BACTERIAL SEROLOGY
Macro-method

Semiquantitative Determination
in Infection test Tubes
of Salmonellosis, Brucellosis
and Rickettsiosis
by Stained Bacterial Suspensions

Available kits:

Salmonella typhi H	REF	6300
Salmonella typhi 0	REF	6301
Salmonella typhi TOTAL	REF	6302
Salmonella paratyphi AH	REF	6303
Salmonella paratyphi AO	REF	6304
Salmonella paratyphi A TOTAL	REF	6305
Salmonella paratyphi BH	REF	6306
Salmonella paratyphi BO	REF	6307
Salmonella paratyphi B TOTAL	REF	6308
Salmonella paratyphi CH	REF	6309
Salmonella paratyphi CO	REF	6310
Salmonella paratyphi C TOTAL	REF	6311
Brucella melitensis	REF	6313
Brucella total/ abortus	REF	6315
Proteus OX 19	REF	6316
Proteus OX 2	REF	6317
Proteus OX K	REF	6318
Multiple 3 macro: OX 19, OX 2, OX K	REF	6321
Multiple 5 macro: H, O, A, B, BRU.TOT.	REF	6320
Multiple 8 macro H, O, AH, AO, BH, BO, C TOT., BRU. TOT.	REF	6322

Other available kits:
Control polyvalent Pos+Neg (4 x 1 ml) REF 6500

PRINCIPLE

The bacterial suspensions are prepared specifically for the detection, identification and semi-quantitation of serum agglutinins developed during infection diseases such as brucellosis, salmonellosis and certain rickettsiosis.
The assay is performed by testing the stained antigens against unknown samples. The presence or absence of a visible agglutination is usually related with presence or absence of the corresponding homologous antibody.
The bacterial suspensions have been stained (somatic blue and flagellar red) to facilitate reading and interpretation of the results.

REAGENTS

Kit components: **from 6300 to 6318** **6321** **6320** **6322**

REAGENT 1 (liquid, white cap) **2x20 ml** **3x20 ml** **5x20 ml** **8x10 ml**
Stained bacterial suspension, ready to use

The reagent contains sodium azide (< 0.1%) as preservative. Do not swallow. Avoid contact with skin and mucous membranes.

STABILITY: the reagents are stable up to stated expiry date when stored at 2-8°C.

SAMPLE

Serum.
STABILITY: 6 days at 2-8°C, 1 month at -20°C.

PREPARATION OF THE REAGENTS

Bring the suspension to room temperature before use. Mix gently the Reagent.

PROCEDURE

Prepare the eight test-tubes necessary for the test. Pour 0.9 ml of saline solution into the first one and 0.5 ml of saline solution into the remaining seven ones.
Add 0.1 ml of serum to the first test-tube and mix, then transfer 0.5 ml of this solution to test-tube 2, mix and transfer 0.5 ml to test-tube 3.
Go on with this procedure till test-tube 7. Discard 0.5 ml of the last solution formed in well 7. Then pour 0.5 ml of Reagent (bacterial suspension) into each test-tube.

SUMMARY TABLE OF DILUTIONS

Test-tube	1	2	3	4	5	6	7	8
Saline sol. (ml)	0.9	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Serum (ml)	0.1	0.5 from tube 1	0.5 from tube 2	0.5 from tube 3	0.5 from tube 4	0.5 from tube 5	0.5 from tube 6 (disc. 0.5)	--
Prepare all dilutions before adding Reagent 1								
Reagent 1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Titer	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Neg.

Mix gently and incubate according to the following scheme:

- 18-20 hours, at 37°C for Salmonella (O) and for Proteus.
- 24 hours, at 37°C for Brucella.

To obtain the best agglutination, place the test-tubes away from the walls of the thermostat. Moreover, avoid placing them on the bottom of it; instead place them on electrically inert material (e.g.: carton).
Avoid any test-tubes vibrations.

READING AND RESULTS

Define the results by observing the test-tubes through a traverse light and avoid any vibration.
NEGATIVE: homogenous suspension without any evident presence of aggregates with eventual presence of a round shaped precipitate, with very defined edges, on the well bottom.
POSITIVE: clear supernatant and presence of irregular agglutinates in the lower or central part of the well.
To confirm the results, shake gently the test-tubes and observe through a transverse light. If the sample is negative, the eventual colored bottom will resuspend and then deposit again slowly. If the sample is positive the agglutinate will remain compact.

REFERENCE VALUES

Salmonella: significant titer ≥1:80
Brucella: significant titer ≥1:100
Proteus: significant titer ≥1:20

Each laboratory should define its own reference values.

NOTES

1. (*) Dangerous reagents are marked by an asterisk. Refer to MSDS.
2. Antibiotic therapies may invalidate the test.
3. Always compare the results with the controls.

REFERENCES

Widal F., Bull. Mem. Soc. Hop de Paris 6,26 (1896)
Weil E. and Felix A., Wein. Klin. Woch 29,974 (1916)

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Manufactured by: **FAR** srl
Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY
Phone +39 045 6700870 – Fax +39 045 7157763
website: