

TORCH IgM

**“Capture” Enzyme Immuno Assay
(ELISA) for the screening of
IgM antibodies to
Rubella, Toxoplasma, CMV and HSV
in human plasma and sera**

- for “in vitro” diagnostic use only -



DIA.PRO

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REF.: TORCHM.CE
96 Tests

TORCH IgM

A. INTENDED USE

The device code TORCHM.CE is intended to be used for the first screening of women in pregnancy, and in other "risk patients" in order to point out those samples that are positive for IgM to any of the TORCH infectious agents (**Toxoplasma gondii**, **Rubella virus**, **Cytomegalovirus** and **Herpes Simplex virus**). In case the assay turns out to be positive, the clinician then on such patient will go throughout specific TORCH tests able to determine which TORCH pathogen is involved in the infection. For "in vitro" diagnostic use only.

B. INTRODUCTION

TORCH microorganisms are ubiquitous human pathogens, whose infections continue to be an important health problem in certain patient populations, such as pregnant women, newborns, graft recipients of solid organs or bone marrow, and AIDS patients. In these last groups, Toxoplasma and CMV are a major cause of morbidity and mortality. The screening of TORCH specific IgM antibodies, even if does not indicate which microorganism is involved, is of great value in the first diagnosis of acute/primary infections or reactivation of a latent one, particularly in the absence of typical clinical symptoms, as usually happens for CMV and for Toxoplasma. Recently developed IgM capture ELISA's for TORCH, taking advantage of specific synthetic antigens or highly purified native ones, provide the clinician with an highly specific and reliable diagnostic test, not affected by Rheumatoid Factor, for the monitoring of such "risk" populations.

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 goat anti hIgM antibody. After washing out all the other components of the sample and in particular IgG antibodies, in the 2nd incubation bound anti TORCH IgM are detected by the addition of a complex composed of TORCH antigens and TORCH conjugate labeled 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 one or more TORCH antigen/s in the sample.

A **Confirmation Test** 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.

It is required to test positive sample in specific: Toxoplasma IgM, Rubella IgM, HSV IgM, CMV IgM in order to know what infection is currently present.

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: **MICROPLATE**

n°1. 12 strips x 8 breakable wells 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 -**

1x2.0 ml/vial. Ready to use control. It contains 1% human plasma negative for TORCH IgM, 2% casein, 10 mM Tris-citrate

buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives. The negative control is yellow color coded.

3. Positive Control: **CONTROL +**

1x2.0 ml/vial. Ready to use control. It contains 1% human plasma positive for TORCH IgM, 2% casein, 10 mM Tris-citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives. The positive control is green color coded.

4. Calibrator CMVM: **CALC ...ml**

n° 1 lyophilized vials. To be dissolved with EIA grade water as reported in the label. It contains anti CMV IgM positive human serum, 10 mM Tris-HCl buffer pH 6.8+/-0.1, 2% bovine proteins, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC 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. Calibrator HSV: **CALH ...ml**

n° 1 lyophilized vials. To be dissolved with EIA grade water as reported in the label. It contains anti HSV IgM positive human serum, 10 mM Tris-HCl buffer pH 6.8+/-0.1, 2% bovine proteins, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC 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.**

6. Calibrator RUBM: **CALR ...ml**

n° 1 lyophilized vials. To be dissolved with EIA grade water as reported in the label. It contains anti RUB IgM positive human serum, 10 mM Tris-HCl buffer pH 6.8+/-0.1, 2% bovine proteins, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC 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.

7. Calibrator TOXOM: **CALT ...ml**

n° 1 lyophilized vials To be dissolved with EIA grade water as reported in the label. It contains anti TOXO IgM positive human serum, 10 mM Tris-HCl buffer pH 6.8+/-0.1, 2% bovine proteins, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC 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.

8. Lyophilized TORCH Ag: **AG TORCH**

n° 6 lyophilized vials. The vials contain lyophilized antigens derived from CMV, Toxoplasma, Rubella and HSV1&2. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC. The content of one vial has to be dissolved with 1.9 ml of Antigen Diluent as reported in the specific section.

9. 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 0.1% Kathon GC.

10. Enzyme conjugate: **CONJ 20X**

1x0.8ml/vial. 20x concentrated solution of anti TORCH specific antibodies, labeled with HRP and diluted in a protein buffer containing 10 mM Tris HCl buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives.

11. Antigen Diluent : AG DIL

1x16ml/vial. Protein buffer solution for the preparation of the Immunocomplex. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code colour with 0.01% red alimentary dye

12. Specimen Diluent : DILSPE

1x50.0ml/container. Protein buffered solution for the dilution of samples. It contains 2% casein, 10 mM citrate buffer pH 6.0+/-0.1, 0.5% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives. This component changes color, from light green to bluish dark green, when the sample is added.

13. Chromogen/Substrate : SUBS TMB

1x16ml/container. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H₂O₂) and 4% dimethylsulphoxide.

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

14. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/container. It contains 0.3 M H₂SO₄ solution.
Attention !: Irritant (Xi R36/38; S2/26/30)

15. Container:

N° 1 to be used for the preparation of the Immunocomplex (Antigen + Conjugate)

16. Plate sealing foils n°2

17. Package insert n°1

18. n°4 caps for the containers.

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 possibly 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.

9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.

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 for up to five days after collection. For longer storage periods, samples 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 0.2-0.8µ filters to clean up the sample for testing.

S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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.

Calibrators CMVM, HSVM, RUBM, TOXOM:

Add the volume of ELISA grade water reported on the label of each specific vial 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 of TORCH Ag 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 TORCH 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 Immunocomplex has to be done 15+/-2 min 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.

Legenda: R 36/38 = Irritating to eyes and skin.

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 and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. **Six (6) washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are required 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 and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ±5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630nm) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "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 Calibrators 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 found in the validation of the instrument for its use with the kit.
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 different from DP1 SScreenEasy, we suggest to make the instrument aspirate 200 ul Sample Diluent and then 10 ul sample.

All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.

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

Dispense 200 ul controls/calibrator in the appropriate control/calibration wells.

Important Note: Visually monitor that samples have been diluted and dispensed into appropriate wells. This is simply achieved by checking that the color of dispensed samples has turned to dark bluish-green while the color of the negative control has remained olive green.

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. Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
2. Dispense 200 ul of Negative Control, 200 ul Calibrator and 200 ul Positive Control in proper wells according to the schemes proposed at page 6. Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !

3. Add 200 ul of Sample Diluent (DILSPE) to all the sample wells; then dispense 10 ul sample in each properly identified well. Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.

Important note: Check that the color of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.

4. Incubate the microplate for **45 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 350ul/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100µl Immunocomplex, prepared in advance as described in the proper section, 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 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 **45 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 15 minutes**.

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

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

Important notes:

1. If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. 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
Controls & Calibrator	200 ul
Samples	200ul dil.+10ul
1st incubation	45 min
Temperature	+37°C
Wash step	6 cycles
Ag/Ab Immunocomplex	100 ul
2nd incubation	45 min
Temperature	+37°C
Wash step	6 cycles
TMB/H ₂ O ₂	100 ul
3rd incubation	15 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

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

The use of Calibrators is recommended when the internal Quality Control procedures of the Laboratory should require it. In such case the scheme becomes the following:

		Microplate											
		1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CALR	S5										
B	NC	CALR	S6										
C	NC	CALT	S7										
D	PC	CALT	S8										
E	CALC	S1	S9										
F	CALC	S2	S10										
G	CALH	S3	S11										
H	CALH	S4	S12										

Legenda: BLK = Blank NC = Negative Control
CAL.. = Calibrators 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

When Calibrators are used verify matching the following results:

Calibrator	Requirements
Rubella IgM	S/Co > 1.1
CMV IgM	S/Co > 1.1
Toxo IgM	S/Co > 1.5
HSV1&2 IgM	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 > 0.050 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.
Calibrators RCH S/Co < 1.1	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred. 5. that it was dissolved in the right volume of EIA grade water reported on the label
Calibrators To S/Co < 1.5	
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:

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

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

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

Q. INTERPRETATION OF RESULTS

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

S/Co	Interpretation
< 1.0	Negative
1.0 - 1.1	Equivocal
> 1.1	Positive

A **negative result** indicates that the patient is not undergoing an acute infection due to TORCH pathogens.

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 collection.

A **positive result** is indicative of an ongoing TORCH infection. In this case the patient should be submitted to specific tests able to point out which TORCH pathogen is responsible of the infection.

An example of calculation is reported below:

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

Negative Control: 0.050 – 0.070 OD450nm
 Mean Value: 0.060 OD450nm
 Lower than 0.200 – Accepted
 Positive Control: 1.850 OD450nm
 Higher than 0.750 – Accepted
 Cut-Off = 0.060+0.250 = 0.310

Sample 1: 0.070 OD450nm
 Sample 2: 1.690 OD450nm
 Sample 1 S/Co < 1 = negative
 Sample 2 S/Co > 1.1 = 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 a TORCH infection 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 case of a confirmed positive result, the patient should be submitted anyway to a further test to determine which TORCH pathogen is responsible of the ongoing infection. The following products can be used for such purpose: code CMVM.CE, code RUBM.CE, code TOXOM.CE and code HSVM.CE.*
5. *In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a device specific for each TORCH microorganism, before taking any preventive medical action. No therapeutic action must be taken only on the basis of the first screening result !*
6. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
7. *Diagnosis of infection has to be made also on the basis of the clinical sign of disease and released to the patient by a suitably qualified medical doctor,*

R. PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

The international standards for Toxoplasma IgM (WHO 3rd int. std.) and for Rubella IgM (PEI) and two internal gold standards (IGS) defined by DiaPro srl in absence of defined available ones for HSV IgM and CMV IgM were used to define the analytical sensitivity of these assays.

In the table below results are reported:

Preparation	Content	Value
WHO TOXO	50 WHO IU/ml	S/Co > 1
PEI RUB	50 PEI IU/ml	S/Co > 1
IGS HSVM	In toto	S/Co ≥ 1.2
IGS CMVM # 2	1:40 final dil.	S/Co ≥ 1.5

2. Diagnostic Sensitivity

Positive samples were collected from patients carrying TORCH infections, confirmed by clinical symptoms and analysis.

An overall value of > 99.5% has been found in the study conducted for the single TORCH markers.

3. Diagnostic Specificity

The diagnostic specificity has been determined on panels of negative samples collected from normal individuals of European origin with no clinical and diagnostic signs of TORCH infection.

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 samples that were clean and free of particles and clots. Some interference was observed on samples showing aggregates, fibrin filaments, heavy particles and visible clots.

A study conducted on potentially cross-reactive samples has not revealed any interference in the system.

No cross reaction were observed.

The Performance Evaluation study conducted in qualified European reference centers has provided a value > 98%.

Some false positive samples were detected. They were all 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 tested in total 144 replicates in different runs. The following average values were found:

Average values N = 144	Negative sample	Low positive	High positive
OD450nm	0.067	0.806	1.888
Std.Deviation	0.007	0.028	0.071
CV %	10.2	3.9	3.8

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates (precipitates, clots and filaments) 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, its symptomatology together with other diagnostic data should be always considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in particular in the follow-up of pregnancy - where a false positive result could lead to abortion - a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of TORCH infection 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 TORCH Antigen lyophilized 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 Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl 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 possibly 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 TORCH Antigens and a crossreaction with the enzymatic tracer 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 1.2 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of TORCH Antigens 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 contamination	False positive	True positive

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