Reactivos GPL

Barcelona, España

CE

-BACTERIAL ANTIGENS-

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Slide and tube agglutination

Store at: +2+8ºC.

Presentation:
Cod. Specified on Reagents composition

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[1] somatic	5 mL.	SE035
Brucella melitensis somatic	5 mL.	SE035-M
Proteus OX19 somatic	5 mL.	SE036
Proteus OX2 somatic	5 mL.	SE037
Proteus OXK somatic	5 mL.	SE038
Control (+) / (-)	1 mL.	

(*): Useful also for *Brucella melitensis* and *Brucella suis* antibodies.

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

REAGENT PREPARATION AND STABILITY

Antigen suspensions:
Controls: Ready to use.
Mix reagents gently before use.

Reagents deterioration:

- Presence of particles and clumps.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8ºC, *protected from light and contaminations.* Don not freeze. Do not freeze. Do not use reagents over the expiration date.

CALIBRATION

There is not any International Reference for the sensitivity standardization of these reagents. We use 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 2-8ºC or 3 months at –20ºC.
The samples with particles or fibrin should be centrifuged to eliminate them. Do not use haemolized or lipemic samples.

MATERIAL REQUIRED BUT NOT PROVIDED

- Mechanical rotator adjustable to 80-100 r.p.m.
- Heater at 37ºC.
- Vortex mixer.
- Pippetes 50 µL.

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. 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.

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 37ºC 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.
All result different from the negative control result, will be considered as a positive.

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 microorganisms.

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

REAGENT PERFORMANCE

All the performance characteristics of the Bacterial Antigens may be found in the corresponding Technical Report and they 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: 48-50ºC 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.

LIMITATIONS OF THE 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).

BIBLIOGRAPHY

1. Edward J Young. Clinical Infectious Diseases 1995; 21: 283-290.
2. Coulter JBS. Current Pediatrics 1996; 6: 25-29.
3. David A et al. Current Opinion in Infectious Diseases 1994; 7: 616-623.
4. David R et al Current Opinion in Infectious Diseases 1993; 6: 54-62.
5. Bradley D Jones. Annu Rev Immunol 1996; 14: 533 – 61.

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