

# CATALASE AND OXIDASE TESTING

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## OBJECTIVE/RATIONALE

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Determination of biochemical reactions is an important step in identification of bacteria. The student will perform the catalase and oxidase tests to identify bacteria.

TEKS 121.14 (c) 1A, 1B, 4B, 4C, 4D, 5B

TAKS ELA 1, 4

Mathematics 8

Science 1, 2, 4

National Science Education Standards A9-12;C9-12

National Health Care Skills Standards .01, .04, .05, .06, .07, .08

National Curriculum Standards for School Mathematics S1; S3

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## KEY POINTS

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- 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

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## ACTIVITIES

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- I. Complete the **Catalase and Oxidase Laboratory Investigation.**

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## MATERIALS/RESOURCES

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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

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

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## ASSESSMENT

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### **Laboratory Investigation Rubric**

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## ACCOMODATIONS

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For reinforcement, the student will review the steps of the catalase and oxidase tests and repeat the laboratory investigation.

For enrichment, the student will identify unknown organisms using catalase and oxidase tests.

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## **REFLECTIONS**

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# 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:

### III. Preparation

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

Catalase procedure:

5. Flame and cool the inoculating loop.
6. Place a small amount of a bacterial colony (18 to 24 hours old) on a clean glass slide.
7. Flame and cool the inoculating loop.
8. Add one to two drops of 3% hydrogen peroxide.

9. Record observations:
  - c. Positive: Rapid bubble formation
  - d. Negative: No bubble formation

**Oxidase procedure:**

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

BACTERIA	CATALASE	OXIDASE

**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.