

Urease Testing

Course

Medical
Microbiology

Unit VII

Laboratory
Investigations &
Identification of
Causative
Agents

Essential Question

How can we
differentiate
between urease
producers and
nonproducers?

TEKS

130.207(c)
1A
2EF
4BCF

Prior Student Learning

Bacterial Growth
Characteristics

Estimated time

60-90 Intro and
lab inoculation,
24h incubation,
30-60 data
gathering and
conclusion
(2-3, 60 min classes)

Rationale

Biochemical reactions are important in the identification of bacteria.

Objectives

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

- Perform the urease test to aid in the identification of unknown bacteria
- Discover how urease testing can be used help identify an unknown patient bacterial infection

Engage

Instructor preparations: Grow sample colonies of *Proteus*, and *E. coli*, or *Morganella*, *Klebsiella*, *Enterobacter* and some non-urease producers for visual comparison. Alternatively find photos of colonies for a quick slide show comparison.

Have students try to identify which colony belongs to each Genus. Discuss how each genus or species of bacterium may have traits that we could test to help us distinguish them from each other.

Key Points

The urease reaction is used to identify the rapid urease producers *Proteus* and *Morganella species*, as well as slower urease producers *Klebsiella* and some species of *Enterobacter*.

- I. The urease reaction
 - A. Principle: Urease is an enzyme that splits urea into alkaline end products of ammonia, carbon dioxide, and water. The ammonia reacts in solution to form ammonium carbonate resulting in a lower pH of the broth. This causes the indicator to change from a buff color to pink-red (fuchsia).
 - B. Purpose: identification of *Proteus*, *Morganella*, *Klebsiella*, and some species of *Enterobacter*.
 - C. Procedure:
 1. Place one single isolated colony into the urea broth with an inoculating loop. The colony should be 18 to 24 hours old.
 2. Replace the cap loosely on the broth or use parafilm over the opening.
 3. Incubate at 35C for 18 to 24 hours.
 4. Tubes may be placed in the refrigerator after incubation until they can be interpreted by the student.

5. Record observations:
 - a. Positive: Pink-red color broth
 - b. Negative: Buff color broth

- II. Quality control
 - A. Positive: *Proteus vulgaris*
 - B. Negative: *Escherichia coli*

Activity

- I. Complete the Urease Laboratory Investigation.

Assessment

Laboratory Investigation Rubric

Materials

TSA plate with 18 – 24 hour cultures

Suggested bacteria:

Urease positive: Nonpathogenic *Proteus vulgaris*

Urease negative: Nonpathogenic *Escherichia coli*

Optional third plate marked as 'patient sample' that is either *P. vulgaris* or *E. coli*

Urea broth tubes

Incubator

Inoculating loops

Bunsen burner

Gloves

Laboratory coat or apron

Bunsen burner

Goggles

Biohazard containers

Surface disinfectant

Paper towels

Accommodations for Learning Differences

For reinforcement, the student will review the steps in the urease procedure and repeat the laboratory investigation. (If supplies are limited have students diagram the procedure with illustrations instead of repeating it.)

For enrichment, the student will research diseases caused by *Proteus vulgaris*, or any other Genus discussed above.

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

UREASE LABORATORY INVESTIGATION

NAME: _____ DATE: _____

PURPOSE:

In this laboratory investigation, the student will learn the steps of urease testing.

BACKGROUND INFORMATION:

MATERIALS:

TSA plate with 18 – 24 hour growth of the following bacteria:

Nonpathogenic *Proteus vulgaris*

Nonpathogenic *Escherichia coli*

Optional third plate marked as 'patient sample'

Urea broth tubes

Incubator

Inoculating loops

Bunsen burner

Gloves

Laboratory coat or apron

Bunsen burner

Goggles

Biohazard containers

Surface disinfectant

Paper towels

PROCEDURE:

1. Wash hands and put on gloves and goggles.
2. Assemble equipment and materials.
3. Prepare work area.
4. Flame and cool the inoculating loop.
5. Place one single isolated colony into the urea broth with an inoculating loop. The colony should be 18 to 24 hours old.
6. Flame and cool the inoculating loop.
7. Replace the cap loosely on the broth or use parafilm over the opening.
8. Incubate at 35C for 18 to 24 hours.
9. Tubes may be placed in the refrigerator after incubation until the student can interpret them.
10. Record observations:

- a. Positive: Pink-red color broth
 - b. Negative: Buff color broth
11. Clean work area with surface disinfectant. Remove goggles and gloves and wash hands.

DATA:

Record the results of your testing by placing a X in the appropriate box.

Urease Results

BACTERIA	POSITIVE	NEGATIVE
<i>Proteus vulgaris</i>		
<i>Escherichia coli</i>		

CONCLUSION QUESTIONS:

1. *Morganella species* infections are most often seen in what type of patients?
2. Diagram the biochemical reaction of the urea test.
3. How are urease positive organisms commonly transmitted? Identify signs and symptoms of infection and the diseases they cause.
4. Which *Proteus species* is most frequently isolated as a human pathogen?

Laboratory Investigation Rubric

Student: _____ **Date:** _____

Scoring Criteria	4 Excellent	3 Good	2 Needs Some Improvement	1 Needs Much Improvement	N/A
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.					