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

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>
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<tr>
<td>Salmonella paratyphi AH</td>
<td>a flagellar</td>
<td>MO-165001</td>
<td>5 mL</td>
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<tr>
<td>Salmonella paratyphi AO</td>
<td>1,2,12, somatic</td>
<td>MO-165002</td>
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<tr>
<td>Salmonella paratyphi BH</td>
<td>b flagellar</td>
<td>MO-165003</td>
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<tr>
<td>Salmonella paratyphi BO</td>
<td>1,4,5,12 somatic</td>
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<td>Salmonella paratyphi CH</td>
<td>c flagellar</td>
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<td>Salmonella paratyphi CO</td>
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<td>Salmonella typhi H</td>
<td>d flagellar</td>
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<td>Salmonella typhi O</td>
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<td>Brucella abortus(*)</td>
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<td>MO-165010</td>
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<td>MO-165011</td>
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<td>Proteus OX19</td>
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<tr>
<td>Control -</td>
<td>MO-165015</td>
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</table>

(*): Useful also for Brucella suis antibodies.

REAGENTS COMPOSITION

- **Bacterial Antigens**: Suspensions of Salmonellas, Brucellas and Proteus in glycine buffer, pH 8.2. Preservative
- **Controls**: Animal serum. Preservative

CALIBRATION

There is not any International Reference for the sensitivity standardization of these reagents. For this reason, Spinreact uses an internal control that contains animal serum with antibodies anti-Salmonellas, Brucellas and Proteus, and titrated with commercial reagents of certified quality.

PREPARATION AND STABILITY

Antigen suspensions: Ready to use. It should be gently mixed before to 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.

ADDITIONAL EQUIPMENT

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

PROCEDURE

A. Slide agglutination method (qualitative test)

1. Prepare 2 tubes for Positive and Negative control: 0.1 mL Control + 0.9 mL NaCl 9 g/L.

2. Prepare 2 tubes for Positive and Negative control: 0.1 mL Control + 0.9 mL NaCl 9 g/L.

3. Add a drop (50 µL) of antigen suspension to each tube.

4. Mix thoroughly and incubate tube test at 37°C for 24 h.

B. Slide agglutination method (titration)

1. Using a micropipette, deliver 80, 40, 20, 10 and 5 µL of undiluted serum into separate circles of the slide test.

2. Place 1 drop (50 µL) 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 2 tubes for Positive and Negative control: 0.1 mL Control + 0.9 mL NaCl 9 g/L.

2. Add a drop (50 µL) of antigen suspension to each tube.

3. Mix thoroughly and incubate tube test at 37°C for 24 h.

READING AND INTERPRETATION (NOTE 4)

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.

REF: MO-165001 + MO-165015

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**REFERENCE RANGES**

| Salmonellas: | Titers ≥ 1/80 (O antibodies) and ≥ 1/160 (H antibodies) indicates recent infection. |
| Brucellas: | Titers ≥ 1/80 indicate infection. |
| Proteus: | OX19 ≥ 1/20, OX2 ≥ 1/20 and OX19 ≥ 1/80 indicate 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.

**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**

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

**LIMITATIONS OF PROCEDURE**

1. When testing for Brucella antibodies it is recommended to reduce the 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, which tends to be difficult to disperse, while flagellar (H) has a characteristic loose, flocculant agglutination.

**NOTES**


**BIBLIOGRAPHY**


**SYMBOLS FOR IVD COMPONENTS AND REAGENTS**

- Manufacturer
- Don't re-use
- Contains sufficient for <n> tests
- Catalogue Code
- Lot Number

For in vitro diagnostic use only
Consult instructions for use
Keep dry
Temperature limitation
Use by

REF: MO-165001 - MO-165015 Manufacturer: Monlab SL Selva de Mar 48 08019 BCN Spain +34 93 433 58 60 Fax +34 93 436 38 94 pedidos@monlab.com www.monlab.com

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