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Europe +800 135 79 135 US 1 855 2360 190

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Stained Proteus Suspensions

EN

REF R30953601
R30953701
R30953801

1. INTENDED USE

Stained Proteus Suspensions are intended for the quantitative detection of rickettsial antibodies in sera for epidemiological and diagnostic purposes in the investigation of rickettsial infections (Weil-Felix test)[1,2].

2. SUMMARY AND EXPLANATION OF THE TEST

The test is based on the fact that somatic constituents of some strains of Proteus are shared with some species of Rickettsia. Sera from people with some rickettsial infections therefore agglutinate suspensions of the Proteus strains.

3. PRINCIPLE OF THE PROCEDURE

In the standard agglutination test diluted patient's serum is mixed with the bacterial suspension. If sufficient homologous antibodies are present they will cause the suspension to agglutinate.

4. REAGENTS

KIT CONTENTS

Stained Proteus Suspensions		5 ml
Proteus OX2	SS16/R30953601	1 dropper bottle
Proteus OX19	SS17/R30953701	1 dropper bottle
Proteus OXK	SS18/R30953801	1 dropper bottle

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**

Stored in the dark at 2 to 8°C the suspensions will retain their reactivity at least until the date shown on the bottle label.

STAINED SUSPENSION

Stained Proteus Suspensions

Standardised smooth suspensions of killed bacteria (approximately 10^{10} bacteria per ml) which have been stained to facilitate reading of agglutination tests. Preserved with 0.25% formalin and 0.01% thiomersal. The suspensions are provided in bottles fitted with teat and dropper.

5. WARNINGS AND PRECAUTIONS

IVD

For *in vitro* diagnostic use only.

For professional use only.

Please refer to the manufacturer's safety data sheet and product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

1. Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for at least 15 minutes at 121°C. Disposables should be autoclaved or incinerated.
2. Spillage of potentially infectious material should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a standard bacterial disinfectant or 70% alcohol. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.
3. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
4. These reagents contain thiomersal and formalin. Although the concentration is low, both are known to be toxic by ingestion and skin contact. Avoid ingestion of the reagents. If any come in contact with skin or eyes wash the area extensively by immediately rinsing with plenty of water.
5. In accordance with the principles of Good Laboratory Practice it is strongly recommended that samples and reagents should be treated as potentially infectious and handled with all necessary precautions.

ANALYTICAL PRECAUTIONS

1. Do not use the reagents beyond the stated expiry date. Microbiological and serological contamination must be avoided as this may cause erroneous results and reduce product life.
2. Do not modify the test procedure, incubation time or temperatures.
3. Allow all suspensions and samples to come to room temperature (18 to 30°C) before use. After use return to 2 to 8°C in the dark.
4. Do not expose reagents to strong light during storage or incubation times.
5. Care must be taken not to cross-contaminate reagents.
6. If the suspension becomes rough or fails to agglutinate with its homologous specific serum it should be discarded.
7. The suspensions must be shaken thoroughly before use to ensure that the organisms are evenly suspended.

6. SPECIMEN COLLECTION, TRANSPORT AND STORAGE

SPECIMEN COLLECTION

Serum samples may be used. Blood collected by venepuncture should be allowed to clot naturally. Care should be taken to ensure that the serum samples are fully clotted. Do not inactivate serum samples.

SPECIMEN TRANSPORT AND STORAGE

Store samples at 2 to 8°C.

7. PROCEDURE

MATERIALS PROVIDED

See **Kit Contents**.

MATERIALS REQUIRED BUT NOT PROVIDED

1. 0.85% saline or 0.25% phenol saline.
2. White card tiles (code RT04/R30368701).
3. Test tubes and racks.
4. Pipettes 5 µl to 50 µl and 50 µl to 1000 µl.
5. Adjustable waterbath.
6. Timer.
7. The following control sera are available in bottles of 2 ml liquid:

Proteus OX2	Code No. ZM14/R30165701
Proteus OX19	Code No. ZM15/R30165801
Proteus OXK	Code No. ZM16/R30165901

TEST PROCEDURE

A. Rapid Screening Test

Step 1 Place two drops (80 µl) of undiluted serum in a 3 cm diameter circle on a white tile.

Step 2 Add one drop of the appropriate well-shaken suspension using the dropper provided.

Step 3 Mix by stirring for a few seconds and spread to fill the whole area of a circle on the tile.

Step 4 Rotate the tile slowly and read agglutination at one minute.

B. Rapid Slide Titration

Step 1 Using a 0.2 ml pipette, deliver 80, 40, 20, 10 and 5 µl of undiluted serum into a row of 3 cm diameter circles on a white tile.

Step 2 Using the dropper provided, add one drop of the appropriate well-shaken suspension to each serum aliquot.

Step 3 Mix by stirring for a few seconds with a wooden applicator stick, proceeding from the mixture containing 5 µl serum to that containing 80 µl serum, spreading the contents to fill the circles.

Step 4 Rotate the tile slowly and **read agglutination at one minute**.

C. Tube Agglutination Test

Step 1 Make one row of serum dilutions for each antigen to be tested as shown in Table 1, using saline or 0.25% phenol saline as diluent. Mix the contents of tube 1 and transfer 1 ml to tube 2. Repeat for each tube, up to but not including tube 8, finally discarding 1 ml from tube 7.

Table 1

Tube No.	1	2	3	4	5	6	7	8
Diluent ml	1.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Patient's serum ml	0.1	——— 1ml serial dilutions ——→						0
Final dilution	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Control

Step 2 Add one drop of the appropriate well-shaken suspension to each tube of a given row using the dropper provided. Do not dilute the suspension before use.

Step 3 Mix and incubate at 50°C for four hours before reading.

Step 4 Examine for agglutination, strong back-lighting is not necessary.

8. RESULTS

READING OF RESULTS

A. **Rapid Screening Test**
If agglutination is visible **within one minute**, a significant titre should be obtained in a confirmatory tube test. The reaction is roughly equivalent to that obtained in a tube agglutination test[3] with a serum dilution of 1 in 20. Smaller volumes of serum (see Section B) may be used if a screening test with sensitivity of 1 in 40 or 1 in 80 is required.

B. **Rapid Slide Titration**
The reactions obtained are roughly equivalent to those which would occur in a tube agglutination test with serum dilutions of 1/20, 1/40, 1/80, 1/160 and 1/320 respectively[3]. If a reaction is found it is advisable to confirm the reaction and establish the titre by a tube test although when experience has been gained this should not be necessary. A tube test is indicated when results do not conform to clinical findings. False results may be obtained if the reagents are not allowed to reach room temperature (18 to 30°C) before use. Also, **false positive reactions are likely if the test is read more than one minute after mixing**.

C. **Tube Agglutination**
In a positive reaction there is obvious granular agglutination. In a negative reaction and the saline control the appearance of the suspension should be unchanged, and show a typical swirl when the tube is flicked. The tube should not be shaken. The titre in each case is the dilution of serum in the last tube showing agglutination. As a positive control for each suspension, a dilution series of Proteus OX2, OX19 or OXK antiserum may be included.

QUALITY CONTROL

It is recommended to test the suspension as described with a known positive serum, for example Proteus OX2, OX19 or OXK antiserum, and negative control serum with each run of test samples. In practice, a run may be defined as a testing period of up to 24 hours. If a suspension agglutinates with a known negative serum or fails to agglutinate with a known positive serum it should be discarded.

These are unabsorbed sera, but are not standard sera and although titres should approach those given on the bottle labels, the exact titre may not always be obtained.

INTERPRETATION OF RESULTS[1,2,4]

Agglutination patterns for several rickettsial diseases are shown in Table 2.

Table 2

Infection	Vector	Proteus Suspension		
		OX19	OX2	OXK
Epidemic typhus	Louse	+++	+	–
Murine typhus	Flea	+++	+	–
Endemic typhus	Flea	+++	+	–
Rocky Mountain Spotted Fever	Tick	+++	+	–
Tsutsugamushi Fever	Mite	–	–	+++
Scrub typhus	Mite	–	–	+++
Boutonneuse fever	Tick	+	+	+
South African tick-bit fever	Tick	+	+	+
Brill's disease	Louse	usually neg.	usually neg.	usually neg.
Trench fever	Louse	–	–	–
Q fever	Tick	–	–	–

The level of agglutinins in "normal" human sera can be 1/40 or more, especially with Proteus OXK suspensions[5] which may give "normal" titres up to 1/160. A rising or falling titre is more significant than a single elevated titre.

9. LIMITATIONS OF THE PROCEDURE

1. Agglutinins tend to fall rapidly within a few months of recovery from an infection and therefore a high titre is a useful indication of recent infection. Positive reactions are sometimes found in unrelated conditions, for example, malaria, infectious mononucleosis, brucellosis, tuberculosis or narcotic addiction and therefore the results must be judged in the context of the clinical findings.
2. Do not inactivate serum samples for use with this test.

10. EXPECTED RESULTS

Visible agglutination in the presence of homologous antibodies.

11. SPECIFIC PERFORMANCE CHARACTERISTICS

See Table 2 for agglutination patterns for several rickettsial diseases.

12. BIBLIOGRAPHY

1. **Felix, A.** (1944). Technique and interpretation of the Weil-Felix test in typhus fever. Trans. *Roy. Soc. Trop. Med. Hyg.*, **37**, 321.
2. **Pedersen, C.E.** (1978). *J. Amer. Med. Technol.*, **40**, 79.
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4. **Wilson, G.S. and Miles, A.A.** (1975). Topley and Wilson's Principles of Bacteriology, Virology and Immunity, 6th Ed., London, E. Arnold, Pages 2355-2357.
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13. PACKAGING

REF SS16/R30953601.....5 ml
SS17/R30953701.....5 ml
SS18/R30953801.....5 ml

Symbol legend

REF	Catalogue Number
IVD	*In vitro* diagnostic medical device
	Consult instruction for use
	Temperature limitation
LOT	Batch code
	Use by
	Manufacturer

CE

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Remel Europe Ltd.
Clipper Boulevard West, Crossways
Dartford, Kent, DA2 6PT
UK

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