

Phadebact® CSF Test 20

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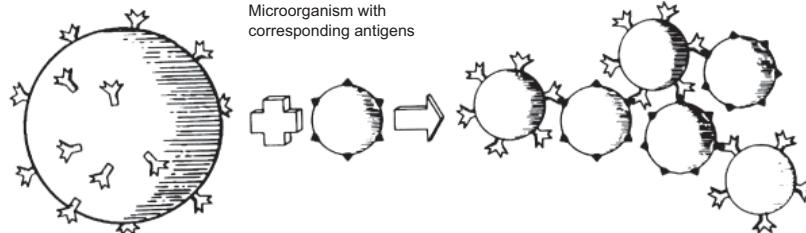
Directions for Use

Bactus AB
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Antibody-coated staphylococci

Co-agglutination



DIRECTIONS FOR USE

Each package of Phadebact® CSF Test contains reagents sufficient for 20 determinations.

INTENDED USE

Phadebact® CSF Test is intended for direct identification of capsular antigens of the following organisms: *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Neisseria meningitidis* groups A, B, C, Y, W135 and *Streptococcus agalactiae* (Strep B) in cerebrospinal fluid. Identification of some of these antigens is also possible with serum and urine samples. Please refer to urine and serum text in this package insert for specific instructions.

SUMMARY AND EXPLANATION OF THE TEST

Acute bacterial meningitis is an infection of the meninges and is caused by a variety of gram-positive and gram-negative microorganisms, predominately *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* (1). Bacterial meningitis also may be secondary to infections in other parts of the body (2), and there is the possibility of the occurrence of simultaneous mixed bacterial meningeal infections (3). *Streptococcus* group B has become the leading etiologic agent for meningitis during the first two months of life (4). The traditional diagnostic methods are microscopy of smears and bacterial cultures. Serological techniques such as counter-current immunoelectrophoresis (CIE) and immunofluorescence as tools for diagnosis of bacterial meningitis require experienced personnel and specially equipped laboratories. Phadebact® CSF Test is based on the co-agglutination technique (5) and allows rapid, direct identification of *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Neisseria meningitidis* and *Streptococcus agalactiae* in cerebrospinal fluid, using a simple slide technique.

PRINCIPLE OF THE PROCEDURE

The co-agglutination technique: Antibodies, raised in rabbit and specific against *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type b or *Streptococcus agalactiae* are bound to Protein A on the surface of non-viable staphylococci (6,7). When a CSF-sample containing microorganisms belonging to one of these groups is mixed with the reagents, the specific antigens on the surface of the microorganisms bind to the corresponding specific antibodies. A co-agglutination lattice is formed, which is visible to the naked eye.

REAGENTS

Each Phadebact® CSF Test package contains reagents sufficient for 20 determinations. The reagents are coloured blue (methylene blue) to facilitate interpretation of results.

Reactive ingredients

- | | |
|---|--------|
| • CSF Pneumococcal Reagent | 1 vial |
| • CSF <i>H. influenzae</i> type b Reagent | 1 vial |
| • CSF Strep B Reagent | 1 vial |
| • CSF Meningococcal Reagent. | 1 vial |

All antibodies raised in rabbit.

READY TO USE

Other components

- Droppers
- Disposable slides
- Directions for Use

Precaution

For *in vitro* diagnostic use.

Warning! The reagents contain sodium azide (NaN₃) 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 microbiology textbook regarding information on collection, handling and transport of cerebrospinal fluid samples (8).

Serum

Collect blood aseptically by venipuncture into a clean tube without anticoagulants. Permit blood to form a clot for at least 10 minutes at room temperature (12-25°C). Centrifuge at 1.000x g for 10 to 15 minutes, or until supernatant fluid is free of red blood cells. Transfer the serum into a clean glass container. Best results will be obtained when fresh serum is tested.

Urine (clean, voided samples)

Please refer to a standard microbiology textbook regarding information on collection and handling of urine samples (8).

PROCEDURE

Materials provided

See under REAGENTS.

Materials required but not provided

- Pasteur pipettes
- Disposable loops
- Incubator 80°C
- Clock with easily read minute indicator

Parameters of the method

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

Preparation of samples

Heat cerebrospinal fluid sample in an incubator or in a waterbath at 80°C for 5 minutes. The heated sample is allowed to cool and used for testing. Red blood cells may be present in a CSF sample due to accidental damage to a blood vessel during lumbar puncture. In such cases centrifugation of the sample is recommended.

Serum

Heat sample in a heating block or water bath at 80°C for 3-5 minutes. Cool. Centrifuge at 1,000x g for 10 minutes to precipitate clot. To prevent excess clotting, dilute serum sample **prior** to heating, 1:4 with 0.1M EDTA solution, pH 7.4. Test supernatant.

Urine

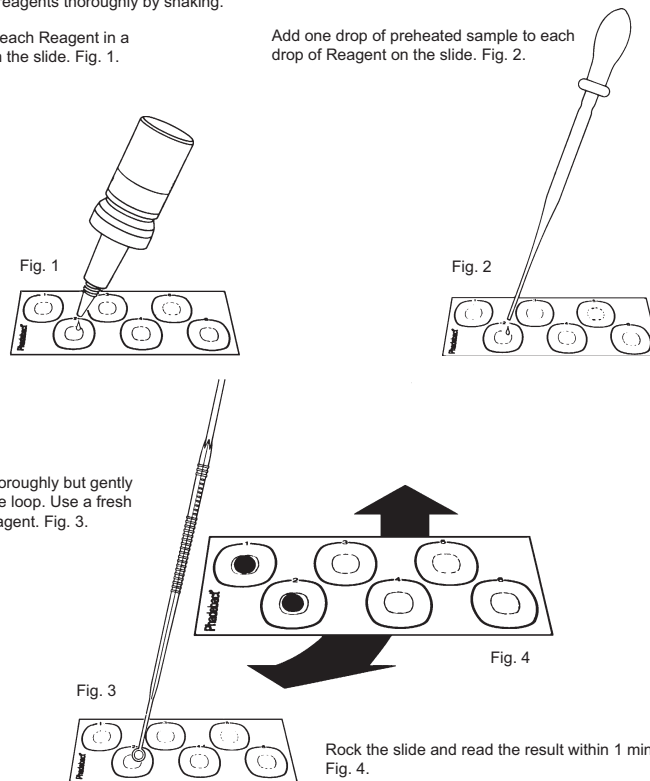
Centrifuge to remove any sediment. For maximum sensitivity, concentrate supernatant to 25x or 50x using Amicon™(or equivalent) concentrator. Heat for 3-5 minutes at 80-100°C. Cool. Perform test.

Test protocol

Note! Suspend reagents thoroughly by shaking.

Put one drop of each Reagent in a separate oval on the slide. Fig. 1.

Add one drop of preheated sample to each drop of Reagent on the slide. Fig. 2.



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

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

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.

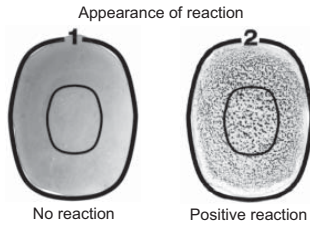
Quality control

Positive control

- Phadebact® CSF Positive Controls are available from Bactus AB. The kit contains separate vials of antigen from pure culture of *S. pneumoniae*, *H. influenzae* type b, *Neisseria meningitidis* and *S. agalactiae* (Strep B).
- These positive controls are tested in the same manner as the heated sample.
- Alternatively, established laboratory strains should be used, e.g. *S. pneumoniae* (ATCC 6303), *H. influenzae* type b (ATCC 10211), *N. meningitidis* (ATCC 13077) and *S. agalactiae* (ATCC 12401).

Negative control

By simultaneous use of all four reagents in testing an unknown sample, there is a built-in negative control since mixed infections are rare.



RESULTS

Positive result

A significantly stronger and more rapid reaction in one of the reagents when compared to the three other reagents constitutes a positive result.

Negative result

No reaction in any of the reagents indicates that the cerebrospinal fluid is not infected by *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Streptococcus agalactiae* or *Neisseria meningitidis* or that the sample does not contain enough antigenic material. In such cases another identification test, culture or CIE should be used.

Non-interpretable results

If co-agglutination of equally strong intensity and speed occurs with more than one reagent, this constitutes a non-interpretable result. The specimen is not identifiable with Phadebact® CSF Tests. Instead another identification test such as culture or CIE should be used.

LIMITATIONS OF PROCEDURE

Immunological methods, such as co-agglutination, used for the identification of *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus agalactiae* and *Neisseria meningitidis*, contain antibodies directed against the capsular antigens of the microorganisms. Therefore, those organisms not possessing a capsule are non-reactive in an immunological test system. Certain types of *Streptococcus pneumoniae* have antigens in common with *Haemophilus influenzae* type b and these will thus cross-react (9). The frequency of such cross-reactions are usually low. Using a concentration of 10⁸ organisms/mL, 4 *H. influenzae* and 9 *S. pneumoniae* samples were tested with Phadebact® CSF Test. No cross-reactions were observed (11). As well as for CIE it must be emphasized that a negative result with Phadebact® CSF Test does not exclude the possibility of bacterial meningitis (12). As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

Specificity and sensitivity

A total of 362 samples of cerebrospinal fluid from patients suspected of meningitis were investigated (10, 11). 324/362 (90%) were correctly identified with a specificity of 99% (155/157 true negatives) and a sensitivity as follows:

<i>H. influenzae</i> type b	74/85	87%
<i>S. agalactiae</i>	14/17	82%
<i>S. pneumoniae</i>	32/38	84%
<i>N. meningitidis</i>	39/65	60%

Testing of serum and/or urine samples from patients with confirmed diagnosis of bacterial meningitis as evidenced by positive CSF culture and/or CIE gave the following results:

Serum samples		
	No. of patients	Positive by co-agglutination
<i>H. influenzae</i> type b	49	41 (84%)
<i>S. pneumoniae</i>	15	10 (67%)
<i>S. agalactiae</i>	12	9 (75%)
<i>N. meningitidis</i>	15	8 (53%)
Urine samples		
	No. of patients	Positive by co-agglutination
<i>H. influenzae</i> type b	83	73 (88%)
<i>S. pneumoniae</i>	19	4 (21%)
<i>S. agalactiae</i>	37	32 (86%)
<i>N. meningitidis</i>	21	3 (14%)

NB: Not all cases of bacterial meningitis will manifest antigen spillover in serum or urine.

Reproducibility

The reagents used in this test are routinely checked against known bacterial standard antigens to ensure reproducibility.

WARRANTY

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

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PRODUCTS**Phadebact® COA System**

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