A rapid slide test for the qualitative and semi-quantitative determination of Toxoplasmosis Antibodies in human serum

**PROCEDURE**

1. **Qualitative method**
   - Bring reagents and specimens to room temperature before use.
   - Gently shake the latex toxo reagent, to disperse the latex particles.
   - Place 50 µl of the sample serum into a circle of the slide.
   - Mix both drops spreading them over the full surface of the circle.
   - Read the presence or absence of visible agglutination in this period of time. Unspecific agglutination could appear if the test is read later than 4 minutes.

2. **Calibration**
   - The Toxo-latex sensitivity is calibrated against the 3rd International Standard for anti-Toxoplasma (WHO)

3. **Reagents**
   - Control +: Animal serum with an antibody anti-Toxoplasma concentration > 4 IU/mL. Preservative.
   - Control -: Animal serum. Preservative.

4. **Precautions**
   - Reagents containing sodium azide may combine with copper and lead plumbing to form highly explosive metal azides. Dispose of reagent by flushing with large amounts of water to prevent azide buildup.

5. **Samples**
   - Use fresh serum. Plasma, lipemic or highly hemolized serum may cause erroneous results. If the test cannot be performed immediately, store the specimen at 2 to 8°C for 7 days. For longer period of time (up to 3 months), the serum sample should be frozen at –20°C. Samples with presence of fibrin should be centrifuged.

6. **Performance characteristics**
   - 1. Analytical sensitivity: 4 (3-7) IU/mL, under the described assay conditions
   - 2. Prozone effect: Up to 200 IU/mL. Occasionally a prozone effect may be observed with strong positive sera. Therefore in these cases where a suspected case of toxoplasmosis gives a negative result, the test should be repeated using 1/5 serum dilution in NaCl 9 g/L.
   - 3. Diagnostic sensitivity: 96.1%
   - 4. Diagnostic specificity: 89.6%

7. **Interferences**
   - Hemoglobin (10 g/l), bilirubin (20 mg/dl), lipemia (10 g/l), and rheumatoid factors (300 IU/ml) do not interfere. Other substances may interfere.

8. **Quality control**
   - Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.

9. **Storage and stability**
   - The latex reagent and control sera are stable up to the expiry date when stored at 2 to 8°C. Do not freeze!
   - The latex reagent, once shaken, must be uniform without visible clumping.

10. **Procedure**
    - Place a drop (50µl) of each Positive and Negative controls onto separate circles of the slide.
    - Add 25 µl of the toxo latex reagent next to the serum.
    - Mix both drops spreading them over the full surface of the circle.
    - Rotate the slide at 80-100 rpm during 4 minutes.
    - Read the presence or absence of visible agglutination in this period of time. Unspecific agglutination could appear if the test is read later than 4 minutes.

11. **Notes**
    - As with all diagnostic methods, the final diagnosis should not be made on the results of a single test, but should be based on a correlation of test results with other clinical findings.
    - False positive results may be obtained with hepato cellular diseases. A 25% of serum containing heterophile antibodies may give false positive results.
    - All positive sera should be tested with a confirmatory test.

**CLINICAL SIGNIFICANCE**

Toxoplasmosis is a parasitic disease caused by the protozoan Toxoplasma gondii. The parasite infects the vast majority of warm-blooded animals, including humans, but the primary host is the cat. The Toxoplasmosis usually has minor symptoms and is self-limiting but it can have serious or even fatal effects on immunocompromised persons (such as those infected with HIV or transplant recipients on immunosuppressive therapy). If infection with T. gondii occurs for the first time during pregnancy, the parasite can cross the placenta and causes congenital toxoplasmosis. The consequences of congenital toxoplasmosis range from spontaneous abortion and prematurity to generalized and neurological symptoms, which often involve ocular complications.

**Semi-quantitative method**

1. **Semi-quantitative method**
   - Will be performed in the same way as the qualitative test but using previous dilution of the serum sample in saline (NaCl 9g/L).

| Dilutions | 1/2  | 1/4  | 1/8  | ...
|-----------|------|------|------|------
| Sample serum | 100µl | 50µl | 25µl | ...
| Saline    | 100µl | 100µl | 100µl | ...
| Volume of the sample | 50µl | 50µl | 50µl | ...

**Reading and interpretation**

- A negative reaction is indicated by a uniform milky suspension with no agglutination as observed with the negative control (2).
- A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared with the positive control (1). The presence of agglutination indicates an antibody concentration equal or greater than 4 IU/ml.

**Notes**

- All positive sera should be tested with a confirmatory test.

- The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

- Concentration will be reciprocal of positive reading dilution.

<table>
<thead>
<tr>
<th>4 x n° of dilution</th>
<th>4 x 2</th>
<th>4 x 4</th>
<th>4 x 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU/ml</td>
<td>8</td>
<td>16</td>
<td>32</td>
</tr>
</tbody>
</table>

**Normal levels**

- Adults < 4 IU/ml