**PURPOSE:**

This document describes the procedure for detection of free coagulase produced by *Staphylococcus aureus*.

**PRINCIPLE:**

*Staphylococcus aureus* can produce two types of coagulase, bound (clumping factor) that remains attached to the cell wall and free (unbound) produced extracellularly. Organisms that do not produce clumping factor must be tested for the ability to produce free coagulase. This is accomplished by inoculating a pure culture of the organism in rabbit plasma and incubated at 35 - 37 C˚. The formation of a clot in the plasma indicates production of free coagulase.

**POLICY:**

Organisms suspected of being Staph aureus that do not produce clumping factor using a commercial slide test will be tested for free coagulase.

**SCOPE:**

This procedure applies to UPMC Hanover Hospital Microbiology laboratory.

**REAGENTS/SUPPLIES:**

Rabbit coagulase plasma with EDTA (3 ml)

* Store unreconstituted reagent at 2 - 8 C˚ until expiry date listed.
* Reconstiututed plasma may be stored at 2 - 8 C˚ for 14 days.
* Do not use reconstituted reagent if it shows evidence of contamination, evaporation or other signs of deterioration, such as cloudiness or partial clotting.

Inoculating loop

Culture tubes 10 x 75 mm

**REAGENT PREPARATION:**

Rehydrate the lyophilized product with 3 ml sterile purified water. Mix by gentle end over end rotation of the vial.

**QUALITY CONTROL:**

* Positive (clot in tube): *Staphylococcus aureus* ATCC 25923
* Negative (no clot in tube): *Staphylococcus epidermidis*  ATCC 14990

Quality control is performed at the time of use to check performance of the coagulase plasma.

**SPECIMEN:**

Well isolated colonies taken from a blood agar plate.

**PROCEDURE:**

1. Using a sterile pipette, add 0.5 ml of rabbit coagulase plasma to a 10 x 75 mm.

2. Using a sterile loop, emulsify several colonies (minimum of 1ul loop) in the plasma.

3. Mix gently.

4. Incubate in at 35 - 37˚ C. for up to 6 hours. If no clot is visible after 6 hours, continue to incubate the tube for up to 24 hours.

5. Examine the tube periodically by gently tipping the tube. Avoid agitating or shaking the tube which could cause breakdown of the clot.

6. If no clot is visible after 6 hours, continue to incubate the tube for up to 24 hours. Many weak producing coagulase strains will coagulate the plasma only after 24 hours incubation.

**RESULTS:**

Positive Any degree of clotting

Negative No evidence of clotting

Note: Any degree of clotting in the coagulase plasma is considered a positive test. Refer to package insert for visual representation of positive tests.

**LIMITATIONS OF THE PROCEDURE:**

Some strains of *S. aureus* may produce staphylokinase, which may lyse clots. If tubes are read until 24 hours, false negatives may occur. Tubes must be monitored the first 6 hours.

**REFERENCE:**

BD BBL Coagulase Plasmas package insert. Becton, Dickinson and Co. 2022.