HSV 1&2 IgM

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

- for "in vitro" diagnostic use only -

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REF HSVM.CE
96 tests

HSV1&2 IgM

A. INTENDED USE

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

B. INTRODUCTION

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

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

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

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

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

C. PRINCIPLE OF THE TEST

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

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

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

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

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

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE

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

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

2. Negative Control: CONTROL -

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

3. Positive Control: CONTROL +

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

The positive control is green colour coded.

4. Calibrator: CAL …ml

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

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

5. Lyophilized HSV1&2 Ag: AG HSV

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

6. Wash buffer concentrate: WASHBUF 20X

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

7. Enzyme conjugate: CONJ 20X

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

8. Antigen Diluent : AG DIL

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

9. Specimen Diluent : DILSPE

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

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

10. Chromogen/Substrate : SUBS TMB

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

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

11. Sulphuric Acid: H_2SO_4 0.3 M

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

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

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

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

F. WARNINGS AND PRECAUTIONS

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

G. SPECIMEN: PREPARATION AND WARNINGS

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

H. PREPARATION OF COMPONENTS AND WARNINGS

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

Microplate:

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

Negative Control:

Ready to use. Mix well on vortex before use.

Positive Control:

Ready to use. Mix well on vortex before use.

Calibrator:

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

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

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

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

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

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

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

Legenda:

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

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

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

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

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

M. ASSAY PROCEDURE

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

M.1 Automated assay:

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

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

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

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

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

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

M. 2 Manual assay:

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

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

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

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

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

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

9. Wash microwells as in section I.3.

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

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

Important notes:

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

N. ASSAY SCHEME

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

(*) Important Notes:

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

An example of dispensation scheme is reported below:

Microplate

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

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

O. INTERNAL QUALITY CONTROL

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

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

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

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

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

**** Important Note:**

If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co ≥ 1.0

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

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

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

P. CALCULATION OF THE CUT-OFF

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

Cut-Off = NC + 0.250

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

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

Q. INTERPRETATION OF RESULTS

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

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

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

An example of calculation is reported below:

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

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

Cut-Off = 0.090+0.250 = 0.340

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

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

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

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

OD450nm values

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

2. Diagnostic sensitivity:

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

3. Diagnostic specificity:

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

3. Precision:

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

Results are reported as follows:

HSVM.CE: lot # RD1

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

HSVM.CE: lot # RD2

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

HSVM.CE: lot # RD3

Negative (N = 16)

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

Low reactive (N = 16)

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

High reactive (N = 16)

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

S. LIMITATIONS

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

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

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

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

T. CONFIRMATION TEST

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

Proceed for confirmation as follows:

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

Interpretation of results is carried out as follows:

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

The following table is reported for the interpretation of results

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

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