STAPHTEX™ BLUE KIT

Cat. no. ST50	StaphTEX™ Blue Kit	50 tests/box
Cat. no. ST200	StaphTEX™ Blue Kit	200 tests/box
Cat. no. ST1000*	StaphTEX™ Blue Kit	1000 tests/box
Cat. no. PL091HD	Wooden Sticks	75 sticks/bag
Cat. no. PL092HD	Latex Agglutination Cards, 10 wells	48 cards/pack
* wooden sticks and latex cards not included, sold separately		

INTENDED USE

Hardy Diagnostics StaphTEX™ Blue Kit is a rapid, latex agglutination slide-card test to detect coagulase and/or protein A characteristics associated with *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*(MRSA). The StaphTEX™ Blue Latex Reagent will react with either or both of these two characteristics.

SUMMARY

Staphylococcus aureus is demonstrated 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 poisonings. It is also a common cause of nosocomial infection.(1)

The coagulase and protein A characteristics associated with *S. aureus* allow for the identification of at least 98% of this species. The StaphTEX™ Blue Latex Reagent is coated with fibrinogen and IgG to aid in the detection of *S. aureus*. Coagulase can either be bound (clumping factor) to the staphylococci or released as a free enzyme. Coagulase converts fibrinogen to form a clot when plasma protein is added to coagulase-positive *S. aureus*. Protein A, on the other hand, is independent from coagulase activity. Protein A is a constituent of the *S. aureus* cell wall that combines with the Fc portion of most IgG immunoglobulins and serves as an additional agglutination marker.(4)

Differential media have 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. In contrast to lengthy culture procedures, the speed, convenience and accuracy of the StaphTEX™ Blue Kit provides an appropriate alternative test. Rapid slide latex agglutination tests have been shown to be as reliable as the tube coagulase system in most cases.(5)

The blue-latex particles used in the StaphTEX™ Blue Latex Reagent are sensitized with specific concentrations of 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/clumping, indicating a positive test for *S. aureus*.

MATERIALS SUPPLIED

StaphTEX™ Blue Latex Reagent:	Blue-latex particles coated with IgG and human fibrinogen. The latex particles are suspended in a buffer containing 0.098% sodium azide as a preservative.
StaphTEX™ Blue Positive Control Reagent:	A formulation of non-viable *S. aureus* in a buffer.
StaphTEX™ Blue Negative Control Reagent:	A formulation of non-viable, coagulase-negative *Staphylococcus* spp. in a buffer containing 0.098% sodium azide as a preservative.
Wooden sticks*	
Disposable, white slide-cards with white reaction circles*	
Instructions for test use	
* Not supplied with Cat. no. ST1000	

MATERIALS REQUIRED BUT NOT PROVIDED

A timing device.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

Do not dilute the StaphTEX™ Blue Latex or StaphTEX™ Blue Control Reagents or interchange with other StaphTEX™ Blue Kit lot numbers. Proper handling and disposal of the used slide-card(s) and other items that come into contact with culture organisms must be employed. Place used slide-card(s) and other disposable items in a container with laboratory disinfectant. The container should be autoclaved.

Reagents contain sodium azide. Sodium azide can react explosively with copper or lead if allowed to accumulate in large concentrations. Although the amount of sodium azide is minimal, large amounts of water should be used when flushing used reagents down the sink.

Human source materials used in the manufacture of the reagents have been tested and found negative for antibody to HIV and HBs Ag. Although, the concentration of such materials in the reagents are very low, no known test

methods can offer complete assurance that infectious agents are absent. Therefore, StaphTEX™ Blue Latex Reagents, StaphTEX™ Blue Positive Control Reagent and StaphTEX™ Blue Negative Control Reagent should be handled using the same safety precautions employed when handling any potentially infectious material.

STABILITY OF THE REAGENTS

Settling of the latex particles may occur when stored at 2-8°C for a period of time. After several inversions of the latex vial, the StaphTEX™ Blue Latex Reagent should appear as a homogenous suspension of blue particles. If non-specific clumping is observed, which is not dispersed by normal resuspension procedures, do not use the reagent. The Positive Control Reagent must agglutinate the Latex Reagent. If either no agglutination using the Positive Control Reagent or agglutination with the Negative Control Reagent is observed after mixing them separately with the Latex Reagent, a loss of reagent stability may have occurred. Contact Hardy Diagnostics in these instances.

The kit should be discarded upon its expiration date.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. For optimal stability, remove only one vial from the refrigerator at a time. **Do not freeze reagents.** Immediately after use, return the reagents to refrigerated storage. Products should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" for more information.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

It is recommended that an overnight (18-24 hour) culture of a primary plated clinical specimen, using Sheep Blood Agar or Nutrient Agar, be used to provide a fresh, sufficient sized (2mm) colony. A secondary subculture to isolate the species of a mixed culture will sometimes be necessary before testing. However, repeated subculturing of staphylococci has been shown to allow these organisms to manifest different characteristics from the original isolate.[6]

For specific procedures regarding specimen collection and preparation refer to the listed references. In general, the colony should be gram stained to confirm the morphology and gram-positive characteristics of the organism being tested.[3,7]

PROCEDURE

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

1. Perform the quality control steps as outlined before testing fresh colony specimens.

2. Resuspend the Latex Reagent by several inversions of the vial. Hold the latex vial in a vertical position just over a white reaction circle on the slide-card. Squeeze the vial to deliver a drop of resuspended Latex Reagent into the reaction circle. Place a drop for each specimen to be tested in separate reaction circles.

3. Use a fresh stick for each specimen. While holding the stick perpendicular to the agar surface, touch one 2mm fresh colony with the flat end of the stick.

4. Thoroughly mix and blend the organisms into the Latex Reagent by lightly rubbing the surface of the slide-card with the stick to the inside limits of the reaction circle.
Note: Avoid damaging the slide-card surface with vigorous rubbing.

5. Discard the stick into disinfectant.

6. For 20 seconds, gently hand-rock the slide-card to agitate the combination. Do not allow the combinations to spill over into adjacent reaction circle.

7. Clumping of the Latex Reagent should be instantaneous with most *S. aureus*strains. Record the results according to the interpretation section below. **Do not read results after 20 seconds from the initial blending.**

8. Follow appropriate procedures for disposal of infectious material.

INTERPRETATION OF RESULTS

A positive test has occurred when agglutination and/or visible clumping of the Latex Reagent/specimen combination is observed within 20 seconds. When obvious latex agglutination is observed within this period, coagulase and/or protein A is present and the specimen is presumed to be *S. aureus*. In a positive reaction, a significantly rapid and strong clumping (within 20 seconds) with the Latex Reagent is observed.

A negative test result has occurred when no agglutination/clumping is observed by the Latex Reagent within the 20 second period. Ordinarily, a homogeneous background should be observed for most negative results. A negative result indicates the absence of coagulase and/or protein A for that specimen. Occasionally, a trace of granularity by the latex particles may be observed with a coagulase-negative specimen.

LIMITATIONS

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 hours. In these instances, the concentration of coagulase and/or protein A may be reduced and consequently produce very weak agglutination patterns.

Stock cultures should be subcultured onto Sheep Blood Agar (Cat. no. A10) overnight before use in the test.

Only 18-24 hour old 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* . Only catalase-positive colonies should be tested with StaphTEX™ Blue.

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.

Although other coagulase-positive staphylococci such as *S. hyicus* and *S. intermedius* can agglutinate the Latex Reagent, they are rarely associated with human infection.[6]

Due to a drying effect, false-positive reactions may occur if reaction times longer than specified are used.

Some streptococci, *Escherichia coli*, *C. albicans* and possibly other organisms that possess immunoglobulin binding factors may also agglutinate Latex Reagents non-specifically.[10] Therefore, test only gram-positive catalase-positive organisms with the StaphTEX™ Blue Kit.

Some rare species of clumping factor-positive *S. lugdunensis* and *S. schleiferi* may yield positive or weakly positive latex agglutination results. Novobiocin resistant strains may also agglutinate with the test reagent and yield a false-positive result. If necessary, further identification using biochemical test procedures should be performed. Confirmation of *S. lugdunensis* may be accomplished through use of the tube coagulate test (negative), Cat. no. Z202; PYR test (positive), Cat. no. Z75; or Rapid Ornithine test (positive), Cat. no. K279.

Refer to the document "Limitations of Procedures and Warranty" for more information.

QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared culture media may be required to perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction (where applicable). Refer to the following documents on the Hardy Diagnostics Technical Document website for more information on QC: " Introduction to Quality Control," " Finished Product Quality Control Procedures," or " The CLSI Standard and Recommendations for User Quality Control of Media." Also see the following reference for more specific information.(1-11)

1. Place a drop of resuspended Latex Reagent in two separately identified reaction circles on the slide-card.

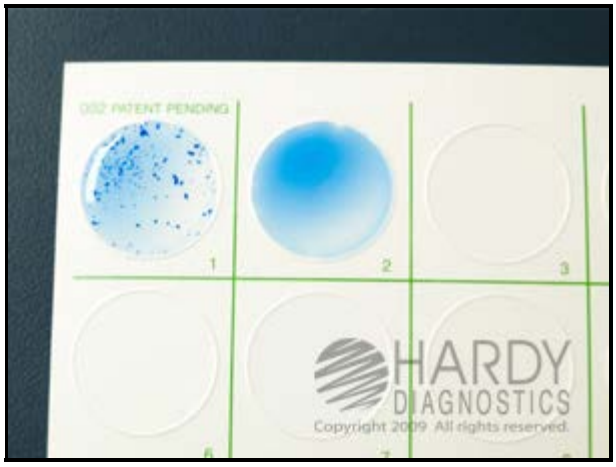
2. Place a drop of the resuspended Positive Control and Negative Control Reagents in their identified reaction circles on the slide-card.

3. See the Procedure section of this document.

4. The Positive Control Reagent must provide obvious agglutination, while the Negative Control Reagent must not produce agglutination within the 20 second period.

5. Notify Hardy Diagnostics if the expected quality control results are not observed.

Hardy Diagnostics' StaphTEX™ Blue Kit (Cat. no. ST50).



Showing positive (left circle) and negative (right circle) agglutination for the StaphTEX™ Blue Kit (Cat. no. ST50).

PERFORMANCE CHARACTERISTICS

The StaphTEX™ Blue Kit was evaluated by an independent laboratory using 100 isolates including 50 *S. aureus*, 30 MRSA isolates, and 20 coagulase-negative *Staphylococcus* (CNS) isolates (including 10 identified by API® as *S. epidermidis*). All testing was performed in parallel using the StaphTEX™ "white" kit and StaphTEX™ Blue Kit. All 50 *S. aureus* isolates were latex positive with both StaphTEX™ "white" and StaphTEX™ Blue Kits. One isolate required repeat testing with the StaphTEX™ "white" kit. All MRSA isolates tested positive in both kits. Negative results were obtained with the CNS isolates using both kits.(11)

The study also attempted to gauge the speed of the reaction using batch sizes of 10 isolates and also determine the average agglutination time for one test. In both instances, the speed of the reaction was faster using the StaphTEX™ Blue Kit. The average agglutination time for single isolates using the StaphTEX™ Blue Kit was more rapid, with agglutination occurring within four to five seconds, and was easily detected within the recommended 20 second testing period. Using StaphTEX™ "white", positive results were seen in eight to nine seconds. The study also showed that the agglutination reaction was visually stronger using the StaphTEX™ Blue Kit.(11)

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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