Coagulase Testing

Rationale
Biochemical reactions are utilized in the identification of bacteria.

Objectives
Upon completion of this lesson, the student will be able to:
- Identify *Staphylococcus aureus* by using the coagulase test
- Explore how enzyme production can lead to bacterial identification

Engage
Show microscope slides or power point slides of photos of example colonies of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Encourage students to brainstorm some ways we could tell these samples apart in the clinical lab. Show samples of colony growth on CNA, Blood agar or TSA plates. Discuss what enzymes are and why they can help us further distinguish them apart.

Key Points
*Teacher Note
Must perform catalase and oxidase test before coagulase test.

I. The coagulase test
   A. Principle: *Staphylococcus aureus* is the only significant human pathogen that produces the enzyme coagulase. Coagulase is an enzyme that changes soluble fibrinogen into soluble fibrin. When *Staphylococcus aureus* is combined with rabbit plasma, soluble fibrin clumps are formed. Bound coagulase is indicated by a positive result in the slide test and free coagulase is indicated by a positive result in the tube test.

   B. Purpose: Differentiate *Staphylococcus aureus* from other *Staphylococcus species*.

   C. Procedure:
      1. Slide test:
         a. Place a drop of rabbit plasma on a clean glass slide.
         b. Place a drop of distilled water on a separate area of the glass slide.
         c. Emulsify a colony of the organism in the distilled water using a sterile inoculating loop. This is the negative control.
         d. Emulsify a colony of the organism in the rabbit plasma using a sterile inoculating loop.
         e. Rock the slide back and forth gently for one minute.
f. Record results:
   a. Positive: white clumps in plasma
   b. Negative: no clumps

2. Tube test:
   a. Place 0.5 mL of rabbit plasma in a test tube.
   b. Place 0.5 mL of distilled water in a test tube.
   c. Add one inoculating loopful of the organism being tested to each tube.
   d. Incubate the test tubes at 37 C for intervals of 30 minutes. Every 30 minutes check the test tubes. Tubes that are negative after 4 hours should be incubated at room temperature for 18 to 24 hours.
   e. Record results:
      a. Positive: fibrin clot formation in the test tube
      b. Negative: no formation of fibrin clot

II. Other information
A. The coagulase tube test detects the free coagulase. Free coagulase is an extracellular toxin that reacts in the presence of CRF (coagulase reacting factor) that resembles thrombin and changes fibrinogen to fibrin.

B. There are several strains of Staphylococcus aureus that produce staphylokinase, a fibrinolysin that slows the fibrin clotting process. Incubation for 18 – 24 hours will catch the slower process and catch false negatives.

C. Other tests for Staphylococcus aureus are growth and fermentation on MSA. S. aureus can grow in 6.5% to 10% NaCl and ferments the mannitol. Fermentation is seen by the yellow color in the agar as the phenol red indicator changes color due to the acid produced.

Activity
I. Complete the Coagulase Test Laboratory Investigation.

Assessment
Laboratory Investigation Rubric
Materials
*Staphylococcus aureus* culture
*Staphylococcus epidermidis* culture
Rabbit plasma anticoagulated with EDTA
Microscope slides
12 x 75 mm test tubes
Distilled water
Test tube racks
Disposable pipettes and bulbs
Gloves
Laboratory coats or aprons
Bunsen burner
Inoculating loops
Goggles
Biohazard containers
Surface disinfectant
Paper towels

Accommodations for Learning Differences
For reinforcement, the student will review the steps of the coagulase test and repeat the laboratory investigation or create an illustrated step-by-step guide.

For enrichment, the student will investigate the following: More than 50% of all clinical isolates contain coagulase negative *Staphylococcus epidermidis*. What is the clinical significance of these findings?

National and State Education Standards
National Healthcare Foundation Standards
HLC01.01 Academic Foundations #1: Health Science 1: Introduction to Health Science Academic Courses: Health Care workers will know the academic subject matter required for proficiency within their area. They will use this knowledge as needed in their role.
HLC06.01 Safety, Health and Environmental #3 Health Science II: Health Safety and Ethics in the Health Environment: Health care workers will understand the existing and potential hazards to clients, co-workers and self. They will prevent injury or illness through safe work practices and follow health and safety policies and procedures

TEKS
130.207(c)(1)(A) Demonstrate safe practices during laboratory and field investigations;
130.207(c)(2)(E) Plan and implement descriptive, comparative and experimental investigations, including asking questions, formulating testable hypotheses, and selecting equipment and technology;
130.207(c)(2)(F) Collect and organize qualitative and quantitative data and
make measurements with accuracy and precision using tools such as calculators, spreadsheet software, various prepared slides, stereoscopes, metric rulers, electric balances, hand lenses, Celsius thermometers, hot plates, lab notebooks or journals, timing devices, Petri dishes, lab incubators, dissection equipment, meter sticks, and models, diagrams, or samples of biological specimens or structures;  
130.207(c)(4)(B) Identify chemical processes of microorganisms; and  
130.207(c)(4)(C) Recognize the factors required for microbial growth.

Texas College and Career Readiness Standards
Mathematics Standards
VI. Statistical Reasoning: A. Data collection, B. Describe Data, C. Read, analyze, interpret and draw conclusions from data
Science Standards
VI. Biology: A. Structure and function of cells, B. Biochemistry 2. Describe the structure and function of enzymes 4. Describe the major features and chemical events of cellular respiration
Cross-Disciplinary Standards
I. Key Cognitive Skills: C. Problem Solving 1. Analyze a situation to identify a problem to be solved 3. Collect evidence and data systematically and directly relate to solving a problem E. Work habits 1. Work independently 2. Work collaboratively
COAGULASE TEST
LABORATORY INVESTIGATION

NAME: ___________________________ DATE: ________________________

PURPOSE:
In this laboratory investigation, the student will be able to identify *Staphylococcus aureus* by using the coagulase test

BACKGROUND INFORMATION:

MATERIALS:
Nonpathogenic *Staphylococcus aureus* culture
Nonpathogenic *Staphylococcus epidermidis* culture
Rabbit plasma anticoagulated with EDTA
Microscope slides
12 x 75 mm test tubes
Disposable pipettes and bulbs
Distilled water
Test tube racks
Gloves
Laboratory coats or aprons
Bunsen burner
Inoculating loops
Goggles
Biohazard containers
Surface disinfectant
Paper towels
PROCEDURE:

Slide test
1. Wash hands and put on gloves.
2. Assemble equipment and materials.
3. Prepare work area.
4. Place a drop of rabbit plasma on a clean glass slide.
5. Place a drop of distilled water on a separate area of the glass slide.
6. Flame and cool inoculating loop.
7. Emulsify a colony of the organism in the distilled water using an inoculating loop. This is the negative control.
8. Flame and cool inoculating loop.
9. Emulsify a colony of the organism in the rabbit plasma using an inoculating loop.
10. Flame and cool inoculating loop.
11. Rock the slide back and forth gently for one minute.
12. Record results:
   a. Positive: white clumps in plasma
   b. Negative: no clumps

Tube test
1. Place 0.5 mL of rabbit plasma in a test tube.
2. Place 0.5 mL of distilled water in a test tube.
3. Flame and cool inoculating loop.
4. Add one inoculating loopful of the organism being tested to each tube.
5. Flame and cool inoculating loop.
6. Incubate the test tubes at 37 C for intervals of 30 minutes. Every 30 minutes check the test tubes. Tubes that are negative after 4 hours should be incubated at room temperature for 18 to 24 hours.
7. Record results:
   a. Positive: fibrin clot formation in the test tube
   b. Negative: no formation of fibrin clot

Clean work area with surface disinfectant. Remove goggles and gloves and wash hands.

DATA:
Record results of your testing.

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>SLIDE TEST RESULT</th>
<th>SLIDE TEST CONTROL RESULT</th>
<th>TUBE TEST RESULT</th>
<th>TUBE TEST CONTROL RESULT</th>
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<tbody>
<tr>
<td>S. epidermidis</td>
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<tr>
<td>S. aureus</td>
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CONCLUSION:
1. What is free coagulase and its clinical significance?

2. Why are the negative tube tests incubated for 18 – 24 hours?

3. What is another test used for the identification of *Staphylococcus aureus*?

4. Explain your findings in complete sentences; use the data table as a guideline.
Laboratory Investigation Rubric

Student: ____________________________           Date: _____________________________

<table>
<thead>
<tr>
<th>Scoring Criteria</th>
<th>4</th>
<th>3</th>
<th>2</th>
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<td>Problem is appropriately identified.</td>
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<td>Problem is precise, clear, and relevant.</td>
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<td>Association between the problem and the predicted results is direct and relevant.</td>
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<td>All variables are clearly operationalized.</td>
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<td>Demonstrates comprehension of the use of scientific concepts and vocabulary.</td>
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<td>All significant data is measured.</td>
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<td>Data is recorded effectively and efficiently.</td>
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<td>Data table is well designed to the task requirements.</td>
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<td>All graphs are appropriate.</td>
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<td>All data accurately plotted.</td>
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<td>Graph visually compelling; highlights conclusions of the study.</td>
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<td>Conclusion relates directly to the hypothesis.</td>
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<td>Conclusion has relevancy in resolution of the original problem.</td>
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<td>Conclusion relates the study to general interest.</td>
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