

**Phadebact® Streptococcus Tests
(A, B, C, G), (A-D, F, G) and (A-D, G)
Phadebact® Strep Respiratory Test (A, C, G)
Phadebact® Strep A Test
Phadebact® Strep B Test
Phadebact® Strep D Tests
Phadebact® Strep F Test**

53-1060-02, Mar-06

Directions for Use

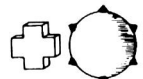
Bactus AB
Lunastigen 3
SE-141 44 Huddinge
Sweden



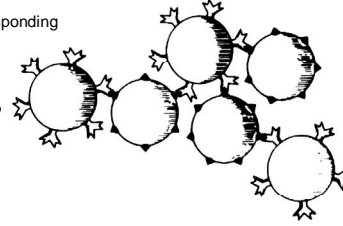
Antibody-coated staphylococci



Microorganism with corresponding antigens



Co-agglutination



INTENDED USE

Phadebact® Streptococcus Tests, Strep Respiratory Test, Strep A Test, Strep B Test, Strep D Tests and Strep F Test are intended for the identification of beta-hemolytic streptococci belonging to groups A, B, C, D, F and G.

SUMMARY AND EXPLANATION OF THE TEST

Differentiation of beta-hemolytic streptococci into the Lancefield groups is important for treatment as well as the prognosis of the patient (1). By providing the physician with information about the serological group, correct treatment will be achieved and unnecessary use of antibiotics may be avoided (2). Streptococci belonging to groups A, B, C, D, F and G are those most often associated with human streptococcal infections (3-9).

Several methods exist for identification of streptococci. Biochemical reactions and the bacitracin disc provide presumptive identification. The Lancefield precipitin technique, immunoelectrophoresis and latex agglutination provide definitive identification. However, with these methods, extraction of the group-specific antigens is necessary. Phadebact® Streptococcus Test, based on the co-agglutination technique (10) allows definitive identification of streptococcal groups A, B, C, D, F and G using a simple and rapid slide technique.

PRINCIPLE OF THE PROCEDURE

The co-agglutination technique: Antibodies, raised in rabbit and specific against groups A, B, C, D, F and G streptococci, are bound to Protein A on the surface of non-viable staphylococci. When a sample containing streptococci belonging to one of these groups is mixed with its reagent, the specific antigens on the surface of the streptococci bind to the corresponding specific antibodies. A co-agglutination lattice, visible to the naked eye, is formed.

REAGENTS

Each of the Phadebact® kits for streptococcal testing contains reagents sufficient for the following number of determinations:

Product	Determinations
Phadebact® Streptococcus Test (A, B, C, G)	300
Phadebact® Strep A-D, F, G Test	300
Phadebact® Strep A-D, G Test	250
Phadebact® Strep Respiratory Test (A, C, G)	200
Phadebact® Strep A Test	100
Phadebact® Strep B Test	100
Phadebact® Strep D 100 Test	100
Phadebact® Strep D Test	50
Phadebact® Strep F Test	50

The reagents are coloured blue (Methylene blue) to facilitate interpretation of results.

Reactive ingredients

Reactive ingredients for the products listed herein are as follows:

Phadebact® Streptococcus Test (A, B, C, G)

Group specific antiserum, raised in rabbit, bound to non-viable staphylococci.

- One vial each of Strep A, B, C and G reagents.

Phadebact® Strep A-D, F, G Test

Group specific antiserum, raised in rabbit, bound to non-viable staphylococci.

- One vial each of Strep A, B, C, D, F and G reagents.

Phadebact® Strep A-D, G Test

Group specific antiserum, raised in rabbit, bound to non-viable staphylococci.

- One vial each of Strep A, B, C, D and G reagents.

Phadebact® Strep Respiratory Test (A, C, G)

Group specific antiserum, raised in rabbit, bound to non-viable staphylococci.

- One vial each of Strep A, C and G reagents.

- Phadebact® Control reagent: Gammaglobulin from non-immunized rabbit, coupled to non-viable staphylococci. 1 vial

Phadebact® Strep A Test

- Strep A Reagent: Specific antibodies, raised in rabbit, coupled to non-viable staphylococci. 1 vial

- Phadebact® Control reagent: Gammaglobulin from non-immunized rabbit, coupled to non-viable staphylococci. 1 vial

Phadebact® Strep B Test

- Strep B Reagent: Specific antibodies, raised in rabbit, coupled to non-viable staphylococci. 1 vial

- Phadebact® Control reagent: Gammaglobulin from non-immunized rabbit, coupled to non-viable staphylococci. 1 vial

Phadebact® Strep D 100 Test

- Strep D Reagent:
Specific antibodies, raised in rabbit, coupled to non-viable staphylococci. 2 vials
- Phadebact® Control reagent:
Gammaglobulin from non-immunized rabbit, coupled to non-viable staphylococci. 2 vials

Phadebact® Strep D Test

- Strep D Reagent:
Specific antibodies, raised in rabbit, coupled to non-viable staphylococci. 1 vial
- Phadebact® Control reagent:
Gammaglobulin from non-immunized rabbit, coupled to non-viable staphylococci. 1 vial

Phadebact® Strep F Test

- Strep F Reagent:
Specific antibodies, raised in rabbit, coupled to non-viable staphylococci. 1 vial
- Phadebact® Control reagent:
Gammaglobulin from non-immunized rabbit, coupled to non-viable staphylococci. 1 vial

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 (18).

Preparation of reagents

The reagents are READY TO USE.

Shelf life and storage

The expiration 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 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
- Enrichment broth (Todd-Hewitt Broth produced by BBL is strongly recommended) needed only for reference broth method
- Pasteur pipettes
- Disposable inoculating loops or equivalent
- Incubator 35-37°C (if using reference broth method)
- Clock with easily read minute indicator

Parameters of the method

Reaction temperature	room temperature
Reaction time	1 minute
Volume of reagents	one drop of each group to be tested

Preparation of samples

Please refer to a standard microbiology textbook regarding detailed information on preparation of primary cultures. There are three alternative procedures for sample preparation to be used with Phadebact® Streptococcus Test.

I. Colony samples

- Inspect the primary growth plate carefully for the presence of beta-hemolytic colonies.
- Remove 1-5 colonies of the beta-hemolytic streptococci with a disposable loop or equivalent in a sufficient number to spread in 2-6 circles selected on the slide.
- These colonies are used for testing.

II. Optional direct colony extraction

An optional direct colony extraction procedure using the Phadebact® Extraction Solutions - available from Bactus AB - may be considered for those occasional situations when:

- Insufficient sample material is present to meet direct colony testing requirements (1-5 colonies per reagent).
- Inconclusive results are obtained with direct colony testing.

Note! Strep D and Strep F are not intended for extraction (broth inoculation recommended).

III. Broth inoculation

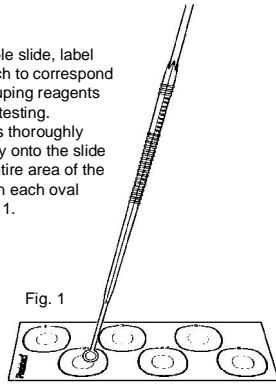
- Inspect the growth plate carefully for the presence of beta-hemolytic colonies.
- Remove 1 or more colonies of the beta-hemolytic streptococci.
- Inoculate into 2 mL enrichment broth.
- Incubate at 35-37°C overnight or until turbidity is evident (equivalent to Mac-Farland Standard Density 2-3). There is usually sufficient growth after only 4 hours incubation, when using 0.5 mL enrichment broth and several colonies.
- Drops of this suspension are used for testing.

Test protocol

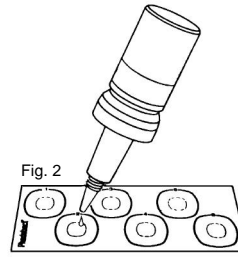
- Mix reagents well by shaking before using.
- Reagents should be at room temperature before using.
- Good laboratory practice suggests the appropriate use of positive and negative controls as with any testing procedure.

I. Direct Colony Test

On disposable slide, label one oval each to correspond with the grouping reagents selected for testing. Rub colonies thoroughly and smoothly onto the slide within the entire area of the inner circle in each oval labelled. Fig. 1.



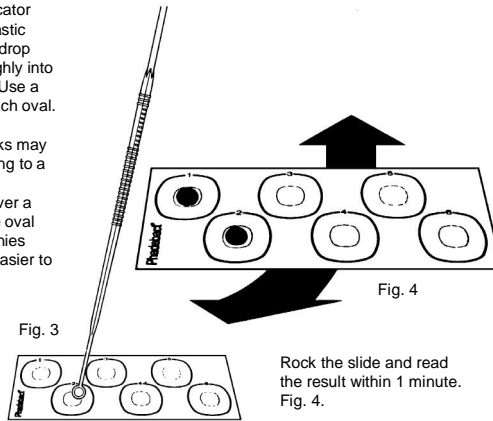
Place one drop of each reagent selected on the spread colonies in each correspondingly labelled oval. Fig. 2



Using bare wood applicator sticks or disposable plastic loops, mix the reagent drop completely and thoroughly into the smeared colonies. Use a new stick or loop for each oval.

CAUTION:

- Paper applicator sticks may adsorb reagent leading to a poor or no reaction.
- Spreading reagent over a larger area within the oval than the spread colonies makes the reaction easier to read. Fig. 3.



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

II. Optional Direct Colony Extraction Procedure

- From the Phadebact® Extraction Solutions kit mix 4 drops of Extraction Solution 1 with 4 drops of Extraction Solution 2 in a small test tube (e.g. size 8 x 50 mm).
- Select 1-3 colonies beta-hemolytic streptococci from the primary plate and suspend them in the Extraction Solution mixture.
- Incubate at room temperature for 10-15 minutes or at 100°C for one minute.
- Using a Pasteur pipette place one drop of extracted colonies in each oval labelled for testing on the disposable slide.
- Add one drop each of Phadebact® reagents selected for testing next to the drops of extracted colonies correspondingly labelled.
- Using a disposable loop or bare wood applicator stick, spread the drops over an area larger than the inner circle within each oval being certain to thoroughly mix reagent with extraction solution while spreading in each oval. Use a new disposable loop or applicator stick to spread each sample.
- Rock the slide and read the result within 1 minute.

III. Reference Broth Test

- On a disposable slide label one oval each to correspond with the grouping reagents selected for testing.
- Place one drop of the broth culture in the small inner circle with each labelled oval.
- Add one drop of each reagent selected next to the broth drop in each correspondingly labelled oval.
- Using a disposable loop or bare wood applicator stick, spread the drops over an area larger than the inner circle being certain to thoroughly mix the reagent and broth drops while spreading in each oval. Use a new disposable loop or applicator stick for spreading each sample.
- Rock the slide and read the result within 1 minute.

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 reaction).

Calibration

No calibration is needed.

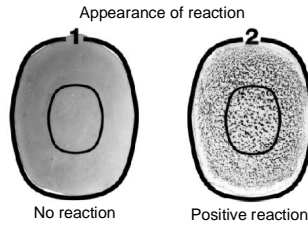
Quality control

Positive control

- Phadebact® Strep Positive controls are available from Bactus AB. The kit contains separate vials of antigen from pure culture of streptococcal groups A, B, C, D, F and G.
- Since these antigen controls are separate and purified they may be used to test Phadebact® Streptococcal reagents for specificity as well as reactivity, thus live organism controls are unnecessary.
- These positive controls are tested in the same manner as unknown bacteria from a broth culture.
- Alternatively, established laboratory strains of streptococcal groups should be used, e.g. Strep A (ATCC 19615), Strep B (ATCC 12401), Strep C (ATCC 9892), Strep D (ATCC 9812), Strep F (ATCC 12392) and Strep G (ATCC 12395).

Negative control

- By simultaneous use of several reagents in testing a control or an unknown specimen, there is a built-in negative control. If the unknown bacteria is a Streptococcus group A, a co-agglutination occurs in the A reagent. Reagents B, C and G show negative results (no or significantly weaker reaction).
- When testing with a single reagent kit such as Phadebact® Strep A Test, Phadebact® Strep B Test, Phadebact® Strep D Tests or Phadebact® Strep F Test, a negative control is another streptococcal group (control or laboratory strain).



RESULTS

Positive result

A significantly stronger and more rapid reaction in one of the reagents when compared to the other reagents constitutes a positive result using the Phadebact® Streptococcus Test. The Phadebact® Control reagent must be used for the other tests.

Negative result

No reaction in any of the reagents strongly indicates that the bacteria tested do not belong to groups A, B, C, D, F or G; or that the sample does not contain enough antigenic material. See under LIMITATIONS OF PROCEDURE.

Non-interpretable results

If co-agglutination of equally strong intensity and speed occurs with more than one streptococcal reagent please refer to LIMITATIONS OF PROCEDURE.

LIMITATIONS OF PROCEDURE

- Streptococcal strains used for producing our antisera are grown in Todd-Hewitt broth manufactured by Difco. Therefore, it is not recommended that Difco's broth be used for growing specimens to be tested with Phadebact® reagents.
- If the results using the Colony Test (I) are inconclusive due to insufficient test material it is recommended that the test be repeated using the Optional Direct Colony Extraction Procedure (II).
- If the results from the 4 hour Broth Test (III) are doubtful due to insufficient growth, it is recommended that the broth be reincubated overnight and retested in the normal manner.
- If co-agglutination of equally strong intensity and speed occurs with more than one streptococcal reagent it is recommended that the test be repeated using the Optional Direct Colony Extraction Procedure.
- Listeria* strains possess similar antigenicity to group B streptococci and may therefore react positively with the group B reagent. To differentiate between *Listeria* and group B streptococci the catalase test can be used: streptococci are catalase negative and *Listeria* are catalase positive.
- Phadebact® Strep D and Phadebact® Strep F are not intended for the Direct Colony Extraction procedure.

PERFORMANCE CHARACTERISTICS

Specificity and sensitivity

I. Colony testing

In clinical studies (11, 12) investigating colony testing, 263/279 (94.3%) of beta-hemolytic streptococcal samples tested by this method were correctly identified.

II. Broth testing - 4 hour incubation

Clinical studies (13, 14, 15) have shown that when compared to the Lancefield technique 272/285 (95.4%) of beta-hemolytic streptococcal samples were identified after 4 hours incubation.

III. Broth testing - 24 hours incubation

Clinical studies (16, 17) have shown that 99% of streptococcal samples were identified following 24 hours (overnight) incubation.

IV. Streptococcus group A

Bacterial specimen	No of strains co-agglutinating	
	Direct colony	4-h broth
Strep A	166/168	
Non-strep A (beta-hemolytic)	1/56	
Strep D	0/13	
<i>S. pneumoniae</i>	1/11	
<i>Klebsiella spp.</i>	0/10	
<i>H. influenzae</i>	0/10	
<i>Staphylococcus spp.</i>	0/20	
Sensitivity: 98.8% Accuracy: 99.1%		
Specificity: 99.4%		

VI. Streptococcus group D

Bacterial specimen	No of strains co-agglutinating	
	Direct colony	4-h broth
Strep D	178/190	226/240
Strep A,B,C,G	0/40	0/40
<i>S. mutans</i>	0/11	0/11
<i>S. sanguis</i>	4/19	-
<i>S. uberis</i>	0/2	0/2
<i>S. mitis</i>	3/4	0/1
<i>S. milleri</i>	0/11	0/11
Sensitivity: 93.4% Accuracy: 93.4%		
Specificity: 92.8%		

V. Streptococcus group B

Bacterial specimen	No of strains co-agglutinating
Strep B	128/130
Non-strep B (beta-hemolytic)	1/18
alpha-hemolytic strep.	0/109
non-hemolytic strep.	0/98
Enterococci	0/28
Staphylococci	0/118
Sensitivity: 98.5% Accuracy: 99.4%	
Specificity: 99.7%	

VII. Streptococcus group F

Bacterial specimen	No of strains co-agglutinating
Strep F	77/81
Strep A	1/26
Strep B	1/24
Strep C	0/21
Strep D	0/7
Strep G	2/19
<i>S. pneumoniae</i>	1/71
Sensitivity: 95.0% Accuracy: 96.4%	
Specificity: 97.0%	

Note! If certain bacteria other than beta-hemolytic streptococci are tested, an incorrect result may be obtained. For instance, strains of *Streptococcus faecalis* may give a weak co-agglutination in the C reagent. *Streptococcus pneumoniae* (*Diplococcus pneumoniae*) may co-agglutinate with group C reagent, which is in accordance with the known similarities between the antigens of *S. pneumoniae* and group C streptococci.

Reproducibility

The reagents used in this test are periodically checked against known strains 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

- 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
- Phadebact® ETEC-LT Test
- Phadebact® Salmonella Test
- Phadebact® Staph Aureus Test

Near Patient Testing

- Phadirect® Strep A
- Phadirect® Rapid CRP Test

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