Bacterial Antigens
Agglutination Slide and Tube Tests

Qualitative test for determination of febrile antibodies

IVD

Brucella Abortus: 101-0060 (20 x 5 mL), 101-0615 (5 mL)
Brucella Mellitensis: 101-0061 (20 x 5 mL), 101-0616 (5 mL)
Salmonella Typhi H (d-H): 101-0062 (20 x 5 mL), 101-0617 (5 mL)
Salmonella Typhi O (1,9,12-O): 101-0063 (20 x 5 mL), 101-0618 (5 mL)
Salmonella Paratyphy AH (a-H): 101-0064 (20 x 5 mL), 101-0619 (5 mL)
Salmonella Paratyphi AO (1,2,12-O): 101-0065 (20 x 5 mL), 101-0630 (5 mL)
Salmonella Paratyphi BH (b-H): 101-0066 (20 x 5 mL), 101-0620 (5 mL)
Salmonella Paratyphi BO (1,4,5,12-O): 101-0067 (20 x 5 mL), 101-0631 (5 mL)
Salmonella Paratyphi CH (c-H): 101-0068 (20 x 5 mL), 101-0632 (5 mL)
Salmonella Paratyphi CO (6,7-0): 101-0069 (20 x 5 mL), 101-0633 (5 mL)
Proteus OX19: 101-0070 (20 x 5 mL), 101-0634 (5 mL)
Proteus OX2: 101-0071 (20 x 5mL), 101-0636 (5 mL)
Proteus OXK : 101-0072 (20 x 5 mL), 101-0635 (5 mL)
Témoin positive: 101-0106 (1 x 1 mL)
Témoin negative: 101-0107 (1 x 1 mL)

Store at 2 - 8º C.

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.

PRINCIPLE
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.

REAGENT COMPOSITION

REAGENT	**Antigen**
Salmonella paratyphi AH	a flagellar
Salmonella paratyphi AO	1,2,12, somatic
Salmonella paratyphi BH	b flagellar
Salmonella paratyphi BO	1,4,5,12 somatic
Salmonella paratyphi CH	c flagellar
Salmonella paratyphi CO	6,7 somatic
Salmonella typhi H	d flagellar
Salmonella typhi O	1,9,12 somatic
Brucella abortus (*)	somatic
Brucella melitensis	somatic
Proteus OX2	somatic
Proteus OX19	somatic
Proteus OXK	somatic
Control +	
Control -	

- 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, Chronolab uses an internal control that contains animal serum with antibodies anti-Salmonellas, Brucellas and Proteus, and titered with commercial reagents of certified quality.

PREPARATION AND STABILITY
Antigen suspensions: Ready to use.
Controls: Ready to use
Mix reagents gently before 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 2-8ºC, **protected from light** and contaminations. Do not freeze.

ADDITIONAL EQUIPMENT
- Mechanical rotator adjustable to 80-100 r.p.m.
- Heater at 37º C.
- Vortex mixer.
- Pippetes 50 µL.

SAMPLE
Fresh serum. Stable 8 days at +2 to +8º C or 3 months at –20º C.
The samples with presence of fibrin should be centrifuged before testing
Do not use highly hemolized or lipemic samples.

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.

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 and 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 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 a row of tube test for each sample as follows:
2.

Dilutions	**1/20**	**1/40**	**1/80**	**1/160**	**1/320**	**1/640**	**…**
Sample (µL)	100	--	--	--	--	--	…
NaCl 9 g/L (mL)	1.9	1	1	1	1	1	…
	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL discard

3. Prepare 2 tubes for Positive and Negative control: 0.1 mL Control + 0.9 mL NaCl 9 g/L.
4. Add a drop (50µL) of antigen suspension to each tube.
5. 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.

REFERENCE RANGES
Salmonellas: Titers ≥ 1/80 (O antibodies) and ≥ 1/160 (H antibodies) indicates recent infection.
Brucellas: Titers ≥ 1/80 indicate infection.
Proteus: 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.
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.

PERFORMACE CHARECTERISTIC
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).

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