

# SALMONELLA TYPHI O SLIDE

## Determination of antibodies associated to salmonella Typhi O by means of coloured bacterial suspension on slide

### TEST SUMMARY

Slide and tube agglutination test for the qualitative and semi-quantitative detection of antibodies associated to Salmonella Typhi O infections.

Samples containing the specific antibody cause the agglutination of inactivate bacteria present in suspension. The intravital coloring allows an easier reading of the formation of the agglutinates. High levels of agglutinating antibodies are indicative of infection by these microorganisms.

### SAMPLES

Fresh clear serum. Stability 7 days at 2-8°C or 3 months at -20°C.

Do not freeze repeatedly.

The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolized or lipemic samples.

Bring to room temperature before analysis..

### REAGENTS

Suspension: Inactivated and intravital colored bacterial suspension in glycine buffer pH 8.2; preservatives.

### MATERIALS REQUIRED BUT NOT SUPPLIED

Saline Solution NaCl 9 g/L. Positive Control serum and Negative Control serum. Slide and stirrer. Automatically micropipette. Mechanical stirrer at 100 r.p.m. Incubator 37°C. Current laboratory instrumentation.

### PRECAUTIONS

The reagent may contain non-reactive components and preservatives of various kinds. For precautionary purposes, however, contact with skin and ingestion should be avoided. Use the normal precautions for behavior in the laboratory.

### REAGENTS PREPARATION

Reagents are ready to use.

Bacterial suspension has to be carefully resuspended shaking it more times for inversion.

Bring to room temperature before analysis Stability: until expiration date on label stored at 2-8°C. Do not freeze.

### PROCEDURE

#### SLIDE AGGLUTINATION (QUALITATIVE)

Reagents	Sample	Positive Control	Negativ Control
Sample	50 µl	--	--
Positive control	--	50 µl	--
Negative control	--	--	50 µl
Suspension	50 µl (1 gtt)	50 µl (1 gtt)	50 µl (1 gtt)

Mix using a disposable stirrer, spread homogeneously over the entire area enclosed by the ring and shake it with a rotary motion or with a mechanical stirrer at 80-100 rpm. **for 1 minute.**

#### SLIDE AGGLUTINATION (TITRATION)

Approximate Titre	1/20	1/40	1/80	1/160	1/320
Sample	80 µl	40 µl	20 µl	10 µl	5 µl
Suspension	50 µl (1 gtt)				

Mix using a disposable stirrer, spread homogeneously over the entire area enclosed by the ring and shake it with a rotary motion or with a mechanical stirrer at 80-100 rpm. **for 1 minute.**

#### TUBE AGGLUTINATION (semiquantitative)

Is suggested the use of LTA Macro suspensions and furthermore LTA Micro suspensions which have buffers purposely studied to guarantee a certain analysis result. The analytical method is anyhow reported to establish the titre with slide suspensions even if this technology has underlining limits.

1. Prepare a row of tube test for each sample as follows:

Titre	1/20	1/40	1/80	1/160	1/320	1/640	--
NaCl 9 g/L	1.9 ml	1 ml	1 ml	1 ml	1 ml	1 ml	--
Sample	100 µl	--	--	--	--	--	--
	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	discharge 1 ml

2. Prepare 1 tube for Positive Control and 1 tube for Negative Control with 0,1 ml of control + 0,9 ml of NaCl 9 g/L each.

3. Add 50 µl (1 gtt) of suspension to each tube.

4. Mix thoroughly and incubate tube test at 37°C for 24 h.

### RESULTS INTERPRETATION

#### SLIDE AGGLUTINATION

Examine macroscopically the absence or presence of agglutination **after 1 minute** by comparing the results with the Positive and Negative control.

Agglutination into time established means positivity. Homogeneous suspension with no visible agglutination is negative.

For each positive result it is advisable to confirm the titre with the test-tube titration.

The results obtained whit slide titration method are roughly equivalent to those which would occur in tube test with serum dilutions. Respectively: 1/20 – 1/40 – 1/160 – 1/320 – 1/640.

#### TUBE AGGLUTINATION

Examine macroscopically the absence or presence of agglutination by comparing the results with the tubes of Positive and Negative control.

Somatic reaction (O) is characterized by coarse, compact agglutination, which tends to be difficult to disperse.

Partial agglutination is a sign of positive reaction.

The titre of the serum examined is due to the most higher dilution in which is showed a feeble positivity.

#### REFERENCE VALUES

For Somatic Antigen (O) Titre  $\geq 1/80$  indicate a recent infection.

In case of a positive result with a low titre, it is significant for the diagnosis verify the increase of titre between samples taken at a distance of days.

If the titre remains unchanged it may be a previous contact or previous vaccination.

A single positive result has less significance than the demonstration of a rising or falling antibodies titre as evidence of infection

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.

#### NOTE

- In some geographical areas with a high prevalence of febrile antibodies, it is recommended to dilute the sample 1:4 with NaCl 9 g/L before to perform the assay.

- As with any diagnostic procedure, if the results are incompatible with the clinical presentation, the physician should evaluate the data obtained using this test by comparing them with other clinical information.

- For in vitro diagnostic use only.

#### CALIBRATION/QUALITY CONTROL

There is not any International Reference for the sensitivity standardization of these reagents. For this reason, LTA uses an internal control that contains animal serum with antibodies anti-Salmonellas, and titred with commercial reagents of certified quality

Use of control sera is recommended as reference; the positive control ought to show a partial or complete agglutination, instead the negative control ought to show no agglutination.

Controls should be ever used to distinguish an eventual agglutination of the bottom of reagent.

Controls should be used as described in procedures or even to be treated as samples (dilution, ecc..).

#### The following controls are available on request:

**BS00011** 3 x 0.5 ml

Febrile Positive Control  
(0.5 ml Salmonella, 0.5 ml Brucella, 0.5 ml Proteus)

**BS00020** 1 x 1 ml

Febrile Negative Control

### TEST PERFORMANCE

#### Sensibility

The method sensibility decrease at low temperature. Better results will be obtained at higher temperature up to 10°C..

#### Interference

No interference was observed by the presence of:

hemoglobin	≤ 1000 mg/dl
bilirubin	≤ 20 mg/dl
lipids	≤ 1000 mg/dl
rheumatic factor	≤ 300 UI/ml

Recent infection, immunodepression or antibiotic treatment can do false negativity.

### WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

### PACKAGING

#### CODE BS00210

Suspension S. typhi O slide 1 x 5 ml

### REFERENCES

- Edward J Young. Clinical Infectious Diseases 1995; 21: 283-290.
- Coulter JBS. Current Pediatrics 1996; 6: 25-29.
- David A et al. Currebt Opinion in Infectious Diseases 1994; 7: 616-623.
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- Bradley D Jones. Annu Rev Immunol 1996; 14: 533-61.

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### SYMBOLS

	Only for IVD use
	Lot of manufacturing
	Code number
	Storage temperature interval
	Expiration date (year, month)
	Warning, read enclosed documents
	Read the directions
	Biological risk

Mod. 01.06 (ver. 3.0 - 02/05/2018)

