



Presentation:

Cod. Specified on Reagents composition

Store at: +2+8°C.

### Procedure

**Diagnostic reagent for qualitative measurement of febrile antigens.**

**Only for in vitro use in clinical laboratory (IVD)**

**TEST SUMMARY**

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.

**REAGENTS COMPOSITION**

REAGENT	SIZE	REF
Salmonella paratyphi AO 1,2,12, somatic	5 mL.	SE027
Salmonella paratyphi AH a flagellar	5 mL.	SE028
Salmonella paratyphi BO 1,4,5,12 somatic	5 mL.	SE029
Salmonella paratyphi BH b flagellar	5 mL.	SE030
Salmonella paratyphi CO 6,7 somatic	5 mL.	SE031
Salmonella paratyphi CH c flagellar	5 mL.	SE032
Salmonella typhi O 1,9,12 somatic	5 mL.	SE033
Salmonella typhi H d flagellar	5 mL.	SE034
Brucella abortus <sup>1</sup> somatic	5 mL.	SE035
Proteus OX19 somatic	5 mL.	SE036
Proteus OX2	5 mL.	SE037
Proteus OXK somatic	5 mL.	SE038
Control (+) / (-)	1 mL.	

(<sup>1</sup>): Useful also for *Brucella melitensis* and *Brucella suis* antibodies.

- **Bacterial Antigens:** Suspensions of Salmonellas, Brucellas and Proteus in glycine buffer, pH 8.2. Sodium azide 0.95 g/L.

- **Controls:** Animal serum. Sodium azide 0.95 g/L.

**PRECAUTIONS**

Good laboratory safety practices should be followed when handling laboratory reagents or human samples.

**REAGENT PREPARATION AND STABILITY**

All the components are ready to use.

Do not use reagents over the expiration date.

Do not freeze; frozen reagents could change the functionality of the test.

If appear particles and clumps do not use.

**All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8°C. Do not use reagents over the expiration date.**

**CALIBRATION**

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

**SPECIMEN**

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

The samples with particles or fibrin should be centrifuged to eliminate them. Do not use haemolized or lipemic samples.

**Discard contaminated specimen.**

**MATERIAL REQUIRED BUT NOT PROVIDED**

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Heater at 37°C.

**General laboratory equipment**

**TEST PROCEDURE**

**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. 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.

**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 min.

**Tube agglutination method**

1. Dilute 1/20 with 9 g/L ClNa (0.1 mL serum+1.9 mL saline) and make serial two-fold dilutions in 9 gr/L saline.
2. Prepare 2 tubes for (+) and (-)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**

**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 serums.

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.

**Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.**

**REFERENCE VALUES**

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

**Brucellas:** Titers ≥ 1/80 indicate infection.

**Proteus:** Titers OX19 ≥ 1/80, 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 suggested that each laboratory establish its own reference range.**

**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 microorganism.

**Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.**

**REAGENT PERFORMANCE**

Details are available on request.

**INTERFERING SUBSTANCES**

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

**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, which tends to be difficult to disperse, while flagellar (H) has a characteristic loose, flocculant agglutination.

**LIMITATIONS OF THE 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).
- 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.

**BIBLIOGRAPHY**

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3. David A et al. Current Opinion in Infectious Diseases 1994; 7: 616-623.
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5. Bradley D Jones. Annu Rev Immunol 1996; 14: 533 – 61.