# BACTERIAL SEROLOGY Micro-method

Semiquantitative Determination on Microplate of Salmonellosis, Brucellosis and Rickettsiosis Infections in Serum by Stained Bacterial Suspensions

Available kits:

Salmonella typhi H	REF 6200
Salmonella typhi O	REF 6201
Salmonella paratyphi A TOTAL	REF 6205
Salmonella paratyphi B TOTAL	REF 6208
Salmonella paratyphi C TOTAL	REF 6211
Brucella total / abortus	REF 6215
Brucella melitensis	REF 6213
Proteus OX 19	REF 6216
Proteus OX 2	REF 6217
Proteus OX K	REF 6218
Multiple 7 micro H, O, AH, AO, BH, BO, BRU	REF 6219
Multiple 3 micro OX 19, OX 2, OX K	REF 6220
Multiple 5 micro H, O, A, B, BRU	REF 6225

#### 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 6200	to 6218	6219	6220	6225
REAGENT 1 (liqui	d, white cap)	3x10 ml	7x10 ml	3x10 ml	5x10 ml
Stained bacterial s	uspension, read	dy to use.			
REAGENT 2		1x0,5 ml	2x0,5 ml	2x0,5 ml	2x0,5 ml
Positive control, to	dilute 1:10 with	h saline solu	tion 0.9 %.		
REAGENT 3		1x1 ml	2 x1 ml	2x1 ml	2x1 ml
Negative control, t	o dilute 1:10 wi	th saline sol	ution 0.9 %.		
MICRO PLATE "L	J". 96 wells				

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

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

#### SAMPLE

Serum: dilute 1:10 with saline solution (ex: 100  $\mu l$  serum + 900  $\mu l$  saline solution).

Stable 6 days at 2-8°C, 4 weeks at -20°C.

#### MANUAL ASSAY PROCEDURE

Bring the test reagents and sample at room temperature. Resuspend the Reagent 1 vial gently. Distribute into micro plate well diluted sample and control as per the following table:

Well	Saline Sol.	Serum	Negative	Positive	Titer
number	(µl)	(µl)	Control (µl)	Control (µl)	
1		100			1:20
2	100	100			1:40
3	100	100 from well 2			1:80
4	100	100 from well 3			1:160
5	100	100 from well 4			1:320
6	100	100 from well 5			1:640
7	100	100 from well 6			1:1280
8	100	100 from well 7			1:2560
9	100	100 from well 8, discard 100			1:5120
10	100				Suspension Control
11			100		Negative Control
12				100	Positive

Add 100 µl of the suspension to each well.

Mix gently for 30 seconds.

Incubate at 37°C for 16-18 hours. Avoid any vibration.

## READING AND RESULTS

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: partial or complete agglutination. A partial or complete agglutination with variable degree of clearing of the supernatant is considered positive.

The serum titer is defined by the highest dilution shown in a positive result.

#### **REFERENCE VALUES**

Salmonellas: titers  $\ \ge 1:80$  (O antibodies) and 1/160 (H antibodies) show recent infection.

Brucellas: titer  $\geq$ 1:80 shows infection.

Proteus: titers OX19  ${\geq}1{:}80,$  OX2  ${\geq}1{:}20$  and OXK ${\geq}1{:}80$  show infection. The level of "normal" agglutination to these organisms varies in each country and community.

Each laboratory should define its own reference values.

#### NOTES

- 1. (\*) Dangerous reagents are marked by an asterisk. Refer to MSDS. Always cvompare the results with the controls.
- 2. "U" or "V" bottomed microplates can be used.
- Bilirubin (20 mg/dl), hemoglobin (10 g/L), lipids (10 g/L) and
  rheumatoid factor (300 Ul/ml) do not interfere.
- Somatic agglutination (O) is thin, granular slow forming and hardly
  separable. The flagellate agglutination (H) is fast forming and easily separable.

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### 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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