Detection of antibodies to *Toxoplasma gondii* in human serum by latex particle agglutination
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1- INTENDED USE

*Toxoplasma gondii* is a protozoa capable of infecting numerous species of mammals and birds. Such infections are very common in man and animals and most often develop without apparent clinical signs. The prevalence of this infection in the population, detected by the presence of specific antibodies in serum, varies with the region and the age.

In the case of a primary infection in pregnant women, it may cause a severe disease of the fetus (birth defects) and even abortion. An immunity of the mother (however old), shown by the presence of IgG antibodies at the beginning of pregnancy, protects the fetus against the infection by this parasite.

The second population sensitive to this infection includes immunodepressed patients, among which are AIDS cases. According to certain studies, 30 to 40 % of the AIDS cases can exhibit brain toxoplasmosis, according to the prevalence of this chronic infection in the population studied. In most cases, these infections are caused by an infection from a quiescent parasitic focus (cyst) in the patient, existing before the infection by the HIV virus.

PASTOREX™ TOXO is a card latex agglutination test for the determination of serum antibodies to *T. gondii*.

2- PRINCIPLE OF THE TEST

Latex particles are coated by a covalent bond with soluble antigens from the membrane and cytoplasm of *T. gondii* (RH strain). This test allows the simultaneous detection of the IgG and/or IgM to *T. gondii*.

The reading of the test is very easy because of the use of suspended red latex particles in a green counterstain. In the case of a negative test, a uniform brown image is obtained. In the case of a positive test, a two color image exhibits a green background containing several red aggregates.
3- COMPOSITION OF THE KIT

<table>
<thead>
<tr>
<th>Label</th>
<th>Reagents</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex</td>
<td>Latex: red latex particles coated with antigens to <em>T. gondii</em> in green counterstain. Preservative: 0.1% proClin 300</td>
<td>1 dropper vial 1.6 ml</td>
</tr>
<tr>
<td>Negative control serum</td>
<td>Negative control serum: Human serum non reactive for antibodies to <em>T. gondii</em> and non reactive for anti-HIV1 and anti-HIV2 antibodies for HBs antigen and for anti-HCV antibodies. Preservative: 0.1% proClinTM 300</td>
<td>1 dropper vial 0.4 ml</td>
</tr>
<tr>
<td>Positive control serum</td>
<td>Positive control serum: Human serum positive for antibodies to <em>T. gondii</em> and non reactive for anti-HIV1 and anti-HIV2 antibodies, for HBs antigen and for anti-HCV antibodies. Preservative: 0.1% proClinTM 300</td>
<td>1 dropper vial 0.4 ml</td>
</tr>
<tr>
<td>Diluent</td>
<td>Diluent: NaCl Solution Preservative: 0.1% proClinTM 300</td>
<td>1 dropper vial 1.8 ml</td>
</tr>
</tbody>
</table>

| Disposable agglutination cards with 8 reaction fields each | 15 |
| Disposable plastic rods     |   |

4- STORAGE

All reagents are stable until the expiry dates indicated on the label, if stored at +2-8°C and in absence of microbial contamination (even when opened). Store the latex reagent bottles upright.

THE LATEX REAGENTS SHOULD NOT BE FROZEN.

5- MATERIALS REQUIRED BUT NOT PROVIDED

- Sodium hypochloride (bleach)
- Disposable latex gloves
- Automatic or semi-automatic pipettes to dispense a volume of 15 µl.
- Biohazardous waste container.
- Mechanical agitator
6- **PRECAUTIONS OF USE**  
The quality of the results depends upon the adherence to good laboratory practices:
- Do not use reagents after their expiration.
- All the reagents and the sample should be used at room temperature (18-30°C).
- Do not mix or associate reagents from kits with different lot numbers during the same manipulation.
- Do not touch the reaction fields of agglutination cards with your fingers.
- Change pipette or sampling tip for each sample tested.
- Shake the latex vial before use.
- Wipe the tip of the reagent dropper bottle in order to obtain well calibrated drops.
- Hold the reagent bottle vertical to deposit drops.
- Change the rod for each reaction.
- Discard all disposable material used in an autoclavable waste bin or disinfectant bath.

**HEALTH AND SAFETY INSTRUCTIONS**
Always observe the current techniques and precautions concerning protection against microbiological hazards.
- All samples taken must be considered potentially infectious.
- Wear disposable gloves to handle reagents and samples and cautiously wash your hands after handling.
- Do not pipette by mouth.
- Human source material used in the preparation of the reagents has been tested and found non-reactive for Hepatitis B surface antigen (HBs Ag), antibodies to Hepatitis C virus (HCV Ab), and antibodies to human immunodeficiency viruses (HIV1 and HIV2 Ab). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious diseases.
- Avoid splashing of samples or solutions.
• Spilled areas should be cleaned with household bleach diluted to 5%. If the contaminating liquid is an acid, spilled areas should be previously neutralized by sodium bicarbonate, washed with bleach, then wiped dry. The material used for cleaning must be thrown away in a biohazardous waste container.

• Samples and reagents of human origin, as well as the contaminated material and products, shall be disposed of after decontamination:
  - either by soaking in bleach at the final concentration of 5% of sodium hypochlorite for 30 minutes.
  - or by autoclaving at +121°C for 2 hours.

**CAUTION: do not place solution containing sodium hypochlorite in the autoclave.**

• Never forget to neutralize and/or autoclave specimens, reagents and washing solutions before disposing in a sink.

• Chemicals should be handled and disposed of in accordance with Good Laboratory Practices.

• Avoid any contact of the reagents with the skin or mucosa (risk of toxicity, irritation or burn).

• 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 is available on www.bio-rad.com.

7- SPECIMENS

1. Serum is the recommended sample type.

2. Observe the following recommendations for the handling, processing, and storing of serum samples:
   - Collect all serum samples observing routine precautions.
   - Allow samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - After centrifugation separate the serum and store it in a tightly stoppered storage tube.
   - The specimens can be stored at +2-8°C if screening is performed within 7 days.
   - If the assay will not be completed within 7 days, or for shipment of samples, freeze at −20°C, or colder.
   - Thaw samples only three times.
   - Previously frozen specimens should be thoroughly mixed after thawing prior to testing.
- Sera should not be contaminated. They must be transported and stored in tightly closed tubes.

3. Samples containing 90 g/l albumin, 100 mg/l bilirubin, lipemic samples containing the equivalent of 36 g/l triolein (triglyceride), and hemolyzed samples containing up to 10 g/l hemoglobin do not affect the result.

4. Do not heat the samples.

8- **ASSAY PROCEDURE**

- Strictly follow the recommended protocol.
- Use the negative and positive control sera for each assay to validate the test quality.
- Apply good laboratory practices.
- Shake the latex before use to obtain an homogenous brown suspension.

1. Apply on different fields of the card
   - A drop of positive control serum,
   - A drop of negative control serum,
   - 15 µl of sera to be examined.

2. Apply on each field, without making them come into contact, a drop of diluent beside the first drop.

3. Apply on each field, without making them come into contact, a drop of latex suspension beside the first two drops.

4. Mix the three drops of each circle using a stirring rod.

5. Place the card on the mechanical agitator and rotate for **5 minutes maximum** until a potential agglutination occurs. Reading should be done between 5 and 7 minutes.

*Caution: do not read after 7 minutes.*

9- **INTERPRETATION OF THE RESULTS**

**Negative reaction:** the suspension remains brown and homogenous. No green color observed. Compare with the negative control serum. In the case of pregnant women, the reaction must be confirmed by another technique.

**Positive reaction:** formation of a green background (more or less clearly marked) with red aggregates. The serum contains antibodies to *T. gondii*. The titer and antibody class must be determined by another technique:

PLATELIA™ TOXO IgG
PLATELIA™ TOXO IgM
PLATELIA™ TOXO IgA
10- QUALITY CONTROL OF THE TEST

After shaking, latex suspension should be perfectly homogenous. The immunoreactivity of the latex can be controlled with positive and negative controls. There should be agglutination with positive control and no agglutination with negative control. The latex suspension should not be used if it does not react with positive control, if it reacts with negative control (these reactions can be due to poor storage conditions or to latex contamination).

11- QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

12 - PERFORMANCE

Pastorex™ Toxo was evaluated on 3 different sites on a total of 732 samples, fresh or frozen, among which 202 sera from blood donors and 530 samples from hospitalized patients and pregnant women. Results with Pastorex™ Toxo were compared to results obtained with Platelia™ TOXO IgG TMB and Platelia™ TOXO IgM TMB (site 1) or with routine method used in the lab (site 2 and 3).

The results are as follow:

<table>
<thead>
<tr>
<th></th>
<th>Site 1</th>
<th>Sites 2 and 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agreement</strong></td>
<td>98.5%</td>
<td>92.9%</td>
<td>96.0%</td>
</tr>
<tr>
<td></td>
<td>[96.8 - 99.5]</td>
<td>[96.8 - 99.5]</td>
<td>[94.4 - 97.3]</td>
</tr>
<tr>
<td></td>
<td>(404/410)</td>
<td>(299/322)</td>
<td>(703/732)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>[98.5 - 100]</td>
<td>[98.1 - 100]</td>
<td>[99.2 - 100]</td>
</tr>
<tr>
<td></td>
<td>(201/201)</td>
<td>(158/158)</td>
<td>(359/359)</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>97.6%</td>
<td>89.8%</td>
<td>94.3%</td>
</tr>
<tr>
<td></td>
<td>[94.5 - 99.2]</td>
<td>[84.0 - 94.1]</td>
<td>[91.3 - 96.4]</td>
</tr>
<tr>
<td></td>
<td>(203/208)</td>
<td>(141/157)</td>
<td>(344/365)</td>
</tr>
</tbody>
</table>
Doubtful results were not included in the calculations. Among the 21 sera negative in Pastorex™ Toxo and found positive with reference method, none was found positive in IgM. 95% of these sera corresponded to weakly positive IgG sera.

Cross reactions:
101 samples positive for different serological or bacterial markers (Measles, Mumps, Rubella, VZV, HSV 1 and 2, CMV, EBV, HIV, HAV, HBV, HCV) and samples positive for rheumatoid factors, ANA autoantibodies, human anti-mouse antibody (HAMA) and with samples from patients with Myeloma were tested with Pastorex™ Toxo and Platelia™Toxo IgG TMB and Platelia™ Toxo IgM TMB.

All the samples found negative with the reference tests were found negative with Pastorex™ Toxo.

Reproducibility:
One negative sample and 2 positive samples were tested 12 times on the same day. These samples were also tested in duplicate during 10 days maximum twice a day per 2 manipulators.

Pastorex™ Toxo shows a good reproducibility in intra-assay and in inter-assay.

13- LIMITS OF USE
1. Some strongly positive sera exceptionally may cause the appearance of a zone phenomenon.
2. Diagnosis of recent infection by the parasite can only be established on the basis of a combination of clinical and serological data.
3. The result of a single serum sample does not constitute sufficient proof for diagnosis of recent infection.
4. A significant increase in anti-\textit{T. gondii} IgG antibody titer in two serum samples obtained at a minimum interval of three weeks and tested within the same run does not permit the diagnosis of a recent infection by the parasite, but instead provides evidence of recent seroconversion.
5. It is essential to detect IgM antibodies to \textit{T. gondii} as part of the serological surveillance of pregnant women, as the appearance of anti- \textit{T. gondii} IgG antibodies may occur slightly later than that of anti-\textit{T. gondii} IgM antibodies (kit PLATELIA™ TOXO IgM).
14- REFERENCES


This product contains human or animal components. Handle with care.
Warning
May cause an allergic skin reaction.
Wear protective gloves/protective clothing/eye protection/face protection. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/ national/international regulations.
nadražaja ili osipa na koži: zatražiti savjet/pomoć liječnika. Odložite sadržaje /spremnike u skladu s lokalnim/regionalnim/nacionalnim/međunarodnim odredbama.

(FI) Figyelem

(IT) Attenzione

(LT) Atsargiai

(NL) Waarschuwing

(NO) Advarsel

(PL) Uwaga
Może powodować reakcję alergiczną skóry.


(PT) Atenção

(RO) Atenție
Poate provoca o reacție alergică a pielii. Purtați mânuși de protecție/imbrăcămintete de protecție/echipament de protecție a ochilor/ chipament de protecție a feței. ÎN CAZ DE CONTACT CU PIELEA: spălați cu multă apă și săpun. În caz de irritare a pielii sau de erupție cutanată: consultați medicul. Aruncați conținutul/ containerul în acord cu regulamentele locale/ regionale/nationale/internaționale.

(SE) Varning

(SI) Pozor

(SK) Pozor