	**1.2**
PHM 925	03	Ind.	n.d.	**1.4**	*0.9*
PHM 934	01	ad	n.d.	**1.0**	0.8

3. Diagnostic Specificity:
It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where more than 5000 negative samples from blood donors (two blood centers), classified negative with a CE marked device in use at the laboratory of collection were examined, the diagnostic specificity was recently assessed by testing a total of 2288 negative blood donors on seven different lots. A value of specificity of 100% was found.
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.

4. Precision:
It has been calculated for the version ULTRA on two samples examined in 16 replicates in 3 different runs for three lots.
Results are reported in the following tables:

Average values Total n = 144	Negative Sample	Calibrator 0.5 IU/ml
OD450nm	0.026	0.332
Std.Deviation	0.004	0.027
CV %	16%	8%

The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS

Repeatable false positive results were assessed on freshly collected specimens in less than 0.1% of the normal population, mostly due to high titers Heterophilic Anti Mouse Antibodies (HAMA).

Interferences in fresh samples were also observed when they were not particles-free or were badly collected (see chapter G).

Old or frozen samples, presenting fibrin clots, crioglobulins, lipid-containing micelles or microparticles after storage or thawing, can 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 approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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HBsAg one

Versión ULTRA

**Ensayo inmunoenzimático de cuarta generación (ELISA)
para la determinación de
antígeno de superficie de la hepatitis B o HBsAg
en plasma y suero humanos**

- Uso exclusivo para diagnóstico “in vitro”-

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REF SAG1ULTRA.CE
96/192/480/960 pruebas

HBsAg One versión ULTRA

A. OBJETIVO DEL EQUIPO.

Ensayo inmunoenzimático de cuarta generación (ELISA) para la determinación en un paso del antígeno de superficie de la hepatitis B o HBsAg en plasma y suero humanos.
El equipo está diseñado para el cribado en unidades de sangre, es capaz de detectar mutantes de HBsAg y puede aplicarse al seguimiento de pacientes infectados con HBV.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.

La Organización Mundial de la Salud (OMS) define la infección con virus de hepatitis B del siguiente modo:

"La Hepatitis B es una de las enfermedades más importantes que aquejan a la humanidad y constituye un problema de salud pública global. El término hepatitis significa inflamación del hígado y la causa más común es la infección por uno de los cinco virus, denominados A, B, C, D y E. Estos virus pueden causar una enfermedad aguda cuyos síntomas persisten por varias semanas, se caracterizan por el color amarillo de la piel y los ojos (ictericia); orina oscura; fatiga extrema; náuseas; vómitos y dolor abdominal. La recuperación puede tardar de varios meses a un año. Los virus de la Hepatitis son causantes de infecciones crónicas en las que el paciente nunca se libera del virus e incluso, años más tarde, desarrolla cirrosis hepática o cáncer de hígado.

El tipo más serio de hepatitis viral es la causada por el HBV, siendo el único tipo, de los que provocan infección crónica, para el cual existe una vacuna disponible. El virus de la Hepatitis B se transmite por contacto con sangre o fluidos corporales de personas infectadas, de la misma forma que el virus de la inmunodeficiencia humana (HIV), agente causal del SIDA. Sin embargo, el HBV es entre 50 y 100 veces más infeccioso que el HIV. Las principales vías de transmisión del HBV son: (a) vía perinatal (transmisión de madre a hijo durante el parto); (b) de niño a niño; (c) mediante inyecciones y transfusiones inseguras (d) por contacto sexual.

A nivel mundial, la mayor parte de las infecciones ocurre de madre infectada a hijo, de niño a niño en hogares y por la reutilización de agujas y jeringuillas sin previa esterilización. En muchos países en desarrollo, casi todos los niños se infectan con el virus. En muchos países desarrollados (Europa Occidental y Norteamérica), el patrón de transmisión es diferente. En estos países, la transmisión de madre a hijo y de niño a niño representaban cerca de un tercio de las infecciones crónicas antes de que se implantara el programa de vacunación infantil. Sin embargo, la mayoría de las infecciones en estos países se adquiere por la actividad sexual durante la adolescencia, y por el consumo de drogas inyectables. Por otra parte, el virus de la hepatitis B constituye el principal riesgo en el trabajo, dentro del colectivo de los profesionales de la salud, motivo por el cual se ha aplicado la vacunación para la protección de los mismos.

El virus de la hepatitis B no se trasmite por la comida o agua contaminadas, ni por contactos casuales en el ámbito laboral. En zonas del Este y Centro de Europa se han encontrado tasas elevadas de infección crónica por HBV. En el Asia Central y en regiones de la India, aproximadamente el 5% de la población está infectada de forma crónica, mientras que en Europa Occidental y Norteamérica, los índices son menores del 1%.

Los niños infectados con HBV, constituyen el grupo más susceptible a la infección crónica. Aproximadamente el 90% de los niños infectados durante el primer año de vida y entre el 30 y el 50% de los niños infectados entre 1 y 4 años, desarrollan este tipo de infección. La mortalidad por cáncer de hígado o cirrosis asociados al HBV es cerca del 25%, entre las personas que han adquirido la infección crónica en la niñez. En ciertos pacientes, la hepatitis B crónica es tratada con interferones o lamivudinas, lo cual puede ayudar en ocasiones. En algunos casos de cirrosis se han realizado trasplantes de hígado, pero el resultado ha sido variable. La prevención de esta enfermedad a través de la vacunación constituye la mejor opción.

La vacuna contra la Hepatitis B tiene índices de seguridad y eficacia demostrados. A partir de 1982, han sido administradas mundialmente alrededor de mil millones de dosis. Se aplica por vía intramuscular en series de tres dosis. Los estudios realizados demuestran un 95% de eficacia en la prevención de la infección crónica en niños y adultos sin infección previa. En muchos países donde el índice de infección crónica en niños oscila entre 8% y 15%, se ha observado una reducción a menos del 1% en grupos de niños inmunizados. Desde 1991, la OMS ha hecho un llamamiento para la introducción de la vacuna contra la hepatitis B en todos los programas nacionales de vacunación."
El antígeno de superficie de la hepatitis B o HBsAg es la proteína más importante de la envoltura del virus, responsable de las hepatitis virales agudas y crónicas.
Contiene el determinante "a", común a todos los subtipos virales conocidos, dividido inmunológicamente en dos subgrupos distintos (ay y ad).
En los últimos años la posibilidad de detectar el HBsAg mediante inmunoensayos altamente sensibles, ha permitido comprender su distribución y epidemiología en el mundo así como la gran disminución del riesgo de infección por transfusiones.

C. PRINCIPIOS DEL ENSAYO.

La superficie de los pocillos está recubierta con una mezcla de anticuerpos monoclonales de ratón específicos para los determinantes "a", "d" e "y" de HBsAg. El suero/plasma del paciente se adiciona al pocillo conjuntamente a una segunda mezcla de anticuerpos monoclonales de ratón conjugada con peroxidasa (HRP) y dirigido contra un epitopo diferente del determinante "a" y contra "preS".
El inmunocomplejo específico, formado en presencia del HBsAg de la muestra, queda capturado en la fase sólida.
Terminada la incubación de un solo paso, los pocillos son lavados para eliminar las proteínas séricas no ligadas y el conjugado HRP.
Después se añade el substrato/ cromogénico, que en presencia del inmunocomplejo de HBsAg capturado, el substrato incoloro es hidrolizado por el conjugado HRP unido, generando un producto final coloreado. Después de bloquear la reacción enzimática, su densidad óptica se mide en un lector ELISA.
La intensidad del color es proporcional a la cantidad de HBsAg presente en la muestra.
La versión ULTRA es especialmente idónea para cribados automatizados y es capaz de detectar mutantes "s".

D. COMPONENTES.

La configuración estándar contiene reactivos suficientes para realizar 192 pruebas y está formada por los siguientes componentes:

1. Microplaca MICROPLATE
n° 2. 12 tiras de 8 pocillos rompibles recubiertos con anticuerpos monoclonales de ratón, purificados por afinidad, anti HBsAg, específicos para determinantes "a", "y" y "d" en una bolsa sellada con desecante.

2. Control negativo CONTROL -
1x4.0 ml/vial. Listo para el uso. Contiene suero de cabra, tampón fosfato 10 mM pH 7.4 +/- 0.1, así como azida sódica 0.09% y ProClin 300 0.045% como conservantes. El control negativo está codificado con color amarillo pálido.

3. Control positivo CONTROL +
1x4.0 ml/vial. Listo para el uso. Contiene suero de cabra, HBsAg recombinante, no infeccioso, tampón fosfato 10 mM pH 7.4 +/- 0.1, además de sulfato de gentamicina 0.02% y ProClin 300 0.045% como conservantes. El control positivo está codificado con el color verde.

4. Calibrador CAL …
n° 2 viales. Calibrador liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero fetal bovino, HBsAg recombinante no infeccioso a 0.5 IU/mL (2° Estándar internacional O.M.S. para HBsAg, NIBSC código

00/588), tampón fosfato 10 mM pH 7.4 +/- 0.1, así como sulfato de gentamicina 0.02% y ProClin 300 al 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. Solución de lavado concentrada WASHBUF 20X
2x60ml/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. Diluente de conjugado CONJ DIL
2x16ml/vial. Listo para el uso y reactivo codificado con color rosa/rojo. Contiene tampón Tris 10mM a pH 6.8 +/- 0.1, 1% de suero de ratón normal, 5% de BSA, además 0.02% de sulfato de gentamicina y ProClin al 300 0.045% como conservantes. La solución es normalmente opalescente.

7. Conjugado CONJ 20X
2x1ml/vial. Reactivo concentrado 20X. Contiene anticuerpos monoclonales de ratón anti HBsAg marcados con peroxidasa (HRP), determinante "a" y preS", tampón Tris 10 mM a pH 6.8+/-0.1, 5% BSA, ProClin 300 al 0.045% y sulfato de gentamicina 0.02% como conservantes.

8. Cromógeno/substrato SUBS TMB
2x25ml/botella. Contiene solución tamponada citrato-fosfato 50 mM a pH 3.5-3.8, dimetilsulfóxido 4%, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02%.
Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

9. Ácido sulfúrico H_2SO_4 O.3 M
1x25ml/botella. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Sellador adhesivo, n° 4

11. Manual de instrucciones

Nota importante:
A solicitud del cliente, Dia.Pro puede suministrar reactivos para 96, 480, 960 pruebas, como se describe a continuación**:**

Microplacas	N°1	N°5	N°10
Control negativo	1x2ml/vial	1x10ml/vial	1x20 ml/vial
Control Positivo	1x2ml/vial	1x10ml/vial	1x20 ml/vial
Calibrador	N° 1 vial	Viales n.º 5	Viales n.º 10
Solución de lavado concentrada	1x60ml/vial	5x60ml/vial	4x150ml/vial
Conjugado	1x0.8ml/vial	1x4ml/vial	2x4ml/vial
Diluente de conjugado	1x16ml/vial	2x40ml/vial	2x80ml/vial
Cromógeno/substrato	1x25 ml/vial	3x42ml/vial	2x125ml/vial
Ácido Sulfúrico	1x15 ml/vial	2x40ml/vial	2x80ml/vial
Sellador adhesivo	N° 2	N° 10	N° 20
Manual de instrucciones	N° 1	N° 1	N° 1
Número de pruebas	96	480	960
Código SAG1ULTRA.CE	.96	.480	.960

E. MATERIALES NECESARIOS NO SUMINISTRADOS.
1. Micropipetas calibradas (150, 100 y 50 µl) y puntas de plástico 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 (en seco o húmedo), capaz de agitar a 1300 rpm+/-150, ajustado 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. Cuando el equipo se utiliza para el cribado de unidades de sangre y componentes sanguíneos, debe utilizarse en un laboratorio certificado y homologado por la autoridad nacional en ese campo (Ministerio de Sanidad o entidad similar) para realizar dicho tipo de análisis.
3. Todo el personal que participe en la realización de los ensayos deberá llevar la indumentaria protectora adecuada de laboratorio, guantes sin talco y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: “Biosafety in Microbiological and Biomedical Laboratories”, ed.1984.
4. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
5. Se debe controlar el entorno del laboratorio para evitar la contaminación por polvo o agentes microbianos en el aire al abrir los viales del equipo y las microplacas, así como durante la realización del ensayo. Evitar la exposición del cromógeno (TMB) a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
6. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
7. No intercambiar componentes de diferentes lotes ni tampoco de diferentes equipos del mismo lote.
8. 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.
9. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
11. No usar el producto después de la fecha de vencimiento indicada en el equipo e internamente en los reactivos. 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 6 meses.
12. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: “Biosafety in Microbiological and Biomedical Laboratories”, ed.1984.
13. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones cruzadas.
14. Los desechos producidos durante el uso del equipo deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser

inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
15. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
16. La solución de parada es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
17. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.
1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según la técnica estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Evitar la adición de conservantes a las muestras, en particular azida sódica, ya que podría afectar a la actividad enzimática del conjugado, generando resultados falsos negativos.
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 en unidades de sangre, se recomienda el uso del código de barras y la lectura electrónica.
4. Las muestras hemolizadas (color rojo) o lipémicas (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 porque pueden dar lugar a fasos positivos. Las muestras con una vía de coagulación alterada, que presentan partículas tras la extracción y preparación de suero/plasma y las que proceden de pacientes hemodializados, pueden originar resultados falsos positivos.
5. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante almenos 12 meses, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
6. Si hay algo de turbidez o se sospecha de la presencia de micropartículas tras descongelar, filtrar la muestra en un filtro de 0.2-0.8u desechable para limpiarla para las pruebas o usar el método alternativo de dos pasos.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.
Estudios de estabilidad realizados en equipos en uso no han arrojado pérdida de actividad significativa en un período de hasta 6 meses.

1. Microplacas:
Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de color verde, lo que indicaría un defecto de conservación.
De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.
Las tiras de pocillos no utilizadas deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. 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.

2. Control negativo:
Listo para el uso. Mezclar bien con un vórtex antes de usar.

3. Control positivo:
Listo para el uso. Mezclar bien con un vórtex antes de usar. El control positivo no contiene ningún HBV infeccioso ya que se compone de HBsAg recombinante sintético.

4. Calibrador:
Añadir al polvo liofilizado, el volumen de agua de calidad ELISA indicado en la etiqueta; dejar disolver completamente y después mezclar cuidadosamente con el vórtex antes de usar. Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.

5. Solución de lavado concentrada:
La solución concentrada 20x debe diluirse con agua de calidad EIA hasta 1200 ml y mezclarse suavemente antes del uso. Dado que pueden existir algunos cristales de sal en el vial, debe prestarse atención a que todo el contenido quede disuelto al preparar la solución.
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 durante una semana a temperaturas entre +2 y 8ºC.

6. Conjugado:
La solución de trabajo se prepara diluyendo el reactivo concentrado 20X con el Diluente de Conjugado.
Mezclar cuidadosamente con el vórtex antes de usar.
Evitar la contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables únicamente.
***Nota importante**: La solución de trabajo no es estable. Preparar solamente el volumen necesario para el trabajo del día. Como un ejemplo cuando el equipo se utiliza en combinación con otros instrumentos o manualmente, diluir 0.1 ml de Conjugado 20X con 1.9 ml de Diluente de Conjugado en un vial de plástico desechable y mezclar cuidadosamente antes de usar.*

7. Cromógeno/ Substrato:
Listo para el uso. Mezclar bien volteando.
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.

8. Ácido Sulfúrico:
Listo para el uso. Mezclar bien volteando.
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 +/- 2%.
2. La **incubadora ELISA** debe ser ajustada a 37ºC (+/- 1ºC de tolerancia) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadores secos o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. En caso de **agitación** durante las incubaciones, el instrumento debe garantizar 350 rpm ±150. La amplitud de la agitación es muy importante ya que si es errónea pueden producirse salpicaduras y por lo tanto falsos positivos.
4. 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.
5. Los tiempos de incubación deben tener un margen de ±5%.
6. El **lector de microplacas** debe estar provisto de un filtro de lectura de 450 nm y, de un segundo filtro de 620-630 nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 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 que se mide la densidad óptica correcta. Periódicamente debe procederse al mantenimiento según las instrucciones del fabricante.
7. En caso de usar **sistemas automatizados ELISA**, los pasos críticos (dispensado, incubación, lavado, lectura, agitación, procesamiento de datos, etc.) 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 comprobando la plena coincidencia de los rendimientos declarados del equipo. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente, prestando particular atención a evitar el arrastre por las agujas de dispensación de muestras y de lavado. Debe estudiarse y controlarse el efecto de arrastre a fin de minimizar la posibilidad de contaminación de pocillos adyacentes debido a muestras muy reactivas, lo que provocaría resultados falsos positivos. 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.
8. Cuando se utilizan instrumentos automáticos, en el caso en que los contenedores para viales del instrumento no se ajusten a los viales del equipo, debe transferirse la solución a contenedores adecuados y marcarlos con la misma etiqueta despegada del vial original. Esta operación es importante para evitar la falta de coincidencia de los contenidos de los viales al transferirlos. Cuando la prueba termine, guardar los contenedores secundarios etiquetados a 2-8°C, firmemente cerrados.
9. 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 esenciales del ensayo. También se ofrece apoyo para la instalación de nuevos instrumentos a usar en combinación con el equipo.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Comprobar la fecha de caducidad indicada en la etiqueta externa de la caja del equipo. No usar si ha caducado.
2. Comprobar que los componentes líquidos no están contaminados con partículas ni agregados observables a simple vista. Comprobar que el cromógeno/substrato es incoloro o azul pálido. Comprobar que no se han producido roturas ni derrames de líquido dentro de la caja 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. Diluir el conjugado concentrado 20X con su diluente, tal y como se describe.
5. Disolver el calibrador como se ha descrito anteriormente.
6. Dejar los componentes restantes hasta alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego según se describe.
7. Ajustar la incubadora de ELISA a 37ºC y alimentar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
8. Comprobar que el lector ELISA ha sido encendido al menos 20 minutos antes de realizar la lectura.
9. Si se utiliza un sistema automatizado, encenderlo y comprobar que los protocolos estén correctamente programados.
10. Comprobar que las micropipetas estén fijadas en el volumen requerido.
11. Asegurarse de que el resto de equipamiento esté disponible y listo para el uso.
12. 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.

Ensayo automatizado:

En caso de que el ensayo se realice automáticamente con un sistema ELISA, se recomienda que el instrumento dispense primero 150 ul de controles y calibrador, después todas las muestras y finalmente 100 ul de conjugado diluido.
Para el paso de pre-lavado (primer punto del procedimiento del ensayo) y 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.

Ensayo manual:

1. Poner el número de tiras necesarias en el soporte de plástico y hacer un ciclo de lavado para hidratar los pocillos. Identificar cuidadosamente los pocillos de controles, calibradores y muestras.

Nota importante: El prelavado (1 ciclo: dispensación de 350 ul de solución de lavado por pocillo además de aspiración) es fundamental para obtener resultados confiables y específicos tanto en el procedimiento automático como en el manual. ¡No omitir!

2. Dejar el pocillo A1 vacío para el blanco.
3. Dispensar 150µl del Control Negativo, por triplicado, 150µl de Calibrador por duplicado y 150 µl del Control Positivo. Posteriormente, añadir 150 µl de cada muestra.
4. Comprobar la presencia de las muestras en los pocillos a simple vista (existe una marcada diferencia de color entre los llenos y los vacíos) o por lectura a 450/620nm. (la densidad óptica de las muestras es superior a 0.100).
5. Dispensar 100 µl del Conjugado diluido en todos los pocillos, excepto en el A1 que se utiliza para operaciones de blanco.

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.*

6. Después de la adición del conjugado comprobar que las muestras han cambiado de color amarillo a rosa/rojo y después incubar la microplaca por **120 min a +37°C**.

Notas importantes:

c. *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.*

d. *Si el proceso es efectuado agitando, asegúrese de tener las mismas rpm de la sección I.3. De lo contrario se podría verificar contaminación dentro el pocillo.*

7. Tras la primera incubación, lavar los pocillos como se ha descrito previamente (sección I.4).
8. Dispensar 200 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.*

9. Incubar la microplaca protegida de la luz a **18-24°C durante 30 minutos.** Los pocillos con control positivo, calibrador y muestras positivas deben pasar de un tono claro a azul.
10. Dispensar 100 µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática usando la misma secuencia que en el paso 8. La adición de la solución de ácido cambiará el color del control positivo, el calibrador y las muestras positivas de azul a amarillo/ marrón.
11. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.6, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas generales importantes:

1. *Asegurarse de que no hay impresiones digitales ni polvo en el fondo externo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*

2. *La lectura debería hacerse inmediatamente después de añadir la solución de ácido y, en cualquier caso, nunca transcurridos más de 20 minutos de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*

3. *Cuando las muestras que se van a analizar no sean seguramente limpias o hayan estado congeladas, se recomienda seguir el procedimiento abajo descrito en cuanto es menos susceptible a la interferencia de la hemólisis, hiperlipemia, contaminación bacteriana y micropartículas de fibrina. El ensayo se realiza en dos pasos a +37°C con agitación a* 350 rpm ±150 *como sigue:*
   a. *dispensar 100 µl de controles, calibradores y muestras*
   b. *incubar 60 min a +37°C con agitación*
   c. *lavar según las instrucciones (sección I.4)*
   d. *dispensar 100 ul de trazador enzimático diluido*
   e. *incubar 30 min a +37°C con agitación*
   f. *lavar*
   g. *dispensar 100 ul de mezcla TMB y H2O2*
   h. *incubar 30 min a t.amb. con agitación*
   i. *parar y leer*

   En este procedimiento se puede omitir el prelavado. Este método muestra un rendimiento similar al método estándar por lo cual puede ser utilizado como alternativa.
4. *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.

Operaciones	Procedimiento
Paso de pre-lavado	ciclo n° 1
Controles&Calibradores& muestras Conjugado diluido	150 µl 100 µl
1ra incubación	**120 min**
Temperatura	+37°C
Pasos de lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Cromógeno/substrato	200ul
2da incubación	**30 min**
Temperatura	temperatura ambiente
Ácido Sulfúrico	100 µl
Lectura D.O.	450nm/620-630nm

En la sección 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 / 620-630 nm o M/Co son los esperados en el análisis.

Asegurar el cumplimiento de los siguientes resultados:

Parámetro	Exigencia
Pocillo blanco	valor < 0.100 DO450nm
Control negativo (CN)	Valor medio < 0.050 de DO450nm después de leer el blanco
Calibrador 0.5 IU/ml	M/Co > 2
Control Positivo	valor > 1.000 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 > 0.100 DO450nm	1. la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control negativo (CN) > 0.050 DO450nm 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. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Calibrador M/Co < 2	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en su distribución (por ejemplo, dispensar control negativo en lugar de 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 < 1.000 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. En este caso, el control negativo tendrá un valor de DO450nm > 0.050). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, después de comprobar, informe al responsable para tomar las medidas pertinentes.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 11.

P. CÁLCULO DEL VALOR DE CORTE.
Los resultados de las pruebas se calculan a partir de un valor de corte determinado con la fórmula siguiente sobre el valor medio de DO450nm/620-630nm del control negativo (CN):

CN + 0.050 = Valor de corte (Co)

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 relación entre el valor de DO450nm/620-630nm de la muestra (M) y el valor de corte (Co), matemáticamente M/Co. Los resultados se interpretan según la siguiente tabla:

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 HBV y la unidad de sangre se puede transfundir.
Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre recogida 1 o 2 semanas después de la inicial. En este caso la unidad de la sangre no debe ser transfundida.
Un resultado positivo es indicativo de infección por HBV y por consiguiente el paciente debe ser tratado adecuadamente o la unidad de sangre debe ser descartada.

Notas importantes:
1. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
2. *Cualquier resultado positivo debe confirmarse antes repitiendo el ensayo sobre la muestra, después de haberla filtrado en un filtro de 0.2-0.8 u para eliminar la interferencia de las micropartículas. Después, si todavía es positivo, la muestra debe someterse a una prueba de confirmación antes de emitir un diagnóstico de hepatitis viral.*
3. *Cuando se transmiten los resultados de la prueba, del laboratorio a otro departamento, 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 describe un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 11).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

***Control negativo**: 0.012 – 0.008 – 0.010 DO450nm*
Valor medio: 0.010 DO450nm
Menor de 0.050 – Válido

***Control positivo**: 2.489 DO450nm*
Mayor de 1.000 – Válido
Valor de corte = 0.010+0.050 = 0.060
Calibrador: 0.350 - 0.370 DO 450nm
Valor medio: 0.360 DO450nm M/Co = 6.0
M/Co Mayor de 2.0 – Válido
Muestra 1: 0.028 DO450nm
Muestra 2: 1.690 DO 450nm
Muestra 1 M/Co < 0.9 = negativa
Muestra 2 M/Co > 1.1 = positiva

R. CARACTERÍSTICAS DEL 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). La versión ULTRA ha demostrado ser al menos equivalente al diseño original en un estudio realizado para la validación de la nueva versión.

1. Sensibilidad analítica
El límite de detección del ensayo se ha calculado sobre el 2º estándar internacional O.M.S., NIBSC código 00/588.
En la siguiente tabla se muestran los resultados de tres lotes (P1, P2 y P3) de la versión ULTRA en comparación con el dispositivo de referencia (Ref.):

O.M.S. IU/ml	Lote P1 M/Co	Lote P2 M/Co	Lote P3 M/Co	Ref. M/Co
0.4	**4.6**	**4.8**	**4.6**	**4.6**
0.2	**2.3**	**2.4**	**2.4**	**2.4**
0.1	**1.4**	**1.4**	**1.5**	**1.2**
0.05	0.8	0.8	***1.0***	0.7
0.025	0.6	0.6	0.6	0.4
SFB (CN)	0.3	0.2	0.3	0.1

El ensayo mostró una sensibilidad analítica mejor a 0.1 O.M.S. IU/ml de HBsAg.

Además se probaron dos paneles de sensibilidad suministrados por EFS, Francia, y por SFTS, Francia, y se obtuvieron los siguientes resultados en condiciones óptimas:

Panel EFS Ag HBs HB1-HB6 lote n° 04

ID muestra	Características	ng/ml	M/Co
HB1	diluente	/	0,2
HB2	adw2+ayw3	0.05	0,6
HB3	adw2+ayw3	0.1	**1,0**
HB4	adw2+ayw3	0.2	**1,8**
HB5	adw2+ayw3	0.3	**2,4**
HB6	adw2+ayw3	0.5	**4,2**

Panel de sensibilidad SFTS, Francia, Ag HBs 2005

ID muestra	Características	ng/ml	M/Co
171	Adw2 + ayw3	2.21 ± 0.15	**15,4**
172	Adw2 + ayw3	1.18 ± 0.10	**8,7**
173	Adw2 + ayw3	1.02 ± 0.05	**6,1**
174	Adw2 + ayw3	0.64 ± 0.04	**4,0**
175	Adw2 + ayw3	0.49 ± 0.03	**3,4**
176	Adw2 + ayw3	0.39 ± 0.02	**2,6**
177	Adw2 + ayw3	0.25 ± 0.02	**2,0**
178	Adw2 + ayw3	0.11 ± 0.02	**1,3**
179	Adw2 + ayw3	0.06 ± 0.01	***0,9***
180	Adw2 + ayw3	0.03 ± 0.01	0,8
181	Adw2	0.5 – 1.0	**4,7**
182	Adw4	0.5 – 1.0	**3,6**
183	Adr	0.5 – 1.0	**4,5**
184	Ayw1	0.5 – 1.0	**5,1**
185	Ayw2	0.5 – 1.0	**6,4**
186	Ayw3	0.5 – 1.0	**7,3**
187	Ayw3	0.5 – 1.0	**5,8**
188	Ayw4	0.5 – 1.0	**6,9**
189	Ayr	0.5 – 1.0	**6,1**
190	diluente	/	0,6

El panel n.º 808, suministrado por Boston Biomedical Inc., Estados Unidos, también se probó para definir el límite de sensibilidad.
Los resultados en condiciones óptimas son los siguientes:

BBI panel PHA 808

ID muestra	Características	ng/ml	M/Co
01	ad	2,49	**10,2**
02	ad	1,17	**4,8**
03	ad	1,02	**4,3**
04	ad	0,96	**3,8**
05	ad	0,69	**2,9**
06	ad	0,50	**2,2**
07	ad	0,41	**1,5**
08	ad	0,37	**1,3**
09	ad	0,30	**1,2**
10	ad	0,23	***1,0***
11	ay	2,51	**11,2**
12	ay	1,26	**5,9**
13	ay	0,97	**4,1**
14	ay	0,77	**3,7**
15	ay	0,63	**2,0**
16	ay	0,48	**2,4**
17	ay	0,42	**2,0**
18	ay	0,33	**1,8**
19	ay	0,23	**1,6**
20	ay	0,13	**1,1**
21	negativo	/	0,6

2. Sensibilidad Diagnóstica:
La sensibilidad diagnóstica ha sido probada según lo establecido en las Especificaciones Técnicas Comunes (ETC) de la Directiva 98/79/CE en IVD para pruebas HBsAg.
Las muestras positivas, incluidos los subtipos de HBsAg y un panel de mutantes “s” de las mutaciones más frecuentes, se recogieron de distintas patologías de HBV (hepatitis B aguda, asintomática y crónica) o producidas sintéticamente, y se detectaron positivas en el ensayo.
Todos los subtipos conocidos de HBsAg, “ay” y “ad”, y los isoformes “w” y “r”, suministrados por CNTS, Francia, se probaron en el ensayo y el equipo los determinó positivos según lo previsto.
Se ha hallado un valor global de 100% en un estudio realizado sobre un número total de más de 400 muestras positivas con la referencia original IVD código SAG1.CE, marca CE.
Se estudiaron 30 sero-conversiones en total, la mayoría producidas por Boston Biomedica Inc., EE.UU.
Los resultados obtenidos al examinar ocho paneles suministrados por Boston Biomedica Inc., EE.UU., se indican abajo para la versión ULTRA en comparación con el dispositivo de referencia código SAG1.CE.

Panel ID	1ª muestra positiva	HBsAg subtipo	HBsAg ng/ml	Versión ULTRA M/Co	Ref. dispositivo M/Co
PHM 906	02	ad	0.5	**3.7**	**1.4**
PHM 907 (M)	06	ay	1.0	**4.4**	**2.9**
PHM 909	04	ad	0.3	**1.2**	0.8
PHM 914	04	ad	0.5	**1.1**	**1.1**
PHM 918	02	ad	0.1	**1.8**	0.5
PHM 923	03	ay	< 0.2	**2.2**	**1.2**
PHM 925	03	Ind.	n.d.	**1.4**	*0.9*
PHM 934	01	ad	n.d.	**1.0**	0.8

3. Especificidad Diagnóstica:
Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico. Además del primer estudio, donde se examinaron más de 5000 muestras negativas de donantes de sangre (dos centros de donación) clasificadas como negativas con un dispositivo con marca CE en uso en el laboratorio de recogida, la especificidad diagnóstica se evaluó recientemente examinando un total de 2288 muestras de donantes de sangre negativas en siete lotes distintos. Se observó un valor de especificidad de 100%.
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 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 agregados.
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. No se detectó reacción cruzada.

4. Precisión:
Ha sido calculada para la versión ULTRA en dos muestras examinadas en 16 réplicas en 3 series diferentes para tres lotes.
Los resultados se indican en la siguiente tabla:

Valores promedio Total n = 144	Negativo Muestra	Calibrador 0.5 IU/ml
DO450nm	0.026	0.332
Desviación estándar	0.004	0.027
CV %	16%	8%

La variabilidad mostrada en las tablas no dio como resultado una clasificación errónea de las muestras.

S. LIMITACIONES.
Se evaluaron resultados falso positivo repetibles en muestras recién recogidas en menos del 0.1% de la población normal, debido principalmente a altos títulos de anticuerpo anti ratón heterofílicos (HAMA).
También se observaron interferencias en muestras frescas cuando no estaban libres de partículas o se recogieron incorrectamente (ver capítulo G).
Las muestras antiguas o congeladas, con coágulos de fibrina, crioglobulinas, micelas que contienen lípidos o micropartículas después de almacenar o descongelar, pueden generar 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 por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (Mi) – Italia

CE
0318

HBsAg

Confirmation

Set of Reagents for the confirmation of HBsAg positivity in human sera or plasma

- for “in vitro” diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
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Phone +39 02 27007161
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e-mail: info@diapro.it

Code SCONF.CE
20/40 tests

HBsAg Confirmation

A. INTENDED USE

In the screening of blood units for Hepatitis B surface Antigen or HBsAg some false positivity may happen, leading to a misinterpretation of the assay results and a misclassification of the blood unit and the donor.
To confirm the positivity of a screened sample or to confirm the presence of an ongoing HBV infection in a hospitalized patient, a confirmatory test has to be run.
A simple procedure based on an immunoreaction of neutralization is used in combination with the HBsAg assay.

B. PRINCIPLE OF THE ASSAY

The device has to be used in combination with the products code SAG1.CE/SAG1ULTRA.CE for the determination of HBsAg in human sera and plasma.
The sample, whose repeatedly positivity for HBsAg has to be confirmed, is premixed with a reagent containing high titer anti HBsAg antibodies that will neutralize the antigen is really present in the sample.
The neutralized sample is then tested for HBsAg according to the procedure reported for the specific device.
If the positivity in the first screening test is specifically related to the presence of HBsAg in the sample, the same will not react any more in the assay having been neutralized by the antibody.
If at contrary the positivity of the sample is not abolished by the neutralization reaction, this reactivity is not due specifically to the presence of HBsAg in the sample, but to some interfering substance.

C. CONTENT OF THE KIT

The set contains the following reagents**.**

1. Neutralizing Reagent SOLN NEUT
It contains high titer human plasma positive for anti HBsAg antibodies, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

2. Control Reagent CONTROL
It contains human plasma negative for anti HBsAg antibodies, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

3. Assay Diluent DILSPE
0.15 M NaCl phosphate buffered solution pH 7.0 ± 0.2 containing 0.1% Kathon GC for the dilution of over ranging samples .

Note: *Reagents have been tested and found negative for HBsAg, HCV Ab and HIV Ab with CE-marked kits.*

Number of tests	**20**	**40**
Code	**SCONF.CE**	**SCONF.CE.40**
Control Reagent (CONTROL)	1x4ml/vial	1x8ml/vial
Neutralizing Reagent (SOLN NEUT)	1x4ml/vial	1x8ml/vial
Phosphate Buffered Saline (DILSPE)	1x30ml/vial	1x60ml/vial
Package insert	N°1	N°1

D. MATERIALS REQUIRED BUT NOT PROVIDED

1. CE marked devices for HBsAg determination:

Product	**Code**	**Tests**
HBsAg one	SAG1.CE	192
	SAG1.CE.96	96
	SAG1.CE.480	480
	SAG1.CE.960	960
HBsAg one version ULTRA	SAG1ULTRA.CE	192
	SAG1ULTRA.CE.96	96
	SAG1ULTRA.CE.480	480
	SAG1ULTRA.CE.960	960

2. Isotonic sterile solution.
3. Calibrated Micropipettes and disposable plastic tips.
4. Timer with 60 minute range or higher.
5. Absorbent paper tissues.
6. Calibrated ELISA microplate thermostatic incubator (dry or wet), capable to provide shaking at 1300 rpm+/-150, set at +37°C.
7. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
8. Calibrated ELISA microplate washer.
9. Vortex or similar mixing tools.
10. Disposable plastic tube of 2-5 ml.

E. WARNINGS AND PRECAUTIONS

1. For “in vitro” diagnostic use only.
2. The Set has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
3. When the device is used for confirmation of a sample repeatedly positive in the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening the vials contained in the set.
5. Upon receipt, store the kit at 2..8°C into a tem perature controlled refrigerator or cold room.
6. Do not interchange Reagents between different lots of the device. It is even recommended that Reagents between two sets of the same lot are not interchanged.
7. Check that the Reagents of the device 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 kit reagents by using disposable tips and changing them between the use of each one.
9. Do not use the set after the expiration date stated on the external container and internal (vials) labels.
10. 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.
11. Waste produced during the use of the set in combination with the devise for HBsAg determination 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..
12. 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.
13. 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.
14. Refer to the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE used in combination for the confirmation assay.

F. SPECIMEN: PREPARATION AND WARNINGS

1. The sample turned out to be repeatedly positive in the first HBsAg determination with HBsAg One has to be used for the test of neutralization. Treat the sample as described in section L.
2. Avoid any addition of preservatives to samples after first screening; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. Haemolysed (red) and lipemic ("milky") samples have to be discarded by definition anyway as they could generate false results in the test for HBsAg.
5. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as well as they could give rise to false positive results both in HBsAg first assay and even in the confirmation one.
6. The assay is not suitable to confirm the negativity of samples that turned out to be negative in the first HBsAg screening test.
7. Sera and plasma can be stored at +2°..8°C for up t o five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months.
8. Any frozen sample should not be frozen/thawed more than once as this may generate particles that could affect the test result. If some turbidity is present or presence of micro particles is suspected after thawing, filter the sample on a disposable 0.2-0.8u filter to clean it up for testing or use the two-steps alternative method.
9. Refer to the Instructions for Use of the products code SAG1.CE/SAG1ULTRA.CE used in combination for the confirmation assay.

G. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The **ELISA incubator** has to be set at +37°C (tolerance of $\pm$1°C) and regularly checked to ensure the correc t 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. In case of **shaking** during incubations, the instrument has to ensure 350 rpm $\pm$150. Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.
4. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
5. **Incubation times** have a tolerance of $\pm$5%.
6. The **microplate reader** has to be equipped with a reading filter of 450nm and with a second filter (620-630nm) strongly recommended for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
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 essential requirements of the assay. Support is also provided for the installation of new instruments to be used in combination with the kit.

H. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the set printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components of the set are not contaminated by naked-eye visible particles or aggregates.
3. Allow components to reach room temperature (about 1 hr) and then mix them on vortex.
4. Check that micropipettes are set to the required volume.
5. Check that equipments and the kits SAG1.CE/SAG1ULTRA.CE used in combination are available and ready to use.
6. In case of problems, do not proceed further with the test and advise the supervisor.
7. Refer to the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE used in combination for the confirmation assay.

I. ASSAY PROCEDURE

The confirmation assay reported below has to be carried out on a sample repeatedly positive for HBsAg, when the product code SAG1.CE/SAG1ULTRA.CE is used for first screening.
The test is not suitable to confirm negative samples.
The Negative Control and the Calibrator of the kit code SAG1.CE/SAG1ULTRA.CE have always to be run whenever the assay of confirmation is used.

Samples with OD450nm < 2

If the sample gave an optical signal < 2 OD450nm in the screening test use the following distribution protocol:

1. Add 50 ul Neutralizing Reagent to 150 ul sample to be confirmed in a disposable test tube (**N**). Mix on vortex.
2. Add 50 ul Control Reagent to 150 ul sample to be confirmed in a second disposable test tube (**C**). Mix on vortex.
3. Incubate both tubes for 30 min at room temperature.
4. Then follow the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE and determine HBsAg reactivity in both N and C.

Samples with OD450nm > 2

If the sample gave an optical signal > 2.000 OD450nm in the screening test, use the following procedure:

1. Dilute the sample 1:100 by dispensing 5 ul of specimen and 495 ul of Assay Diluent in a disposable tube (S01K). Mix on vortex.
2. Dilute further 1:10,000 the sample by dispensing 5 ul of the 1:100 solution and 495 ul of Assay Diluent in a disposable tube (S10K). Mix on vortex.
3. In a first test tube dispense 150 ul S01K and add 50 ul control Reagent (**C01K**). Mix on vortex.
4. In a second test tube dispense again 150 ul S01K and add 50 ul Neutralizing Reagent (**N01K**). Mix on vortex.
5. In a third test tube dispense 150 ul of solution S10K and add 50 ul Control Reagent (**C10K**). Mix on vortex.
6. In a fourth test tube dispense again 150 ul of solution S01K and add 50 ul Neutralizing Reagent (**N10K**). Mix on vortex.
7. Incubate all these tubes for 30 min at room temperature.
8. Then follow the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE and determine HBsAg reactivity in all the tubes (C01K, C10K, N01K and N10K).
9. If the OD450nm value of the 1:10,000 dilution is still greater than 2 for the non-neutralized sample (control well), repeat the test after further diluting the sample 1:100,000.

An example of dispensation scheme is reported in the following table:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2 C										
B	NC	S2 N										
C	NC	S3 C										
D	NC	S3 N										
E	CAL C	S4 C										
F	CAL N	S4 N										
G	S1 C	S5 C										
H	S1 N	S5 N										

Legenda : BLK = Blank NC = Negative Control CAL = Calibrator S = Sample C = Control N = Neutralizing Reagent

L. CALCULATION OF RESULTS

The positivity of the specimen is confirmed if the ratio between the OD450nm value for the control well (**C**) and the OD450nm value for the neutralization well (**N**) is higher than 2, that is formulated mathematically as follows:

$$C / N > 2$$

If a HBsAg positive sample shows a ratio C/N < 2 in the neutralization assay it is considered false positive.

M. INTERNAL QUALITY CONTROL

A check is performed any time the kit is used in combination with the device for HBsAg determination (code SAG1.CE/SAG1ULTRA.CE) in order to assure full matching the expected performances.

In particular ensure that the following results are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC) of product code SAG1.CE/SAG1ULTRA.CE	< 0.050 mean OD450nm value after blanking
Calibrator (CAL) of product code SAG1.CE/SAG1ULTRA.CE treated with CONTROL	S/Co $\geq$ 2
Calibrator (CAL) of product code SAG1.CE/SAG1ULTRA.CE treated with SOLN NEUT	C/N > 2
Sample to be confirmed treated with CONTROL	S/Co > 1.1

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	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.050 OD450nm after blanking	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 the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of 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 (CAL) Treated with CONTROL S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the Calibrator (dispensation of negative control instead) or the CONTROL (dispensation of the SOLN NEUT instead). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination has occurred.
Calibrator (CAL) treated with SOLN NEUT C/N < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the Calibrator (dispensation of negative control instead) or the SOLN NEUT (dispensation of the CONTROL instead). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination has occurred.
Sample to be confirmed treated with CONTROL S/Co < 1.1	1. the sample to be confirmed was mishandled or confused with a negative one. 2. that the SOLN NEUT was dispensed instead of the CONTROL. 3. that the washing procedure and the washer settings are as validated in the pre qualification study.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

N. EXAMPLE OF RESULTS

Below an example of calculation and interpretation of results is reported:

Sample # 1

Control well C: 1.000 OD450nm
Neutralization well N: 0.100 OD450nm
Ratio C/N: 10
Result of confirmation: **true positive**

Sample # 2

Control well C: 1.000 OD450nm
Neutralization well N: 0.800 OD450nm
Ratio C/N: 1.25
Result of confirmation: **false positive**

Important note:

*If the OD450nm value for the control well (**C**) of the 1:100,000 dilution of the sample is still higher than the upper limit of detection of the microplate reader, the specimen is confirmed positive if the value for the neutralization well (**N**) is equal or less than 50% of the maximum optical density of the reader. An example of such case is reported below:*

Upper limit of detection of the reader: 2.000

Sample # 1 diluted 1:100,000

Control well C: >2.000 OD450nm
Neutralization well N: 0.800 OD450nm
50% of *the upper limit of detection of the reader*:: 1.000
Result of confirmation: ***true positive***

Sample # 2 diluted 1:100,000

Control well C: >2.000 OD450nm
Neutralization well N: 1.850 OD450nm
50% of *the upper limit of detection of the reader*::1.000
Result of confirmation: ***false positive***

O. TEST PERFORMANCES

Sensitivity:

A total of 300 samples positive for HBsAg in HBsAg One, including standards for HBsAg provided by WHO, NIBSC and PEI, were examined. In the study also 15 panels of HBsAg seroconversion were included.
All the positive samples were confirmed positive providing a value of 100% sensitivity.

Specificity:

A total of 20 false positive samples (prevalently HAMA positive), obtained from a population examined without the HAMA blocker with the kits SAG1.CE/SAG1ULTRA.CE, were tested.
All of them, again tested with a SAG1.CE/SAG1ULTRA.CE device lacking the HAMA blocker, were not confirmed for HBsAg presence and therefore defined false positives.
In addition, even if the assay is not suitable to test negative samples, a total of 50 specimens negative in HBsAg One first screening, coming from hospitalized patients with pathologies different from HBV infection, showed a mean C/N value < 2 in the confirmation assay, therefore proving the validity of the above calculation and not to generate interferences in the confirmation test.

P. LIMITATIONS OF THE TEST

All the limitations reported in the kit HBsAg One apply to the above described assay, as they are conducible to the HBsAg assay itself.
Please read with attention the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE before carrying out the test of neutralization.

In particular the product must not be applied to those specimens showing particles or aggregates, unless the sample is cleaned before use by filtration on 0.2-0.8 u disposable filters.

The confirmation assay for HBsAg positivity is not suitable to confirm negativity on negative samples and therefore must not be used for such analysis.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Produced by
Dia.Pro. Diagnostic Bioprobes Srl.
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CE
0318

TOXO IgG

Enzyme Immunoassay for the quantitative/qualitative determination of IgG antibodies to Toxoplasma gondii in human serum and plasma

- for “in vitro” diagnostic use only -

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Code: TOXOG.CE
96 Tests

TOXO IgG

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Toxoplasma gondii in plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Toxoplasma gondii is an obligate intracellular protozoan parasite that is probably capable of infecting all species of mammals, including man. The detection of IgM antibodies to T.gondii is particularly helpful for the diagnosis of acute infections in "risk" individuals, in association with AIDS, organ transplantation and pregnancy. As most of T.gondii infections are mild or asymptomatic in otherwise healthy individuals, the detection of T.gondii specific IgM antibodies, in absence of detectable specific IgG, has become important for the monitoring of acute infections in pregnant women, as the parasite can lead to severe birth defects. Moreover, as T.gondii infections are most severe in immunocompromised patients, where the disease can be fatal, acute infections due to this parasite have to be distinguished from other disorders.
Recently developed IgM capture assays provide the clinician with a helpful and reliable test, not affected by the rheumatoid factor as it happens to be in classic sandwich tests.

C. PRINCIPLE OF THE TEST
Microplates are coated with native T. gondii antigens, highly purified by sucrose gradient centrifugation and inactivated.
The solid phase is first treated with the diluted sample and IgG to T. gondii are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti Toxoplasma gondii IgG are detected by the addition of polyclonal specific anti human IgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti Toxoplasma gondii IgG antibodies present in the sample. A Calibration Curve, calibrated against the W.H.O 3nd international standard , makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS
Each kit contains sufficient reagents to perform 96 tests.

1. : **Microplate**: MICROPLATE
12 strips x 8 microwells coated with purified and gamma-irradiation inactivated Toxoplasma gondii in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° ….
Ready to use and colour coded, calibrated against the 3nd international standard produced by the World Health Organization (WHO). The calibration curve range is as follows:
4ml CAL 1 = 0 WHO IU/ml
4ml CAL 2 = 50 WHO IU/ml
2ml CAL 3 = 100 WHO IU/ml
2ml CAL 4 = 250 WHO IU/ml
2ml CAL 5 = 500 WHO IU/ml
4ml CAL 6 = 1000 WHO IU/ml.
It contains Toxo IgG positive plasma titrated against WHO 3rd international standard code TOXM, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 2% casein, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. Standards are blue colored.

3. Control Serum: CONTROL ….
n° 1 vial - Lyophilized. To be dissolved with the volume of EIA grade water reported on the label. It contains fetal bovine serum, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives and human plasma positive to T.gondii calibrated at 250 IU/ml +/-10%, whose content is calibrated on 3nd international standard produced by the World Health Organization (WHO - TOXM).
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 10 mM Tris buffer pH 6.8+/-0.1,5% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives. Coded with 0.01% red alimentary dye

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide or H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vialIt contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. To be used to dilute the sample.

9. Plate sealing foils n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices

should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-T.gondii IgG antibody concentration is expected to be higher than 1000 IU/ml should be diluted before use, either 1:10 or 1:100 in the Calibrator 0 IU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M.1 Quantitative analysis

Automated assay:
In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute controls/calibrator as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:
1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the 1st and 2nd wells (positions A1 and B1 of the microplate) empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of 1:101 diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the 1st and the 2nd blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important notes:
1. *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*
2. *Mix thoroughly the Enzyme Conjugate on vortex before its use !!!*
7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M.2 QUALITATIVE ANALYSIS

If only a qualitative determination is required, proceed as described below:

Automated assay:
Proceed as described in section M1.

Manual assay:
1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the 1st well (positions A1 of the microplate) empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 IU/ml and 100 µl of Calibrator 50 IU/ml in duplicate, and 100 µl of Calibrator 1000 IU/ml in single. Then dispense 100 µl of 1:101 diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important notes:
1. *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*
2. *Mix thoroughly the Enzyme Conjugate on vortex before its use !!!*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *The Control Serum (CS) does not affect the test results calculation. The Control Serum may be used only when a laboratory internal quality control a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control Samples diluted 1:101	100 µl 100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS	S 7									
H	CAL3	CS	S 8									

Legenda: BLK = Blank CAL = Calibrator CS = Control Serum S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrators
CS = Control Serum S = Sample

O. INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether the performances of the assay are as expected and required by the IVDD directive 98/79/EC. Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
Calibrator 0 IU/ml (CAL1)	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
Calibrator 50 IU/ml	OD450nm > OD450nm CAL1 + 0.100
Calibrator 1000 IU/ml	OD450nm > 1.000
Control Serum	250 WHO IU/ml +/-10%

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not got contaminated during the assay
Calibrator 0 IU/ml > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

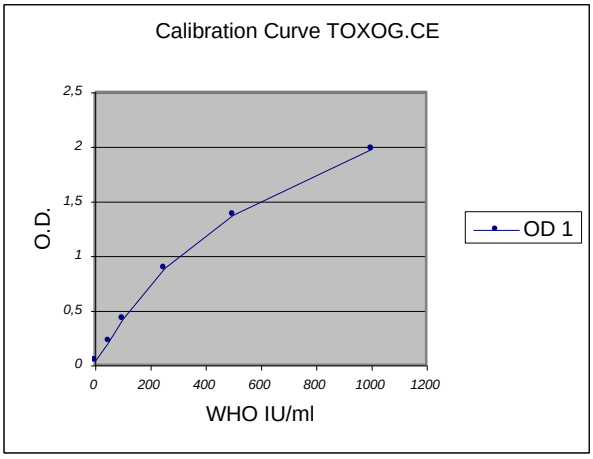
Calibrator 50 IU/ml OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 1000 IU/ml < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (e.g..: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 11.

P. RESULTS

P.1 Quantitative method
If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Toxoplasma gondii IgG antibody in samples.
An example of Calibration curve is reported in this page.



Important Notes:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 50 IU/ml and then check that the assay is valid.

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 11).

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 IU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 50 IU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted
Calibrator 1000 IU/ml: 2.845 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS
Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of Toxoplasma gondii due to the risk of severe neonatal malformations.
The cut-off of the device has been set at 50 IU/ml, and not lower as some other devices present on the market do, in order to assure the highest diagnostic value to the test, in particular when the assay is applied in pregnancy monitoring.
Upon infection, in fact, a part from the very first time of seroconversion, patients develop a strong immunological response to Toxoplasma gondii, far exceeding 50 IU/ml.
Low titer antibodies (below 50 IU/ml) mostly show low avidity to the infective agent and may represent a diagnostic marker of a recent infection, in combination with IgM.
Pregnant women, with antibodies concentrations below 50 IU/ml are by the devise considered negative in order to make the clinician consider them "risk" patients and follow them up for both IgG and IgM along pregnancy.
Samples with a concentration higher than 50 WHO IU/ml are considered positive for anti Toxoplasma gondii IgG antibody, surely able to provide immunity against the infection.
This titer is considered the lowest concentration of IgG to provide an effective immunological protection against a second infection of Toxoplasma gondii by NCCLS, USA.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *In the follow-up of pregnancy for Toxoplasma Gondii infection a positive result (presence of IgG antibody > 50 IU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what indicated in the standard prEN 13612.

1. Limit of detection

The limit of detection of the assay has been calculated by means of the 3nd international standard produced by the World Health Organization (WHO).

The limit of detection has been calculated as mean OD450nm Calibrator 0 IU/ml + 5 SD.

The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay.

OD450nm values

WHO IU/ml	TOXOG.CE Lot # 0503	TOXOG.CE Lot # 0403	TOXOG.CE Lot # 0303
250	0.816	0.853	0.974
100	0.365	0.398	0.445
50	0.209	0.244	0.246
10	0.094	0.125	0.108
Std 0	0.033	0.031	0.056

The assay shows a limit of detection better than 10 IU/ml.

2. Diagnostic Sensitivity:

The diagnostic sensitivity has been tested in a Performance Evaluation trial on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of Toxoplasma gondii Virus infection were tested.

The value, obtained from the analysis of more than 300 specimens, has been > 98%.

3. Diagnostic Specificity:

The diagnostic specificity has been determined on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.

Frozen specimens have been tested, as well, to check for interferences due to collection and storage.

No interference was observed.

Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested. No crossreaction was observed.

An overall value > 98% of specificity was found when examined on more than 100 specimens.

4. Precision:

It has been calculated on three Calibrators, examined in 16 replicates in three separate runs with three lots.

Results are reported as follows

TOXOG.CE: lot 0503

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.067	0.066	0.070	0.067
Std.Deviation	0.006	0.005	0.006	0.006
CV %	9.3	7.7	9.0	8.7

Calibrator 50 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.276	0.259	0.268	0.267
Std.Deviation	0.025	0.006	0.010	0.014
CV %	9.1	2.4	3.6	5.0

Calibrator 1000 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.768	2.657	2.707	2.711
Std.Deviation	0.118	0.098	0.101	0.106
CV %	4.3	3.7	3.7	3.9

TOXOG.CE: lot # 0403

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.067	0.065	0.068	0.066
Std.Deviation	0.003	0.004	0.006	0.004
CV %	5.2	6.3	8.3	6.6

Calibrator 50 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.270	0.262	0.265	0.265
Std.Deviation	0.012	0.009	0.008	0.010
CV %	4.5	3.4	3.1	3.7

Calibrator 1000 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.765	2.652	2.718	2.712
Std.Deviation	0.115	0.101	0.092	0.103
CV %	4.2	3.8	3.4	3.8

TOXOG.CE: lot # 0303

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.068	0.067	0.069	0.068
Std.Deviation	0.004	0.004	0.006	0.004
CV %	5.1	6.1	8.0	6.4

Calibrator 50 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.268	0.261	0.265	0.265
Std.Deviation	0.012	0.009	0.008	0.010
CV %	4.6	3.3	3.2	3.7

Calibrator 1000 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.766	2.651	2.719	2.712
Std.Deviation	0.115	0.100	0.091	0.102
CV %	4.2	3.8	3.3	3.8

The variability shown in the tables above did not result in sample misclassification.

5. Accuracy
The assay accuracy has been checked by the dilution and recovery tests. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled out up to 4.000 IU/ml.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 11.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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TOXO IgG

Ensayo inmunoenzimático para la determinación cualitativa/cuantitativa de anticuerpos IgG frente a *Toxoplasma gondii* en plasma y suero humano

Uso exclusivo para diagnóstico “in vitro”

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96 pruebas

TOXO IgG

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos IgG frente a *Toxoplasma gondii*, en plasma y suero humanos.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
Toxoplasma gondii es un protozoo, parásito intracelular obligado, que puede infectar probablemente a todas las especies de mamíferos, incluido el hombre.
La detección de anticuerpos IgM contra *T. gondii* es particularmente útil en el diagnóstico de la infección aguda, ya sea en los individuos "de riesgo", durante el embarazo, en personas sometidas a trasplante de órganos, o en pacientes con SIDA.
Gran parte de las infecciones por *T. gondii* en individuos sanos son leves o asintomáticas. La detección de anticuerpos IgM al mismo, en ausencia de anticuerpos detectables de clase IgG, es de gran importancia en el seguimiento de infecciones agudas durante el embarazo ya que el parásito puede ocasionar severos trastornos en el noenato. Por otra parte, como las infecciones agudas por *T. gondii* son severas en pacientes inmunocomprometidos, deben ser diferenciadas de otros tipos de trastornos.
El sistema ELISA de captura de IgM constituye una prueba diagnóstica potente y confiable, sobretodo porque no se ve afectada en presencia del factor reumatoideo como ha sucedido en los ensayos clásicos tipo "sandwich".

C. PRINCIPIOS DEL ENSAYO.
Los pocillos de la placa están recubiertos con antígenos nativos de *T. gondii*, purificados por gradiente de centrifugación con sacarosa e inactivados.
Se añade la muestra diluída, y los anticuerpos IgG contra *T. gondii* presentes en la misma son capturados por los antígenos de la fase sólida.
Después del lavado, en la 2ª incubación, los anticuerpos IgG anti *Toxoplasma gondii* son detectados mediante anticuerpos policlonales específicos anti-IgG humana, conjugados con Peroxidasa (HPR).
La enzima capturada en la fase sólida, combinada con la mezcla substrato/cromógeno, genera una señal óptica proporcional a la cantidad de anticuerpos IgG anti-*T. gondii* presentes en la muestra. Posteriormente, con la ayuda de una Curva de Calibración contra el 3er estándar internacional de la Organización Mundial de la Salud (O.M.S), es posible determinar cuantitativamente los anticuerpos IgG contenidos en la muestra.

D. COMPONENTES.
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
12 tiras de 8 pocillos recubiertos con *T. gondii*, altamente purificado e inactivado por radiaciones gamma en presencia de proteínas del suero bovino.
Las placas están almacenadas en bolsas selladas con desecante. Se deben poner las mismas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y conservar a 2-8ºC.

2. Curva de Calibración: CAL N° …
6x2.0 ml/vial. Listo para el uso y curva con código estándar de color, calibrada contra el 3er estándar International producido por la O.M.S. con rangos:

4ml CAL 1 = 0 O.M.S IU/ml
4ml CAL 2 = 50 O.M.S IU/ml
2ml CAL 3 = 100 O.M.S IU/ml
2ml CAL 4 = 250 O.M.S IU/ml
2ml CAL 5 = 500 O.M.S IU/ml
4ml CAL 6 = 1000 O.M.S IU/ml.
Contiene plasma positivo a Toxo IgG, titulado contra el 3er estándar internacional O.M.S. (código: TOXM), caseína al 2%, tampón Citrato de sodio 10 mM pH 6.0+/-0.1, 0.1% de Tween 20, así como azida sódica 0.09% y ProClin 300 0.045% como conservantes. Los estándar son de color azul.

3. Suero Control: CONTROL …ml
1 vial. Liofilizado. Para ser disuelto en el volumen de agua de calidad EIA indicado en la etiqueta. Contiene proteínas del suero bovino fetal, plasma humano positivo a *T. gondii a* 250 IU/ml +/-10% calibrado contra el 3er estándar internacional O.M.S. (código: TOXM), contiene además sulfato de gentamicina 0.2 mg/ml y ProClin 300 0.045% como conservantes.
Nota: El volumen necesario para disolver el contenido del frasco varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

4. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%

5. Conjugado: CONJ
2x8ml/vial. Solución lista para el uso. Codificado con el color rojo. Contiene anticuerpos policlonales anti-IgG humanos conjugados con Peroxidasa (HRP), BSA 5%, tampón Tris 10 mM pH 6.8+/-0.1, además sulfato de gentamicina 0.02% y ProClin 300 0.045% como conservantes.

6. Cromógeno/Substrato. SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM pH 3.5-3.8, dimetilsulfóxido 4%, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02%.
Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

7. Ácido Sulfúrico: H_2SO_4 O.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Diluente de muestras: DILSPE
2x60ml/vial. Contiene proteínas del suero de cabra, 2% de caseína, tampón Citrato de sodio 10 mM pH 6.0 +/-0.1, 0.1% de Tween 20, azida sódica al 0.09% y ProClin 300 0.045% como conservantes. Se utiliza para diluir las muestras.

9. Sellador adhesivo, n° 2

10. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.
1. Micropipetas calibradas (1000µl, 100µl y 10µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (tolerancia+/-1°C).
6. Lector calibrado de microplacas de ELISA con filtros de 450 nm (lectura) y filtros de 620-630 nm.

7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar los indumentos protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbicas cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contengan precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas depués de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de vencimiento indicada en el equipo e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 3 meses.
11. Tratar todas las muestras como potencialmente infectivas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infectivos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según la técnica estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben ser identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante al menos 12 meses, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.
6. Aquellas muestras, cuya concentración de IgG anti-*T. gondii* se sospeche mayor de 1000 IU/ml, deben diluirse a 1:10 o 1:100 antes del uso, con ayuda del Calibrador 0 IU/ml. Las diluciones deben efectuarse en tubos limpios desechables añadiendo 50 µl de la muestra y 450 µl del Cal 0 (1:10), después 50 µl de la dilución 1:10 y 450 µl del Cal 0 (1:100). Mezclar los tubos en el vortex y después proseguir con los pasos indicados en la sección M.

PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 3 meses.

Microplaca:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Curva de Calibración:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Suero Control:

Añadir al polvo liofilizado el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver totalmente y mezclar delicadamente en el vórtex.

Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada y mezclarse delicadamente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*
Conjugado:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Diluente de muestras :
Solución lista para el uso. Mezclar bien con un vórtex antes de usar.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra (etanol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450 nm y de un segundo filtro de 620-630 nm obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10 b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medida de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y las de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no sean contaminados con partículas o agregados visibles.
3. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico.
4. Compruebe que no han ocurrido roturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
5. Disolver el Suero Control liofilizado, como se ha descrito anteriormente.
6. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
7. Dejar los componentes restantes hasta alcanzar la temperatura ambiente (aprox. 1 hora), mezclar después suavemente en el vórtex todos los reactivos líquidos.

8. Ajustar la incubadora de ELISA a 37ºC y alimentar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
9. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
10. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
11. Comprobar que las micropipetas estén fijadas en el volumen requerido.
12. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
13. En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

El equipo puede usarse tanto para la determinación cuantitativa como cualitativa.

M1. DETERMINACIÓN CUANTITATIVA:

Ensayos Automatizados.

En caso de que el ensayo se realice de manera automatizada con un sistema ELISA, se recomienda programar el equipo para aspirar 1000µl de Diluente de Muestras, y posteriormente 10µl de muestra (factor de dilución 1:101).

La mezcla debe ser dispensada cuidadosamente en un tubo de dilución. Antes de aspirar la muestra siguiente, las agujas deben lavarse debidamente para evitar cualquier contaminación cruzada entre las muestras. Una vez diluídas las mismas, programar el equipo para dispensar 100 µl de cada una en los pocillos correspondientes.

Este procedimiento puede realizarse además en dos pasos de dilución 1:10 (90 µl Diluente de Muestras + 10 µl Muestra) en una segunda plataforma de dilución. Después, se recomienda programar el equipo para aspirar 100µl de Diluente de Muestras y 10µl de la primera dilución en la plataforma, posteriormente dispensar el contenido total en los pocillos correspondientes.

No es necesario diluir el Calibrador ni el Suero Control (ya diluído) pues están listos para el uso.

Dispensar 100µl de controles/calibradores en los pocillos correspondientes.

Para las operaciones siguientes, consulte las instrucciones que aparecen a continuación para el Ensayo Manual.

Es muy importante comprobar que el tiempo entre el dispensado de la primera y la última muestra sea calculado por el instrumento y considerado para los lavados.

Ensayo Manual.

1. Diluir las muestras 1:101 en un tubo de dilución apropiado (ejemplo: 1000 de Diluente de Muestras+10µl de muestra). No diluir el Panel de Calibración, ya que los calibradores están listos para el uso. Mezclar cuidadosamente, con ayuda de un vórtex, todos los componentes líquidos y continuar como se describe a continuación.
2. Poner el número de tiras necesarias en el soporte de plástico. Dejar vacíos los pocillos A1 y B1 para el blanco.
3. Dispensar 100 µl de Calibradores y 100 µl de Suero Control, por duplicado, después dispensar 100 µl de cada muestra diluída en su pocillo correspondiente.
4. Incubar la microplaca **60 min a +37°C**.

***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el ensayo manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

5. Lavar la microplaca con el lavador automático dispensando y aspirando 350µl/pocillo de solución de lavado diluída, segun según se indica (section I.3).
6. Dispensar 100µl del Conjugado en todos los pocillos, excepto en A1 y B1, después cubrir con el sellador. Compruebe que este reactivo de color rojo ha sido añadido en todos los pocillos excepto A1 y B1.

***Notas importantes**:*
Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.
Mezclar el Conjugado en el vórtex antes de usarlo!

7. Incubar la microplaca durante **60 minutos a +37ºC.**
8. Lavar los pocillos de igual forma que en el paso 5.
9. Dispensar 100µl de TMB/H_2O_2 en todos los pocillos, incluidos los del blanco. Controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca por **20 minutos a temperatura ambiente (18-24°C).**

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

10. Dispensar 100µl de Ácido Sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición del ácido cambia el color de los calibradores positivos, el suero control y las muestras positivas de azul a amarillo.
11. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco B1 (blanco).

Notas generales importantes:

1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de stop y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*
3. *El suero de control (CS) no afecta al cálculo de los resultados de la prueba. El suero de control (CS) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

M2. DETERMINACIÓN CUALITATIVA:

Si se requiere solamente un análisis cualitativo, proceda como se indica a continuación.

Ensayo automatizado:

Proceder según la sección M1.

Ensayo Manual.

1. Diluir las muestras 1:101 en un tubo de dilución apropiado (ejemplo: 1000 de Diluente de Muestras+10µl de muestra). No diluir el Panel de Calibración, ya que los calibradores están listos para el uso. Mezclar cuidadosamente, con ayuda de un vórtex, todos los componentes líquidos y continuar como se describe a continuación.
2. Poner el número de tiras necesarias en el soporte de plástico. Dejar vacío el pocillo A1 para el blanco.
3. Dispensar 100 µl del Calibrador 0 IU/ml y 100 µl del Calibrador 50 IU/ml por duplicado, y 100 µl del Calibrador 1000 IU/ml sencillo. Después dispensar 100 µl de cada muestra diluída 1:101 en su pocillo correspondiente.
4. Incubar la microplaca **60 min a +37°C**.***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo*

cuando se hace el ensayo manualmente. No sellar cuando se emplean equipos automatizados de ELISA.

5. Lavar la microplaca con el lavador automático dispensando y aspirando 350µl/pocillo de solución de lavado diluída, según se indica (section I.3).
6. Dispensar 100µl del Conjugado en todos los pocillos, excepto en A1, después cubrir con el sellador. Compruebe que este reactivo de color rojo haya sido añadido en todos los pocillos excepto A1.

***Notas importantes**:*
Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.
Mezclar el Conjugado en el vórtex antes de usarlo!

7. Incubar la microplaca durante **60 minutos a +37ºC.**
8. Lavar los pocillos de igual forma que en el paso 5.
9. Dispensar 100µl de TMB/H_2O_2 en todos los pocillos, incluido el del blanco. Controlar que los reactivos hayan sido correctamente añadidos. Incubar la microplaca durante **20 minutos a temperatura ambiente (18-24°C).**

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

10. Dispensar 100µl de Ácido Sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición del ácido cambia el color de los calibradores positivos, el suero control y las muestras positivas de amarillo a azul.
11. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

N. ESQUEMA DEL ENSAYO.

Método	**Operaciones**
Calibradores & Control Muestras diluídas 1:101	100 µl 100 µl
1ra incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2da incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
TMB/H2O2	100 µl
3ra incubación	20 min
Temperatura	t.a.*
Ácido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado en el análisis cuantitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	CAL4	M 1									
B	BL	CAL4	M 2									
C	CAL1	CAL5	M 3									
D	CAL1	CAL5	M 4									
E	CAL2	CAL6	M 5									
F	CAL2	CAL6	M 6									
G	CAL3	SC	M 7									
H	CAL3	SC	M 8									

Leyenda: BL = Blanco // CAL = Calibradores // M = Muestra // SC = Suero Control

A continuación se describe un ejemplo del esquema de dispensado en el análisis cualitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 3	M 11									
B	CAL1	M 4	M 12									
C	CAL1	M 5	M 13									
D	CAL2	M 6	M 14									
E	CAL2	M 7	M 15									
F	CAL6	M 8	M 16									
G	M 1	M 9	M 17									
H	M 2	M 10	M 18									

Leyenda: BL = Blanco // CAL = Calibradores //M = Muestra

O. CONTROL DE CALIDAD INTERNO.
Se realiza un grupo de pruebas de validación con los controles cada vez que se usa el equipo para verificar si el funcionamiento del ensayo es correcto, según las directivas IVDD 98/79/EC.
Asegurar el cumplimiento de los siguientes parámetros :

Parámetro	**Exigencia**
Pocillo Blanco	< 0.100 DO450nm
Calibrador 0 IU/ml (CAL1)	< 0.150 valor medio DO450nm después de leer el blanco Coeficiente de variación < 30%
Calibrador 50 IU/ml	DO450nm > DO450nm CAL1 + 0.100
Calibrador 1000 IU/ml	DO450nm > 1.000
Suero Control	250 O.M.S. IU/ml +/-10%

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.
En caso contrario, detenga el ensayo y compruebe:

Problema	**Compruebe que**
Pocillo blanco > 0.100 DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Calibrador 0 IU/ml > 0.150 DO450nm después de leer el blanco Coeficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido alimentado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensado de un calibrador positivo en lugar del negativo). 4. no ha existido contaminación del Cal negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.

Calibrador 50 IU/ml DO450nm < DO450nm CAL1 + 0.100	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Calibrador 1000 IU/ml < 1.000 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Suero Control Valor distinto al esperado	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar una muestra equivocada). 3. el proceso de lavado y los parámetros del lavador son correctos. 4. no ha ocurrido contaminación externa de los controles. 5. el Suero Control ha sido disuelto con el volumen correcto indicado en la etiqueta Si se indica un error, el ensayo debe repetirse tras eliminar la causa del mismo. En caso de no encontrar un error, procédase como sigue: a) si se obtiene un valor hasta +/-20%: la precisión global del laboratorio podría no permitir alcanzar +/-10% del valor esperado. Comunicar el problema al responsable para aceptar ó rechazar este resultado. b) si se obtiene un valor superior a +/-20%: en este caso el test es inválido y hay que avisar al servicio de atención al cliente de DiaPro

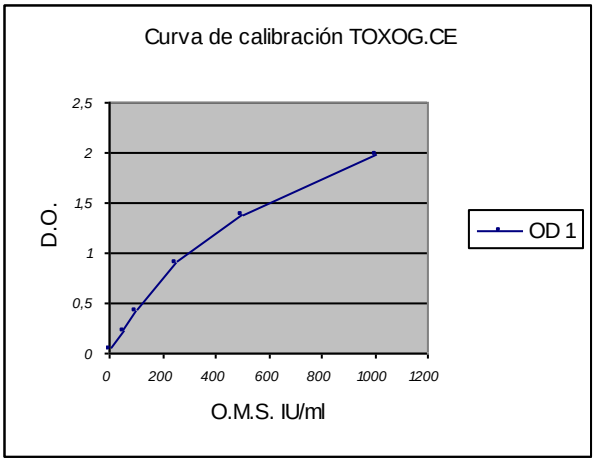
De presentarse alguno de los problemas anteriores, después de comprobar, avisar al responsable para tomar las medidas pertinentes.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 11.

P. RESULTADOS.

P.1 Método cuantitativo.
Si el ensayo resulta válido, usar para el método cuantitativo un sistema de ajuste de curva para diseñar la curva de calibración con los valores obtenidos en la lectura a 450nm (se sugiere interpolar 4 parámetros).
Posteriormente, calcular sobre la curva de calibración la concentración de anticuerpos IgG contra el *T. gondii* presentes en la muestra.

A continuación, un ejemplo de curva de calibración:



Nota Importante:
No usar la curva anterior para formular los cálculos.

P.2 Método cualitativo.
En el método cualitativo, calcular los valores medios de DO450nm para los Calibradores 0 y 50 IU/ml, después comprobar que el ensayo es válido.

A continuación, un ejemplo de los cálculos a realizar para el método cualitativo: (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 11).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Calibrador 0 IU/ml: 0.020 – 0.024 DO450nm
Valor medio : 0.022 DO 450nm
Menor de 0.150 – Válido

Calibrador 50 IU/ml: 0.250 – 0.270 DO 450nm
Valor medio : 0.260 DO 450nm
Mayor que Cal 0 + 0.100 – Válido

Calibrador 250 IU/ml: 2.845 DO 450nm
Mayor que 1.000 – Válido

Q. INTERPRETACIÓN DE LOS RESULTADOS.
Debe ponerse particular atención a la interpretación de los resultados en el seguimiento del embarazo, debido a que la infección por *T. gondii* puede provocar malformaciones en el neonato.
El cut-off del producto ha sido posizionado a 50 IU/ml, y no debajo de esta, en modo de assigurar al test un mayor valor diagnostico, en particular sobre todo cuando il dosaje es aplicado durante il monitorage de las mujeres embarazadas.
Debido a la infecciòn, de echo a parte del primerisimo periodo di sieroconversiòn, el paciente desarrolla una fuerte respuesta immunologica contra l'agente infectante, que sobrepasa di bastante las 50 IU/ml.
Anticuerpos con titulo bajo(debajo de 50 IU/ml) muestran prevalentemente una baja reactividad contra el Toxoplasma gondii y pueden asi representar un marcador diagnostico de una reciente infecciòn, en combinaziòn con los IgM.
Las muestras con una concentración menor de 50 OMS IU/ml son consideradas negativas a anticuerpos IgG contra *Toxoplasma gondii,* en modo de inducir el medico a considerar tales sujetos 'a riesgo' y de proseguir el monitorage sea de los IgG que de los IgM durante el embarazo.

Las muestras con una concentración mayor de 50 OMS IU/ml se consideran positivas a anticuerpos IgG contra el Virus Toxoplasma gondii y del punto de vista inmunológico protejidos contra la infecciòn. Este título es considerado, según NCCLS Estados Unidos, la menor concentración de IgG que ofrece una protección inmunológica efectiva contra una segunda infección por *Toxoplasma gondii*.

Notas importantes:

1. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
2. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
3. *En el monitoreo de infección por Toxoplasma Gondii durante el embarazo, un resultado positivo (presencia de anticuerpos IgG > 50 IU/ml) debe ser confirmado para eliminar cualquier riesgo de falso positivo o falsa definición de protección.*

R. FUNCIONAMIENTO.

La evaluación del funcionamiento ha sido realizada según lo establecido en el estándar prEN 13612.

1. Límite de detección.

El límite de detección del ensayo ha sido calculado por medio del 3er estándar internacional producido por la Organización Mundial de la Salud (O.M.S).

El límite de detección ha sido calculado como valor medio de DO450nm del Calibrador 0 OMS U/ml + 5 SD.

La siguiente tabla muestra los valores medios de DO450nm del estándar, diluído en plasma negativo y examinado en el ensayo.

Valores DO450nm

O.M.S. IU/ml	TOXOG.CE Lote # 0503	TOXOG.CE Lote # 0403	TOXOG.CE Lote # 0303
250	0.816	0.853	0.974
100	0.365	0.398	0.445
50	0.209	0.244	0.246
10	0.094	0.125	0.108
Est 0	0.033	0.031	0.056

El ensayo demuestra un límite de detección superior a 10 IU/ml.

2. Sensibilidad Diagnóstica:

La sensibilidad diagnóstica se ha estudiado en un ensayo clínico externo utilizando paneles de muestras, clasificadas como positivas mediante un equipo de referencia US FDA. Se probaron muestras positivas correspondientes a diferentes etapas de la infección por *Toxoplasma gondii.*

El valor obtenido del análisis de más de 300 muestras fue > 98%.

3. Especificidad Diagnóstica::

La especificidad diagnóstica ha sido determinada en el mismo centro, utilizando paneles de muestras provenientes de individuos sanos, clasificadas como negativas mediante un equipo de referencia US FDA.

Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos para determinar la especificidad. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

Las muestras congeladas han sido analizadas para comprobar si la colección y la conservacion interfiere con el procedimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.

Se analizaron muestras de potencial interferencia derivadas de pacientes con diversas patologías (mayormente positivos a ANA, AMA y RF) y de mujeres embarazadas. No se observaron reacciones cruzadas.

Se obtuvo un valor de especificidad total > 98% al examinar más de 100 muestras.

4. Precisión:

Ha sido calculada a partir de tres Calibradores examinados en 16 réplicas en tres corridas separadas, para 3 lotes.

Los resultados son los siguientes:

TOXOG.CE: lote 0503

Calibrador 0 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.067	0.066	0.070	0.067
Desviación estándar	0.006	0.005	0.006	0.006
CV %	9.3	7.7	9.0	8.7

Calibrador 50 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.276	0.259	0.268	0.267
Desviación estándar	0.025	0.006	0.010	0.014
CV %	9.1	2.4	3.6	5.0

Calibrador 1000 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	2.768	2.657	2.707	2.711
Desviación estándar	0.118	0.098	0.101	0.106
CV %	4.3	3.7	3.7	3.9

TOXOG.CE: lote # 0403

Calibrador 0 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.067	0.065	0.068	0.066
Desviación estándar	0.003	0.004	0.006	0.004
CV %	5.2	6.3	8.3	6.6

Calibrador 50 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.270	0.262	0.265	0.265
Desviación estándar	0.012	0.009	0.008	0.010
CV %	4.5	3.4	3.1	3.7

Calibrador 1000 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	2.765	2.652	2.718	2.712
Desviación estándar	0.115	0.101	0.092	0.103
CV %	4.2	3.8	3.4	3.8

TOXOG.CE: lote # 0303

Calibrador 0 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.068	0.067	0.069	0.068
Desviación estándar	0.004	0.004	0.006	0.004
CV %	5.1	6.1	8.0	6.4

Calibrador 50 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.268	0.261	0.265	0.265
Desviación estándar	0.012	0.009	0.008	0.010
CV %	4.6	3.3	3.2	3.7

Calibrador 1000 IU/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	2.766	2.651	2.719	2.712
Desviación estándar	0.115	0.100	0.091	0.102
CV %	4.2	3.8	3.3	3.8

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

5. Exactitud.
La exactitud del ensayo ha sido comprobada mediante diluciones y pruebas de recuperación. Cualquier "efecto gancho", estimación errónea que puede presentarse a elevadas dosis del analita, no se manifiesta hasta 4.000 IU/ml.

Nota importante:
Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 11.

S. LIMITACIONES DEL PROCEDIMIENTO.
La contaminación bacterica o la inactivación por calor de la muestra pueden afectar los valores de DO y por tanto alterar los niveles del analita.
Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.
El ensayo es útil solo para probar muestras independientes y no mezclas.
El diagnóstico de una enfermedad infecciosa no debe establecerse en base a un solo resultado, sino que deben tenerse en consideración la historia clínica del paciente, la sintomatología, así como otros datos diagnósticos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni
(Milán) - Italia

CE
0318

Toxo IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM antibodies to Toxoplasma gondii in human plasma and sera

- for "in vitro" diagnostic use only -

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REF TOXOM.CE
96 tests

Toxo IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Toxoplasma gondii or T.gondii in human plasma and sera with the "capture" system.
The devise is intended for the follow-up of T.gondii infected patients and for the monitoring of risk of neonatal defects due to T.gondii infection during pregnancy.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that is probably capable of infecting all species of mammals, including man.
The detection of IgM antibodies to T.gondii is particularly helpful for the diagnosis of acute infections in “risk” individuals, in association with AIDS, organ transplantation and pregnancy.
As most of T.gondii infections are mild or asymptomatic in otherwise healthy individuals, the detection of T.gondii specific IgM antibodies, in absence of detectable specific IgG, has become important for the monitoring of acute infections in pregnant women, as the parasite can lead to severe birth defects.
Moreover, as T.gondii infections are most severe in immunocompromised patients, where the disease can be fatal, acute infections due to this parasite have to be distinguished from other disorders.
Recently developed IgM capture assays provide the clinician with a helpful and reliable test, not affected by the rheumatoid factor as it happens to be in classic sandwich tests.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a preparation of inactivated T.gondii, labeled with a specific monoclonal antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of peroxidase the colorless substrate is hydrolysed to a colored end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to T.gondii present in the sample.
A system is described how to control whether the positivity shown by a sample is true or not (Confirmation Test), helpful for the clinician to make a correct interpretation of results.

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with anti human IgM affinity purified goat antibody, in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -
1x4.0 ml/vial. Ready to use control. It contains 1% human plasma negative for T.gondii IgM, 2% casein, 10 mM Tris-citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The negative control is colorless.

3. Positive Control: CONTROL +
1x4.0 ml/vial. Ready to use control. It contains 1% human plasma positive for T.gondii IgM, 2% casein, 10 mM Tris-citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The positive control is green colour coded.

4. Calibrator: CAL …ml
N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti T.gondii IgM at 200 WHO IU/ml +/-10% (3rd WHO International Standard for T.gondii IgG&IgM), fetal bovine serum, 0.2 mg/ml gentamicine sulphate and ProClin 300 0.045% as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Lyophilized T.gondii Ag: AG TOXO
N° 6 lyophilized vials.
The vials contain lyophilized gamma ray inactivated Toxoplasma gondii in a protein buffer. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300. To be dissolved with 1.9 ml of Antigen Diluent as reported in the specifi section.

6. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300

7. Enzyme conjugate: CONJ 20X
1x0.8 ml/vial. 20x concentrated solution of a T.gondii-specific monoclonal antibody, labeled with HRP and diluted in a protein buffer containing 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives.

8. Antigen Diluent : AG DIL
n° 1 vial of 16 ml. Protein buffer solution for the preparation of the Immunocomplex. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code colored with 0.01% red alimentary dye

9. Specimen Diluent : DILSPE
2x60.0 ml/vial. Proteic buffered solution for the dilution of samples. It contains 2% casein, 10 mM citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The reagent is color coded with 0.01% blue alimentary dye.

10. Chromogen/Substrate : SUBS TMB
1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use.

Calibrator:
Add the volume of ELISA grade water reported on the label to the lyophilized powder. Let fully dissolve and then gently mix on vortex.

***Important Note**: The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
***Note:** Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Ag/Ab Immunocomplex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved T.gondii antigen and mix gently on vortex.

***Important Notes**:*
1. *Dissolve and prepare only the number of vials necessary to the test. The Immunocomplex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.*
2. *The preparation of the Immucomplex has to be done **right before** the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.*

Specimen Diluent:
Ready to use. Mix well on vortex before use

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check

that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

M.1 Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Specimen Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl diluted samples into the proper wells of the microplate.

This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Specimen Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.

Do not dilute controls/calibrator as they are ready to use.

Dispense 100 µl calibrators/control in the appropriate calibration/control wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

M. 2 Manual assay:

1. Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
2. Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
3. Dispense 100 µl of Negative Control in triplicate and 100 µl of Calibrator in the proper wells in duplicate. Dispense 100 µl of Positive Control in single into the proper well. Do not dilute controls and the calibrator as they are ready to use !
4. Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue colored and that controls and calibrator have been dispensed.
5. Incubate the microplate for **60 min at +37°C** .

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer as reported previously (section I.3).
7. Pipette 100 µl Ag/Ab Immunocomplex into each well, except the blanking well A1, and cover with the sealer. Check that all wells are red colored, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Ag/Ab Immunocomplex . Contamination might occur.*

8. Incubate the microplate for **60 min at +37°C** .
9. Wash microwells as in step 6.
10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 (mandatory).

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Controls&calibrator Samples diluted 1:101	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Immunocomplex	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A quality control check is performed on the controls/calibrator any time the kit is used in order to verify whether the performance of the assay matches the requirements reported in table below.

Parameter	Requirements
Blank well	< 0.050 OD450nm value
Negative Control mean value (NC)	< 0.150 OD450nm value after blanking coefficient of variation < 30%
Calibrator	S/Co > 1.5
Positive Control	> 0.750 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 1.5	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 0.750 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

Cut-Off = NC + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 - 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient is not undergoing an acute infection of Toxoplasma gondii.
Any patient showing an equivocal result, should be re-tested by examining a second sample taken from the patient after 1-2 weeks from first testing.
A positive result is indicative of a Toxoplasma gondii infection.

An example of calculation is reported below:

***Important Note:** The following data must not be used instead or real figures obtained by the user.*

Negative Control: 0.050 – 0.060 – 0.070 OD450nm
Mean Value: 0.060 OD450nm
Lower than 0.150 – Accepted
Positive Control: 1.850 OD450nm
Higher than 0.750 – Accepted

Cut-Off = 0.060+0.250 = 0.310

Calibrator: 0.550 - 0.530 OD450nm
Mean value: 0.540 OD450nm S/Co = 1.7
S/Co higher than 1.0 – Accepted

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1 = negative
Sample 2 S/Co > 1.2 = positive

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of Toxoplasma gondii due to the risk of severe neonatal malformations.*
3. *Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, leading to a misinterpretation of the analytical result, can be revealed and then ruled out.*
4. *In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a different device for T.gondii IgM detection, before taking any preventive medical action.*
5. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
6. *Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

Dia.Pro Diagnostic BioProbes s.r.l. has defined the 3rd WHO International Standard for TOXO IgG (Coded TOXG), positive also for IgM anti Toxoplasma Gondii, as an Internal Gold Standard (or IGS).
Results of Quality Control are given in the following table:

The limits of detection of this material, when diluted first in negative serum and then in the sample diluent to generate dilutions tested in four replicates, are reported in the following table for three lots of the device:

OD450nm values

WHO (IGS) IU/ml	TOXOM.CE Lot # 0703	TOXOM.CE Lot # 0603	TOXOM.CE Lot # 0503
3000 IU/ml	2.936	3.005	2.983
1500 IU/ml	2.547	2.615	2.589
750 IU/ml	2.350	2.433	2.378
375 IU/ml	1.368	1.452	1.377
188 IU/ml	0.911	1.125	0.968
94 IU/ml	0.522	0.637	0.561
47 IU/ml	0.271	0.338	0.285
23 IU/ml	0.176	0.171	0.115
Negative	0.060	0.055	0.052

In addition the preparation Accurun n° 136 supplied by Boston Biomedica Inc., USA, has been also used to detect the sensitivity of the devise. The preparation was examined on three lots in 4 replicates. Results, expressed as S/Co values, are reported in table below:

ACCURUN # 136	TOXOM.CE Lot # 0703	TOXOM.CE Lot # 0603	TOXOM.CE Lot # 0503
1X	0.808	0.957	0.796
2X	0.389	0.468	0.369
4X	0.169	0.228	0.188
8X	0.065	0.078	0.059
Negative	0.051	0.063	0.044

2. Diagnostic Sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from patients carrying T.gondii acute infection, confirmed by clinical symptoms and analysis.
An overall value > 98% has been found in the study conducted on a total number of more than 60 samples.
The Performance Panel code PTT 201, supplied by Boston Biomedica Inc. USA, has been also evaluated. Data are reported below:

BBI Performance Panel code PTT 201

Sample ID	TOXOM.CE OD450nm	S/Co	REF BioMerieux VIDAS S/Co	Sample ID	TOXOM.CE OD450nm	S/Co	REF BioMerieux VIDAS S/Co
1	0.052	0.1	0.3	14	0.082	0.2	0.2
2	0.048	0.1	0.1	15	0.121	0.3	0.2
3	0.078	0.2	0.1	16	0.049	0.1	0.1
4	0.072	0.2	0.4	17	0.476	**1.4**	**1.5**
5	0.048	0.1	0.1	18	0.057	0.1	0.1
6	0.044	0.1	0.1	19	0.185	0.5	0.2
7	0.045	0.1	0.1	20	0.092	0.2	0.4
8	1.134	**3.5**	**3.5**	21	0.165	0.5	0.1
9	0.126	0.3	0.1	22	0.084	0.2	0.1
10	0.047	0.1	0.1	23	3.181	**9.8**	**10.3**
11	1.232	**3.8**	**2.4**	24	0.137	0.4	0.2
12	0.088	0.2	0.1	25	1.007	**3.1**	**1.8**
13	3.166	**9.8**	**7.3**				

3. Diagnostic Specificity:

The diagnostic specificity has been determined on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
A study conducted on more than 60 potentially cross-reactive samples has not revealed any interference in the system.
No cross reaction were observed.
The Performance Evaluation study conducted in a qualified external reference center on more than 400 total samples has provided a value > 98%.
False positive reactions may be anyway pointed out and then ruled out in the interpretation of results with the procedure reported in section T, able to verify whether or not a positive result is real.

4. Precision:
It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs. Results are reported as follows:

TOXOM.CE: lot # 0703

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.058	0.072	0.076	0.069
Std.Deviation	0.005	0.006	0.007	0.006
CV %	8.9	8.3	9.1	8.7

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.583	0.567	0.579	0.576
Std.Deviation	0.040	0.049	0.056	0.048
CV %	6.8	8.6	9.7	8.4

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.754	2.625	2.625	2.668
Std.Deviation	0.247	0.214	0.126	0.196
CV %	9.0	8.2	4.8	7.3

TOXOM.CE: lot # 0603

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.063	0.064	0.061	0.063
Std.Deviation	0.008	0.012	0.009	0.010
CV %	13.2	18.2	15.3	15.6

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.641	0.651	0.644	0.645
Std.Deviation	0.038	0.042	0.042	0.041
CV %	5.9	6.5	6.6	6.3

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.889	2.830	2.879	2.866
Std.Deviation	0.122	0.123	0.074	0.106
CV %	4.2	4.4	2.6	3.7

TOXOM.CE: lot # 0403

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.057	0.060	0.060	0.059
Std.Deviation	0.006	0.007	0.006	0.007
CV %	11.1	12.4	10.5	11.3

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.544	0.556	0.520	0.540
Std.Deviation	0.040	0.078	0.058	0.058
CV %	7.3	14.0	11.1	10.8

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.850	2.866	2.846	2.854
Std.Deviation	0.139	0.122	0.126	0.129
CV %	4.9	4.3	4.4	4.5

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of Toxoplasma gondii is released to the doctor.
Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section. This reagent is called Solution A.
2. Then 25 ul concentrated Enzymatic Conjugate are diluted in 500 ul Antigen Diluent and mixed gently on vortex. Do not use any lyophilized vial of T.gondii for this procedure ! This solution is called Solution B.
3. The well A1 of the strip is left empty for blanking.
4. The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and S/Co values.
5. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
6. The strip is incubated for 60 min at +37°C.
7. After washing, the blank well A1 is left empty.
8. 100 µl of Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl of Solution B are added to well E1.
10. The strip is incubated for 60 min at +37°C.
11. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
12. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 1.0 a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of T.gondii and a crossreaction with the monoclonal antibody, labeled with HRP, has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value lower than 1.2 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of T.gondii and not due to any crossreaction.

The following table is reported for the interpretation of results:

Well	S/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.2
Interpretation	Problem of contam.	False positive	True positive

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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Toxo IgM

Ensayo inmunoenzimático (ELISA) de "captura" para la determinación de anticuerpos IgM frente a *Toxoplasma gondii* en plasma y suero humanos

- Uso exclusivo para diagnóstico "in vitro"-

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REF TOXOM.CE
96 pruebas

Toxo IgM

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático (ELISA) para la determinación de anticuerpos IgM frente a *Toxoplasma gondii* en plasma y suero humanos, mediante un sistema de "captura".
El equipo ha sido concebido para el seguimiento de pacientes infectados por *T. gondii* y de la infección durante el embarazo, causa de riesgo de malformaciones en el neonato.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
Toxoplasma gondii es un protozoo, parásito intracelular obligado, que puede infectar probablemente a todas las especies de mamíferos, incluido el hombre.
La detección de anticuerpos IgM frente a *T. gondii* es particularmente útil en el diagnóstico de la infección aguda, ya sea en los individuos "de riesgo", durante el embarazo, en personas sometidas a trasplante de órganos, o en pacientes con SIDA.
Gran parte de las infecciones por *T. gondii* en individuos sanos son leves o asintomáticas. La detección de anticuerpos IgM al mismo, en ausencia de anticuerpos detectables de clase IgG, es de gran importancia en el seguimiento de infecciones agudas durante el embarazo ya que el parásito puede ocasionar severos trastornos en el neonato. Por otra parte, como las infecciones agudas por *T. gondii* son graves en pacientes inmunocomprometidos, deben ser diferenciadas de otros tipos de trastornos.
El sistema ELISA de captura de IgM constituye una prueba diagnóstica potente y segura, sobretodo porque no se ve afectada en presencia del factor reumatoide como ha sucedido en los ensayos clásicos tipo "sandwich".

C. PRINCIPIOS DEL ENSAYO.
El ensayo se basa en el principio de "captura de IgM", donde los anticuerpos de esta clase presentes en la muestra son capturados por la fase sólida recubierta con un anticuerpo anti-IgM humana.
Depués del lavado, que elimina el resto de los componentes de la muestra en particular los anticuerpos IgG, se adiciona una preparación inactivada de *T. gondii*, marcado con un anticuerpo monoclonal conjugado con Peroxidasa (HRP), lo cual permite detectar los anticuerpos IgM inmovilizados en la fase sólida.
Posteriormente a la incubación, los pocillos se lavan para eliminar cualquier traza de conjugado en exceso y se añade el substrato cromogénico. En presencia del conjugado el substrato es hidrolizado generándose una señal de color proporcional a la cantidad de anticuerpos IgM a *Toxoplasma gondii* presentes en la muestra.
La Prueba de Confirmación controla la presencia de falsos positivos, lo cual permite a los clínicos una correcta interpretación de los resultados.

D. COMPONENTES.
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
12 tiras de 8 pocillos recubiertos con anticuerpos monoclonales de cabra anti-IgM humana, purificados por afinidad, en presencia de proteinas de bovino.
Las placas están en una bolsa sellada con desecante. Se deben poner las mismas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y conservar entre 2 y 8ºC.

2. Control Negativo: CONTROL -
1x4.0 ml/vial. Listo para el uso. Contiene 1% de plasma humano negativo a IgM-*T. gondii*, 2% de caseína, tampón Tris-citrato 10 mM pH 6.0+/-0.1, 0.1% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como conservantes.
El control negativo es incoloro.

3. Control Positivo: CONTROL +
1x4.0 ml/vial. Listo para el uso. Contiene 1% de plasma humano positivo a IgM-*T. gondii*, 2% de caseína, tampón Tris-citrato 10 mM pH 6.0+/-0.1, 0.1% de Tween 20, y tambien azida sódica 0.09% y ProClin 300 0.045% como conservantes.
El control positivo está codificado con el color verde.

4. Calibrador: CAL …ml
n° 1 vial. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene IgM anti *T. gondii* a 200 O.M.S. IU/ml +/-10% (3er Estándar Internacional de la O.M.S. para *T. gondii* IgG&IgM), contiene además suero fetal bovino, sulfato de gentamicina 0.2 mg/ml y ProClin 300 0.045% como conservantes.
Nota: El volumen necesario para disolver el contenido del frasco varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Antígenos liofilizados de *T.gondii* : AG TOXO
N° 6 viales liofilizados. Contienen *T. gondii* liofilizado, inactivado por radiaciones gamma, diluído en un tampón proteico. Contienen además 2% de proteinas de bovino, tampón Tris HCl 10 mM pH 6.8+/-0.1, sulfato de gentamicina 0.2 mg/ml y ProClin 300 0.045% como conservantes.
Debe disolverse con 1.9 ml de Diluente de Antígeno, según se indica más adelante.

6. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%

7. Conjugado: CONJ 20X
1x0.8 ml/vial. Solución concentrada 20x de un anticuerpo monoclonal anti-*T. gondii*, conjugado con peroxidasa (HPR) diluído en un tampón proteico. Contiene tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, y tambien 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservantes.

8. Diluente de Antígeno: AG DIL
n° 1 vial de 16 ml. Solución tamponada proteica para la preparación del Inmunocomplejo. Contiene tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, además 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservantes. El reactivo está codificado con el color rojo (0.01% de colorante rojo).

9. Diluente de muestras DILSPE
2x60ml. Solución tamponada proteica para la dilución de las muestras. Contiene 2% de caseína, tampón Citrato 10mM a pH 6.8 +/- 0.1, 0.1% de Tween 20, además azida sódica al 0.09% y 0.045% de ProClin 300 como conservantes.
El reactivo está codificado con el color azul (0.01% de colorante azul).

10. Cromógeno/Substrato SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02% así como dimetilsulfóxido 4%.
Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

11. Ácido Sulfúrico: H_2SO_4 0.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Sellador adhesivo, n° 2

13. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (1000µl, 100µl y 10µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (+/-0.5°C tolerancia).
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar los indumentos protectores adecuados de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbicos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contengan precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente y reemplazar el equipo.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas depués de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de vencimiento indicada en el equipo e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 3 meses.
11. Tratar todas las muestras como potencialmente infectivas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infectivos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según la técnica estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben ser identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Se recomienda el uso del código de barras.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante varios meses, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Estudios de estabilidad realizados en equipos en uso no han arrojado pérdida de actividad significativa en un período de hasta 3 meses.

Microplacas:
Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.
Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el

desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Control Negativo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Control Positivo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Calibrador:
Añadir al polvo liofilizado, el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver completamente y después mezclar cuidadosamente con el vórtex antes de usar.
Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada y mezclarse delicadamente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

Inmunocomplejo Antígeno/Anticuerpo :
Proceder cuidadosamente según se indica:
1. Disolver el contenido de un vial liofilizado utilizando 1.9 ml de Diluente de Antígeno. Dejar disolver completamente y después mezclar cuidadosamente con el vórtex.
2. Mezclar el Conjugado concentrado con ayuda del vórtex. Añadir después 0.1 ml del mismo al vial del Ag de *T. gondii* disuelto y mezclar suavemente en el vórtex.

Notas Importantes*:*
1. *Disolver y preparar solamente los viales necesarios para la prueba. El inmunocomplejo obtenido no es estable. Almacenar la solución sobrante en alícuotas a –20°C.*
2. *La preparación del inmunocomplejo debe realizarse **justo antes** de dispensar las muestras y los controles en la placa. Mezclar nuevamente en vórtex justo antes de usar.*

Diluente de muestras :
Listo para el uso. Mezclar bien con un vórtex antes de usar.

Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.
1. Las micropipetas deben estar calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (acohol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periodicamente los residuos de los componentes del equipo.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC de tolerancia) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10nm b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medida de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por

muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.

7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no seancontaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido roturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Disolver el Calibrador como se ha descrito anteriormente y mezclar suavemente.
4. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
5. Dejar los componentes restantes hasta alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37ºC y alimentar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
11. En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

M.1 Ensayo automatizado:

En caso de que el ensayo se realice de manera automatizada con un sistema ELISA, se recomienda programar el equipo para aspirar 1000µl de Diluente de Muestras, y posteriormente 10µl de muestra (factor de dilución 1:101).

La mezcla debe ser dispensada cuidadosamente en un tubo de dilución. Antes de aspirar la muestra siguiente, las agujas deben lavarse debidamente para evitar cualquier contaminación cruzada entre las muestras. Cuando todas las muestras han sido diluídas, programar el equipo para dispensar 100 µl de las mismas en los pocillos correspondientes.

Este procedimiento puede realizarse en dos pasos de dilución de 1:10 cada uno (90 µl de Diluente de Muestras + 10 µl de muestra) en una segunda plataforma de dilución. Programar el equipo para aspirar primeramente 100 µl de Diluente de Muestras, después 10 µl de la primera dilución en la plataforma y finalmente dispensar todo el contenido en los pocillos apropiados de la microplaca.

No diluir el Calibrador ni los controles, ya que están listos para el uso.

Dispensar 100µl de controles/calibrador en los pocillos correspondientes.

Para las operaciones siguientes, consulte las instrucciones que aparecen debajo para el Ensayo Manual.

Es muy importante comprobar que el tiempo entre el dispensado de la primera y la última muestra sea calculado por el instrumento y considerado para los lavados.

M. 2 Ensayo Manual.

1. Diluir las muestras 1:101 dispensando primeramente 10 µl de muestra y después 1 ml de Diluente de Muestras en un tubo de dilución, mezclar bien con vórtex.
2. Poner el número de tiras necesarias en el soporte plástico. Dejar el pocillo A1 vacío para el blanco.
3. Dispensar 100 µl del Control Negativo por triplicado y 100µl de Calibrador por duplicado, después dispensar 100µl del Control Positivo, sencillo, en los respectivos pocillos. No diluir los controles ni el calibrador ya que están listos para el uso!
4. Dispensar 100 µl de las muestras diluídas en los pocillos correspondientes y chequear después que estos pocillos son de color azul y que los controles y el calibrador han sido añadidos.
5. Incubar la microplaca **60 min a +37°C**.

***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el ensayo manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Lavar la microplaca con el lavador automático según se indica (section I.3).
7. Dispensar 100µl del **Inmunocomplejo Antígeno/Anticuerpo** en todos los pocillos, excepto en el A1 y cubrir con el sellador. Compruebe que este reactivo de color rojo hyaa sido añadido en todos los pocillos excepto el A1.

***Nota importante**: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el **Inmunocomplejo Antígeno/Anticuerpo**. Podría producirse contaminación.*

8. Incubar la microplaca **60 min a +37°C**.
9. Lavar la microplaca, de igual forma que en el paso 6.
10. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1. Incubar la microplaca a **temperatura ambiente (18-24°C) por 20 minutos**.

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

11. Dispensar 100µl de Ácido Sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición del ácido cambia el color de los controles positivos y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo), calibrando el instrumento con el pocillo A1 (blanco, obligatorio).

Notas generales importantes:

1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de stop y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo*

3. *El calibrador (CAL) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba. El calibrador (CAL) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

N. ESQUEMA DEL ENSAYO.

Controles&Calibrador Muestras diluídas 1:101	100 µl 100 µl
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Inmunocomplejo	100 µl
2da incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H2O2	100 µl
3ra incubación	**20 min**
Temperatura	t.a.*
Ácido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 2										
B	CN	M 3										
C	CN	M 4										
D	CN	M 5										
E	CAL	M 6										
F	CAL	M 7										
G	CP	M 8										
H	M 1	M9										

Leyenda: BL = Blanco CN = Control Negativo
CAL = Calibrador CP = Control Positivo M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un control de validación sobre los controles y el calibrador cada vez que se usa el equipo, para verificar si el performance del ensayo es el esperado.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	< 0.050 DO450nm
Control Negativo, valor medio (CN)	< 0.150 DO450nm valor después de leer el blanco Coeficiente de variación < 30%
Calibrador	M/Co > 1.5
Control Positivo	> 0.750 DO450nm

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.
En caso contrario, detenga el ensayo y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.050DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control Negativo (CN) > 0.150 DO450nm después de leer el blanco Coefficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido alimentado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Calibrador M/Co < 1.5	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Control Positivo < 0.750 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, después de comprobar, informe al responsable para tomar las medidas pertinentes.

P. CÁLCULO DEL VALOR DE CORTE.

Los resultados de la prueba se calculan a partir de un valor medio de DO450nm del control Negativo (CN), mediante un valor de corte (Co) hallado con la siguiente fórmula:

Valor de corte = CN + 0.250

El valor encontrado en la prueba es utilizado para la interpretación de los resultados, según se describe a continuación.

***Nota Importante**: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS.

La interpretación de los resultados se realiza mediante la relaión entre las DO a 450nm de las muestras (M) y el Valor de corte (Co).
Los resultados se interpretan según la siguiente tabla:

(M/Co)	Interpretación
< 1.0	Negativo
1.0 – 1.2	Equívoco
> 1.2	Positivo

Un resultado negativo indica que el paciente no está padeciendo infección aguda por *T. gondii*.
Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial.
Un resultado positivo es indicativo de infección por *T. gondii*.

A continuación, un ejemplo de los cálculos a realizar:

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Control Negativo: 0.050 – 0.060 – 0.070 DO 450nm
Valor medio: 0.060 DO 450nm
Menor de 0.150 – Válido
Control Positivo: 1.850 DO 450nm
Mayor de 0.750 – Válido
Valor de corte = 0.060+0.250 = 0.310

Calibrador: 0.550 - 0.530 DO 450nm
Valor medio: 0.540 DO 450nm M/Co = 1.7
M/Co Mayor de 1.0 – Válido

Muestra 1: 0.070 DO 450nm
Muestra 2: 1.690 DO 450nm
Muestra 1 M/Co < 1 = negativa
Muestra 2 M/Co > 1.2 = positiva

Notas importantes:
1. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
2. *Debe ponerse particular atención a la interpretación de los resultados ante sospecha de infección primaria por* T. gondii *en el embarazo, debido al riesgo de malformaciones en el neonato.*
3. *Antes de emitir un criterio de positividad, cada muestra positiva debe ser sometida a la Prueba Confirmatoria reportada en la sección T. Mediante la misma es posible descartar cualquier error en la interpretación del resultado analítico producido por una falsa reactividad de la muestra.*
4. *En el monitoreo de infección por* T. gondii *durante el embarazo, se recomienda, antes de tomar cualquier decisión médica preventiva, confirmar cualquier resultado positivo, primero con el procedimiento descrito y después con un sistema de detección de IgM.*
5. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
6. *El diagnóstico de infección debe ser evaluado y comunicado al paciente por un médico calificado.*

R. CARACTERíSTICAS DEL PERFORMANCE.

1. Límite de detección.

Dia.Pro Diagnostic BioProbes s.r.l. ha definido como Gold Standard Interno (IGS) el 3er Estándar Internacional O.M.S. para TOXO IgG (Código TOXG), positivo además para IgM anti *Toxoplasma Gondii*.
En la siguiente tabla se muestran los resultados, para tres lotes del producto, después de una primera dilución en suero negativo y posteriormente en Diluente de Muestras con el objetivo de generar un sistema de diluciones a probar en cuatro réplicas.

Valores DO450nm

O.M.S (IGS) IU/ml	TOXOM.CE Lote # 0703	TOXOM.CE Lote # 0603	TOXOM.CE Lote # 0503
3000 IU/ml	2.936	3.005	2.983
1500 IU/ml	2.547	2.615	2.589
750 IU/ml	2.350	2.433	2.378
375 IU/ml	1.368	1.452	1.377
188 IU/ml	0.911	1.125	0.968
94 IU/ml	0.522	0.637	0.561
47 IU/ml	0.271	0.338	0.285
23 IU/ml	0.176	0.171	0.115
Negativo	0.060	0.055	0.052

Para detectar la sensibilidad del equipo se probó además la preparación Accurun n° 136 suministrada por Boston Biomedical Inc., Estados Unidos. La preparación ha sido examinada en tres lotes en cuatro réplicas. En la siguiente tabla se relacionan los resultados expresados como valores de M/Co:

ACCURUN # 136	TOXOM.CE Lote # 0703	TOXOM.CE Lote # 0603	TOXOM.CE Lote # 0503
1X	0.808	0.957	0.796
2X	0.389	0.468	0.369
4X	0.169	0.228	0.188
8X	0.065	0.078	0.059
Negativo	0.051	0.063	0.044

2. Sensibilidad Diagnóstica

La sensibilidad diagnóstica se ha estudiado utilizando paneles de muestras, clasificadas como positivas mediante un equipo de referencia US FDA.
Las muestras positivas se obtuvieron de pacientes con infección aguda por *T. gondii*, confirmada mediante análisis clínicos y la observación de los síntomas.
El valor del análisis obtenido después del estudio de más de 60 muestras, fue > 98%.
Se evaluaron además el Performance Panel PTT 201, suministrado por BBI, Estados Unidos.
Los valores se muestran a continuación.

BBI Performance Panel PTT 201

Muestra ID	TOXOM.CE DO450nm	M/Co	REF BioMerieux VIDAS M/Co	Muestra ID	TOXOM.CE DO450nm	M/Co	REF BioMerieux VIDAS M/Co
1	0.052	0.1	0.3	14	0.082	0.2	0.2
2	0.048	0.1	0.1	15	0.121	0.3	0.2
3	0.078	0.2	0.1	16	0.049	0.1	0.1
4	0.072	0.2	0.4	17	0.476	**1.4**	**1.5**
5	0.048	0.1	0.1	18	0.057	0.1	0.1
6	0.044	0.1	0.1	19	0.185	0.5	0.2
7	0.045	0.1	0.1	20	0.092	0.2	0.4
8	1.134	**3.5**	**3.5**	21	0.165	0.5	0.1
9	0.126	0.3	0.1	22	0.084	0.2	0.1
10	0.047	0.1	0.1	23	3.181	**9.8**	**10.3**
11	1.232	**3.8**	**2.4**	24	0.137	0.4	0.2
12	0.088	0.2	0.1	25	1.007	**3.1**	**1.8**
13	3.166	**9.8**	**7.3**				

3. Especificidad Diagnóstica:

La especificidad diagnóstica ha sido determinada utilizando paneles de más de 300 muestras provenientes de individuos sanos de origen europeo, clasificadas como negativas mediante un equipo de referencia.

Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos para determinar la especificidad. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

Las muestras congeladas han sido probadas para comprobar si la colección y la concervacion interfiere con el procedimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.

Un estudio realizado con más de 60 muestras que pudieran presentar potencialmente reactividad cruzada, no reveló interferencia alguna en el sistema. No se detectó reacción cruzada.

El estudio para evaluar el performance, realizado en un centro de referencia externo con más de 400 muestras totales, reveló un valor > 98%.

El procedimiento reportado en la sección T permite detectar y descartar los falsos positivos en la interpretación de los resultados y por tanto verificar si un resultado positivo es real.

La Prueba de Confirmación es un sistema que permite estimar, con un 100% de confiabilidad, la especificidad de una prueba (ya que en ausencia de un antígeno específico, un resultado positivo no es posible).

4. Precisión:

Ha sido calculada a partir de tres muestras, una negativa, una debilmente positiva y una positiva, examinadas en 16 réplicas en tres series separadas.

Los resultados son los siguientes:

TOXOM.CE: lote # 0703

Negativa (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.058	0.072	0.076	0.069
Desviación estándar	0.005	0.006	0.007	0.006
CV %	8.9	8.3	9.1	8.7

Débil reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.583	0.567	0.579	0.576
Desviación estándar	0.040	0.049	0.056	0.048
CV %	6.8	8.6	9.7	8.4

Altamente reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	2.754	2.625	2.625	2.668
Desviación estándar	0.247	0.214	0.126	0.196
CV %	9.0	8.2	4.8	7.3

TOXOM.CE: lote # 0603

Negativa (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.063	0.064	0.061	0.063
Desviación estándar	0.008	0.012	0.009	0.010
CV %	13.2	18.2	15.3	15.6

Débil reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.641	0.651	0.644	0.645
Desviación estándar	0.038	0.042	0.042	0.041
CV %	5.9	6.5	6.6	6.3

Altamente reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	2.889	2.830	2.879	2.866
Desviación estándar	0.122	0.123	0.074	0.106
CV %	4.2	4.4	2.6	3.7

TOXOM.CE: lote # 0403

Negativa (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.057	0.060	0.060	0.059
Desviación estándar	0.006	0.007	0.006	0.007
CV %	11.1	12.4	10.5	11.3

Débil reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	0.544	0.556	0.520	0.540
Desviación estándar	0.040	0.078	0.058	0.058
CV %	7.3	14.0	11.1	10.8

Altamente reactiva (N = 16)

Valores medios	1ra serie	2da serie	3ra serie	Valor promedio
DO 450nm	2.850	2.866	2.846	2.854
Desviación estándar	0.139	0.122	0.126	0.129
CV %	4.9	4.3	4.4	4.5

S. LIMITACIONES.

La contaminación bacterica o la inactivación por calor de la muestra pueden afectar los valores de DO y por tanto alterar los niveles del analito.

Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.

El ensayo es útil solo para probar muestras independientes y no mezclas.

El diagnóstico de una enfermedad infecciosa no debe establecerse en base a un solo resultado, sino que deben tenerse en consideración la historia clínica del paciente, la sintomatología, así como otros datos diagnósticos.

T. PRUEBA DE CONFIRMACIÓN.

Se realiza esta prueba con el propósito de garantizar la mayor precision del ensayo en el seguimiento del embarazo, donde un resultado falso positivo puede conducir a un aborto. La misma debe realizarse a cada una de las muestras positivas, antes de emitir un diagnóstico de infección por *Toxoplasma gondii*.

Proceder para la confirmación como sigue:

1. Preparar el Inmunocomplejo Antígeno/Anticuerpo según se describe en la sección. Este reactivo se denomina Solución A.
2. Diluir 25 µl del Conjugado concentrado en 500 µl de Diluente de Antígeno, mezclar sdelicadamente con ayuda del vórtex. No usar para este procedimiento ningún vial liofilizado de *T. gondii*! Este reactivo se denomina Solución B.
3. Dejar vacío el pocillo A1 para el blanco.

4. Dispensar el Control Negativo en las posiciones B1+C1, el mismo se usa para calcular el valor de corte y los valores M/Co.
5. La muestra positiva a confirmar, diluída 1:101, se añade en las posiciones D1+E1.
6. Incubar la tira durante 60 min a +37°C.
7. después del lavado el pocillo A1 queda vacío.
8. Dispensar 100 µl de la Solución A en los pocillos B1+C1+D1.
9. Dispensar 100 µl de la Solución B en el pocillo E1.
10. Incubar la tira durante 60 min a +37°C.
11. Después del lavado, adicionar a todos los pocillos 100 µl del Cromógeno/Substrato, posteriormente incubar la tira durante 20 minutos a t.a.
12. Dispensar 100 µl de Ácido sulfúrico en todos los pocillos, medir después la intensidad del color utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

La interpretación de los resultados se realiza de la siguiente forma:

1. Si la muestra en posición D1 tiene un valor de M/Co menor de 1.0, probablemente en el primer ensayo haya ocurrido un error en el dispensado o alguna contaminación. Debe repetirse el Procedimiento del Ensayo, sección M.
2. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en posición E1 el valor de M/Co es todavía mayor de 1.2, la muestra se considera un **falso positivo**. La reactividad de la muestra, en este caso, no depende de la presencia específica de *T.gondii*, por lo tanto ha ocurrido una reacción cruzada con el anticuerpo monoclonal conjugado con HRP.
3. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en la posición E1 el valor M/Co es menor de 1.2 se considera **realmente positiva**. La reactividad de la muestra, en este caso se debe a la presencia específica del protozoo y no a reacciones cruzadas.

En la siguiente tabla se muestra la interpretación de los resultados:

Pocillo	**M/Co**		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.2
Interpretación	Probl. de contam.	Falso positivo	Realmente positivo

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

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