

## HAEMOPHILUS INFLUENZAE ANTISERA

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

The *H. influenzae antisera* are liquid products that contain type-specific agglutinins used for the serotyping of *Haemophilus influenzae* capsules by slide agglutination. The antisera are prepared by hyperimmunising rabbits with bacterial strains from each of the six type-specific capsules a, b, c, d, e, and f. The serum is separated, heated at 56°C for 30 minutes, absorbed to remove the non-specific agglutinins and sterilised by filtration. Sodium azide is added as a preservative, at 0.08% w/v.

### PRODUCTS

The following serum types are provided as 2mL volumes in vials with a dropper attachment, ready to use.

Set containing 6 vials, capsule types a-f  
Individual vials for capsule types a-f, available separately.

### INTENDED USE

The *Haemophilus influenzae* Antisera are intended for the qualitative serological identification and screening of prepared *Haemophilus influenzae* serotype bacterial cultures, using slide agglutination to detect the presence of bacterial antigens. *Haemophilus influenzae* Antisera are intended for use by trained laboratory personnel.

### PRINCIPLE OF MEASUREMENT

When this reagent is mixed with an *H. influenzae* strain which has corresponding antigens, 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 an amount of fresh bacterial growth (3-5 times the amount of a match head) on a chocolate agar plate in 0.5mL physiological saline and use as an antigenic suspension.
- 2) Place a drop each of a-f antiserum and physiological saline (30µL) as a control onto a cleaned glass slide partitioned into several parts with a glass pencil.
- 3) Place antigenic suspension (5-10µL) onto the serum and physiological saline on the glass slide.
- 4) Mix the reagents by tilting the glass slide back and forth for one 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 between the antigenic suspension and physiological saline. Only strong agglutination observed within one minute 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 *H. influenzae*, e.g., chocolate agar media. If selective media are used, antigen production may be insufficient or autoagglutination may occur.
2. When antigenic suspension and serum are mixed during the procedure of slide agglutination, the microbiological loop should be sterilised with a flame before each serum to avoid cross contamination among sera.
3. Use fresh bacterial growth for the agglutination test because *H. influenzae* easily 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 by 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. Handling precautions

- 1) All specimens, materials and containers coming into contact with samples should be treated as infectious.
- 2) If reagent comes into contact with skin, mucous membranes, or 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 0.08% w/v sodium azide. Sodium azide may react with lead or copper pipes to form explosive heavy metal azides so the reagent should be disposed of with a large amount of water.
- 2) All specimens, 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.

### REFERENCES

- 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)

	Manufacturer
	Consult instruction for use
	For in vitro diagnostic use only
	Catalogue number
	Batch code
	Storage temperature limitation
	Use by
	Contains or presence of natural rubber latex



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