

# Coagulase Testing

## Course

Medical  
Microbiology

## Unit VII

Laboratory  
Investigations &  
Identification of  
Causative  
Agents

## Essential Question

How can the  
enzyme  
coagulase be  
used to help  
distinguish  
bacteria  
species?

## TEKS

130.207(c)

1A

2EF

4BCF

## Prior Student Learning

Bacterial Growth

## Estimated time

Tube Test: 4hr

Slide Test: 5min

1, 60 min class  
to explain and  
perform slide.

Idea: Use back  
to back classes  
to perform tube  
test.

## 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.

BACTERIA	SLIDE TEST RESULT	SLIDE TEST CONTROL RESULT	TUBE TEST RESULT	TUBE TEST CONTROL RESULT
<i>S. epidermidis</i>				
<i>S. aureus</i>				

**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:** \_\_\_\_\_

<b>Scoring Criteria</b>	<b>4</b> <b>Excellent</b>	<b>3</b> <b>Good</b>	<b>2</b> <b>Needs Some Improvement</b>	<b>1</b> <b>Needs Much Improvement</b>	<b>N/A</b>
Problem is appropriately identified.					
Problem is precise, clear, and relevant.					
Association between the problem and the predicted results is direct and relevant.					
All variables are clearly operationalized.					
Demonstrates comprehension of the use of scientific concepts and vocabulary.					
All significant data is measured.					
Data is recorded effectively and efficiently.					
Data table is well designed to the task requirements.					
All graphs are appropriate.					
All data accurately plotted.					
Graph visually compelling; highlights conclusions of the study.					
Conclusion relates directly to the hypothesis.					
Conclusion has relevancy in resolution of the original problem.					
Conclusion relates the study to general interest.					