Platelia™ TOXO IgM

1 plate - Σ 96 REF 26211

KIT FOR THE QUALITATIVE DETECTION OF ANTI-*Toxoplasma Gondii* IGM IN HUMAN SERUM OR PLASMA BY ENZYME IMMUNOASSAY

IVD

881170 - 2015/01

BIO-RAD

TABLE OF CONTENTS

1. INTENDED USE 3
2. SUMMARY AND EXPLANATION 3
3. PRINCIPLES OF THE PROCEDURE 4
4. PRODUCT INFORMATION 5
5. WARNINGS AND PRECAUTIONS 7
6. SPECIMEN COLLECTION, PREPARATION AND STORAGE 9
7. PROCEDURE 10
8. INTERPRETATION OF RESULTS 13
9. LIMITATIONS OF THE PROCEDURE 14
10. EXPECTED VALUES 17
11. SPECIFIC PERFORMANCE CHARACTERISTICS 18
12 REFERENCES 24

1 - INTENDED USE

The Platelia™ Toxo IgM kit is an *in vitro* diagnostic test kit allowing the qualitative detection of anti-*Toxoplasma gondii* in human serum or plasma (EDTA, Heparin, Citrate).

Note:

- ***Patient testing with the Platelia™ Toxo IgM assay must be performed in conjunction with an anti-Toxoplasma gondii IgG antibody assay.***
- ***The Platelia™ Toxo IgM assay is presumptive for the detection of anti-Toxoplasma gondii IgM antibodies and presumptive for the diagnosis of acute, recent, or reactivated Toxoplasma gondii infection.***
- ***The performance of the Platelia™ Toxo IgM assay has not been established for neonate testing.***
- ***The Platelia™ Toxo IgM assay has not been cleared/approved by the FDA for blood/plasma donor screening.***

2 - SUMMARY AND EXPLANATION

Toxoplasmosis, caused by the parasite *Toxoplasma gondii* (*T. gondii*), has been implicated in serious congenital abnormalities following maternal infections just prior to or during pregnancy. Usually a mild or even asymptomatic disease in adults, toxoplasmosis acquired *in utero* has resulted in blindness, encephalomyelitis, impaired brain functions, convulsions, and death to infected neonates. Also predisposition to severe toxoplasmosis infection is common in persons known to have Acquired Immune Deficiency Syndrome (AIDS), or who are otherwise immunocompromised.

Toxoplasmosis can be treated with antibiotics but early detection of the disease is crucial to effective therapy. Complete eradication of the parasite becomes difficult after long-term, chronic infection. Specific diagnosis of *T. gondii* infection can be complicated and isolation of the parasite is rare. Serologic confirmation of *T. gondii* antibody is indicative of exposure to the parasite and has become widely accepted as a means to determine immune status and susceptibility to infection. *T. gondii* IgM antibody testing can be used to verify early infections caused by *T. gondii* in conjunction with conventional diagnostic procedures.

Diagnostic serology for Toxoplasmosis has progressed from the use of a specialized dye test to immunofluorescence and enzyme immunoassay (EIA) techniques. The Platelia™ Toxo IgM kit utilizes EIA technology for the determination of IgM antibodies to *T. gondii*. The kit includes two levels of control sera positive for *T. gondii* IgM antibody and a negative control serum. The test utilizes a monoclonal antibody to *T. gondii* conjugated with peroxidase for enzymatic assay signal development.

3 - PRINCIPLES OF THE PROCEDURE

The Platelia™ Toxo IgM assay utilizes an immunoenzymatic double sandwich method. Diluted samples and controls are placed into microplate wells coated with antibodies to human IgM. The IgM antibodies present in the samples are captured by the solid phase. Remaining antibodies (including any of the IgG class) and other serum proteins are removed by washing. A solution containing *T. gondii* antigen and conjugate (monoclonal antibody to *T. gondii* labeled with horseradish peroxidase) is placed into each well. *T. gondii* IgM antibodies in the sample that are captured on the solid phase bind the *T. gondii* antigen-conjugate complex. Excess *T. gondii* antigen and conjugate are removed by washing. A chromogen solution is added which reacts with the conjugate to initiate a color development reaction. This reaction is stopped by the addition of an acid. The optical density readings for the samples are obtained with a spectrophotometer set at a wavelength of 450 nm. The presence of *T. gondii* IgM antibodies in an individual sample is determined by comparing the optical density reading for the sample to the optical density reading of the calibrator serum.

4 - PRODUCT INFORMATION

The kit should be stored at +2-8°C until the expiry date mentioned on the package.

Note: before use, allow reagents to reach room temperature (+18-30°C).

Label		Reagents	Quantity
R1	**Microplate**	**Microplate :** 12 strips with 8 break-away wells each, coated with antibodies to human IgM. Return unused strips to the bag immediately. Re-seal the bag carefully and store it at +2-8°C. Providing that strips are stored correctly, they are stable for 8 weeks once the bag has been opened. Tabs are labeled "73"	1 plate
R2	**Concentrated washing solution (20X)**	**Concentrated washing solution (20X):** TRIS NaCl buffer pH 7.4, 2% Tween® 20, Concentrated 20X. Preservative: < 1.5% ProClin 300. Prepare the working strength (diluted) Washing Solution by diluting the Washing Solution Concentrate (R2) 1:20 in distilled water. Prepare 350 mL for one plate of 12 strips if washing manually. Diluted Washing Solution may be stored at +2-30°C for up to 2 weeks. The concentrated solution can be stored at +2-30°C.	1 vial 70 mL
R3	**Negative control**	**Negative control:** Human serum negative for IgM antibodies to *T. gondii*, and negative for HBs antigen, anti-HIV1, anti-HIV2 and anti-HCV Preservative: < 1.5% ProClin 300 Dilute 1:21 as for patient samples, for example: 15 µL to 300 µL.	1 vial 0.75 mL
R4	**Calibrator**	**Calibrator:** Human serum reactive for IgM antibodies to *T. gondii*, and negative for HBs antigen, anti-HIV1, anti-HIV2 and anti-HCV Preservative: < 1.5% ProClin 300. Dilute 1:21 as for patient samples, for example: 15 µL to 300 µL.	1 vial 0.75 mL
R5	**Positive control**	**Positive control:** Human serum reactive for IgM antibodies to *T. gondii*, and negative for HBs antigen, anti-HIV1, anti-HIV2 and anti-HCV Preservative: < 1.5% ProClin 300. Dilute 1:21 as for patient samples, for example: 15 µL to 300 µL.	1 vial 0.75 mL

Label		Reagents	Quantity
R6a	**Antigen**	***T. gondii* antigen:** Inactivated *T. gondii* antigen (RH strain); lyophilized. Reconstitute the lyophilized Antigen (R6a) before use by adding 14 mL diluent (R7) to one vial of antigen. The reconstituted *T. gondii* Antigen solution must be completely clear and used immediately after reconstitution. **Note**: cloudiness indicates the reagent is unusable.	2 vials 14 mL
R6b	**Conjugate (101X)**	**Concentrated conjugate (101X):** Murine monoclonal antibody to *T. gondii* (P30) conjugated with horseradish peroxidase; concentrated (101X). Preservative: < 1.5% ProClin 300. ***To prepare Working Conjugate Solution:*** Add 140 µL of conjugate (R6b) to each vial of reconstituted *T. gondii* antigen (R6a). Mix thoroughly. The Working Conjugate Solution must be prepared at least 1 hour before use. Once reconstituted, the Working Conjugate Solution is stable for 8 hours at room temperature (+18-30°C) or 4 weeks at +2-8°C.	1 vial 0.4 mL
R7	**Diluent**	**Diluent:** TRIS-NaCl buffer (pH 7.7 ± 0.15), bovine serum albumin, 0.1% Tween® 20, phenol red. Preservative: < 1.5 ProClin 300. Ready to use.	1 vial 80 mL
R9	**Chromogen TMB**	**Chromogen TMB:** 3.3'.5.5' tetramethylbenzidine (< 0.1%), H_2O_2 (<1%). Ready to use.	1 vial 28 mL
R10	**Stopping solution**	**Stopping solution:** 1N sulfuric acid (H_2SO_4) solution. Ready to use.	1vial 28 mL
		Plate sealers	4

5 - WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

The reliability of the results depends on correct implementation of the following Good Laboratory Practices:

- Do not use expired reagents.

REMARK: For Washing Solution (R2, label identification: 20X colored green), Chromogen (R9, label identification: TMB colored turquoise) and Stopping Solution (R10, label identification: 1N colored red), it is possible to use other lots than those contained in the kit, provided these reagents are strictly equivalent and the same lot is used within a given test run.

REMARK: In addition, the Washing Solution (R2, label identification: 20X colored green) can be mixed with the 2 other washing solutions included in various Bio-Rad reagent kits (R2, label identifications: 10X colored blue or 10X colored orange) when properly reconstituted, provided only one mixture is used within a given test run.

- Allow all kit components, including the microplate, to warm to room temperature (18-30°C) for a minimum of 30 minutes prior to use.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde) or dust that could alter the enzyme activity of the conjugate.
- Use disposable reagent containers, or alternately, glassware that has been thoroughly washed and rinsed with deionized water.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- The Chromogen TMB must be colorless. The appearance of blue color indicates that the reagent cannot be used and must be replaced.
- Use a new pipette tip for each sample.
- Washing the microplate is a critical step in the procedure; follow the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and development solution.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.
- The concentration of anti-*Toxoplasma gondii* IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity

HEALTH AND SAFETY PRECAUTIONS

- **For Professional use only**.
- Wear protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) and handle kit reagents and patient samples with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
- Do not pipette by mouth. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.

- The positive control, calibrator and negative control are manufactured from human serum that has been tested and found non-reactive for Hepatitis B Surface Antigen (HBs Ag), antibodies to Hepatitis C (HCV) and to Human Immunodeficiency Virus (anti-HIV1 and anti-HIV2) with CE marked tests. The *T. gondii* Antigen has been treated to reduce the potential for *T. gondii* infectivity. Handle these reagents as though capable of transmitting infection. All testing should be conducted in accordance with the OSHA Standard on Bloodborne Pathogens, Biosafety Level 2 outlined in the current edition of the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, or other appropriate biosafety practices.
- Any material, including washing solution, which comes directly in contact with samples and reagents containing materials of human origin, should be considered as if capable of transmitting infectious disease.
- Avoid spilling samples or solutions containing samples.
- Biological spills not containing acid should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), 70% ethanol, or 0.5% Wescodyne Plus. Materials used to wipe up spills may require biohazardous waste disposal.
- Spills containing acid, must be initially neutralized with sodium bicarbonate, and then the area cleaned with disinfectant and dried. The material used for cleaning must be discarded in a contaminated residue container.
- Samples, reagents containing materials of human origin, as well as contaminated material and products should be discarded after decontamination, either chemically by immersion in a 1:10 dilution of household bleach for 30 minutes or equivalent by autoclaving at 121°C for 2 hours minimum.
- Autoclaving for at least one hour at 121°C is the best method to inactivate the HIV viruses and the HB viruses.

CAUTION: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.

CAUTION: Avoid contact with all kit reagents. The following is a list of potential chemical hazards contained in some kit reagents (refer to section 4-PRODUCT INFORMATION):

- **ProClin 300 < 1.5 %**: A biocidal preservative that is irritating to eyes and skin, may be detrimental if enough is ingested, and may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
- **1N Sulfuric acid**: The 1N Sulfuric Acid (H_2SO_4) Stopping Solution is irritating to skin and severely irritating or corrosive to eyes, depending on the amount and length of exposure; greater exposures can cause eye damage,

including permanent impairment of vision. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Keep away from strong bases and reducing agents; do not pour water or bleach into this component. Waste from this material is considered hazardous acidic waste, however if permitted by local, regional, and national regulations, it might be neutralized to pH 5-8 for non-hazardous disposal.

Laboratory Chemicals and biohazardous wastes must be handled and disposed of in accordance with Good Laboratory Practices and in compliance with all local, regional and national regulations.

For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of instruction for use.

The Safety Data Sheet (SDS) is available upon request.

6 - SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Collect blood samples according to standard laboratory procedures. Separate the serum or plasma (collected with EDTA, heparin or citrate) from the clot or red cells as soon as possible to avoid any hemolysis. Extensive hemolysis may affect test performance. Specimens with aggregates should be clarified by centrifugation prior testing. Suspended fibrin particles or aggregates may yield false positive results.

The specimens can be stored at +2-8°C if screening is performed within 5 days or they may be frozen at -20°C for several months. Avoid repeated freeze / thaw cycles. If the specimens are to be shipped, they must be packaged in accordance with current regulations regarding the transport of etiological agents.

DO NOT USE CONTAMINATED, HYPERLIPEMIC OR HYPERHEMOLYZED SERUM OR PLASMA.

7 - PROCEDURE

1 - MATERIALS PROVIDED

R1: Microplate
R2: Concentrated Washing Solution (20X)
R3: Negative Control
R4: Calibrator
R5: Positive Control
R6a: Antigen (lyophilized)
R6b: Concentrated Conjugate (101X)
R7: Diluent
R9: Chromogen TMB
R10: Stopping Solution
Plate Sealers

2 - MATERIALS REQUIRED BUT NOT PROVIDED

- Vortex mixer.
- Microplate reader equipped with 450 nm and 620-630 nm reference filters.
- Water bath or equivalent microplate incubator, thermostatically set at 37±1°C.
- Container for biohazard waste.
- Sodium hypochlorite (bleach) and sodium bicarbonate.
- Distilled or deionized water.
- Graduated cylinders of 25, 50, 100 and 1000 mL capacity.
- Disposable latex gloves.
- Goggles or safety glasses.
- Absorbent paper.
- Automatic or semi-automatic, adjustable or preset, pipettes or multi-channel pipettes to measure and dispense 10 to 1000 µL and 1, 2 and 10 mL.
- Manual, semi-automatic or automatic microplate washer.
- Disposable tubes.

3 - PROCEDURE

Strictly follow the recommended procedure.
Use the controls with each run to validate the test results.
Apply Good Laboratory Practice.

1. Carefully document the sample distribution and identification plan.
2. Dilute the Concentrated Washing Solution (R2) as directed. (*Refer to section 4*).
3. Take the carrier tray and the strips (R1) out of the protective pouch.
4. Prepare the conjugate working solution R6 (R6a+R6b) (*Refer to section 4*).
5. Dilute test sera by adding 15 µL sample to 300 µL Diluent (R7). Controls and calibrator are diluted in the same manner to give a 1:21 dilution. Vortex diluted samples.
6. For manual performance of the assay, add 200 µL of diluted controls, calibrator and test samples to the wells as suggested below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	R3	S5	S13									
B	R4	S6										
C	R4	S7										
D	R5	S8										
E	S1	S9										
F	S2	S10										
G	S3	S11										
H	S4	S12										

7. Cover the microplate with adhesive plate sealer or other means to prevent evaporation; pressing firmly onto the plate to ensure a tight seal. Incubate the microplate in a thermostat controlled water-bath or microplate incubator at 37±1°C for 1 hour ± 5 minutes.
8. Remove adhesive plate sealer. Aspirate the contents of all wells into a container for biohazards waste (containing sodium hypochlorite). Wash microplate 4 times using 350 µL of diluted Washing Solution. Invert microplate and gently tap on absorbent paper to remove remaining liquid .
9. Add 200 µL of the Conjugate Working Solution to all wells. The Conjugate Working Solution must be mixed gently before use.
10. Cover the microplate with adhesive plate sealer or other means to prevent evaporation; pressing firmly onto the plate to ensure a tight seal. Incubate the microplate in a thermostat controlled water-bath or microplate incubator at 37 ± 1°C for 1 hour ± 5 minutes.
11. Remove the adhesive plate sealer, aspirate all wells, and wash 4 times as described above. Invert microplate and gently tap on absorbent paper to remove remaining liquid.
12. Add 200 µL of the Chromogen TMB (R9) to each well. Allow the reaction to develop in the dark for 30 minutes ± 5 minutes at room temperature (+18-30°C). Do not use adhesive film during this incubation.
13. Add 100 µL of Stopping Solution (R10) to each well.
14. Carefully wipe the plate bottom. Read the optical density at 450/620 nm using a plate reader within 30 minutes of stopping the reaction (the strips must always be kept away from light before reading).
15. Check all results for agreement between the Optical Density reading and visual inspection of the plate, the sample distribution, and the plate identification plan.

8 - INTERPRETATION OF RESULTS

1 - DETAILS OF CALIBRATION

The presence or absence of IgM antibodies to *T. gondii* in the test is determined by comparing the optical density of each patient specimen to a cut-off value. Two comparison studies were performed to establish the cut-off value of the Platelia™ Toxo IgM assay. In the first study, 104 serum samples were evaluated on the Platelia™ Toxo IgM assay and on an IgM indirect fluorescent antibody test. In the second study, 120 serum samples were evaluated on the Platelia™ Toxo IgM assay and on another IgM EIA commercialized assay.

2 - QUALITY CONTROL

The calibrators are appropriate for both serum and plasma sample testing. Include all the controls for each test run. For validation of the assay, the following criteria must be met.

Optical density values:

- Negative Control OD ≤ 0.150 (OD R3 ≤ 0.150)
- Mean of Calibrator OD ≥ 0.300 (OD_MR4 ≥ 0.300)

The cut-off value OD_MR4 corresponds to the mean value of the optical densities of the Calibrator R4.

- 0.80 x CO < OD R4 Repl.1 < 1.20 x CO
- 0.80 x CO < OD R4 Repl.2 < 1.20 x CO

(Individual OD of each replicate of the Calibrator (R4) must not differ more than 20% of the CO value).

Ratios:

- Negative Control OD / Mean Calibrator ≤ 0.30 (OD R3 / OD_MR4 ≤ 0.30)
- Positive Control OD / Mean Calibrator OD ≥ 1.80 (OD R5 / OD_MR4 ≥ 1.80)

If the quality control criteria are not met, the test run should be repeated.

Note: Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. For additional guidance on internal quality control testing, please refer to CLSI document C24-A2 Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Second Edition.

3 - CALCULATION OF THE CUT-OFF VALUE AND OF THE SAMPLE RATIO

- The cut-off value OD_MR4 corresponds to the mean value of the optical densities of the Calibrator R4.
- The result can also use the following ratio:

Sample Ratio = Sample OD / Mean Calibrator OD (Sample OD / OD_MR4)

4 - INTERPRETATION OF THE RESULTS

Using OD cut-off value	Using Sample Ratio	Result	Interpretation / Recommendations
Sample OD < OD_MR4 x 0.80	Sample Ratio < 0.8	Negative sample	Probably no primary infection due to *T. gondii.*
Sample OD ≥ OD_MR4 x 0.80	Sample Ratio ≥ 0.8	Equivocal sample	Result to be confirmed 10-20 days later on a second serum sample. Quantitative examination of the anti-*T. gondii* IgG antibodies needed.
Sample OD < OD_MR4	Sample Ratio < 1		
Sample OD ≥ OD_MR4	Sample Ratio ≥ 1	Positive sample	Result to be confirmed 10-20 days later on a second serum sample. Quantitative examination of the anti-*T. gondii* IgG antibodies needed.

Note: The magnitude of the measured result, above the cut-off is not indicative of the total antibody present.

5 - INTERPRETATION OF TOXO IgM RESULTS IN CONJUNCTION WITH TOXO IgG RESULTS

Anti-*T. gondii* IgM results	Anti-*T. gondii* IgG results	Report / Interpretation
Negative	**Negative**	It is presumed the patient has not been infected with and is not undergoing an acute infection with *Toxoplasma gondii*. If symptoms persist, submit a new specimen within 3 weeks.
Negative	**Positive**	From this testing it cannot be determined whether the patient is or is not undergoing a reactivated *Toxoplasma gondii* infection. It appears the patient has been previously infected with *Toxoplasma gondii*. Infection occurred more than one year ago.
Negative	**Equivocal**	Obtain a new specimen for further testing. Patient may not be undergoing an acute infection with *Toxoplasma gondii*. Determining whether the patient has been previously infected with *Toxoplasma gondii* is not possible.
Equivocal	**Negative**	Obtain a new specimen for determination of IgM antibodies to *Toxoplasma gondii*. It cannot be determined if the patient is undergoing an acute *Toxoplasma gondii* infection. It appears the patient has not been previously infected with *Toxoplasma gondii*. If the new specimen result is positive or equivocal for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Equivocal	**Positive**	Obtain a new specimen for determination of IgM antibodies to *Toxoplasma gondii*. It cannot be determined if the patient is undergoing or has undergone an acute *Toxoplasma gondii* infection. It appears the patient has been previously infected with *Toxoplasma gondii*. If the new specimen result is equivocal or positive for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Equivocal	**Equivocal**	Obtain a new specimen for further testing. It cannot be determined if the patient is undergoing an acute infection or has been previously infected with *Toxoplasma gondii*. If the new specimen result is equivocal or positive for IgM antibodies, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

Anti-*T. gondii* IgM results	Anti-*T. gondii* IgG results	Report / Interpretation
Positive	**Negative**	Obtain a new specimen for further testing. The patient may or may not be acutely infected with *Toxoplasma gondii*. Since the IgG antibodies to *Toxoplasma gondii* are negative, the specimen may have been obtained too early in the disease process for an accurate determination. Retest the new specimen with a different anti-*Toxoplasma gondii* IgM assay. If the new specimen result is still positive for IgM and IgG antibodies to *Toxoplasma gondii*, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	**Positive**	The patient may or may not be acutely infected with *Toxoplasma gondii*. Obtain a new specimen for further testing. Since the IgG antibodies to *Toxoplasma gondii* are positive, it appears the patient may be acutely infected with *Toxoplasma gondii*. The new specimen should be repeated with a different anti-*Toxoplasma gondii* IgM assay. If the new specimen result is still positive for IgM and IgG antibodies to *Toxoplasma gondii*, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.
Positive	**Equivocal**	It cannot be determined if the patient is acutely infected with *Toxoplasma gondii*. Obtain a new specimen for further testing. Determining whether the patient has been previously infected with *Toxoplasma gondii* is not possible. The specimen may have been collected too early during the disease process for an accurate determination. Retest the new specimen with a different anti-*Toxoplasma gondii* IgM assay. If the new specimen result is still positive for IgM and the IgG is positive / negative / equivocal for antibodies to *Toxoplasma gondii*, the specimen should be sent to a reference laboratory with experience in the diagnosis of toxoplasmosis for further testing.

6 - TROUBLE SHOOTING GUIDE

Non-valid or non-repeatable reactions are often caused by:

- Inadequate microplate washing,
- Contamination of negative samples by serum or plasma with a high antibody titer,
- Contamination of the development solution by oxidizing agents (bleach, metal ions...),
- Contamination of the stopping solution.

7 - EXAMPLE CALCULATION

Note: The following data are given for example purposes only

RESULTS

Controls and Patient samples	OD 450 nm - 620 nm	Sample Ratio	Result
R3	0.058		
R4	0.691		
R4	0.700		
R5	1.494		
Sample n° 1	0.109	0.15	Negative
Sample n° 2	1.096	1.57	Positive
Sample n° 3	2.096	3.01	Positive

VALIDATION OF THE TEST

Optical density values:

- Negative Control OD = 0.058 ($N \leq 0.150$) Accepted

Calculation of the cut-off value

- OD_M R4 = 0.696
- OD R4 Repl.1 = 0.691 ($N = 0.696 \pm 20\%$) Accepted
- OD R4 Repl.2 = 0.700 ($N = 0.696 \pm 20\%$) Accepted

Ratios:

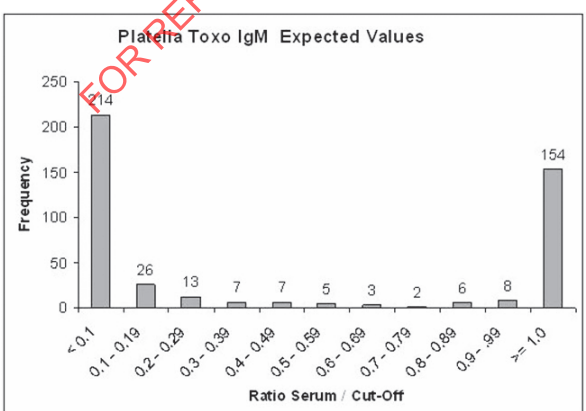
- OD R3 / OD_MR4 = 0.083 ($N \leq 0.30$) Accepted
- OD R5 / OD_MR4 = 2.147 ($N \geq 1.80$) Accepted

9 - LIMITATIONS OF THE PROCEDURE

1. Diagnosis of recent infection by *T. gondii* can only be established on the basis of a combination of clinical and serological data.
2. The result of a single serum sample does not constitute sufficient proof for diagnosis of recent infection.
3. If a serum sample was collected too soon after infection, IgM antibodies to *T. gondii* may be absent. If this is suspected, a second serum sample should be collected 15 days later and the test repeated.
4. The results in HIV patients, in patients undergoing immunosuppressive therapy, or in patients with other disorders leading to immunosuppression, should be interpreted with caution.
5. Heterophile antibodies in the patient samples may interfere with the assay performance.
6. The performance of the assay has not been established for cord blood testing.
7. As with any low prevalence analyte, there is the increased possibility that a positive result may actually be false, reducing the assay's positive predictive value.

10 - EXPECTED VALUES

A total of 445 fresh and frozen serum samples obtained from pregnant women during routine laboratory activities in the area of Paris, France were tested with the Platelia™ Toxo IgM assay. The distribution of serum / cut-off ratio values is shown in the following chart.



11 - SPECIFIC PERFORMANCE CHARACTERISTICS

Note: Due to the apparent low prevalence of anti-Toxoplasma gondii IgM in the United States, specimens used to establish performance characteristics of this assay may not be representative of specific populations.

1 - COMPARATIVE STUDY

The performance characteristics of the Platelia™ Toxo IgM assay, summarized below, have been established using frozen serum samples collected from pregnant women during routine laboratory activities.

The Platelia™ Toxo IgM assay was compared to another commercially available enzyme immunoassay (EIA) procedure for detection of IgM antibodies to *T. gondii*. A total of 348 prospective samples and 97 retrospective specimens from 40 seroconversion panels were tested by the two procedures. The Platelia™ Toxo IgM assay demonstrated an overall agreement of 99.53% with the reference assay. Results from these studies are summarized in the following tables.

Platelia™ Toxo IgM vs. IgM EIA Correlation Table (prospective samples)

N = 348		Platelia™ Toxo IgM		
		Neg	Equivocal	Pos
IgM EIA	Neg	261	0	0
	Equivocal	4	3	0
	Pos	1	9	70

Excluding equivocal samples, the results of comparative prospective testing demonstrated the following:

Negative agreement: 100.00% (261/261). The 95% confidence interval[26] is 99.81% - 100.00%.

Positive agreement: 98.59% (70/71). The 95% confidence interval[26] is 95.06% - 100.00%.

Overall agreement: 99.70% (331/332). The 95% confidence interval[26] is 98.96% - 100.00%.

Platelia™ Toxo IgM vs. IgM EIA Correlation Table (retrospective specimens)

N = 97		Platelia™ Toxo IgM		
		Neg	Equivocal	Pos
IgM EIA	Neg	10	0	0
	Equivocal	0	0	0
	Pos	1	2	84

Excluding equivocal specimens, the results of comparative retrospective testing demonstrated the following:

Negative agreement: 100.00% (10/10). Note: the 95% confidence interval[26] cannot be calculated for the retrospective negative agreement due to low sample size.

Positive agreement: 98.82% (84/85). The 95% confidence interval[26] is 95.88 – 100.00%.

Overall agreement: 98.95% (94/95). The 95% confidence interval[26] is 96.32% - 100.00%.

Platelia™ Toxo IgM vs. IgM EIA Correlation Table (combined results)

	N = 445	Platelia™ Toxo IgM		
		Neg	Equivocal	Pos
IgM EIA	Neg	271	0	0
	Equivocal	4	3	0
	Pos	2	11	154

Excluding equivocal samples / specimens, the combined results of comparative prospective and retrospective testing demonstrated the following:

Negative agreement: 100.00% (271/271). The 95% confidence interval[26] is 99.82% - 100.00%.

Positive agreement: 98.72% (154/156). The 95% confidence interval[26] is 96.61% - 100.00%.

Overall agreement: 99.53% (425/427). The 95% confidence interval[26] is 98.76% - 100.00%.

2 - CDC TOXOPLASMA 1998 HUMAN SERUM PANEL

The following information is from a serum panel obtained from the CDC and tested by Bio-Rad Laboratories. The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

The panel consists of 32 positive and 65 negative samples. The Platelia™ Toxo IgM assay demonstrated 100% agreement with the positive specimens and 100% agreement with the negative specimens.

Please Note: There should be no other statistical calculation or inferences drawn from the panel results.

3 - PRECISION

Inter-assay and intra-assay reproducibility were determined by assaying two *T. gondii* IgM negative samples and four *T. gondii* IgM positive samples in triplicate, on three different days at three laboratory sites.

SITE 1	NEG 1		NEG 2		POS 1		POS 2		POS 3		POS 4	
	OD	S/CO	OD	S/CO	OD	S/CO	OD	S/CO	OD	S/CO	OD	S/CO
N	9	9	9	9	9	9	9	9	9	9	9	9
Mean	0.027	0.05	0.022	0.04	0.742	1.44	0.660	1.28	0.825	1.60	1.477	2.86
Within Run (intra-assay)												
SD	0.001	0.003	0.002	0.003	0.011	0.021	0.011	0.022	0.016	0.031	0.017	0.034
%CV	**5.2%**	**5.1%**	**7.5%**	**7.6%**	**1.5%**	**1.4%**	**1.7%**	**1.7%**	**1.9%**	**1.9%**	**1.2%**	**1.2%**
Total (inter-assay)												
SD	0.002	0.005	0.001	0.003	0.020	0.041	0.013	0.038	0.013	0.048	0.032	0.050
%CV	**8.1%**	**9.7%**	**6.7%**	**6.3%**	**2.6%**	**2.8%**	**2.0%**	**3.0%**	**1.6%**	**3.0%**	**2.1%**	**1.7%**

SITE 2	NEG 1		NEG 2		POS 1		POS 2		POS 3		POS 4	
	OD	S/CO	OD	S/CO	OD	S/CO	OD	S/CO	OD	S/CO	OD	S/CO
N	9	9	9	9	9	9	9	9	9	9	9	9
Mean	0.031	0.05	0.029	0.05	0.746	1.21	0.669	1.08	0.776	1.26	1.511	2.44
Within Run (intra-assay)												
SD	0.007	0.011	0.007	0.011	0.025	0.040	0.015	0.024	0.009	0.015	0.051	0.082
%CV	**22.5%**	**23.0%**	**22.6%**	**23.2%**	**3.3%**	**3.3%**	**2.3%**	**2.3%**	**1.2%**	**1.2%**	**3.4%**	**3.4%**
Total (inter-assay)												
SD	0.009	0.016	0.007	0.012	0.024	0.061	0.015	0.066	0.012	0.063	0.046	0.151
%CV	**29.9%**	**32.8%**	**22.3%**	**24.4%**	**3.2%**	**5.0%**	**2.2%**	**6.1%**	**1.5%**	**5.0%**	**3.0%**	**6.2%**

SITE 3	NEG 1		NEG 2		POS 1		POS 2		POS 3		POS 4	
	OD	S/CO	OD	S/CO	OD	S/CO	OD	S/CO	OD	S/CO	OD	S/CO
N	9	9	9	9	9	9	9	9	9	9	9	9
Mean	0.032	0.06	0.024	0.04	0.769	1.39	0.705	1.28	0.684	1.23	1.346	2.43
Within Run (intra-assay)												
SD	0.004	0.007	0.006	0.011	0.055	0.104	0.023	0.044	0.011	0.020	0.061	0.107
%CV	**11.6%**	**12.1%**	**24.0%**	**26.3%**	**7.1%**	**7.5%**	**3.3%**	**3.5%**	**1.6%**	**1.6%**	**4.5%**	**4.4%**
Total (inter-assay)												
SD	0.014	0.022	0.010	0.016	0.073	0.203	0.077	0.212	0.119	0.150	0.098	0.150
%CV	**43.7%**	**38.7%**	**41.2%**	**36.8%**	**9.5%**	**14.6%**	**11.0%**	**16.6%**	**17.4%**	**12.2%**	**7.3%**	**6.2%**

In addition, inter-assay and intra-assay reproducibility with plasma samples were determined by assaying three additional samples (one *T. gondii* IgM negative sample and two *T. gondii* IgM positive samples) in triplicate, on three different days, at one laboratory site.

SERUM	NEG 1		POS 1		POS 2	
	OD	S/CO	OD	S/CO	OD	S/CO
N	9	9	9	9	9	9
Mean	0.022	0.04	0.710	1.26	1.083	1.92
Within Run (intra-assay)						
SD	0.002	0.003	0.019	0.034	0.029	0.052
%CV	**8.7%**	**8.8%**	**2.7%**	**2.7%**	**2.7%**	**2.7%**
Total (inter-assay)						
SD	0.003	0.006	0.035	0.077	0.051	0.074
%CV	**14.5%**	**14.9%**	**4.9%**	**6.1%**	**4.8%**	**3.9%**

EDTA	NEG 1		POS 1		POS 2	
	OD	S/CO	OD	S/CO	OD	S/CO
N	9	9	9	9	9	9
Mean	0.024	0.04	0.761	1.35	1.063	1.88
Within Run (intra-assay)						
SD	0.002	0.004	0.019	0.034	0.019	0.033
%CV	**8.3%**	**8.3%**	**2.5%**	**2.6%**	**1.7%**	**1.8%**
Total (inter-assay)						
SD	0.002	0.004	0.036	0.030	0.056	0.076
%CV	**8.5%**	**9.4%**	**4.7%**	**2.2%**	**5.2%**	**4.0%**

CITRATE	NEG 1		POS 1		POS 2	
	OD	S/CO	OD	S/CO	OD	S/CO
N	9	9	9	9	9	9
Mean	0.022	0.04	0.674	1.19	1.075	1.91
Within Run (intra-assay)						
SD	0.001	0.002	0.017	0.029	0.020	0.036
%CV	**5.0%**	**5.0%**	**2.5%**	**2.5%**	**1.9%**	**1.9%**
Total (inter-assay)						
SD	0.002	0.004	0.031	0.038	0.043	0.091
%CV	**9.9%**	**10.6%**	**4.5%**	**3.2%**	**4.0%**	**4.8%**

HEPARIN	NEG 1		POS 1		POS 2	
	OD	S/CO	OD	S/CO	OD	S/CO
N	9	9	9	9	9	9
Mean	0.022	0.04	0.719	1.27	1.034	1.83
Within Run (intra-assay)						
SD	0.001	0.002	0.012	0.020	0.022	0.039
%CV	**4.4%**	**4.4%**	**1.6%**	**1.6%**	**2.1%**	**2.1%**
Total (inter-assay)						
SD	0.002	0.003	0.024	0.031	0.059	0.115
%CV	**11.3%**	**8.7%**	**3.3%**	**2.4%**	**5.7%**	**6.3%**

4 - CROSS REACTIVITY

239 specimens with the following characteristics, which could potentially result in non-specific reactions, were studied.

Specimen known to be positive for	N° of serum samples	N° of false positive results
Human Immunodeficiency Virus (HIV)	63	0
Epstein Barr Virus (EBV IgM)	9	0
Epstein Barr Virus (EBV IgG)	10	0
Herpes Simplex Virus (HSV IgM)	10	0
Herpes Simplex Virus (HSV IgG)	10	0
Varicella Zoster Virus (VZV IgG)	10	0
Varicella Zoster Virus (VZV IgM)	10	0
Rubeola IgM	10	0
Rubeola IgG	10	0
Mumps IgM	8	0
Mumps IgG	10	0
Rubella IgM	5	0
Rubella IgG	5	0
Cytomegalovirus (CMV IgM)	5	0
Cytomegalovirus (CMV IgG)	5	0
Rheumatoid factors	37	0
Human Anti-Mouse Antibodies (HAMA)	2	0
Anti-Nuclear Antibodies (ANA)	10	0
Myeloma	10	0
Total	**239**	**0**

12 - REFERENCES

1. ANDERSON S.E., and REMINGTON J.S. The diagnosis of toxoplasmosis. Southern Med. J. 1975; 68, 1433-1443
2. BELANGER F., DEROUIN F., GRANGEOT-KEROS L., MEYER L. and the HEMOCO and SEROCO Study Groups Incidence and risk factors of toxoplasmosis in a cohort of Human immunodeficiency virus- infected patients (1988-1995). Clinical Infectious Diseases 1999; 28, 575-581
3. BULLOCK S.L., and WALLS, K.W., Evaluation of some of the parameters of the enzyme-linked immunospecific assay. J. Inf. Dis. (Suppl.) 136: 2, S279-S285

4. DAFFOS, FORESTIER F., CAPELLA-PAVLOVSKY M., THULLIEZ P., AUFRANT C., VALENTI D. and COX L. Prenatal management of 746 pregnancies at risk for congenital toxoplasmosis. New Eng. J. Med. 1988; 318, 271-275
5. DECOSTER A., DARCY F., CARON A., VINATIER D., HOUZE DE L'AULNOIS D., VITTU G., NIEL G., HEYER F., LECOLIER B., DELCROIX M., MONNIER J.C., DUHAMEL M. and CAPRON A. Anti-P30 IgA antibodies as prenatal markers of congenital Toxoplasma infection. 1992; 87, 310-315
6. DEROUIN F., LEPORT C., PUEYO S., MORLAT P., LETRILLART B., CHENE G., ECOBICHON J.L., LUFT B., AUBERTIN J., HAFNER R., VILDE J.L., SALAMON R. and ANRS 005/ACTG 154 Trial Group. Predictive value of *Toxoplasma gondii* antibody titres on the occurrence of toxoplasmic encephalitis in HIV-infected patients. AIDS 1996; 10, 1521-1527
7. DESMONTS G., COUVREUR J., THULLIEZ Ph., SAINT JOIGNY G. and COLIN J. Sérodiagnostic de la Toxoplasmose, des méthodes simples, pour des questions précises. Le concours médical 1985; 107-03, 227-234
8. DUBEY J.P. and BEATTIE C.P. Toxoplasmosis of animal and man. CRC Press (1988).
9. JENUM P.A., STRAY-PEDERSEN B., MELBY K.K., KAPPERUD G., WHITELAW A., ESKILD A. and ENG J. Incidence of *Toxoplasma gondii* infection in 35.940 Pregnant Women in Norway and Pregnancy Outcome for Infected Women. J. Clin. Microbiol. 1998; 36, 2900-2906
10. JENUM P.A. and STRAY-PEDERSEN B. Development of specific immunoglobulins G, M, and A following *Toxoplasma gondii* infection in pregnant women in Norway and pregnancy outcome for infected women. J. Clin. Microbiol. 1998; 36, 2907-2913
11. JENUM P.A., STRAY-PEDERSEN B. and GUNDERSEN A.G. Improved Diagnosis of primary *Toxoplasma gondii* infection in early pregnancy by determination of anti-Toxoplasma immunoglobulin G avidity
12. LAPPALAINEN M., KOSKELA P., KOSKINIEMI M., AMMALA P., HILESMAA V., TERAMO K., RAIVIO K.O., REMINGTON J.S., HEDMAN K. Toxoplasmosis acquired during pregnancy: improved serodiagnosis based on avidity of IgG. J. Infect. Dis. 1993; 41, 155-158
13. LECOLIER B. and PUCHEU. Usefulness of IgG avidity analysis for the diagnosis of toxoplasmosis. Path. Biol. 1993; 42, 2 155-158
14. LEBECH M., JOYNSON D.H.M., SEITZ H.M. THULLIEZ P., GILBERT R.E., DUTTON G.N., OVLISEN B. and PETERSEN E., Classification system and case definition of *Toxoplasma gondii* infection in immunocompetent pregnant women and their congenitally infected offspring. Eur. J. Microbiol. Infect. Dis. 1996; 15, 799-805

15. LUFT B.J. and REMINGTON J.S. Toxoplasmic encephalitis. Clinical Infectious Diseases 1992; 15, 211-222
16. NAESSENS A., HEUNINCKX W., FOULON W. and LAUWERS S. Evaluation of seven commercially available enzyme immunoassays for immunoglobulin G and M antibody detection of *Toxoplasma gondii*. Immunol. Infect. Dis. 1993; 3, 258-262
17. PETITHORY J.C., REITER-OWONA I., BERTHELOT F., MILGRAM M., DE LOYE J. and PETERSEN E. Performance of European laboratories testing serum samples for *Toxoplasma gondii*. Eur. J. Microbiol. Infect. Dis. 1996; 15, 45-49
18. REMINGTON J.S. and DESMONTS G. Toxoplasmosis in infectious disease of the fetus and newborn infant. JS Remington and JO Klein, eds. WB Saunders Co., Philadelphia 1976; 191-332
19. SABIN A.B., and FELDMAN H.A. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (Toxoplasma), Science 1948; 108: 660 – 663
20. SANTORO F., AFCHAIN D., PIERCE J.R., CESBRON J.Y., OVLAQUE G. AND CAPRON A. Serodiagnosis of Toxoplasma infection using a purified parasite protein P30. Clin. Exp. Immunol. 1885; 62, 262-269
21. SHARMA S.D., MULLENAX J., ARAUJO F.G., ERLICH H.A. and REMINGTON J.S. Western Blot analysis of the antigens of *Toxoplasma gondii* recognized by human IgM and IgG antibodies. J. Immunol. 1983; 131: 977-983
22. SULHANIAN A., NUGUES C., GARIN J.F., PELLOUX H., LONGUET P., SLIZEWICZ B. and DEROUIN F. Serodiagnosis of toxoplasmosis in patients with acquired or reactivating toxoplasmosis and analysis of the specific IgA antibody response by ELISA, agglutination and immunoblotting. Immunol. Infect. Dis. 1993; 3, 63-69
23. SULZER A.J. and HALL EC. Indirect fluorescent antibody tests for parasitic diseases : IV Statistical study of variation in the indirect fluorescent antibody (IFA) test for toxoplasmosis. Amer. J. Epidemiol. 1967; 86: 401-407
24. WILSON M., REMINGTON J.S., CLAVET C., VARNEY G., PRESS C., WARE D. and the FDA TOXOPLASMOSIS AD HOC WORKING GROUP. Evaluation of six commercial kits for detection of human immunoglobulin M antibodies to *Toxoplasma gondii*. J. Clin. Microbiol. 1997; 35, 3112-3115
25. WONG S.Y. and REMINGTON J.S. Biology of *Toxoplasma gondii*. AIDS 1993; 7, 299-316
26. BLYTHE C.R., STILL H.A. Binomial confidence intervals. J. American Statistical Assoc., 1983; 78, 108-116

SUMMARY OF THE ASSAY PROCEDURE

1. Regulate the thermostat of the incubator at 37±1°C.
2. Dilute the Concentrated Washing Solution (R2)1:20 in distilled water.
3. Prepare the conjugate working solution R6 (R6a+R6b) (*Refer to section 4*).
4. Dilute the controls and samples 1:21 in R7.
5. Distribute 200 µL of diluted controls and samples into the wells as suggested in the plan below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**R3**	**S5**										
B	**R4**	**S6**										
C	**R4**	**S7**										
D	**R5**	**S8**										
E	**S1**	**S9**										
F	**S2**	**S10**										
G	**S3**	**S11**										
H	**S4**	**S12**										

6. Incubate for 1hour ± 5 minutes at 37±1°C.
7. Aspirate then wash 4 times with diluted Washing Solution.
8. Add 200 µL of Conjugate Working Solution to all wells.
9. Incubate for 1 hour ± 5 minutes at 37±1°C.
10. Aspirate then wash 4 times with diluted Washing Solution.
11. Add 200 µL of Chromogen TMB (R9) to all wells.
12. Incubate for 30 minutes ± 5 minutes at room temperature (+18-30°C), in the dark.
13. Add 100 µL Stopping Solution (R10) to all wells.
14. Read the absorbance with a spectrophotometer at 450/620 nm.

(EN) Caution, consult accompanying materials.
This product contains human or animal components. Handle with care.

ProClin 300
H317

1 N H_2SO_4
H314

H314 - H317

P280 - P305+P351+P338 - P301+P330+P331 - P303+P361+P353 - P333+P313 - P501

(EN)
Danger
Causes severe skin burns and eye damage. May cause an allergic skin reaction.
Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.

NOTES

Distributed in the U.S. by:
Bio-Rad Laboratories
6565 185th Avenue NE
Redmond, WA 98052, U.S.A.

DIST.

Manufactured in France by:
Bio-Rad
3, boulevard Raymond Poincaré
92430 Marnes-la-Coquette, France
Tel. : +33 (0) 1 47 95 60 00
Fax.: +33 (0) 1 47 41 91 33
www.bio-rad.com

2015/01
code: 881170

For Customer Orders and Technical Service Call: 1-800-2-BIO-RAD (1-800-224-6723)