PROVIDENCE
Sacred Heart
Medical Center &
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StaphTEX Blue™ Rapid Latex Agglutination for *Staphylococcus aureus*

Department of Microbiology

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1.0 Purpose

Hardy Diagnostics StaphTEX™ Blue Kit is a rapid, latex agglutination test to detect coagulase and/or protein A produced by *Staphylococcus aureus*. The detection of coagulase and protein A permits the identification of at least 98% of *S. aureus* isolates. Coagulase is most commonly produced as a bound enzyme referred to as clumping factor. Coagulase converts fibrinogen to form a clot when plasma protein is added. Protein A is a cell wall constituent independent of coagulase. Protein A combines with the Fc portion of most IgG immunoglobulins and serves as an additional agglutination marker. The StaphTEX™ Blue Latex Reagent is coated with fibrinogen and IgG. Colonial growth is harvested from culture and mixed with the reagent on a

reaction card. If the test isolate possesses coagulase and/or protein A the bacterial cells will interact with the sensitized particles to produce visible agglutination, indicating a positive test for *S. aureus*.

2.0 Clinical Significance

Staphylococcus aureus is clinically the most important species, capable of causing a wide range of human diseases. In addition, the pathogen has become resistant to many of the therapeutic agents available. Diseases caused by *S. aureus* can be broadly divided into toxin-mediated diseases and suppurative infections comprising skin and soft tissue infections (SSTIs), systemic infections, and foreign-body-related infections. The spectrum of SSTIs ranges from superficial (impetigo, folliculitis, furuncles/carbuncles, hydradenitis suppurativa, pyoderma, and wound infections) to deep (abscesses, mastitis, cellulitis, and pyomyositis) to life-threatening necrotizing fasciitis and myositis. SSTIs are the most frequent infections associated with community-acquired MRSA. *S. aureus* is the most common cause of nosocomial pneumonia and skin and soft tissue infections. Infection of deep sites may involve any body compartments and organ systems resulting in empyemas, osteomyelitis, arthritis, endocarditis, pneumonia, otitis media, sinusitis, mastoiditis, and parotitis. Any localized *S. aureus* infection can become invasive and lead to bacteremia. Systemic infections comprise primary and secondary bacteremia, meningitis, and endocarditis. Bacteremia may be complicated by metastatic foci (e.g., vertebral osteomyelitis).

3.0 Scope

This procedure is classified under CLIA as highly complex. It should be carried out by technical personnel familiarized and trained on culture testing and interpretation. Testing includes but is not limited to: culture examination and workup, Quality Control, record keeping, and technical proficiency. Employee records are to be kept within the employee's file in the department documenting continued competence and proficiency for culture testing. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety & Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All cultures must be handled as potentially infectious material as outlined in the Microbiology Biohazards and Safety document. Follow proper handling and disposal of the used reaction card(s) and other items that come into contact with culture organisms. Place used reaction card(s) and other disposable items in a biohazardous waste container.

The reagent used in this procedure contains no hazardous ingredients, or the concentrations of the individual reagents are lower than the regulatory threshold limits. However, human source materials are used in the manufacturing of these reagents and should be treated as potentially infectious.

This procedure will expose you to:

- Pathogenic microorganisms

To perform this procedure, you must use:

- Laboratory Coat

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

5.0 Specimen Requirements

Isolates from overnight (18-24 h) cultures on sheep blood agar are recommended. Isolates growing on CHROMagar™ MRSA II may also be used for testing.

6.0 Materials

6.1 Consumables

- Wooden applicator sticks
- Disposable, white reaction cards with white reaction circles

6.2 Reagents & Control Materials

StaphTEX™ Blue Kit: Store at 2-8˚C. Do not freeze reagents. Immediately after use, return reagents to refrigerated storage. Products should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed.

- StaphTEX™ Blue Latex Reagent (Catalog Number ST1000), 1000 Reactions.
- StaphTEX™ Blue Positive Control Reagent
- StaphTEX™ Blue Negative Control Reagent

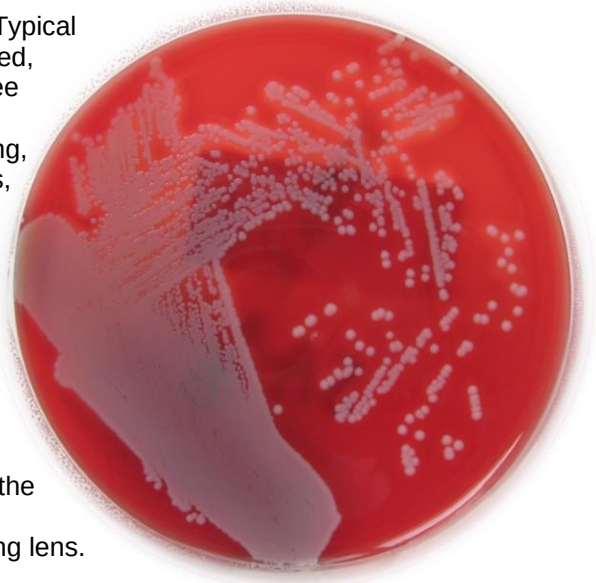
Control organisms: Maintain control strains as outlined in the QC Organism Maintenance Procedure.

- *Staphylococcus aureus* ATCC 25923
- *Staphylococcus epidermidis* ATCC 12228

7.0 Procedure

The Latex Reagent should be at room temperature prior to use. Remove the reagent from the refrigerator at least 10 min prior to use. Do not allow the tip of the latex vial to touch a specimen.

1. Perform the quality control on new lots or shipments as described before testing isolates from patient cultures.
2. Resuspend the Latex Reagent by inverting the vial several times. Hold the latex vial in a vertical position just over a white reaction circle on the reaction card. Squeeze the vial to deliver a drop of resuspended Latex Reagent into the reaction circle. Place a drop for each isolate to be tested in separate reaction circles.
3. Select colonies consistent with *Staphylococcus* species. Typical *S. aureus* colonies growing on blood agar are medium-sized, cream to yellow, smooth, slightly raised, and hemolytic (see image to the right). The typical appearance of coagulase-negative staph species is nonpigmented, smooth, glistening, and opaque. There are coagulase-negative staph species, including *S. lugdunensis* and *S. haemolyticus*, that may appear beta-hemolytic.



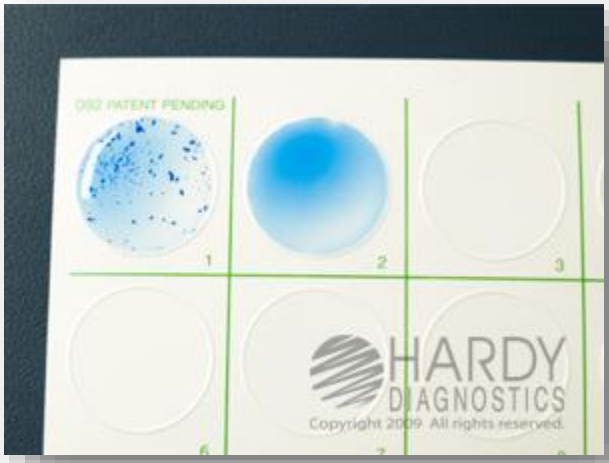
4. Use a separate wooden stick for each test isolate. Sample a colony with the flat end of the stick.
5. Thoroughly mix and blend the organisms into the Latex Reagent by lightly rubbing the surface of the reaction card inside limits of the reaction circle. Avoid damaging the reaction card surface with vigorous rubbing.
6. Discard the stick into a biohazard waste container.
7. Gently hand-rock the reaction card for 20 s. Do not allow the mixtures to spill over into adjacent reaction circles.
8. Examine mixture for agglutination. Do not use a magnifying lens. **Do not read results after 20 s.**
9. If the test isolate does not emulsify, or clumping is observed without a clearing of the background, check for autoagglutination by mixing the test isolate in a drop of water on a glass slide. Most staph isolates will emulsify into a smooth milk-colored suspension. If the inoculum clumps in the water control, the slide test cannot be interpreted and a tube coagulase test should be performed.

8.0 Interpretation & Reporting of Results

8.1 Positive Reaction

Agglutination and/or visible clumping of the Latex Reagent within 20 s after mixing the test isolate into the reagent indicates the presence of coagulase and/or protein A. Clumping of the Latex Reagent will be instantaneous with most *S. aureus* strains. Document the test result in the isolate workup. Report as *Staphylococcus aureus* if the test is positive and no autoagglutination is observed. If the test is positive but the colony morphology of the isolate is inconsistent with *S. aureus*, Report *Staphylococcus* species and confirm with a tube coagulase test.

Positive (left circle) and negative (right circle) agglutination for the StaphTEX™ Blue



8.2 Negative Reaction

A negative result is indicated when the particles do not agglutinate and the appearance of the reagent remains unchanged throughout the test. Document the test result in the isolate workup. Report the isolate as coagulase-negative staphylococci. When appropriate, screen for *Staphylococcus lugdunensis* as indicated in the Isolate Workup Charts.

9.0 Quality Control & Quality Assurance

Quality Control should be performed on each lot or shipment received using positive and negative control reagents and ATCC control strains. Quality Control with the manufacturer's reagents complies with the package insert while QC with the ATCC strains provides lot to lot comparison of the latex StaphTEX™ Blue Latex Reagent.

9.1 QC with Control Reagents

1. Place a drop of resuspended Latex Reagent in two, separately identified reaction circles on the reaction card.
2. Place a drop of the resuspended Positive Control and Negative Control Reagents in their identified reaction circles on the reaction card.
3. Use a wooden applicator stick to thoroughly mix the reagents by lightly rubbing the surface of the reaction card inside limits of the reaction circle.
4. Gently hand-rock the reaction card for 20 s.
5. Examine mixture for agglutination. Do not use a magnifying lens.
6. The Positive Control Reagent must provide obvious agglutination, while the Negative Control Reagent must not produce agglutination within the 20 s.

7. Notify the supervisor and/or technical specialist if the expected quality control results are not observed.
8. Document QC results in the LIS.

9.2 QC with Control Organisms

1. Use fresh 18-24 h old cultures of the control organisms. If necessary, prepare subcultures and test the new lot/shipment on the following day.
2. Test the control organisms as outlined above under the Procedure section.
3. *Staphylococcus aureus* ATCC 25923 must provide obvious agglutination, while *Staphylococcus epidermidis* ATCC 12228 must not produce agglutination within the 20 s.
4. Notify the supervisor and/or technical specialist if the expected quality control results are not observed.
5. Document QC results in the LIS.

10.0 Limitations

1. Do not perform testing from growth on mannitol salt agar. Rough, stringy and non-interpretable results may occur when isolates have been grown on high salt-containing media.
2. Only 18-24 h old colonies should be used with the test. Some streptococci, *Escherichia coli, C. albicans* and possibly other organisms that possess immunoglobulin binding factors may also agglutinate Latex Reagents non-specifically. Colonies with atypical morphology should be Gram stained to confirm that the organism cells are gram-positive cocci in clusters. Only catalase-positive colonies should be tested with StaphTEX™ Blue.
3. If the suspension of organism used is not heavy enough, the reaction may be weak and slow in agglutinating, and may lead to erroneous results.
4. Due to a drying effect, false-positive reactions may occur if reaction times longer than specified are used.
5. The advantage of agglutination assays is that they are very sensitive and rapid in separating *S. aureus* from other coagulase-negative staphylococci. False-positive reactions do occur with strains of *S. capitis, S. saprophyticus,* and, *S. warneri*, since these species possess protein A. *S. saprophyticus* is most commonly isolated in urine cultures and can be differentiated by the rose-colored colonies it produces on CHROMagar Orientation. *S. lugdunensis* and *S. schleiferi* can also give a positive result, since they possess clumping factor. Use other methods, such as PYR, to detect these strains in invasive sites. Positive tests from isolates that do not resemble *S. aureus* should be confirmed by a tube coagulase.
6. *S. hyicus* and *S. intermedius* may also agglutinate the Latex Reagent, but it is clinically not important to separate these animal pathogens from *S.* aureus, because they are rarely found in humans (< 0.1%) and they are considered as pathogenic as *S. aureus.*
7. A false-negative test, especially for MRSA with capsular antigens, can result. The tube coagulase test will detect these strains and should be performed on isolates that produce colonies consistent with *S. aureus* but produce a negative slide coagulase result.

11.0 Verification Information

The StaphTEX™ Blue (Hardy Diagnostics) was evaluated and compared with Staphaurex® (Remel) using a total of 61 staph isolates, including 28 *S. aureus* and 33 coagulase-negative staph. The 28 *S. aureus* included 23 clinical isolates and 5 ATCC strains (25923, 29213, 43300, BAA-976, and BAA-977). The 33 coagulase-negative staph included 31 clinical isolates and 2 ATCC strains (*S. epidermidis* 12228 and *S. saprophyticus* 15305). Isolates were tested from either BBL™ TSA II™ with 5% Sheep Blood Agar (BAP) or BBL™ CHOMagar™ MRSA II. The table below summarizes the results from the study. No false positives were encountered with either reagent. However, the StaphTEX Blue reagent detected 1 *S. aureus* isolate (4%) that was missed by the Staphaurex® reagent. The coagulase reaction of this isolate was confirmed with a tube coagulase test. The StaphTEX Blue reagent also produced reactions that were stronger, faster, and easier to interpret.

	Isolates Tested	No. (%) of isolates correctly identified	
		Staphaurex	StaphTEX
Staphylococcus aureus			
MRSA	14	14	14
MSSA	14	13	14
Subtotal	**28**	**27 (96)**	**28 (100)**
Coagulase-negative Staph			
S. lugdunensis	4	4	4
S. saprophyticus	2	2	2
Other CoNS	27	27	27
Subtotal	**33**	**33 (100)**	**33 (100)**

12.0 References

1. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C
2. Package insert: Hardy Diagnostics, StaphTEX™ Blue Kit Instructions for Use, 11/08/2011.

13.0 Document Control History

Adopted/Reviewed by director (AR) 03/29/2013

Supervisor reviews: Jerry Claridge 04/01/2013, Jason Ammons 05/2015