5  KIT CONTENTS, PREPARATION FOR USE AND STORAGE

5.1 Stained Salmonella O and H Suspensions

Stained Salmonella O and H Suspensions

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Description</th>
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<tbody>
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<td>5 ml dropper bottle (red cap)</td>
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<tr>
<td>SS02/R30855101</td>
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<td>SS09/R30955401</td>
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</tr>
</tbody>
</table>

5.2 Stained Salmonella Suspensions

Standardised smooth suspensions of killed bacteria (approximately 10^9 bacteria per ml) which have been stained to facilitate reading of agglutination tests. Preserved with 0.25% formalin. Each bottle, fitted with a cap, contains 5 ml liquid and is ready to use.

6  PRECAUTIONS

6.1 Non-disposable apparatus should be sterilised by any appropriate method or autoclave for at least 15 minutes at 121°C. Disposables should be autoclaved or incinerated.

6.2 Spillage of potentially infectious material should be removed immediately with absorbent paper tissue and disposed of as biohazardous waste.

6.3 Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the test. Wash hands thoroughly when finished.

6.4 These reagents contain formalin. Although the concentration is low, formalin is 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.

6.5 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

6.6 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.

6.7 Do not modify the test procedure, incubation times or temperatures.

6.8 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.

6.9 Do not expose reagents to strong light during storage or incubation times.

6.10 Care must be taken not to cross-contaminate reagents.

6.11 If the suspension becomes rough or fails to agglutinate with its homologous specific serum it should be discarded.

6.12 The suspension must be shaken thoroughly before use to ensure that organisms are evenly suspended.

7  SPECIMEN COLLECTION, TRANSPORT AND STORAGE

7.1 SPECIMEN COLLECTION

Serum samples should 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.

7.2 SPECIMEN TRANSPORT AND STORAGE

Store samples at 2 to 8°C.

8  TEST PROCEDURE

8.1 REQUIRED MATERIALS PROVIDED

See Kit Contents, section 5

MATERIALS REQUIRED BUT NOT PROVIDED

0.85% saline or 0.25% phenol saline

White card tiles (code RTO4/R30368701)

Test tubes and rack

Pipettes to cover the range 5 µl to 1000 µl

Adjustable waterbath with thermometer

Timer

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 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 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 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 mixing 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.

9  QUALITY CONTROL

It is recommended to test the suspension as described with a known positive serum, for example the appropriate Salmonella antisera as shown in Table 2, and a 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.

With the exception of the S. typhi Vi standard serum, the sera listed are absorbed, and are not, strictly speaking, standard sera. The titres obtained with the control sera should be within two serial dilutions of that stated on the bottle label. A prozone phenomenon may sometimes be encountered.

Reagents may be selected from the table below as positive control reagent for rapid screening, rapid slide and tube agglutination tests.

10  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 routinely 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 for the screening test if the titre considered to be significant is greater than 1 in 20. It is not possible to screen sera at a level equivalent to a tube dilution of 1 in 10.
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 where 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 O reaction there is obvious granular agglutination; H agglutination has a characteristic floccular appearance. In a negative reaction and in the saline control the appearance of the suspension should be unchanged, and show a typical swirl when the tube is flicked. The tubes should not be shaken. The titre in each case is the dilution of the serum in the last tube showing agglutination. As a positive control for each suspension, a dilution series of the appropriate Salmonella Agglutinating Serum, as shown in Table 2, may be included.

INTERPRETATION OF RESULTS
Many types of Salmonella have somatic antigens in common. Agglutination of any of the suspensions by the patients' serum cannot therefore be taken as proof of infection by that particular organism but only of infection by an organism of similar antigenic constitution. Significant titres of agglutinins e.g. greater than 1 in 80, usually reflect recent infection, but low titres are often found in normal individuals. In Britain the usual limits of such normal titres are, using Standard O or H Suspensions, S. typhi and S. paratyphi B, 1 in 40 and S. paratyphi A, 1 in 10. The level of 'normal' agglutinins to these organisms varies in different countries and different communities.

12 EXPECTED RESULTS
Visible agglutination in the presence of homologous antibodies.

13 PERFORMANCE CHARACTERISTICS
See Interpretation of Results, section 10.

14 BIBLIOGRAPHY