

D Deben Diagnostics Ltd,
***Haemophilus influenzae* Antisera**

Haemophilus influenzae is a short, non-motile, capsulated bacillus which does not form endospores.

These are antisera used for the serotyping of *Haemophilus influenzae* capsules and are liquid products that contain type-specific agglutinins.

When these products are used, the capsular type can be identified by slide agglutination.

The antisera are prepared by hyper-immunizing rabbits with bacterial strains with each of the 6 type specific capsules a, b, c, d, e, and f. After bleeding, the serum is separated, heated at 56°C for 30 minutes, absorbed to remove the non-specific agglutinins and sterilized by filtration. As a preservative, sodium azide is added at 0.08 w/v%.

PRODUCT

Haemophilus influenzae Antisera are produced from rabbits and contain 0.08% sodium azide as a preservative. The following sera types are provided in 2mL vials with dropper attachment, ready to use.

Set: 6 vials, capsule type a - f

INTENDED USE

Determination of the *Haemophilus influenzae* capsule serotype.

PRINCIPLE OF MEASUREMENT

When this reagent is mixed with *H. influenzae* strain which has antigens correspondent to the reagent, the antigen antibody reaction occurs to produce agglutination. This reaction is macroscopically observed to determine each serotype.

PROCEDURES

1. Material required but not provided

Glass slide, glass pencil, small test tubes, pipette and micropipette, microbiological loop, physiological saline.

2. Preparation of reagents

The antisera are ready for use.

3. Specimen

Cultures of organisms which are derived from a pure culture and identified as *H. Influenzae* by biochemical tests should be serotyped. If the specimen consists of multiple strains, the serotype may not be correctly identified.

4. Procedures

- 1) Suspend a certain amount of fresh bacterial growth (3-5 times the amount of a match head) on the chocolate agar plate in 0.5 mL physiological saline and use antigenic suspension.
- 2) Place a drop each of a-f antiserum and physiological saline (30 µL) as a control onto a cleaned glass slide partitioned into several parts with a glass pencil.
- 3) Place a antigenic suspension (5-10 µL) onto the serum and physiological saline on the glass slide.
- 4) Mix the reagents by tilting the glass slide back and forth for 1 minute and the agglutination pattern should be observed. Agglutination is grossly observed with light through the slide including fluorescent light. It should be first confirmed that no agglutination is found with the reaction with antigenic suspension and physiological saline. Only strong agglutination observed within one minutes of the reaction with each serum should be regarded as positive. Delayed or weak agglutination is regarded as negative.

PRECAUTIONS

1. Bacteria culture should be performed using indicated media for *Haemophilus influenzae* e.g. chocolate agar media. If selected media are used, antigen production may be insufficient or autoagglutination may occur.
2. When antigenic suspension and serum are mixed as a procedure of slide agglutination, the microbiological loop should be sterilized with a flame for each serum to avoid

cross contamination among sera.

3. Use fresh bacterial growth for agglutination test, because *H. Influenzae* is easy to lose their capsular antigen.
4. If positive agglutination is observed, the isolate possesses the specific antigen group. If agglutination is not observed in the mixture with any serotype a - f, the isolate may possess capsules other than a - f, or lack capsule antigens.
5. If agglutination is observed in the physiological saline control, the test should be repeated selecting another colony.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

When one drop of the antiserum is allowed to react on a slide with a known serotype of the reference strain, granular agglutination is observed macroscopically.

2. Specificity

In tests performed in a similar manner to the sensitivity test, the antiserum agglutinates only with the reference strain corresponding to the serotype, while in reactions with non-corresponding reference strains, macroscopic agglutination is not observed.

PRECAUTION FOR USE AND HANDLING

1. General precautions

- 1) This test is for in vitro diagnostic use only.
- 2) This reagent should only be used from sufficiently trained lab staff.
- 3) *H. Influenzae* lose their capsular antigen easily, so the selection of a colony which has a larger amount of capsular antigen is very important.

2. Precautions of handling

- 1) All specimens, samples and containers coming into contact with samples should be treated as infectious.
- 2) If reagent come into contact with skin, mucous membranes of eyes, wash immediately with plenty of water.
- 3) Do not freeze the reagents nor use past the expiration date as this may result in poor reagent performance.
- 4) The reagent should be allowed to stand at 15-25°C for at least 30 minutes before use.
- 5) Used containers should not be used for other purposes.
- 6) Sera with different production numbers should not be mixed.
- 7) The reagents should be used according to the described procedures.
- 8) The reagents should only be used for the intended use.
- 9) Special precautions should be taken to ensure that the reagent caps are not exchanged.

3. Precautions for disposal

- 1) The reagent contains 0.08w/v% sodium azide. Sodium azide may react with lead or copper to form explosive heavy metal azides. The reagent should be disposed of with a large amount of water.
- 2) All specimen, spills, inoculated product, and equipment used in this test should be treated with one of the following methods.

[1] Soaking in 0.1 w/v% hypochlorite for 1 hour or more.

[2] Autoclaving at 121°C for 20 minutes or more.

STORAGE AND SHELF LIFE

Storage: 2-10°C

Shelf life: Up to the expiry date on the label.

PACKAGE

Haemophilus influenzae Antisera: Each type in a 2mL vial with a pipette.









Set: 6 vials 1 package

Each serum is separately available.

REFERENCE

- 1) Wallace, R. J., et al.: *Haemophilus influenzae* infections in adults : Characterization of strains by serotypes, biotypes, and β-lactamase production, J.Inf. Dis., 144, 101 (1981).
- 2) Mason, E.O., et al. : Serotype and ampicillin susceptibility of *Haemophilus influenzae* causing systemic infections in children : 3 years of experience, J Clin. Microbial., 15,543(1892)

Please feel free to contact us at the following with your questions or comments:
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	Manufacturer		Batch Code
	Consult instruction for use		Temperature Limitation
	For In vitro diagnostic Use Only		Use By
	Catalogue Number		Contains sufficient for <n> tests



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