Stained Proteus Suspensions

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. 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

4.1. KIT CONTENTS

- SS16/R30953601 1 dropper bottle
- Proteus OX19 SS17/R30953701 1 dropper bottle
- SS18/R30953801 1 dropper bottle
- Proteus OXK

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also Warnings and Precautions

Stained Proteus Suspensions

Standardised smooth suspensions of killed bacteria (approximately 10\(^8\) 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 a teat and dropper.

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.

5. WARNINGS AND PRECAUTIONS

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 handled with all necessary precautions. 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. Do not modify the test procedure, incubation time or temperature. The reaction is roughly equivalent to that obtained in a tube agglutination test! 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. 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°C to 30°C) before use. Also, false positive reactions are likely if the test is read more than one minute after mixing.

A. Rapid Screening Test

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

**Step 2** Add one drop of the appropriate dilution of serum 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. Proceed 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**

<table>
<thead>
<tr>
<th><strong>Tube No.</strong></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 ml</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>1 ml</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Patient’s serum ml</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final dilution</td>
<td>1/20</td>
<td>1/40</td>
<td>1/80</td>
<td>1/160</td>
<td>1/320</td>
<td>1/640</td>
<td>1/1280</td>
<td>Control</td>
</tr>
</tbody>
</table>

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

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.

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! 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. 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°C 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 Test

A positive reaction 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 antisera 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 antisera, 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 label, the exact titre may not always be obtained.
Agglutination patterns for several rickettsial diseases are shown in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Infection</th>
<th>Vector</th>
<th>Proteus Suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OK19</td>
<td>OK2</td>
</tr>
<tr>
<td>Epidemic typhus</td>
<td>Louse</td>
<td>+++</td>
</tr>
<tr>
<td>Murine typhus</td>
<td>Flea</td>
<td>+++</td>
</tr>
<tr>
<td>Endemic typhus</td>
<td>Flea</td>
<td>+++</td>
</tr>
<tr>
<td>Rocky Mountain</td>
<td>Tick</td>
<td>+++</td>
</tr>
<tr>
<td>Spotted Fever</td>
<td>Mite</td>
<td>–</td>
</tr>
<tr>
<td>Scrub typhus</td>
<td>Mite</td>
<td>–</td>
</tr>
<tr>
<td>Boutonneuse fever</td>
<td>Tick</td>
<td>+</td>
</tr>
<tr>
<td>South African tick-bit fever</td>
<td>Tick</td>
<td>+</td>
</tr>
<tr>
<td>Brill's disease</td>
<td>Louse</td>
<td>–</td>
</tr>
<tr>
<td>Q fever</td>
<td>Tick</td>
<td>–</td>
</tr>
</tbody>
</table>

The level of agglutinins in "normal" human sera can be 1/40 or more, especially with Proteus OKX suspensions 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