Phadebact®
Staph Aureus Test

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Directions for Use

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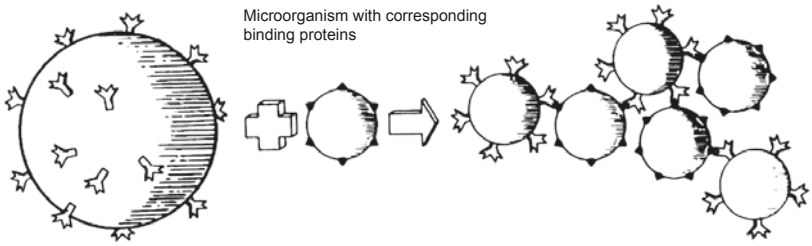
MKL Diagnostics

Sensitized latex particles with human and porcine plasma proteins

Microorganism with corresponding binding proteins

Agglutination



INTENDED USE
Phadebact® Staph Aureus Test is intended for the detection of coagulase (clumping factor) and/or Protein A characteristics associated with *Staphylococcus aureus* obtained from primary cultures. The Phadebact® Staph Aureus reagent will react with either or both of these two characteristics.
Each package of Phadebact® Staph Aureus Test contains reagents for 100, 200 or 400 determinations.

SUMMARY AND EXPLANATION OF THE TEST
Staphylococcus aureus has been shown to be a pathogenic bacterial species. Since it is an organism commonly found on the skin, nasal passages and mucous membranes, an injury to these sites provides an opportunity for these agents to produce an infection. *S. aureus* is responsible for most superficial suppurative infections and food poisoning and is also a cause of nosocomial infection (1).
The coagulase and Protein A characteristics associated with *S. aureus* allows for the identification of at least 98% of this species. Indeed, coagulase-negative *Staphylococcus* species induce infection as well. Coagulase can be either bound (clumping factor) to the staphylococci or released as a free enzyme. Coagulase converts fibrinogen to form a clot when EDTA-plasma is added to coagulase-positive *S. aureus*. Differential medium has been described for growth of coagulase-positive staphylococci (2). In addition, various media have been described for other individual properties of pathogenic staphylococci (3). Most of the above identified culture efforts require many hours of testing and evaluation before the results become available. Independent from coagulase activity is Protein A substance. Protein A is a constituent of the *S. aureus* cell wall. It combines with the Fc portion of most IgG immunoglobulins and serves as another marker (4). In contrast to lengthy culture procedures, the speed, convenience and accuracy of the Phadebact® Staph Aureus system provides for an appropriate alternative test. Rapid slide agglutination tests have been shown to be as reliable as the tube coagulase system in most cases (5).

PRINCIPLE OF THE PROCEDURE
The red-latex particles used in the The Phadebact® Staph Aureus latex reagent are sensitized with specific concentrations of human and porcine plasma proteins. When coagulase and/or Protein A is provided by the culture specimen at detectable levels they will interact with the sensitized particles to produce visible agglutination. This is a positive result.

REAGENTS
The Phadebact® Staph Aureus Test exists in kits with 100, 200 or 400 determinations respectively. The reagents are coloured red to facilitate interpretation of results.

Reactive ingredients

Phadebact® Staph Aureus 100 Test

- Phadebact® Staph Aureus Latex Reagent: 1 vial
  Red-latex particles coated with human and porcine proteins suspended in a buffer containing a preservative.
- Phadebact® Staph Aureus Positive Control: 1 vial
  A formulation of non-viable *S. aureus* (ATCC 25923) in a buffer containing a preservative.
- Phadebact® Staph Aureus Negative Control: 1 vial
  A formulation of non-viable, *S. epidermidis* (CDC 3258) in a buffer containing a preservative.
- Phadebact® Staph Aureus Latex Reagent: 1 vial
  Red-latex particles coated with human and porcine proteins suspended in a buffer containing a preservative.

Phadebact® Staph Aureus 200 Test

- Phadebact® Staph Aureus Latex Reagent: 2 vials
  Red-latex particles coated with human and porcine proteins suspended in a buffer containing a preservative.
- Phadebact® Staph Aureus Positive Control: 1 vial
  A formulation of non-viable *S. aureus* (ATCC 25923) in a buffer containing a preservative.
- Phadebact® Staph Aureus Negative Control: 1 vial
  A formulation of non-viable, *S. epidermidis* (CDC 3258) in a buffer containing a preservative.

Phadebact® Staph Aureus 400 Test

- Phadebact® Staph Aureus Latex Reagent: 4 vials
  Red-latex particles coated with human and porcine proteins suspended in a buffer containing a preservative.
  READY TO USE

Other components

- Droppers
- Disposable slides
- Directions for Use

Note! Phadebact® Staph Aureus 400 Test only includes specific Staph Aureus reagent 4 vials.
Phadebact® Staph Aureus 100 Test Reagent only includes specific Staph Aureus reagent 1 vial.

Precautions
For *in vitro* diagnostic use.
Please refer to decontamination procedures as outlined by e.g. CDC.
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.
The human sera components have been tested negative for HIV-1, HIV-2, HCV antibodies and HBsAg. This does not ensure absence of these organims.The controls have been tested negative for growth of staphylococci.

THE REAGENTS SHOULD BE HANDLED AS IF THEY WERE POTENTIALLY INFECTIOUS.

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. Overnight culture of a primary plate will provide a fresh, sufficient sized colony specimen (approximately 2 mm). Sample the colony with a sterile stick-end. It is preferable to use nutrient or sheep blood agar for any subculture which is to be performed if contamination of the culture is suspected. An identical sampling manoeuvre should be followed for any subcultured colony. The colony should be gram-stained to confirm the morphology and gram-positive characteristics of the organism.

PROCEDURE
Materials provided
See under REAGENTS.

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

Parameters for the method

Reaction temperature	room temperature
Volume of Staph Aureus reagent	one drop
Volume of Controls	one drop
Total reaction time	60 seconds (10 seconds of blending and 50 seconds of hand rocking) or shorter if agglutination occurs.

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

Test protocol
Note! Allow the reagents to achieve room temperature. Suspend the latex reagent thoroughly by shaking before using.

Mark one oval each to correspond with control and samples to be tested. Place a drop of latex and one drop of controls in the corresponding marked ovals. Fig. 1.

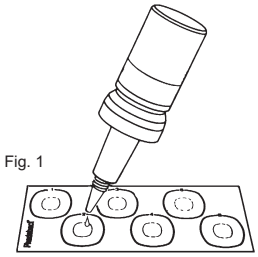


Fig. 1

Using a disposable inoculating loop or equivalent, pick a fresh colony. Fig. 2.

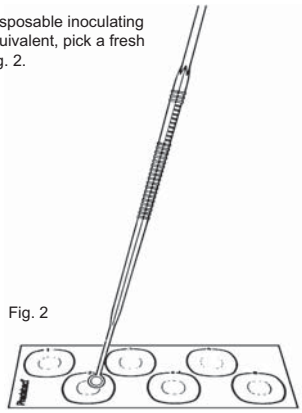


Fig. 2

Blend the fresh colony into the latex on the surface of the oval, mix them during 10 seconds. Avoid excessive rubbing of the card surface during the blending of the colonies and latex. Fig. 3.

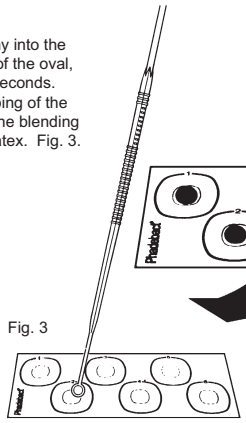


Fig. 3

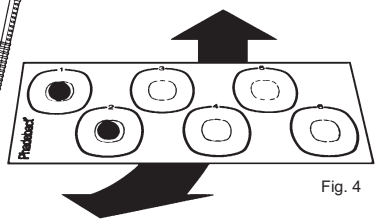


Fig. 4

After the culture specimen and latex have been blended in the oval, hand rock the card to mix each latex and specimen combination. When obvious agglutination is observed within 60 seconds, from the start of blending, a positive result is recorded for that specimen. Fig. 4.

Stability of the final reaction mixture
The agglutination is stable, but good laboratory practice dictates that the result be read within 60 seconds (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 and negative control
A positive and a negative control are provided with the kit for 100 and 200 determinations (not in Phadebact® Staph Aureus 100 Test Reagent).

Quality Control of the Latex with Positive and Negative Control:

1. Place a drop of resuspended latex reagent in two, separately identified ovals on the card.
2. Place a drop of the resuspended, positive control and negative control reagent in separate ovals on the card.
3. Using a disposable inoculating loop or equivalent for each combination, blend in each control with the red latex for 10 seconds inside their ovals.
4. Hand rock the slide for 50 seconds.
5. During the rocking, observe the two ovals for agglutination.
6. The positive control must provide obvious agglutination. The negative control must not produce agglutination within the 60 second period.
7. Notify your distributor or MKL Diagnostics AB if the expected quality control results are not observed.
8. This Quality Control procedure should be performed as often as the needs of the laboratory dictate.

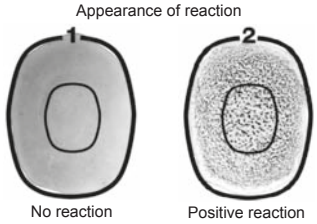
Good Laboratory Practices to Follow:

1. Use the Directions for Use as provided.
2. Allow the reagents to achieve room temperature before using.
3. RESUSPEND the reagents before dispensing them into the ovals.
4. Do not reuse an oval on the card.
5. Use a fresh stick to deliver each specimen.
6. Follow appropriate microbiological procedures in handling and disposing of the material used in the performance of the test.
7. Replace the proper droppers on their respective vials.
8. It is recommended that inoculated plates with known strains of *S. aureus* (e.g. ATCC 25923) and *S. epidermidis* (e.g. ATCC 12228) are to be used in parallell with the enclosed controls for 100 and 200 tests. For 400 tests and Phadebact® Staph Aureus 100 Test Reagent this is strongly recommended.

PROTOCOL TECHNIQUES

(ENSURE THAT THE REAGENTS HAVE REACHED ROOM TEMPERATURE)

1. Perform the Quality Control steps as outlined before testing fresh colony specimens.
2. Suspend the latex reagent thoroughly by shaking before using. Hold the latex vial in a vertical position just over an identified oval on the card. Squeeze the vial to deliver a drop of resuspended latex reagent into the oval. Place a drop for each specimen to be tested in separate ovals.
3. Use a fresh stick-end for each specimen. While holding the stick PERPENDICULAR to the agar surface, touch one, 2 mm fresh colony with the flat end of the stick.
4. For 10 SECONDS, thoroughly mix and blend the organisms into the latex reagent by lightly rubbing the surface of the card with the stick-end, to the inside limits of the oval. **NOTE!** Avoid damaging the card surface with vigorous rubbing.
5. Discard the stick into disinfectant after this step.
6. For 50 SECONDS, gently hand-rock the card to agitate the combination. Do not allow the combinations to spill over into adjacent ovals.
7. Clumping of the red latex should be instantaneous with most *S. aureus* strains. Ordinarily agglutination/clumping of the latex will progressively increase during the rocking of the card within its 50 SECOND period if *S. aureus* is the specimen being tested (see RESULTS section below). Record the results.
8. Dispose of the card into a disinfectant.



RESULTS

Positive result

A positive test occurs when agglutination and/or visible clumping, with simultaneous clearing of latex background, is observed by the red latex reagent on the white background in combination with a SPECIMEN within 60 SECONDS after the initial blending of a SPECIMEN and the detection latex. Although agglutination with *S. aureus* may be evident after blending, agglutination/clumping will progressively increase during the fifty seconds of rocking the card. When obvious latex agglutination is observed within this period, coagulase and/or Protein A was presented by the specimen and presumed to be *S. aureus*. The red latex will be obviously clumped which in turn will clear the red background to a very faint pink colour.

Negative result

A negative test result has occurred when no agglutination/clumping is observed by the red latex reagent within the 60 second period. A trace of granularity by the latex particles may be observed in combination with a coagulase-negative specimen. Ordinarily, a homogeneous background of red colour should be observed for most all negative results. A negative result indicates the absence of coagulase and Protein-A for that specimen.

LIMITATIONS OF THE PROCEDURE

1. Rough, stringy and non-interpretable results may occur when specimens have been grown on high salt-containing media and the culture is older than 48 hrs or when too much material is used. In these instances, the concentration of coagulase and Protein A may be reduced and consequently produce very weak agglutination patterns (alternatively obscured by non-specific interactions).
2. Stock cultures should be subcultured on sheep blood agar overnight before use in the test.
3. Continuous subculting of Staphylococci species has been shown to induce biological changes to the organisms. Thus, subsequent test results are produced which are different from the true results produced by the organism(s) when tested as the original clinical isolate.
4. Only fresh colonies should be used with the test. The colony should be gram-stained to confirm the morphology and gram-positive characteristics of *S. aureus*. Other tests should include acid production by the specimen in coloured agar plates containing carbohydrate(s) and anaerobic acid production using mannitol, as a substrate, can be performed (3,6).
5. Although other coagulase-positive staphylococci such as *S. hyicus* and *S. intermedius* can agglutinate the latex reagent, they are rarely associated with human infection. These species can be differentiated by growth on modified nutrient agars (7).

EXPECTED VALUES

A positive result has occurred when agglutination/clumping is observed by the red latex reagent in combination with fresh SPECIMEN within 60 seconds after the initial blending. When the specimen being tested is *S. aureus*, progressive agglutination should be observed during the hand rocking manoeuvre. If no agglutination is observed by the 60 second end point after the initial blending, coagulase and/or Protein A associated with *S. aureus*, is not present at detectable levels. This event is a negative test result.

PERFORMANCE CHARACTERISTICS

The Phadebact® Staph Aureus latex reagent was evaluated by independent laboratories using 836 cultured specimens with suspected staphylococci (8). The evaluation consisted of the Phadebact® Staph Aureus System compared to the classical tube coagulation. The specimens were both fresh isolates and stored cultures. Included among the staphylococcal specimens tested were methicillin-resistant or methicillin-sensitive *S. aureus* and coagulase-negative staphylococci. This characteristic of resistance or sensitivity did not interfere with the detection of coagulase or Protein A positive or negative staphylococci. Several media types, such as tryptic-soy agar, chocolate agar, sheep or horse blood agar, Mueller-Hinton agar and colistin-nalidixic acid agar were used for overnight culture. The results of the evaluation are summarized in the Table below:

		Coagulase Test	
		+	-
Phadebact®	+	549	3
Staph Aureus	-	5	279

The Phadebact® Staph Aureus latex reagent produced a relative sensitivity of 99.1% and a relative specificity of 98.9%. The red latex detection reagent provides an extremely usable format to more easily interpret latex clumping produced by coagulase-positive staphylococci.

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.

Bibliography:

1. *Kloos W J and Smith P B*: Staphylococci, 1980. In Manual of Clinical Microbiology, 3ed, Lennette E H, Balows A, Hausler Jr W J and Truant J P, ed., ASM, Washington, D.C.
2. *Finegold S M and Sweeney E E*, 1961. New selective and differential medium for coagulase-positive staphylococci allowing rapid growth and stain differentiation. J. Bacteriol. 81: 636-641.
3. *Kloos W E and Schleifer K H*, 1975. Simplified scheme for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. 1:82-88.
4. *Forsgren A and Sjöquist J*, 1967. "Protein A" from *Staphylococcus aureus.* Reaction with Rabbit Gamma-globulin. J. Immunol. 99:19-24.
5. *Aldridge K E, Kogos C, Sanders C V and Marier R L*, 1984. Comparison of Rapid Identification Assays for *Staphylococcus aureus*, J. Clin. Microbiol. 19:703-704.
6. *Hajek V*, 1976. *Staphylococcus intermedius*, a new species isolated from animals, Int. J. Syst. Bacteriol. 26:401-408.
7. *Roberson J R, Fox L A, Hancock D D and Bessor T E*, 1992. Evaluation of Methods for Differentiation of Coagulase-Positive Staphylococci. J. Clin. Microbiol. 30:3217-3219.
8. 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
Phadebact® ETEC-LT Test
Phadebact® Salmonella Test
Phadebact® Staph Aureus

Near Patient Testing

Phadirect® Strep A
Phadirect® Rapid CRP Test

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