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1.0 Principle

Coagulase is a thermostable thrombin-like substance that activates fibrinogen to form fibrin, resulting in a fibrin clot. This is demonstrated in the test tube by the formation of a clot when plasma is inoculated with the staphylococcus. The substance is known as free coagulase, since it is liberated by the cell. In most, but not all, *S. aureus* organisms, a fibrinogen binding cell surface receptor is also present in cell wall, called “bound coagulase” or “clumping factor.” Clumping factor is demonstrated by the ability of the organism to act directly on the fibrinogen in the plasma to clump it in a slide assay. The test for clumping factor is rapid but requires several colonies, and as stated above, the factor is not always present in all *S. aureus* isolates. In addition, clumping factor can be masked by cell surface capsular polysaccharides and can be present in other species, namely, *Staphylococcus lugdunensis* and *Staphylococcus schleiferi*.

Negative slide tests on isolates with colonies resembling *S. aureus* must be followed with a confirmatory tube test.

2.0 Clinical Significance

Staphylococcus aureus, the most pathogenic of the staphylococci, is separated from other species by the presence of coagulase.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained to identify *Staphylococcus* spp. Testing includes but is not limited to: morphologic recognition, confirmatory testing, and Quality Control testing of reagents.

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 Providence Sacred Heart Microbiology Safety Guidelines.

More extensive information concerning the safe handling of the reagents used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

To perform this procedure, you must use:

- Laboratory Coat – must be worn when handling cultures and reagents.

Disinfectant following procedure:

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

Reference for spill/decontamination:

- MSDS/SDS

5.0 Microorganisms/Samples Tested

- Colonies of gram-positive cocci in clusters which are catalase positive, as part of the identification of *S. aureus*.
- Positive blood cultures containing gram-positive cocci in clusters for rapid detection of *S. aureus*.

6.0 Materials

6.1 Consumables

- Loops or wooden applicators
- Glass slides or black coated cards
- Glass test tubes

6.2 Reagents

- Frozen rabbit plasma with EDTA ([Hardy Diagnostics Coagulase Cryo™](#), Cat. No. Z202), 20 vials/package. Store below -2°C. Product can be shipped without refrigeration if transit time is less than 1 week. Do not thaw and refreeze more than once. Reagent should not be used if there is any sign of deterioration, or if the expiration date has passed. Product is light and temperature sensitive.

7.0 Procedure

To avoid misidentifications, only perform this test on characteristic white to yellow, creamy, opaque, hemolytic colonies of gram-positive cocci in clusters that are catalase positive. Hemolysis should only be observed on a fresh BAP at 18- 24 h.

Remove Coagulase Cryo™ from freezer and thaw to room temperature. Remove only the number of vials that are required for testing that day to avoid thawing and refreezing.

7.1 Slide Coagulase Test Procedure

1. Place a drop of coagulase plasma on a clean, dry glass slide.
2. Place a drop of distilled water or saline near the drop of plasma as a control.
3. With a sterile loop or wooden stick, select colonies to be tested, and emulsify the isolate into each drop, inoculating the water or saline first. Try to create a smooth suspension.
4. Observe for clumping in the coagulase plasma and a homogenous suspension in the control.

7.2 Tube Coagulase Test Procedure

1. Using a culture that is less than 24 h old, inoculate the Coagulase Cryo™ by emulsifying one loop full (2-4 colonies) of bacteria into the liquid. For positive blood cultures, inoculate the plasma reagent with 2 drops of blood culture broth.
2. Incubate the inoculated tube at $35 \pm 2^\circ\text{C}$ without CO_2 for up to 4 h, and observe for clot formation hourly. Do not agitate the tube during observations. Gently tilt the vial to observe for clot formation. Negative tests at 4 h should be held at room temperature for a total of 24 h before reporting results.

Note: Prolonged incubation at $35 \pm 2^\circ\text{C}$ for more than 4 h may allow some strains to produce fibrinolysin, which will break up the clot resulting in a false-negative reaction. Incubation at room temperature after the initial 4 h will prevent fibrinolysin production.

8.0 Interpretation of Results

8.1 Slide Coagulase Interpretation

Clumps that will not mix uniformly into coagulase plasma represent a positive slide coagulase test and are indicative of *S. aureus*. Colonies that mix smoothly into the plasma indicate a negative slide coagulase test. Clumping in both the coagulase and control indicate that the organism autoagglutinates and is unsuitable for the slide coagulase test. When autoagglutination is observed, the tube coagulase test should be employed as an alternative to the slide agglutination test.

8.2 Tube Coagulase Interpretation

Any degree of clotting of the plasma reagent before 24 h indicates a positive test. A flocculent or fibrous precipitate is not a true clot and should be regarded as negative.

9.0 Quality Control & Quality Assurance

Perform QC of coagulase plasma on new lots/shipments prior to use for testing patient isolates. If testing with the following control strains does not produce the expected results, do not use reagents for testing patient isolates. Results should be documented in LIS.

- *S. aureus* ATCC 25923 – coagulase positive (slide and tube)
- *S. epidermidis* ATCC 12228 – coagulase negative (slide and tube)

10.0 Limitations

1. MRSA can be deficient in bound coagulase, which results in a negative slide test.
2. *S. intermedius* and *S. hyicus* maybe positive in the tube test. These species are generally found only in dogs and pigs, respectively, but are as infectious as *S. aureus* when they infect humans.

3. *S. lugdunensis* and *S. schleiferi* produce clumping factor resulting in a positive slide test and a negative tube coagulase test.
4. It is not recommended that colonies from high-salt containing agars, such as Mannitol Salt Agar, be used with the slide coagulase test.

11.0 Verification Information

Rabbit plasma with EDTA has been historically used for slide and tube coagulase testing on isolated colonies. However, it was not previously utilized to test positive blood cultures. An evaluation was performed to determine the performance of rabbit plasma with instrument-positive blood cultures demonstrating gram-positive cocci in clusters in order to shorten the time to identification. Bottles that produce a positive tube coagulase result within 4 h can be further characterized as MRSA vs. MSSA using other rapid techniques.

A total of 31 blood cultures, demonstrating staph on the Gram stain, were tested by adding 2 drops of the blood culture broth to 0.5 mL of plasma. The tubes were then incubated at $35 \pm 2^\circ\text{C}$ for up to 4 h. The tubes were examined for clot formation after each hour of incubation. Twenty-two of the cultures produced *S. aureus* and nine produced coagulase-negative staphylococcus species. The table below summarizes the results.

	n	No. Positive (%)				Total
		1 h	2 h	3 h	4 h	
<i>Staphylococcus aureus</i>	22	5 (23)	6 (27)	3 (14)	4 (18)	18 (82)
Coagulase-Negative Staph	9	0	0	0	0	0

A total of 18 (82%) of the *S. aureus* isolates produced a positive tube-coagulase within 4 h of incubation. None of the coagulase-negative staph isolates produced a clot.

The tube coagulase test offers an economical method to screen positive blood cultures for *S. aureus*. This would permit rapid identification of *S. aureus* and help target the use of more expensive tests that differentiate MRSA from MSSA.

12.0 References

1. Instructions for use: Hardy Diagnostics Coagulase Cryo™, 011311jf.
2. www.hardydiagnostics.com
3. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

13.0 Document Control History

Medical Director: Dr. Joseph Schappert 03/10/2010

Microbiology Director Approval: Dr. Ann Robinson 02/02/2006

Microbiology Supervisor Reviews: Jerry Claridge 02/01/2006, 11/2006, 10/2007, 05/2008, 05/2009, 04/01/2011, 03/2013

Revisions: 02/03/2014 Combined slide and tube coagulase procedures into one. Added information for testing positive blood cultures with GPC in clusters. Changed reagent from BBL Coagulase Plasma to Hardy Coagulase Cryo.