Haemophilus influenzae Agglutinating Sera

**INTENDED USE**
Haemophilus influenzae agglutinating sera are intended for use in slide agglutination test to identify serologically the type antigen of pathogenic strains of H influenzae (types a to f) for epidemiological and diagnostic purposes.

**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 counter current immunoelectrophoresis (CIE) is also described in literature 1-3.

**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.

**REAGENTS**

4.1. kit contents

Haemophilus influenzae Agglutinating Sera 2 ml

Type a (ZM20/R30166001) 1 dropper bottle
Type b (ZM21/R30166001) 1 dropper bottle
Type c (ZM22/R30166201) 1 dropper bottle
Type d (ZM23/R30166301) 1 dropper bottle
Type e (ZM24/R30166401) 1 dropper bottle
Type f (ZM25/R30166501) 1 dropper bottle

4.2. Description, Preparation for Use and Recommended Storage Conditions

See also Warnings and Precautions

**AGGLUTINATING SERUM**

H. influenzae Agglutinating Sera

Produced in rabbits and are preserved with 0.5% phenol. Each bottle, fitted with a heat and dropper, contains 2 ml liquid and is supplied ready to use.

On storage some sera become slightly turbid. This does not necessarily indicate deterioration and normally it will not interfere with the results, but the sera may be clarified before use by centrifugation or membrane filtration (0.45 µm). Gross turbidity indicates contamination and such sera should be discarded.

**5. WARNINGS AND PRECAUTIONS**

For in vitro diagnostic use only.

For professional use only.

Caution: This product contains dry natural rubber.

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

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 autoclaving for at least 15 minutes at 121°C. Disposables should be autoclaved or incinerated.

5.1.3. Spillage of potentially infectious material should be removed immediately with absorbent paper towel and the contaminated area swabbed with a standard bactericidal disinfectant or 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 should be treated as potentially infectious and handled with all necessary precautions.

5.2. Analytical Precautions

5.2.1. Do not use antisera beyond the stated expiry date. Microbiological contamination of the antiserum 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. 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 white light is transmitted obliquely through a culture growing on Levinthal’s agar 1.

For details on specimen collection and preparation a standard test book should be consulted. The use of fresh cultures grown on Levinthal’s agar is recommended.

7. PROCEDURE

Materials Provided

See Kit Contents.

Materials Required but not Provided

1. 0.85% saline.

2. Glass slides.

3. Microbiological loop and bunsen burner.

4. Light source over dark background.

5. Timer.

6. Pasteur pipette.

8. TEST PROCEDURE

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.

9. RESULTS

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.

10. QUALITY CONTROL

It is recommended to test the product, throughout its use, with know 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

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.

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 1,4. 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 if the concentration of the antigen is not sufficient in the specimen then a negative result will be obtained. Antiserum provide serological identification only; full identification of an organism must only be made in conjunction with biochemical testing.

13. EXPECTED RESULTS/PERFORMANCE CHARACTERISTICS

Visible agglutination in the presence of homologous antigens (refer to bottle label for specificity of the antiserum). See limitations of the procedure.

14. BIBLIOGRAPHY


15. PACKAGING

Catalogue Number

Temperature Limitations (Storage temp.)

Contains sufficient for <N> tests

Batch Code (Lot Number)

Use By (Expiration Date)

Manufactured by

www.oxoid.com/ifu

For technical assistance please contact your local distributor.