Rationale
Determination of biochemical reactions is an important step in identification of bacteria.

Objectives
Upon completion of this lesson, the student will be able to:

- Perform the catalase and oxidase tests to identify bacteria

Engage
Show examples of colonies of gram stains of Micrococcus, Streptococcus and Staphylococcus cultures. Discuss with students ways that they can be differentiated. Discuss enzymes and how we can use what a bacterium produces to distinguish it from others. Remind students about the importance of patients receiving the proper antibiotic for their infection and how all samples in the lab are unknowns that we must identify.

Key Points
I. The catalase test
   A. Principle: Catalase is an enzyme that converts hydrogen peroxide into water and oxygen. The bacteria that contain this enzyme are usually aerobic (need oxygen) or facultative anaerobes (can live with or without oxygen). A positive reaction is indicated by a continuous bubble formation when the catalase is introduced to bacterial colonies.

   B. Purpose: Differentiate Staphylococcus from Streptococcus species of bacteria.

   C. Procedure:
      1. Place a small amount of a bacterial colony (18 to 24 hours old) on a clean glass slide.
      2. Add one to two drops of 3% hydrogen peroxide.
      3. Record observations:
         a. Positive: rapid bubble formation
         b. Negative: no bubble formation
      4. Possible false positives
         a. The order of the procedure is reversed.
         b. Bacterial colonies are contaminated with red blood cells from the blood agar.
II. The oxidase test
A. Principle: *Micrococcus species* contain cytochrome C, a component of the cytochrome oxidase system. This test differentiates between *Micrococcus* and *Staphylococcus* species. Additional testing may need to be performed for positive identification. When the oxidase reagent is added to a colony of bacteria, a dark blue to purple color is formed.

B. Purpose: Differentiation of *Micrococcus* from *Staphylococcus* species of bacteria.

C. Procedure:
1. Place one drop of oxidase reagent on an 18 to 24 hour colony of bacteria grown on a TSA plate.
2. Observe for color change:
   a. Positive: blue to dark purple color
   b. Negative: no color change

Activity
I. Complete the Catalase and Oxidase Laboratory Investigation.

Assessment
Laboratory Investigation Rubric

Materials
TSA plate with 18 – 24 hour growth of cocci
Suggested nonpathogenic specimens:
   Catalase positive: *Staphylococcus epidermis*
   Catalase negative: *Enterococcus faecalis*
   Oxidase positive: *Micrococcus luteus*
   Oxidase negative: *Staphylococcus epidermidis*
Optional – one extra culture for each test labeled as a 'patient sample' to have students test and draw conclusions about.
3% Hydrogen Peroxide
Microscope slides
Oxidase reagent (6% tetramethylphenylenediamine hydrochloride in dimethyl sulfoxide)
Gloves
Laboratory coat or apron
Bunsen burner
Eye dropper
Inoculating loops
Goggles
Biohazard containers
Surface disinfectant
Paper towels
Accommodations for Learning Differences
For reinforcement, the student will review the steps of the catalase and oxidase tests and repeat the laboratory investigation or create a step-by-step illustration.

For enrichment, the student will research the impact of poorly matched antibiotics with infections of *Micrococcus*, *Streptococcus* and *Staphylococcus* species.

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
CATALASE AND OXIDASE

NAME: ___________________________ DATE: _________________________

PURPOSE:
In this laboratory investigation, the student will learn the steps in performing the catalase and oxidase tests.

BACKGROUND INFORMATION:

MATERIALS:
TSA plate with 18 – 24 hour growth of the following bacteria:
- Staphylococcus epidermis
- Enterococcus faecalis
- Micrococcus luteus
- Staphylococcus epidermidis
3% Hydrogen Peroxide
Microscope slides
Oxidase reagent (6% tetramethylphenylenediamine hydrochloride in dimethyl sulfoxide)
Gloves
Laboratory coat or apron
Bunsen burner
Eye dropper
Inoculating loops
Goggles
Biohazard containers
Surface disinfectant
Paper towels

PROCEDURE:
Preparation
1. Wash hands and put on gloves and goggles
2. Assemble equipment and materials
3. Prepare work area

Catalase procedure
1. Flame and cool the inoculating loop.
2. Place a small amount of a bacterial colony (18 to 24 hours old) on a clean glass slide.
3. Flame and cool the inoculating loop.
4. Add one to two drops of 3% hydrogen peroxide.
5. Record observations:
   a. Positive: rapid bubble formation
   b. Negative: no bubble formation

**Oxidase procedure**
1. Place one drop of oxidase reagent on an 18 to 24 hour colony of bacteria grown on a TSA plate.
2. Observe for color change:
   a. Positive: blue to dark purple color
   b. Negative: no color change

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

**DATA:**
Record the results of your testing as positive (+) or negative (-).

**Catalase and Oxidase Results**

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>CATALASE</th>
<th>OXIDASE</th>
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**CONCLUSION:**
1. List the colony characteristics of bacteria used in laboratory investigation.

2. Compare and contrast colony characteristics of bacteria used in this laboratory investigation.

3. A hospital client recovering from open heart surgery (a triple bypass) develops tenderness, swelling, and redness at the incision site on the chest. The client’s temperature is 101F and has a blood pressure of 179/92. The physician orders aerobic and anaerobic cultures of the incision site. The gram stain shows many gram-positive cocci in clusters with many white blood cells. Determine the media for inoculation and the biochemical tests needed to identify this organism.
# Laboratory Investigation Rubric

Student: ____________________________  Date: ____________________________

<table>
<thead>
<tr>
<th>Scoring Criteria</th>
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<th>3</th>
<th>2</th>
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<th>N/A</th>
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<tbody>
<tr>
<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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