**SALMONELLA PARATYPHI AO SLIDE**

Determination of antibodies associated to salmonella paraTyphi AO by means of coloured bacterial suspension on slide

**TEST SUMMARY**
Slide and tube agglutination test for the qualitative and semi-quantitative detection of antibodies associated to Salmonella paraTyphi AO infections. Samples containing the specific antibody cause the agglutination of inactivate bacteria present in suspension. The intravitral coloring allows an easier reading of the formation of the agglutinates. High levels of agglutinating antibodies are indicative of infection by these microorganisms.

**SAMPLES**
Fresh clear serum. Stability 7 days at 2-8°C or 3 months at –20°C.
Do not freeze repeatedly.
The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolized or lipemic samples.

**MATERIALS REQUIRED BUT NOT SUPPLIED**
Is suggested the use of LTA Macro suspensions and LTA suspensions. The Intravitral coloring allows an easier reading of the formation of the agglutinates.

**PRECAUTIONS**
The reagent may contain non-reactive components and preservatives of various kinds. For precautionary purposes, however, contact with skin and ingestion should be avoided. Use the normal precautions for behavior in the laboratory.

**REAGENTS PREPARATION**
Reagents are ready to use.
Bacterial suspension has to be carefully resuspended shaking it more times for inversion.

**PROCEDURE**

**SLIDE AGGLUTINATION (QUALITATIVE)**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Positive Control</th>
<th>Negative Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
<tr>
<td>Positive control</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
<tr>
<td>Negative control</td>
<td>50 μl (1 gtt)</td>
<td>50 μl (1 gtt)</td>
</tr>
</tbody>
</table>

Mix using a disposable stitter, spread homogeneously over the entire area enclosed by the ring and shake it with a rotary motion or with a mechanical stitter at 80-100 rpm, for 1 minute.

**SLIDE AGGLUTINATION (TITRATION)**

Approximate titre

<table>
<thead>
<tr>
<th>Sample</th>
<th>1/20</th>
<th>1/40</th>
<th>1/80</th>
<th>1/160</th>
<th>1/320</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>80 μl</td>
<td>50 μl</td>
<td>50 μl</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
<tr>
<td>Suspension</td>
<td>80 μl</td>
<td>50 μl</td>
<td>50 μl</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
</tbody>
</table>

Mix using a disposable stitter, spread homogeneously over the entire area enclosed by the ring and shake it with a rotary motion or with a mechanical stitter at 80-100 rpm, for 1 minute.

**TUBE AGGLUTINATION (semiquantitative)**

Is suggested the use of LTA Macro suspensions and furthermore LTA Micro suspensions which have buffers purposely studied to guarantee a certain analysis result. The analytical method is anyhow reported to establish the titre with slide suspensions even if this technology has underlining limits.

1. Prepare a row of tube test for each sample as follows:
   - NaCl 9 g/L: 1.9 ml
   - 1 ml
   - 1 ml
   - 1 ml
   - 1 ml
   - Sample: 100 μl
   - 1 ml
   - 1 ml
   - 1 ml
   - 1 ml
   - 1 ml
   - 1 ml

2. Prepare 1 tube for Positive Control and 1 tube for Negative Control with 0.1 ml of control + 0.9 ml of NaCl 9 g/L each.
3. Add 50 μl (1 gtt) of suspension to each tube.
4. Mix thoroughly and incubate tube test at 37°C for 24 h.

**RESULTS INTERPRETATION**

**SLIDE AGGLUTINATION**
Examine macroscopically the absence or presence of agglutination after 1 minute by comparing the results with the Positive and Negative control.
Agglutination into time established means positivity.

**TUBE AGGLUTINATION**
Examine macroscopically the absence or presence of agglutination by comparing the results with the tubes of Positive and Negative control.

**REFERENCE VALUES**
For Somatic Antigen (O) Titre ≥ 1/80 indicate a recent infection.
In case of a positive result with a low titre, it is significant for the diagnosis verify the increase of titre between samples taken at a distance of days.
If the titre remains unchanged it may be a previous contact or previous vaccination.
A single positive result has less significance than the demonstration of a rising or falling antibodies titre as evidence of infection.
The level of “normal” agglutinins to these organisms varies in different countries and different communities. It is recommended that each laboratory establish its own reference range.

**SYMBOLES**

- IVD: Only for IVD use
- LOT: Lot of manufacturing
- REF: Code number
- Storage temperature interval
- Exp: Expiration date (year, month)
- Warn: Warning, read enclosed documents
- Read: Read the directions
- Bio: Biological risk

**REFERENCES**