LABORATORY PROCEDURE
BBL™ Staphyloslide™ Latex Test for *Staphylococcus aureus*

I. INTENDED USE

The **BBL™ Staphyloslide™** Latex Test is a latex slide agglutination test for the differentiation of staphylococci which possess clumping factor and/or Protein A, usually present with S*taphylococcus aureus*, from staphylococci that do not possess these properties.[1]

II. SUMMARY AND EXPLANATION

It has been reported that approximately 97 % of human strains of *S. aureus* possess both bound coagulase and extracellular staphylocoagulase. Protein A is found on the cell surface of about 95 % of human strains of *S. aureus* and has the ability to bind the Fc portion of immunoglobulin G (IgG).[2]

III. PRINCIPLES OF THE PROCEDURE

Traditionally, differentiation between coagulase positive and negative staphylococci has been performed with the tube coagulase test which detects extracellular staphylocoagulase or the slide coagulase test that detects the clumping factor (bound coagulase) present on the bacterial cell surface. Several other differentiation tests are also available, including the passive hemagglutination test and the DNase test.

The **BBL™ Staphyloslide ™** Latex Test consists of blue latex particles coated with human fibrinogen and IgG. On mixing the latex reagent with colonies of staphylococci which have clumping factor or Protein A present, cross-linking will occur giving visible agglutination of the latex particles. Such agglutination will occur notably with *S. aureus.* If neither clumping factor nor Protein A are present, no agglutination will occur and the result will be regarded as negative. The most frequent coagulase and Protein A negative isolates of staphylococci are *Staphylococcus epidermidis.*

IV. REAGENTS

		100 Tests	500 Tests
BBL™Staphyloslide™ Test Latex	Blue latex particles coated with human fibrinogen and IgG, with 0.1% sodium azide (preservative).	2x2.5mL	5x2.5mL
BBL™ Staphyloslide™ Control Latex	Blue unsensitized latex particles, with 0.1% sodium azide (preservative).	2x2.5mL	5x2.5mL
BL™ Staphyloslide™ Latex Reaction Cards	Disposable: each card may be used for 6 tests.	35 cards	175 cards

Precautions: For *in vitro* Diagnostic Use.

This product contains dry natural rubber.

Observe established precautions against microbiological hazards throughout all procedures. After use, contaminated materials must be sterilized by autoclaving for 15 min at 121°C.

REAGENTS: The test latex can become contaminated if the dropper tip is allowed to touch the specimen on the reaction card. Ensure caps on reagent bottles are securely fitted after each use to prevent contamination and drying out of the reagents.

WARNING: The reagents contain material of human origin. The human plasma proteins used in the manufacture of the reagent have been tested for the presence of the antibody to HIV (Human Immunodeficiency Virus), and Hepatitis C, HBsAG (Hepatitis B surface Antigen) and found not to be reactive. Because no test method can offer complete assurance that infectious agents are absent, SPECIMENS AND THESE REAGENTS SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING AN INFECTIOUS DISEASE. The U.S. Food and Drug Administration (FDA) recommends such materials be handled at a Biosafety Level 2. BSL 2 is referenced in the U.S. Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) manual. *Biosafety in Microbiological and Biomedical Laboratories.*

Reagents contain sodium azide, which is very toxic by inhalation, in contact with skin, and if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water. 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.

CARDS: Cards must be flat for proper reactions. If necessary, flatten cards by bowing back in a direction opposite to that of the curl. Care should be taken not to finger-mark the test areas, since this may result in an oily deposit and improper test result. Use each card once and discard. Store cards in the original package in a dry area at room temperature.

Storage: Upon receipt, store the kit at 2-8°C. DO NOT FREEZE. Under these conditions the reagents will retain their activity until the date shown on the bottle labels. After use, return the kit to the refrigerator, storing bottles in an upright position.

V. SPECIMEN COLLECTION AND PREPARATION

Staphylococcal isolates grown on nonselective media, such as blood agar, should be used.[3] Selective media such as Baird-Parker or mannitol salt agar may also give satisfactory results, but interpretation is more difficult due to the weak positive reactions and the tendency to produce stringy reactions. Subculture of isolates onto nonselective media is recommended. Test only fresh cultures or subcultures (18-36h incubation).

VI. PROCEDURES

Review "Precautions" and "Specimen Collection and Preparation" prior to performing procedures. The testing area, reagents, test specimens and test components should be at room temperature when used.

Materials Provided: All materials listed under "Reagents", and test disposables and accessories.

Materials Required But Not Provided: Timer, microbiological loop, or wooden mixing sticks. Bunsen burner, disinfectant, and Gram stain reagents and quality control organisms.

Also required are the necessary equipment and labware used for preparation, storage and handling of specimens.

Test Method: If fewer than six tests are performed, the card maybe cut with scissors and the unused portion saved for later use.

1. Mix the latex reagent by shaking; expel any latex from the dropper for complete mixing.

2. Dispense 1 drop of Test Latex onto one of the circles on the reaction card and add 1 drop of Control Latex onto another circle.

3. Using a microbiological loop pick up and smear 5 suspect colonies onto the Test Latex-containing circle and mix this into the Test Latex reagent. Spread to cover the circle.

4. Repeat step 3 for the Control Latex.

5. Pick up and hand rock the card for up to 20 sec and observe for agglutination under normal lighting conditions. Read macroscopically; do not use a magnifying glass.

6. Dispose of the reaction card in an appropriate biohazard container.

7. Re-cap the bottles and return to the refrigerator.

User Quality Control: Perform Daily Quality Control each day the kit is used. Follow methods given in "Test Method."

1. *Positive Control:* Use a known *S. aureus* strain such as ATCC™ 25923. Ensure agglutination occurs within 20 sec.
2. *Negative Control:* Use a known *S. epidermidis* strain such as ATCC™ 12228. Ensure that the reagent remains smooth and unagglutinated for the entire 20 sec of the test.

NOTE: Do not use the reagents if reactions with the control organisms are incorrect.

VII. INTERPRETATION OF TEST RESULTS

Positive Results: A positive result is obtained if agglutination of the blue latex particles occurs within 20 sec in the test circle, with no agglutination in the control circle. The result is positive when there is noticeable clearing of the blue background in the test latex. This indicates the presence of *S. aureus*.

Negative Results: A negative result is obtained if no agglutination occurs and a smooth suspension remains at 20 sec in the test circle. The result is negative when there is no noticeable clearing of the blue background in the test latex

Reactions occurring after 20 sec should be ignored.

Uninterpretable Results: The test is uninterpretable if the control reagent shows agglutination or autoagglutination.

Occasional granular or stringy reactions may be seen due to the particular nature of the test. If granular or stringy reactions occur, they should be interpreted using the following criteria: (1) The result is POSITIVE when there is a noticeable clearing of the blue background. (2) The result is NEGATIVE when there is no noticeable clearing of the blue background.

VII. LIMITATIONS OF THE PROCEDURE

Some staphylococci other than *S. aureus* may give positive coagulase results. These strains include *S. hyicus, S. intermedius, S. lugdunensis*, and *S. schleiferi*, which may also react in rapid latex tests. If necessary, these species would require identification using biochemical test procedures. Both *S. intermedius* and *S. hyicus* are rarely isolated from human specimens.[1]

Studies have shown that a limited number of MRSA strains may produce weak levels of clumping factor and protein A. These strains may fail to react in the latex test.[4]

Staphylococci isolated from urine specimens which give a weak positive[5] or stringy result with the **BBL™ Staphyloslide™** Latex Test may be *Staphylococcus saprophyticus*.

Further identification of such isolates may be conducted using biochemical tests and novobiocin sensitivity (*S. saprophyticus* is resistant to novobiocin).

Autoagglutination, where there is a partial clearing of the blue background, may occur if rough strains of staphylococci are tested or if a culture is incubated beyond 36 h. If the test colonies appear rough or autoagglutination is suspected, then the organism should be subcultured and retested at 18-24 h. If the organism continues to produce auto-agglutination, the test result is uninterpretable.

Some streptococci and possibly other organisms possessing immunoglobulin or Plasma-binding factors may react in the latex test and some species such as *Escherichia coli* are able to nonspecifically agglutinate latex particles.[6,7] To overcome these potential nonspecific results, a Gram stain should be performed so only typical staphylococci are tested.

The amount of protein A expressed by *S. aureus* is dependent on the medium, and other growth conditions. Rare strains of *S. aureus* that do not produce coagulase but still express some protein A on their cell surface may yield a weak positive reaction with the **BBL™ Staphyloslide™** Latex Test. If such a strain is suspected, alternate identification methods should be used.

The use of high salt media may result in a weaker reaction. Colonies taken from these media may be more difficult to emulsify and may therefore give rise to slightly stringy reactions.

IX. PERFORMANCE CHARACTERISTICS

In three clinical comparisons of the performance of the **BBL™ Staphyloslid**e™ Latex Test, the sensitivity of the Latex Test was 100% (530/530) and the specificity was 99% (379/383).

	BBL™ Staphyloslide™ Latex		**Commercial Latex Test**		**Tube Coagulase**		**No. of Strains Tested**
	+	-	+	-	+	-	
S. aureus[a]	**530**	**0**	**530**	**0**	**526**	**4**[c]	**530**
Non-*S. aureus*	**4**[b]	**379**	**4**[b]	**379**	**0**	**383**	**383**

a. To be classified as *S. aureus*, a positive result must be obtained in two or more established methods; e.g., slide coagulase, tube coagulase, DNase or biochemical tests.
b. All strains were identified biochemically as *S. saprophyticus*.
c. All discrepant strains were identified as *S. aureus* as determined by two or more of the following tests: slide coagulase, red cell agglutination, latex agglutination or biochemical methods.

X. AVAILABILITY

Cat. No.	Description
240952	**BBL™ Staphyloslide™** Latex Test Kit, 100 Tests
240953	**BBL™ Staphyloslide™** Latex Test Kit, 500 Tests
237052	**QualiSwab™**, *S. aureus*, ATCC™ 25923, one Swab.
237055	**QualiSwab™**, *S. epidermidis*, ATCC™ 12228, one Swab.

X. REFERENCES

1. Kloos, W.E., and T. L. Bannerman. 1995. *Staphylococcus* and *Micrococcus*, p. 282-298. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology. Washington, D.C.
2. Essers, L., and K. Radebold. 1980. Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test. J. Clin. Microbiol.*12*:641-643.
3. Taussig, M.J. 1984. Processes in pathology and microbiology . 2nd ed. Oxford; Boston: Blackwell Scientific Publ., St. Louis, Mo., Blackwell Mosby Book Distr. 520-530.
4. Roberts, J.I.S., and M.A. Gaston. 1987. Protein A and coagulase expression in epidemic and non-epidemic *Staphylococcus aureus*. J. Clin. Pathol. *40*:837-840.
5. Philips, W.E., and W.E. Kloos. 1981. Identification of coagulase-positive *Staphylococcus intermedius* and *Staphylococcus hyicus* subsp*. hyicus* isolates from veterinary clinical specimens. J. Clin. Microbiol. *14*:671-673.
6. Myhre, E.B., and P. Kuusela. 1983. Binding of human fibrinogen to group A, C, and G streptococci. Infect. Immun. *40*:29-34.
7. Runehagen, A., C. Schonbeck, U. Hedner, B. Hessel, and G. Kronvall 1981.Binding of fibrinogen degradation products to *S.aureus* and β-hemolytic streptococci group A, C and G. Acta Pathol. Microbiol. Scand. Sect B. *89*:49-55.

Technical Information: In the United States, telephone BD Diagnostic Systems Technical Services, toll free (800) 638-8663.

Approved by:

Supervisor: _____ Date: _____

Director: _____ Date: _____

Reviewed:

PI Rev. 11/99
Rev. 06/02