

Phadebact®

Monoclonal GC Test

53-1054-03, Mar-06

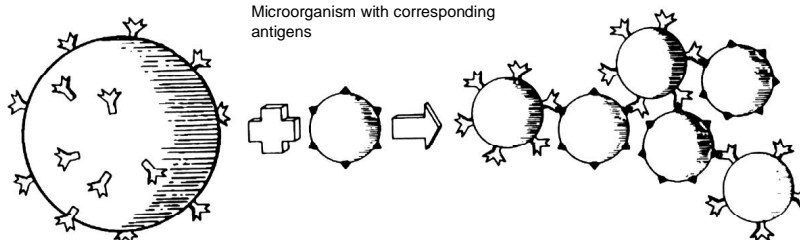
Directions for Use

Bactus AB
Lunastigen 3
SE-141 44 Huddinge
Sweden



Antibody coated staphylococci

Co-agglutination



INTENDED USE

Phadebact® Monoclonal GC Test is intended for the definitive identification of *Neisseria gonorrhoeae*.

SUMMARY AND EXPLANATION OF THE TEST

The causative agent of the sexually transmitted disease Gonorrhoea is *Neisseria gonorrhoeae*. A number of organisms other than *Neisseria gonorrhoeae* belong within the *Neisseria* genus. These other *Neisseriae* are:

1. *N. meningitidis* (1), a cause of epidemic meningitis and meningococcaemia, is also occasionally found in venereal discharges. In addition, it is often found in the naso-pharynx without causing clinical symptoms.
2. *N. sicca* (1), *N. subflava* (1), *N. flavescens* (1), *N. mucosa* (1), and *N. lactamica* may all be found in the naso-pharynx without causing clinical symptoms. These are opportunistic pathogens. In addition *Moraxella (Branhamella) catarrhalis* may be found on mucous membranes, including the pharynx, and occasionally in venereal discharges. This is also an opportunistic pathogen.

In order to differentiate *Neisseria gonorrhoeae* from these other *Neisseria* it is necessary to perform definitive testing. Various test methods may be used to provide this definitive identification. The Sugar Utilization test is the standard reference method today. The Immuno-fluorescent technique can also be used for definitive identification of *N. gonorrhoeae*. However, technical and practical limitations in both these procedures have restricted their application and use for the definitive identification of *N. gonorrhoeae*.

The co-agglutination technique has been applied to the definitive identification of *N. gonorrhoeae* (2). Phadebact® Monoclonal GC Test is based on this co-agglutination technique.

PRINCIPLE OF THE PROCEDURE

The WI and WII/III Gonococcal Reagents are composed of two pools of murine monoclonal antibodies, reacting with a gonococcal specific membrane protein called protein I (3). Gonococci harbouring protein IA are classified to serogroup WI, whereas gonococci containing protein IB are referred to serogroup WII/III (4). The monoclonal antibodies of WI and WII/III Gonococcal Reagents are coupled to the protein A of non-viable staphylococci.

When a sample containing gonococci is mixed with the WI and WII/III Gonococcal Reagent, the specific protein I antigens of the cell bind to the corresponding specific monoclonal antibodies. In this way a co-agglutination lattice is formed, which is visible to the naked eye.

REAGENTS

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

Reactive ingredients

- WI Gonococcal Reagent 1 vial
Murine monoclonal antibodies to protein IA,
bound to non-viable staphylococci.
- WII/III Gonococcal Reagent 1 vial
Murine monoclonal antibodies to protein IB,
bound to non-viable staphylococci.

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 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 for growth and identification, the swab should be inoculated or immersed in a transport medium such as Transgrow or Stuart's. The sample must be protected from extremes of temperature and should reach the laboratory within 24 hours (5). Alternatively, the swab can be used to inoculate a plate of GC growth medium, which is then incubated, and after growth sent on to the laboratory for identification (6). 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 with oxidase positive, gram-negative diplococcal organisms.
- Inoculating loops
- Waterbath
- Saline solution 0.9%

Parameters of the method

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

Preparation of samples

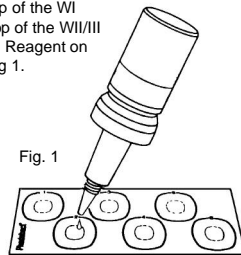
Please refer to a standard microbiology textbook regarding detailed information on preparation of primary cultures. After inoculation, the media should be incubated at 35-37°C, in a humid atmosphere containing 3-7% CO₂, for a period of 16-24 hours (7). Colonies that have been presumptively identified as *N. gonorrhoeae* (7) may then be tested by Phadebact® Monoclonal GC Test for definitive identification:

- Remove colonies of the presumptively identified *N. gonorrhoeae* from the primary plate making a light suspension in 0.5 ml 0.9% saline.
- Heat the suspension in a boiling waterbath for at least 5 minutes. To avoid the risk of evaporation, cover the opening of the tube.
- Cool suspension to room temperature.
- Drops of the heat treated suspension are used for testing.

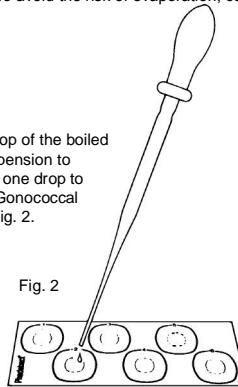
Test protocol

Note! Suspend reagents thoroughly by shaking.

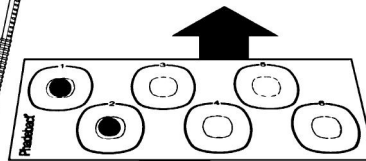
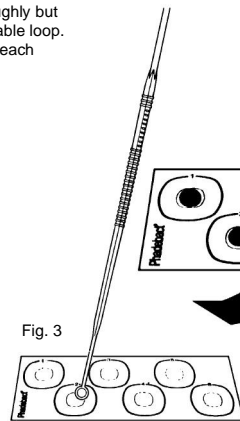
Put one drop of the WI and one drop of the WII/III Gonococcal Reagent on the slide. Fig 1.



Add one drop of the boiled colony suspension to the WI and one drop to the WII/III Gonococcal Reagent. 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

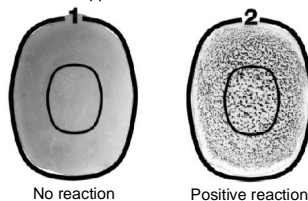
Positive control

Phadebact® GC Positive Controls (ready to use extracts from pure cultures *N. gonorrhoeae*) or use freshly grown reference strains as ATCC 19424 (serogroup WI) and ATCC 49498 (serogroup WII/III).

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 belong to *N. gonorrhoeae* group WI, co-agglutination occurs with the WI Gonococcal Reagent and the WII/III Gonococcal Reagent will show a negative result.

Appearance of reaction



RESULTS

Positive result

A reaction in either WI or WII/III Gonococcal Reagent constitutes a positive result. A positive result provides definitive identification of *N. gonorrhoeae* (see under PERFORMANCE CHARACTERISTICS). A positive reaction of the same strength in both reagents is an equivocal result. Dilute the treated suspension 2-4x with 0.9% saline and test again.

Negative result

Lack of reaction in both WI and WII/III Gonococcal Reagents constitutes a negative result. A negative result strongly suggests that the bacteria tested are not *N. gonorrhoeae* (see under PERFORMANCE CHARACTERISTICS).

LIMITATIONS OF PROCEDURE

Use another identification test (preferably biochemical) when presumptive isolates are weak or equivocal in co-agglutination test, positive results are obtained with isolates from extragenital sites of persons at low risk for gonococcal infection, or positive tests are obtained with isolates from both genital and extragenital sites of children.

PERFORMANCE CHARACTERISTICS

Specificity and sensitivity

In total 1366 clinical isolates from female genital and rectum, male urethra and rectum and throat samples were investigated (8). Identity of isolates was determined by sugar fermentation and/or immuno-fluorescent techniques.

Positive by reference method	Positive by co-agglutination
1080	1077
Sensitivity:	99.7%
Negative by reference method	Negative by co-agglutination
286	286
Specificity:	100%

Note! False positive reactions with *N. meningitidis* have been reported on two occasions, i.e. specificity is not 100%.

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.

Bibliography:

1. Murray P R, Baron E Jo, Pfaller M A, Tenover J C, Tenover F C: Manual of Clinical Microbiology. 8th ed. ASM Press, Washington DC, 2003.
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3. Tam M R et al: Serological classification of Neisseria gonorrhoeae with monoclonal antibodies. Infect and Immun 36: pp 1042-53, 1982.
4. Sandström E G et al: Serology of Neisseria gonorrhoeae: Coagglutination Serogroups WI and WII/III correspond to different outer membrane protein I molecules. Infect and Immun 38: pp 462-470, 1982.
5. Danielsson D & Johansson G: Culture diagnosis of gonorrhoea. Acta Derm Venereol 53 (1973), p 75.
6. USDHEW: Criteria and Techniques for the Laboratory Diagnosis of Gonorrhoea. CDC Atlanta 1975.
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8. Young H, Moyes A: Utility of monoclonal antibody coagglutination to identify Neisseria gonorrhoeae. Genitourin Med. 65 (1989), pp 8-13.
9. Data on file, Bactus 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
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Phadebact® Monoclonal GC Test
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Phadebact® Salmonella Test
Phadebact® Staph Aureus Test

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