PROTEUS OX2 SLIDE

Determination of antibodies associated to rickettsie 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 rickettsie 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	Negative Control
Sample	20 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	1/80	1/160	1/320
Sample	20 µl	10 µl	5 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.**			

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.
Homogeneous suspension with no visible agglutination is negative.

Agglutination into time established means positivity Reaction, but since a great number of false positive reactions have been reported in healthy individual with Proteus antigens especially in slide agglutination test, it is advisable to confirm the titre with the test-tube titration.

The method for the titration on slides is however reported, which allows obtaining results approximately equivalent to those obtained by titrating the tube.
The "approximate titre" indicated in the Slide Titration was determined by comparing the results obtained between the two methods.

TUBE AGGLUTINATION

Examine macroscopically the absence or presence of agglutination by comparing the results with the tubes of Positive and Negative control.
Partial agglutination is a sign of positive reaction.
The title of the serum examined is due to the most higher dilution in which is showed a feeble positivity.

REFERENCE VALUES

Titre ≥ 1/160 indicate a recent infection.
A titre of less 1/160 should not be considered significant.

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-Proteus, 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 BS01010
Suspension Proteus OX2 slide 1 x 5 ml

REFERENCES

1. Edward J Young. Clinical Infectious Diseases 1995; 21: 283-290.
2. Coulter JBS. Current Pediatrics 1996; 6: 25-29..
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5. Bradley D Jones. Annu Rev Immunol 1996; 14: 533–61.

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SYMBOLS

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

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