**Haemophilus influenzae**

**Agglutinating Sera**

1. **INTENDED USE**

*Haemophilus influenzae* Agglutinating Sera are intended for use in the qualitative slide agglutination test and in counterimmunoelectrophoresis (CIE) to identify serologically the type antigen of pathogenic strains of *H. influenzae* (types a to f) for epidemiological and diagnostic purposes.

2. **SUMMARY AND EXPLANATION OF THE TEST**

The pathogenic strains possess capsules and are classified serologically into six types according to the chemical structure of the capsular antigen. Strains possessing these antigens are specifically agglutinated by the homologous antiserum, and a capsulated culture may therefore be typed by slide agglutination tests. An alternative method of typing, counterimmunoelectrophoresis (CIE), is based on the fact that under appropriate conditions antibodies migrate towards the cathode under the influence of an electric current, whereas antigens migrate towards the anode. Suspensions of bacteria from solid or liquid culture and antisera are placed in wells cut in a layer of agar such that when an electric current is passed through the agar the antigens and antibodies migrate towards each other. The ability of the antigens to form precipitates depends upon the chemical characteristics of the antigen and of the antibodies and also on the conditions of the test. Serological typing gives reliable results only if the culture possesses capsules. It is advisable to type a strain as soon as possible after isolation because the ability to produce capsules is lost with time. Capsulated strains can be recognised by the characteristic iridescence which is seen when a strong white light is transmitted obliquely through a culture growing on Levinthal's agar.

3. **PRINCIPLE OF THE PROCEDURE**

Serological tests are based on the fact that antibodies in serum, produced in response to exposure to bacterial antigens, will agglutinate with bacteria carrying homologous antigens.

4. **REAGENTS**

4.1. **kit contents**

*Haemophilus influenzae Agglutinating Sera* 2 ml

<table>
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<tr>
<th>Type</th>
<th>Code</th>
<th>Description</th>
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<tbody>
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<td>a</td>
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<td>1 dropper bottle</td>
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<td>b</td>
<td>ZM21/R30166101</td>
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<td>c</td>
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<tr>
<td>f</td>
<td>ZM25/R30166501</td>
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5. **WARNINGS AND PRECAUTIONS**

5.1. **Health and Safety Information**

5.1.1. Handle all bacteria according to appropriate local and statutory guidelines.

5.1.2. Non-disposable apparatus should be sterilised by any appropriate method after use, although the preferred method is to autoclave for at least 15 minutes at 121°C.

5.1.3. Spillage of potentially infectious material should be removed immediately with absorbent paper and the contaminated areas swabbed with a standard disinfectant and 70% alcohol. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.

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

5.1.5. These reagents contain phenol. Although the concentration is low, phenol is known to be toxic by ingestion and skin contact. Avoid ingestion of the reagents. If any come into contact with skin or eyes wash the area extensively by immediately rinsing with plenty of water.

5.1.6. In accordance with the principles of Good Laboratory Practice it is strongly recommended that samples and reagents are treated as potentially infectious and handled with all necessary precautions.

5.2. **Analytical Precautions**

5.2.1. Do not use antiseria beyond the stated expiry date. Microbiological contamination of the antiseria must be avoided as this may cause erroneous results and reduce product life.

5.2.2. Do not modify the test procedure, incubation time or temperatures. Do not dilute the agglutinating sera.

5.2.3. After use return sera to recommended storage temperature.

5.2.4. Do not use a microbiological loop to dispense the antiserum. Use the dropper provided.

6. **SPECIMEN COLLECTION, TRANSPORT AND STORAGE**

Serological typing gives reliable results only if the culture possesses capsules. Antigens are specifically agglutinated by the homologous antiserum.

7. **PROCEDURE**

**Materials Provided**

For use in vitro diagnostic use only.

For professional use only.

**Materials Required but not Provided**

- 0.85% saline
- Glass slides
- Microbiological loop and bunsen burner
- Light source over dark background
- Timer
- Pasteur pipette
- Barbitone-HCl buffer, pH 8.6

8. **TEST PROCEDURE**

8.1. **Slide Agglutination Test**

**Step 1** Put two separate drops (40 µl each) of saline on a glass slide. Emulsify portions of the culture under test with a loop in each drop of saline, to give a smooth, fairly dense suspension.

**Step 2** To one suspension add one drop (40 µl) of saline as a control and mix. To the other suspension add one drop (40 µl) of undiluted antiserum and mix.

**Step 3** Rock the slide for one minute and observe for agglutination, which can be more easily seen by viewing against a dark background using indirect lighting. Discard the used slide for safe disinfection and disposal.

8.2. **Counterimmuno-electrophoresis**

**Step 1** Fill the electrophoretic tank with barbitone-HCl buffer to the required level.

**Step 2** Punch wells in the agarose and remove the core from each well by careful aspiration with a Pasteur pipette attached to suction apparatus. Avoid damaging the walls of the wells. A single test unit consists of two wells 3 mm in diameter placed 5 mm apart along the electrophoretic axis. Several of these units can be cut in each slide: an 81 x 81 mm slide can accommodate 18 pairs of wells. (See figure 1)

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**Figure 1**

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**Step 3** With a fine Pasteur pipette place sufficient antiserum in the anodal well of each pair to fill it without spilling over.

**Step 4** Similarly, place the test sample in the cathodal well of each pair.

**Step 5** Without delay, place the slide in position in the electrophoresis tank and make connections between the ends of the slide and the buffer compartments with wicks of soft filter paper soaked in buffer. Only the end 10 mm of agarose should be covered by the wicks. Smooth down gently so that the agarose air bubbles are removed. Replace the lid of the tank.

**Step 6** Check that the power supply is connected with the correct polarity (antiserum anodic). Switch on and adjust to a steady current of 2.5 mA/cm² width of gel.

**Step 7** After one hour, switch off the power and remove the slide from the tank. View in a dark-background illuminator or by oblique lighting against a dark background. Reading is often easier if the slide is immersed in saline in a Petri dish whilst viewing.
9. RESULTS

Slide Agglutination
Agglutination should be strong and clearly visible within one minute. There should be no visible agglutination in the control suspension; if agglutination is seen in the control, the suspension is not suitable for testing by this method.

Counterimmunoelectrophoresis
In a positive result, a white line of precipitation will be seen between a pair of wells, perpendicular to the axis of electrophoresis (figure 2). In a negative reaction, no precipitation is visible between the wells.

Figure 2

10. QUALITY CONTROL

Slide Agglutination
It is recommended to test the product, throughout its use, with known positive and negative cultures. Homologous cultures should be used for positive control organisms. For a negative control culture use Neisseria lactamica. Strains with the appropriate serotypes may be obtained from a recognised culture collection such as NCTC or ATCC.

11. INTERPRETATION OF RESULTS

Slide Agglutination
Agglutination of type “e” strains are usually finer than the others. Slide agglutination reactions which are weak or which take longer than one minute to appear are not significant. If agglutination is seen in the control suspension, the culture is not suitable for testing.

Counterimmunoelectrophoresis
If the antigen concentration is low, the line may be faint and in this case evaluation may be facilitated by the use of a hand lens. The reactions are reasonably stable and although the slides should be inspected immediately after electrophoresis is completed, the results should remain unchanged for several hours. If a permanent record is required the slides can be washed, stained and dried by conventional methods.

12. LIMITATIONS OF THE PROCEDURE

H. influenzae Agglutinating Sera have been absorbed as necessary to render them specific within the species H. influenzae. However, cross-reactions have been reported to occur with organisms of other species,1-6. It is important to confirm the species of the organism under test by the established morphological, cultural and biochemical techniques. This cautionary note applies to all serological test methods and it emphasises the fact that CIE should complement rather than replace conventional techniques. If the concentration of the antigen is not sufficient in the specimen then a negative result will be obtained. Antisera provide serological identification only; full identification of an organism must only be made in conjunction with biochemical testing.

13. EXPECTED RESULTS

Visible agglutination or precipitation in the presence of homologous cultures and antigens.

14. SPECIFIC PERFORMANCE CHARACTERISTICS

The ZM20/R30166001 to ZM25/R30166501 antisera should show visible agglutination in the slide agglutination test and a precipitation line in the CIE test with H. influenzae type a, b, c, d, e and f capsular antigen respectively.

15. BIBLIOGRAPHY


16. PACKAGING

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<th>Symbol</th>
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