**Bacterial Antigens MonlabTest®**

**A slide and tube agglutination test**

**Qualitative determination of febrile antibodies**

Only for professional in vitro diagnostic use. Store at 2 - 8°C.

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**PRI NCI PLE OF THE METHOD**

The Bacterial Antigens is a slide and tube agglutination test for the qualitative and semi-quantitative detection of antibodies against 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.

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**CLI NI CAL SI GNI FI CANCE**

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.

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**REAGENTS**

- **Bacterial Antigens**: Suspensions of Salmonellae, Brucellae and Proteus in glycerin buffer, pH 8.2. Preservative.
- **Controls**: Animal serum. Preservative.

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**PREPARATION AND STABILITY**

Antigen suspensions: Ready to use. It should be gently mixed before use. Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.

Controls: Ready to use.

**Reagents deterioration**: Presence of particles and clumps.

All the components of the kit are stable until the expiration date on the label when stored at 2-8°C. Do not freeze.

**ADDITI ONAL E QIP MENT**

- Mechanical rotator adjustable to 80-100 r.p.m.
- Heater at 37°C. / - Vortex mixer. / - Pipettes 50 µL

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**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. Do not use highly hemolized or lipemic samples.

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**PROCEDURE**

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

1. Bring 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, 2) and 1 drop of each control into separate circles on the slide test.
3. Mix the antigen vial vigorously or on a vortex mixer 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. Using a micropipette, deliver 40, 20, 10 and 5 µL of undiluted serum into separate circles of the slide test.
2. Place 1 drop (50µL) of the antigen to each circle next to the sample to be tested.
3. Mix with a disposable stirrer and spread over the entire area enclosed by the circle.
4. Place the slide on a mechanical rotator at 80-100 r.p.m., for 1 minute.

**C. Tube agglutination method**

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

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>Sample (µL) NaCl 9 g/L (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/20</td>
<td>1/40</td>
</tr>
<tr>
<td>100 µL</td>
<td>1mL</td>
</tr>
<tr>
<td>19 µL</td>
<td>1mL</td>
</tr>
<tr>
<td>1 µL</td>
<td>1mL</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 6)**

**Slide agglutination method**

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. All result different from the negative control result, will be considered as a positive.

**REFERENCE RANGES**

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

Brucellas: Titer ≥ 1/80 indicate infection.

Proteus: A great number of false positive reactions has 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.

The level of "normal" agglutinins to these organisms varies in different countries and different communities. It is recommended that each laboratory establishes 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/dL), 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 (Brucellosis), 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).

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, if the test is recommended to dilute the sample 1/4 in NaCl 9 g/L before to perform the assay.
3. The incubation procedure may be accelerated incubating as follows:
   - Somatic (O) and Proteus antigens: 48-50ºC for 4 h.
   - Flagellar (H) antigens: 48-50ºC 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, which tends to be difficult to disperse, while flagellar (H) has a characteristic loose, flocculant agglutination.

**BI BILIOGRAPHY**


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**SYMBOLS FOR IVD COMPONENTS AND REAGENTS**

- Manufacturer
- Catalogue Code
- Temperature limitation
- Use by

For in vitro diagnostic use only

Consult instructions for use

Keep dry

Use by