COAGULASE TEST

Principle and Purpose
Staphylococcus aureus is the major pathogen among species of Staphylococcus. It possesses several traits that enhance this bacterium’s ability to cause disease. These traits, termed virulence factors, include toxins as well as two types of coagulase enzymes. One type of coagulase remains attached to the cell wall of the bacterium, whereas the other enzyme is a secreted product. Both coagulase enzymes bind to fibrinogen, a soluble blood protein involved in the clotting of blood. Hence, when present, coagulase promotes the attachment of S. aureus to blood clots and damaged tissues, as well as indwelling medical devices. In addition, coagulase converts fibrinogen to fibrin, which coats and protects S. aureus from attack by the immune system. Hence, coagulase fosters the survival and propagation of this pathogenic microbe.

The presence of coagulase is considered to be solely associated with S. aureus and is used to differentiate this species from other staphylococci. In the following exercise, students will use a commercial reagent to detect the presence of coagulase. Specifically, the reagent Coagulase Cryo™ (Hardy Diagnostics) contains rabbit plasma which tests for the presence of coagulase (Fig. 1). This reagent can be used either in a tube test or as a slide test. The tube coagulase test is generally used to assess the presence of secreted enzyme, whereas the slide test detects the presence of cell-wall bound enzyme. Students shall perform both methods as described below.

Learning Objectives
Upon completion of this exercise, a student should be able to:
• Understand the biochemical basis of the coagulase test;
• Properly perform both the tube and slide coagulase tests; and
• Discern how this information can be used to differentiate staphylococcal species.

Materials Required
The following materials are necessary to successfully conduct this exercise:
Organisms - The following organisms should be provided as 18-24 hour-old TSA plate cultures:
• Staphylococcus aureus (ATCC 25923) [abbreviated as S. aureus]
• Staphylococcus epidermidis (ATCC 12228) [abbreviated as S. epidermidis]

Students are encouraged to learn more about the physiology of coagulase and the basis of testing for this enzyme by viewing the following video: https://youtu.be/6xMclwJGjDo.

Figure 1. Frozen Tube of Coagulase Cryo™ Reagent. (www.hardydiagnostics.com)
Materials

- Sterile plastic bulb pipettes
- Sterile water or sterile saline
- Sterile toothpicks
- Coagulase Cryo™ reagent (Cat. No. Z202; Hardy Diagnostics, Santa Maria, CA; [https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/CoagulaseCryo.htm](https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/CoagulaseCryo.htm))

**Note:** The Coagulase Cryo™ reagent must be kept at -20°C until needed. The laboratory instructor will retrieve the reagent from storage when needed by students.

Procedures

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

Students shall perform two different coagulase tests simultaneously and compare the results.

**Tube Coagulase Method**

1) Remove two tubes of Coagulase Cryo™ from the freezer and permit them to thaw to room temperature (Fig. 1).

2) When thawed, label one tube as *S. aureus* and one tube as *S. epidermidis*.

3) Using a microbiological loop or sterile toothpick, aseptically transfer 2-4 colonies of *S. aureus* grown overnight on a TSA plate to the appropriately labeled Coagulase Cryo™ tube being sure to thoroughly emulsify the colonies in the reagent. Similarly, aseptically transfer 2-4 colonies of *S. epidermidis* to the second labeled tube of reagent.

**Option for Broth Cultures of Test Organisms:** If the organisms to be tested are suspended in a broth medium, the following inoculation procedure can be used.

Using a sterile plastic bulb pipet, add approximately 2 drops (about 0.05 ml) of an overnight TSB culture of *S. aureus* to the appropriately labeled tube. Similarly, use a separate sterile plastic bulb pipet to inoculate the second tube of reagent with *S. epidermidis*. Gently mix both tubes by ‘flicking’ them several times.

4) Incubate both tubes at 37°C for 4 hours. Although positive results may occur before this incubation period is concluded, results are best read at 4 hours.

**Figure 2. Coagulase Positive (lower tube) and Negative (upper tube) Results.** A loopful of *Staphylococcus epidermidis* and *Staphylococcus aureus* were emulsified in Coagulase Cryo™ reagent (upper and lower tubes, respectively). The tubes were incubated for four hours at 37°C. The lower tube shows the ‘gelling’ of the reagent by *S. aureus*, whereas upper tube remains fluid depicting a negative coagulase reaction.
Interpretation of Results: A positive test for coagulase production results in the clotting ('gelling') of the rabbit plasma (Fig. 2). Any degree of clotting is considered a positive test. A positive coagulase reaction can be determined by turning the tube at an angle. If the liquid in the tube does not move, it has gelled/clotted. If the liquid remains fluid, then the result is negative for coagulase production.

Record any observations on the data report sheet attached to this document.

Note: Negative tests at 4 hours should be held at room temperature for a total of 24 hours before recording the results. Prolonged incubation at 37°C may cause 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 hours will prevent fibrinolysin from forming.

Slide Coagulase Test

Students are encouraged to the following video that depicts the procedure for conducting the slide coagulase test: https://youtu.be/NBcMypWZo18.

1) Remove a single tube of Coagulase Cryo™ from the freezer and permit it to thaw to room temperature.
2) To two separate clean, dry glass slides, label one S. aureus and the other S. epidermidis.
3) Using a sterile plastic bulb pipet, place a drop of coagulase reagent to one side on each glass slides.
4) Using a second sterile plastic bulb pipet, place a drop of sterile distilled water or saline on the side opposite of the reagent on both slides. This drop of water/saline shall serve as a negative control.
5) With a microbiological loop or sterile toothpick, aseptically pick a portion of an isolated colony of S. aureus from the agar plate culture provided. To the appropriately labeled slide, transfer these cells to the water/saline drop and create a smooth suspension.
6) Likewise, use the microbiological loop to aseptically pick the remaining portion of the previously selected colony of S. aureus. To the appropriately labeled slide, transfer these cells to the to the drop of coagulase reagent and create a smooth suspension.
7) Similarly, repeat steps 5 and 6 to create suspensions of S. epidermidis in the saline/water and coagulase reagent drops on the appropriately labeled slide. Pick the colony from the agar plate culture provided.
8) Keep the slides at room temperature. Observe these slides for 5-10 minutes looking for clumping in the coagulase reagent and a homogenous suspension in the water/saline drop.

**Interpretation of Results:** Clumps that form in the coagulase reagent indicate a positive test, whereas a uniform suspension is indicative of a negative test. The cells in the control (water/saline drop) should always remain uniformly suspended. Clumping in both drops indicate that the organism autoagglutinates and is unsuitable for the slide coagulase test.

*Record any observations on the data report sheet attached to this document.*
Observe and record the results of coagulase production by *Staphylococcus aureus* and *Staphylococcus epidermidis* when assessed by the tube and slide methods. Clotting in the tube test is considered positive, whereas clumping in the slide test is a positive result.

### Results of Coagulase Test*

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Tube</th>
<th>Slide</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td></td>
<td></td>
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</tbody>
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*Denote a positive coagulase reaction with a ‘+’ symbol and a negative results with a ‘-’ symbol.

### Discussion Question:

Coagulase is a known factor in the ability of *S. aureus* to cause infection. However, coagulase-negative staphylococci may be pathogenic as well. Briefly, **LIST** some of the factors that contribute to virulence of these coagulase-negative bacteria. This may require some additional research. Be sure to cite your sources.