Instruction Manual

Toxoplasma IgM ELISA

Enzyme immunoassay based on microtiter plate for the detection and quantitative determination of human IgM antibodies against Toxoplasma gondii in serum and plasma

Cat. No.: 40-375-380074
Storage: 2-8°C
For research use only

September 2009
Contents

1. Intended Use 3
2. General Information 3
3. Principle of the Test 3
4. Limitations, Precautions and General Comments 4
5. Reagents Provided 4
6. Materials Required but not Provided 5
7. Specimen Collection and Handling 5
8. Assay Procedure 5
9. Evaluation 6
10. Assay Characteristics 7
11. References 8

Symbols and Translations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
<th>French</th>
<th>German</th>
<th>Italian</th>
<th>Spanish</th>
<th>Greek</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL</td>
<td>Calibrator</td>
<td>Étalon</td>
<td>Standard</td>
<td>Calibratore</td>
<td>Calibrador</td>
<td>Πρότυπο</td>
</tr>
<tr>
<td>CONJ</td>
<td>Conjugate</td>
<td>Conjugué</td>
<td>Konjugat</td>
<td>Coniugato</td>
<td>Conjugado</td>
<td>Διάλυμα</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate (&lt;n&gt;-fold)</td>
<td>Concentré (&lt;n&gt; fois)</td>
<td>Konzentrat (&lt;n&gt;-fach)</td>
<td>Concentrado (&lt;n&gt;-veces)</td>
<td>Συμπύκνωση (&lt;n&gt; φορές)</td>
<td></td>
</tr>
<tr>
<td>SAMP DIL</td>
<td>Sample Diluent</td>
<td>Diluant échantillon</td>
<td>Proben verdünner</td>
<td>Diluente del campione</td>
<td>Diluyente de muestra</td>
<td>Διάλυμα Αραίωσης Δειγμάτων</td>
</tr>
<tr>
<td>STOP</td>
<td>Stop Solution</td>
<td>Solution d’arrêt</td>
<td>Stopp-Lösung</td>
<td>Soluzione d’arresto</td>
<td>Solución de parada</td>
<td>Διάλυμα Αναστολής</td>
</tr>
<tr>
<td>SUBS</td>
<td>Substrate</td>
<td>Substrat</td>
<td>Substrat</td>
<td>Substrato</td>
<td>Sustrato</td>
<td>Διάλυμα Υποστρώ ματώς</td>
</tr>
<tr>
<td>MT PLATE</td>
<td>Microtiter plate</td>
<td>Microplaque</td>
<td>Mikrotiteplate</td>
<td>Piastre</td>
<td>Placa microtiter</td>
<td>Μικρόπλακα</td>
</tr>
<tr>
<td>WASH BUF</td>
<td>Wash buffer</td>
<td>Tampon de lavage</td>
<td>Waschpuffer</td>
<td>Soluzione di lavaggio</td>
<td>Tampón de lavado</td>
<td>Πλυστικό Διάλυμα</td>
</tr>
</tbody>
</table>

For research use only.
1. Intended Use

The GenWay Toxoplasma IgM Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgM antibodies against Toxoplasma gondii in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of GenWay.

This assay is intended for research use only.

2. General Information

Toxoplasmosis is an infection, which is caused by the parasite toxoplasma gondii. Up to 20% of the population carry the pathogenic agent, but only a few feel symptoms, because the immune system hinders the illness to break out. Pregnant women should however be very cautious, as an infection can be detrimental to the foetus.

Toxoplasma gondii belongs to the protozoae and is a parasite, which can infect many different species of mammals, amongst others human beings. Animals like cats, pigs and sheep spread oocysts as well as tissue cysts, which after ingestion by humans are converted into tachyzoites, and these can be found afterwards in nerve and muscle tissue. When a pregnant woman becomes infected, the tachyzoites can reach the foetus via the placenta.

The symptoms of toxoplasmosis are very different, and most of the infected persons do not really feel ill. A typical sign of the disease is a flu-like sensation with swollen lymph nodes and muscle pain, which may last for months. The severe form of toxoplasmosis causes damages to the brain, the eyes and further organs. Even when the illness has ceased, a reactive chronic type can develop. The transmission of the disease takes primarily place by the ingestion of cat feces, e.g. during work in the garden or in the course of the pet care. But infective agents can also appear in raw meat or contaminated water.

Normally the clinical symptoms disappear after some weeks even without treatment, but for pregnant women and individuals with reduced immune system a therapy with drugs should be initiated.

The following laboratory methods are available: Complement fixation (CF), immunofluorescence (IFT) or ELISA. The determination of virus-specific IgM antibodies in fresh or reactivated infections is of special importance, the IgG test is used for the detection of immunity. If there is a severe connatal illness, an identification of the parasite out of peripheral blood, amniotic liquid or tissue samples can be tried.

3. Principle of the Test

The GenWay Toxoplasma IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Toxoplasma gondii antigen is bound on the surface of the microtiter strips. Diluted serum or ready-to-use calibrators are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Toxoplasma gondii antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgM peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgM antibodies is directly proportional to the intensity of the color.

For research use only.
4. Limitations, Precautions and General Comments

- Only for research use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.

- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.

- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.

- All reagents have to be brought to room temperature (18-25°C) before performing the test.

- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.

- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.

- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.

- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.

- No reagents from different kit lots have to be used, and they should not be mixed with one another.

- All reagents have to be used within the expiry period.

- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to micropipets and washing or reading (ELISA-Reader) instrumentation.

- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

5. Reagents Provided

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume / Qty.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT PLATE Toxoplasma gondii antigen coated microtiter strips</td>
<td>12</td>
</tr>
<tr>
<td>CAL Calibrators with: 1, 10, 30, 120 U/mL</td>
<td>4 x 2 mL</td>
</tr>
<tr>
<td>CONJ Enzyme Conjugate</td>
<td>15 mL</td>
</tr>
<tr>
<td>SUBS Substrate</td>
<td>15 mL</td>
</tr>
<tr>
<td>STOP Stop Solution</td>
<td>15 mL</td>
</tr>
<tr>
<td>SAMP DIL Sample Diluent</td>
<td>60 mL</td>
</tr>
<tr>
<td>WASH BUF CONC Washing Buffer (10×)</td>
<td>60 mL</td>
</tr>
<tr>
<td>Plastic foils</td>
<td>2</td>
</tr>
<tr>
<td>Plastic bag</td>
<td>1</td>
</tr>
</tbody>
</table>

Storage and limitation of usage (expiry dates are printed on the labels)
Store the components of the kit at 2-8°C. After usage put the plate in the plastic bag, close the bottles with their screw caps, and again store the kit at 2-8°C. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.

5.1. MT PLATE Microtiter Strips
12 strips with 8 breakable wells each, coated with a Toxoplasma gondii antigen (strain RH, isolated from infected mice). Ready-to-use.

For research use only.
5.2. CAL Calibrators A-D
4 x 2 mL, human serum diluted with PBS, with 1, 10, 30, 120 U/mL of IgM antibodies against Toxoplasma gondii. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.3. CONJ Enzyme Conjugate
15 mL, anti-human-IgM-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone, 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

5.4. SUBS Substrate
15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.5. STOP Stop Solution
15 mL, 0.5 M sulfuric acid. Ready-to-use.

5.6. SAMP DIL Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

5.7. WASH BUF CONC Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.8. Plastic Foils
2 pieces to cover the microtiter strips during the incubation.

5.9. Plastic Bag
Resealable, for the dry storage of non-used strips.

6. Materials Required but not Provided
• 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
• Microtiter Plate Reader (450 nm)
• Microtiter Plate Washer
• Reagent tubes for the serum dilution
• Bidistilled water

7. Specimen Collection and Handling
Principally serum or plasma (EDTA, citrate) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the calibrators) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

Important note: To avoid false positive results, positively determined samples should be confirmed generally by the use of an appropriate RF-absorbent (e.g. cat. no. ILE-MJS01).

8. Assay Procedure
8.1. Preparation of Reagents
Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
• Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.

For research use only.
8. All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.

9. Calibrators and samples should be assayed in duplicates.

10. A calibration curve should be established with each assay.

11. Return the unused microtiter strips to the plastic bag and store them with desiccant at 2-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the calibrators and samples in duplicate as well as for a substrate blank.

2. Pipet 100 µL each of the diluted (1:101) samples and the ready-to-use calibrators respectively into the wells. Leave one well empty for the substrate blank.

3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.

4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.

6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.

7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.

9. Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).

10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.

11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

9. Evaluation

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

<table>
<thead>
<tr>
<th></th>
<th>OD Value</th>
<th>corrected OD</th>
<th>Mean OD Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate Blank</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator A (1 U/mL)</td>
<td>0.033 / 0.029</td>
<td>0.013 / 0.009</td>
<td>0.011</td>
</tr>
<tr>
<td>Calibrator B (10 U/mL)</td>
<td>0.380 / 0.508</td>
<td>0.400 / 0.488</td>
<td>0.444</td>
</tr>
<tr>
<td>Calibrator C (30 U/mL)</td>
<td>1.060 / 1.080</td>
<td>1.040 / 1.060</td>
<td>1.050</td>
</tr>
<tr>
<td>Calibrator D (120 U/mL)</td>
<td>2.181 / 2.153</td>
<td>2.161 / 2.133</td>
<td>2.147</td>
</tr>
</tbody>
</table>

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently no reference values which have to be found in other laboratories in the same way.

9.1. Qualitative Evaluation

The calculated absorptions for the sample sera, as mentioned above, are compared with the value for the cut-off calibrator (10 U/mL). If the value of the sample is higher, there is a positive result. For a value below the cut-off calibrator, there is a negative result. It seems reasonable to define a range of +/-20 % around the value of the cut-off as a grey zone. In such a case the repetition of the

For research use only.
test with the same serum or with a new sample of the same individual, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

9.2. Quantitative Evaluation

The ready-to-use calibrators of the Toxoplasma gondii antibody kit are defined and expressed in Units per millilitre (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given sample follow-up controls become possible. The values for the calibrators in International Units are printed on the labels of the vials. For a quantitative evaluation the absorptions of the calibrators are graphically drawn against their concentrations. From the resulting calibration curve the concentration values for each sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

10. Assay Characteristics

<table>
<thead>
<tr>
<th>Toxoplasma gondii ELISA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay-Precision</td>
<td>4.3 - 6.2 %</td>
</tr>
<tr>
<td>Inter-Assay-Precision</td>
<td>2.7 - 18.1 %</td>
</tr>
<tr>
<td>Inter-Lot-Precision</td>
<td>0.2 - 23.7 %</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>1.1 U/mL</td>
</tr>
<tr>
<td>Recovery</td>
<td>107 - 112 %</td>
</tr>
<tr>
<td>Linearity</td>
<td>76 - 123 %</td>
</tr>
<tr>
<td>Cross-Reactivity</td>
<td>No cross-reactivity to rubella, cytomegaly, bordetella, borrelia, brucella and helicobacter. Interferences of samples of donors suffering from an acute EBV infection cannot totally be excluded.</td>
</tr>
<tr>
<td>Interferences</td>
<td>No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL</td>
</tr>
<tr>
<td>Clinical Specificity</td>
<td>99 %</td>
</tr>
<tr>
<td>Clinical Sensitivity</td>
<td>100 %</td>
</tr>
</tbody>
</table>
11. References


