**BACTERIAL ANTIGENS**

**Slide and tube agglutination**

**Qualitative determination of febrile antibodies**

**IVD**

Store at 2 - 8°C.

**PRINCIPLE OF THE METHOD**

The Bacterial Antigens is a slide and tube agglutination test for the qualitative and semi-quantitative detection of antibodies anti-Salmonella, Brucella and certain Rickettsias in human serum. The reagents, standardized suspensions of killed and stained bacteria, agglutinate when mixed with samples containing the homologous antibody.

**CLINICAL SIGNIFICANCE**

Febrile diseases diagnostic may be assessed either by microorganism isolation in blood, stools or urine, or by titration of specific antibodies, somatic (O) and flagellar (H). The detection of these antibodies forms the basis for the long-established Widal test. This test dictates that a serum with high levels of agglutinating antibodies to O and H >1/100 is indicative of the infection with these microorganisms.

### REAGENTS

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>Antigen</th>
<th>Ref.</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella paratyphi</em> A4</td>
<td><em>a</em> flagellar</td>
<td>1205011</td>
<td>1 mL</td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em> AO</td>
<td>1,2,12, somatic</td>
<td>1205021</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em> BH</td>
<td><em>b</em> flagellar</td>
<td>1205031</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em> BO</td>
<td>1,4,5,12 somatic</td>
<td>1203041</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em> CH</td>
<td><em>c</em> flagellar</td>
<td>1205051</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em> CO</td>
<td>6,7 somatic</td>
<td>1205061</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhi</em> H</td>
<td><em>d</em> flagellar</td>
<td>1205071</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhi</em> O</td>
<td>1,9,12 somatic</td>
<td>1205081</td>
<td></td>
</tr>
<tr>
<td>Brucella abortus (*)</td>
<td>somatic</td>
<td>1205091</td>
<td></td>
</tr>
<tr>
<td>Brucella melitensis</td>
<td>somatic</td>
<td>1205097</td>
<td></td>
</tr>
<tr>
<td>Proteus OX2</td>
<td></td>
<td>1205101</td>
<td></td>
</tr>
<tr>
<td>Proteus OX19</td>
<td></td>
<td>1205111</td>
<td></td>
</tr>
<tr>
<td>Proteus OXK</td>
<td>somatic</td>
<td>1205121</td>
<td></td>
</tr>
</tbody>
</table>

(*) Use also for Brucella suis antibodies.

**ADDITIONAL EQUIPMENT**

- Mechanical rotator adjustable to 80-100 r.p.m.
- Heat at 37°C.

**SAMPLES**

Fresh serum. Stable 8 days at 2-8°C or 3 months at -20°C.

The samples with presence of fibrin should be centrifuged before testing.

**PROCEDURE**

**A. Slide agglutination method (qualitative test)**

1. Arrange the reagents and samples to room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 50 µL of the sample to be tested (Note 1 and 2) and 1 drop of each control into separate circles on the slide test.
3. Swirl the antigen vial gently before using. Add 1 drop (50 µL) of antigen to each circle next to the sample to be tested.
4. Mix with a disposable stirrer and spread over the entire area enclosed by the circle.
5. Place the slide on a mechanical rotator at 80-100 r.p.m., for 1 minute.

**B. Slide agglutination method (titration)**

1. Place 1 drop (50 µL) of antigen to each circle next to the sample to be tested.
2. Mix with a disposable stirrer and spread over the entire area enclosed by the circle.
3. Place the slide on a mechanical rotator at 80-100 r.p.m., for 1 minute.

**C. Tube agglutination test**

1. Prepare a row of tube test for each sample as follows:

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>Sample µL</th>
<th>NaCl 9 g/L (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/20</td>
<td>1</td>
<td>1 mL</td>
</tr>
<tr>
<td>1/40</td>
<td>1</td>
<td>1 mL</td>
</tr>
<tr>
<td>1/80</td>
<td>1</td>
<td>1 mL</td>
</tr>
<tr>
<td>1/160</td>
<td>1</td>
<td>1 mL</td>
</tr>
<tr>
<td>1/320</td>
<td>1</td>
<td>1 mL</td>
</tr>
<tr>
<td>1/640</td>
<td>1</td>
<td>1 mL discard</td>
</tr>
</tbody>
</table>

2. Prepare 2 tubes for Positive and Negative control: 0.1 mL Control + 0.9 mL NaCl 9 g/L.
3. Add a drop (50µL) of antigen suspension to each tube.
4. Mix thoroughly and incubate tube test at 37°C for 24 h (Note 3).

**READING AND INTERPRETATION (Note 4)**

Examine macroscopically the presence or absence of clumps within 1 minute after removing the slide from the rotator comparing test results with control sera.

The reactions obtained in the slide titration method, are roughly equivalent to those which would occur in tube test with serum dilutions of 1/20, 1/40, 1/80, 1/160 and 1/320 respectively. If a reaction is found it is advisable to confirm the reaction and establish the titer by a tube test.

**TUBE AGGLUTINATION TEST**

Examine macroscopically the pattern of agglutination (Note 5) and compare the results with those given by all control tubes.

Positive control should give partial or complete agglutination. Negative Control should not give visible clumping.

Partial or complete agglutination with variable degree of clearing of the supernatant fluid is recorded as a positive.

The serum titer is defined as the highest dilution showing a positive result.

**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.

**REFERENCE RANGES**

Salmonellas: Tilters ≥ 1/80 (O antibodies) and ≥ 1/160 (H antibodies) indicates recent infection.

Brucellas: Tilters ≥ 1/80 indicate infection.

Proteus: Tilters OX19 ≥ 1/80, OX2 ≥ 1/20 and OX19 ≥ 1/80 indicate infection.

The level of "normal" agglutinations to these organisms varies in different countries and different communities. It is recommended that each laboratory establish its own reference range.

**PERFORMANCE CHARACTERISTICS**

All the performance characteristics of the Bacterial Antigens may be found in the corresponding Technical Report and they are available on request.

**INTERFERENCES**

Bilirubin (20 mg/dL), hemoglobin (10 g/L), lipids (10 g/L) and rheumatoid factors (300 IU/mL), do not interfere.

**LIMITATIONS OF PROCEDURE**

False negative results can be obtained in early disease, immune-unresponsiveness, prozone (Brucelosis), and antibiotic treatment (somatic).

- Serological cross-reactions with Brucella have been reported in cases of infection or vaccination with some strains of Vibrio cholerae, Pasteurella, Proteus OX19 and Y. enterocolitica (serotype 9).

A great number of false positive reactions have been reported in healthy individuals with Proteus antigens, especially in slide agglutination test. A titer of less than 1/160 should not be considered significant.

**NOTES**

1. When testing for Brucella antibodies it is recommended to reduce sample volume to 20 µL in order to avoid prozone.
2. In some geographical areas with a high prevalence of febrile antibodies, it is recommended to dilute the sample ¼ en NaCl 9 g/L before to perform the assay.
3. The incubation procedure may be accelerated incubating as follows:
   - Somatic (O) and Proteus antigens: 48-50°C for 4 h.
   - Flagellar (H) antigens: 48-50°C for 2 h.
4. A single positive result has less significance than the demonstration of a rising or falling antibodies titer as evidence of infection. A clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.
5. A somatic reaction (O) is characterized by coarse, compact agglutination. The reactions obtained in the slide titration method, are roughly equivalent to those which would occur in tube test with serum dilutions of 1/20, 1/40, 1/80, 1/160 and 1/320 respectively. If a reaction is found it is advisable to confirm the reaction and establish the titer by a tube test.

**REFERENCES**

- Edward J Young. Clinical Infectious Diseases 1995; 21: 283
- David A et al. Current Opinion in Infectious Diseases 1994; 7; 616-623
- David R al Current Opinion in Infectious Diseases 1993; 6: 54-62