Phadebact®

Haemophilus Test

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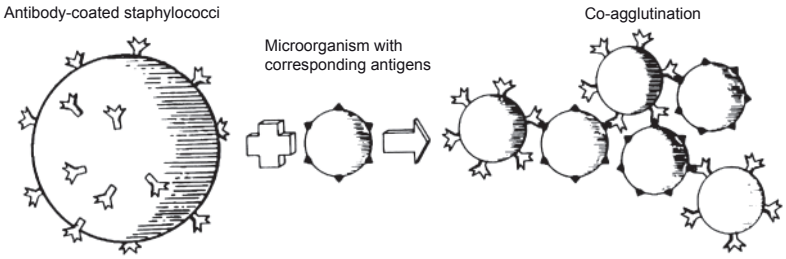
Directions for Use

MKL Diagnostics AB
Kung Hans Väg 3
SE-192 68 Sollentuna
Sweden

CE

MKL Diagnostics



INTENDED USE
Phadebact® Haemophilus Test is intended for the identification of *Haemophilus influenzae* type b and types a, c-f.

SUMMARY AND EXPLANATION OF THE TEST
Most *Haemophilus influenzae* infections are caused by *H. influenzae* type b (1). The virulent variants are almost always encapsulated and in general strict parasites of man and animals (2). *H. influenzae* plays an important etiological role in acute respiratory tract infections, meningitis, otitis media and bacteremia. Subacute bacterial endocarditis and septic arthritis are occasionally caused by these organisms. *H. influenzae* type b is responsible for up to 95% of infections caused by *Haemophilus* spp. in children from 6 months to 3 years of age (3).
Differentiation of *Haemophilus* species is commonly based on organims´ability to hemolyze, accessory growth factor requirements and enhancement of growth in an atmosphere of increased CO_2 tension (5-10%). Phadebact® Haemophilus Test is based on the co-agglutination technique and allows rapid identification of typeable *Haemophilus influenzae* and differentiates type b from types a, c-f using a simple slide technique.

PRINCIPLE OF THE PROCEDURE
Phadebact® Haemophilus Test is a co-agglutination test containing Type b Reagent consisting of specific anti-type b antibodies and Types a, c-f Reagent consisting of a pool of specific antitype a, c, d, e and f antibodies, coupled to protein A of non-viable staphylococci (4). This coupling technique allows the antigen binding part, the Fab part, of the antibody to be directed outwards giving optimal reaction conditions.
When a sample containing *H. influenzae* type b is mixed with the Type b Reagent, the specific antigens on the cell surface bind to the corresponding specific antibodies. In this way, a co-agglutination lattice is formed which is visible to the naked eye. No reaction should be obtained with the Types a, c-f Reagent. Similarly, when a sample containing *H. influenzae* of types a, c-f is mixed with the Types a, c-f Reagent, co-agglutination will occur and no reaction will be obtained with the Type b Reagent. A sample containing none of the specific antigens of types a, b, c, d, e or f will not react with any of the reagents.

REAGENTS
Each Phadebact® Haemophilus Test package contains reagents sufficient for 50 determinations. The reagents are coloured blue (Methylene blue) to facilitate interpretation of results.

Reactive ingredients
- Type b Reagent — 1 vial
  Type b specific antiserum, raised in rabbit, bound to non-viable staphylococci.
- Types a, c-f Reagent — 1 vial
  A pool of specific a, c, d, e and f antisera raised in rabbit, bound to non-viable staphylococci.
  READY TO USE

Other components
- Droppers
- Disposable slides
- Directions for Use

Precaution
For *in vitro* diagnostic use.
Warning! The reagents contain sodium azide (NaN_3) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC.

Preparation of reagents
The reagents are READY TO USE.

Shelf life and storage
The expiry date is stated on the outer label and the vial labels. It is recommended that the kit be stored at 2-8°C. Reagents must be protected from freezing.

SPECIMEN COLLECTION AND HANDLING
Please refer to a standard microbiolology textbook regarding information on specimen collection and handling.
Samples for investigation can be taken from any part of the body where viable organisms are present. If the sample is to be transported to the laboratory the swab should be immersed in a transport medium such as Stuart´s. The swab should reach the laboratory within 24-48 hours. No additives or preservatives need to be used during transport or culturing.
Samples from patients taking antibiotics may contain very few or no viable bacteria.

PROCEDURE
Materials provided
See under REAGENTS.

Materials required but not provided
- Primary culture
- Disposable inoculating loops or equivalent
- Clock with easily read minute indicator

Parameters of the method

Reaction temperature	room temperature
Volume of reagents	one drop
Reaction time	1 minute

Preparation of samples
Please refer to a standard microbiology textbook regarding detailed information on preparation of primary cultures.

Test protocol
Note! Suspend reagents thoroughly by shaking.

On disposable slide, for each sample to be tested, label one oval each with Type b Reagent and Types a, c-f Reagent.

Using a disposable loop or equivalent, collect colonies from the culture plate. Spread the colonies smoothly and thoroughly in correspondingly marked ovals on the slide. Fig. 1.

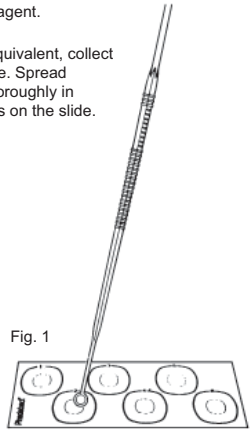


Fig. 1

Add one drop of Type b Reagent and one drop of Types a, c-f Reagent to the corresponding marked oval. Fig. 2.

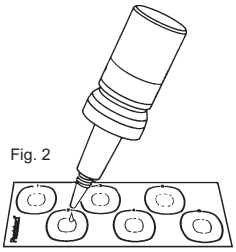


Fig. 2

Mix the drops thoroughly but gently with a disposable loop or equivalent. Use a fresh loop for each reagent. Fig. 3.

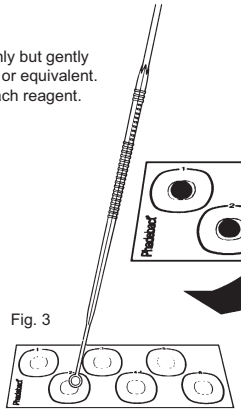


Fig. 3

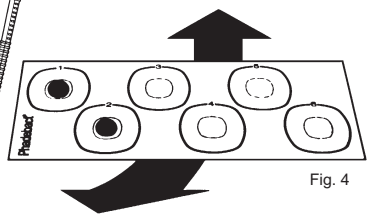


Fig. 4

Rock the slide and read the result within 1 minute. Fig. 4.

Direct testing of cerebrospinal fluid (CSF)
For identification of *H. influenzae* by direct testing of cerebrospinal fluid please refer to Phadebact® CSF Test.

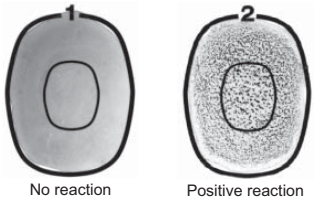
Stability of the final reaction mixture
The co-agglutination reaction is stable, but good laboratory practice dictates that the result be read within 1 minute (observe the risk of drying out of the reagents which may be misinterpreted as a positive reaction).

Calibration
No calibration is needed.

Quality control
Positive control
As a control, established laboratory strains of *H. influenzae* type b (e.g. ATCC 10211) and *H. influenzae* types a, c-f (e.g. ATCC 9006 (type a)) should be used. The control strains are treated in an identical manner as the unknown bacteria in the test procedure.

Negative control
By simultaneous use of both Reagents in testing an unknown sample, there is a built-in negative control since mixed infections are rare. If the unknown bacteria is *Haemophilus influenzae*, type b, co-agglutination occurs with the Type b Reagent. The Types a, c-f Reagent will show a negative result (no or significant weaker reaction).

Appearance of reaction



No reaction Positive reaction

RESULTS
Positive result, Type b
A significantly stronger reaction in the Type b Reagent compared to the Types a, c-f Reagent identifies the organism as *Haemophilus influenzae* type b.

Positive result, Types a, c-f
A significantly stronger reaction in the Types a, c-f Reagent compared to the Type b Reagent identifies the organism as *Haemophilus influenzae* types a, c-f.

Negative result
If no co-agglutination occurs in both Reagents, the specimen is either not a typeable *H. influenzae*, or there is insufficient antigenic material in the sample. The test should be repeated using a larger quantity of the specimen.

Non-interpretable results
A reaction of equal strenght in both Reagents constitutes a non-interpretable result. The specimen is then not identifiable with Phadebact® Haemophilus Test. Instead another identification test (biochemical) may be used.

LIMITATIONS OF PROCEDURE
Immunological methods, such as co-agglutination, used for the identification of *H. influenzae*, contain antibodies directed against the capsular antigens of *H. influenzae*. Therefore, those *Haemophilus* spp. not possessing a capsule are non-reactive in an immunological test system. *H. influenzae* type b has antigens in common with certain types of *S. pneumoniae* and these will thus cross-react.

PERFORMANCE CHARACTERISTICS
Specificity and sensitivity
The specificity and sensitivity of *Haemophilus influenzae* Type b Reagent and Types a, c-f Reagent were investigated using a total of 859 specimens including stock cultures (5).

Type b Reagent	Sensitivity: 98.8% (No tested=83)	Specificity: 97.6% (No tested=776)
Types a, c-f Reagent	Sensitivity: 97.3% (No tested=37)	Specificity: 96.6% (No tested=822)

Bacterial Species	No tested	No of strains aggl. in both reagents	No. of strains co-aggl. typ b	Types a, c-f
H. influenzae, Type b	83	0	82	1
H. influenzae, Types a, c-f	37	0	1	36
H. influenzae, untypeable	156	5	0	0
H. parainfluenzae	210	0	0	6
H. parahemolyticus	4	0	0	0
H. aphrophilus	1	0	0	0
Streptococci, groups A;B,C,G	54	9	0	0
Streptococci, group D	58	1	0	0
Streptococci, alpha-hemolytic	83	1	0	0
S. pneumoniae	93	0	0	3*
N. meningitidis	14	1	0	0
Neisseria spp	2	0	0	0
Moraxella spp	1	0	0	0
S. aureus	34	0	0	0
S. epidermidis	29	1	0	0

*S. pneumoniae types 11b, 11c and 38

WARRANTY
The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by MKL Diagnostics AB may affect the results, in which event MKL Diagnostics AB disclaims all warranties, expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. MKL Diagnostics AB and its authorized distributors, in such event, shall not be liable for any damages, whether direct, indirect or consequential.

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1. *Weinstein, L:* Haemophilus infections. Principles of internal medicine. Ed. Harrison, T R New York (McGraw-Hill), 1977.
2. *Murray P R, Barron E Jo, Pfaller M A, Tenover F C, Yolken R H:* Manual of Clinical Microbiology. 6th ed. ASM Press, Washington DC, 1995.
3. *Weinstein L:* Type b *Haemophilus influenzae* infections in adults. N Eng J Med 4, (1970), pp 221-222.
4. *Christensen P, Kahlmeter G, Jonsson S & Kronvall G:* New method for the serological grouping of streptococci with specific antibodies absorbed to Protein-A containing staphylococci. Infect Immun 7 (1973), pp 881-885.
5. Data on file, MKL Diagnostics AB.

PRODUCTS
Phadebact® COA System
Phadebact® Streptococcus Tests
Phadebact® Streptococcus Respiratory Test
Phadebact® Strep A Test
Phadebact® Strep B Test
Phadebact® Strep D Tests
Phadebact® Strep F Test
Phadebact® Strep Positive Controls
Phadebact® Pneumococcus Test
Phadebact® Haemophilus Test
Phadebact® GC Positive Controls
Phadebact® CSF Test
Phadebact® CSF Positive Controls
Phadebact® Extraction Solutions
Phadebact® Monoclonal GC Test
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