

“The Art of Streaking” (For Isolation)

Course

Medical
Microbiology

Unit V

Microorganisms

Essential Question

How do you
streak an agar
plate in order to
isolate individual
colonies of
bacteria?

TEKS

130.207 1A, 1B,
2A-H, 3D,
4C,E,F

Prior Student Learning

Basic
understanding of
bacteria

Estimated time

4-6 hours

Rationale

To identify microorganisms from a lab specimen it is necessary to isolate individual colonies.

Objectives

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

- Describe media and its function in bacterial growth
- Become familiar with and perform the quadrant streaking method for bacterial cell plating
- Streak an agar plate to isolate bacterial colonies

Engage

Before the lesson is to be taught, streak an agar plate with bacteria from the doorknob of the classroom. Show the class the bacteria that have grown on the agar plate.

- What do you notice about the plate?
- Are they all the same type of bacteria?
- What makes the areas of growth different from each other?
- What are some ways we could group the different areas of growth?

Key Points

- I. Key terms
 - A. Agar – is a nutrient medium or culture medium that is commonly used to culture bacteria in the lab. Agar is also called AGAR-AGAR, a gelatin-like product made primarily from red algae and brown algae (e.g., kelps); Kingdom Protista)
 - B. Broth – liquid nutrient- rich medium used for cultivating microorganisms
 - C. Inoculating loop – made of platinum or wire used to streak, smear or take a sample from a culture
 - D. Streaking – a petri dish is streaked in manner such that individual colonies may be isolated
 - E. Microbial growth – increase in numbers, not increase in size
 - F. Culture medium – nutrients prepared for microbial growth
 - G. Inoculum – microbes introduced into a medium to initiate growth
 - H. Culture – microbes growing in/on culture medium

- I. Colony – population of cells arising from a single cell or spores or group of attached cells
- J. Solid medium – media containing agar or some other, mostly inert solidifying agent
- K. Culture medium – solution containing all of the nutrients and necessary physical growth parameters necessary for microbial growth
- II. Streak Plate Method – Spreads the colonies throughout four quadrants in such a way that the fourth quadrant will have the least amount of bacteria within it, therefore isolating colonies.
- III. The Pour plate and Spread Plate methods of isolation
 - A. Pour plate – bacteria are inoculated into a tube of melted agar cooled to a temperature of 45 degrees C to 47 degrees C, mixed, and poured into a sterile petri plate
 - B. Spread plate -- liquid cultures are *spread* on the center of an agar surface using a bent sterile glass rod
- IV. Use of specialized media
 - A. Selective media -- media that suppresses the growth of some microorganisms while allowing the growth of others
 - B. Differential media – are designed to separate organisms based on type of growth or end products of metabolic reactions
 - C. Enrichment media -- used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium
- V. Streaking procedure
 - A. Hold the inoculating loop handle in one hand as you would a pencil; hold the agar plate in the opposite hand
 - B. Open the lid of the agar plate enough to insert the swab and spread the inoculum over the surface of one quadrant of the agar plate; do not streak over previously streaked lines
 - C. Flame and cool the inoculating loop
 - D. Turn the plate so section two is in position; streak the second quadrant of the plate by touching the loop into the first quadrant and streaking all the way across the second quadrant, making six to eight strokes
 - E. Flame and cool the loop
 - F. Streak the third quadrant by touching the loop into the second quadrant and streaking into the third quadrant, making six to eight

strokes

- G. Flame and cool the loop
- H. Streak the fourth quadrant in a manner to produce isolated colonies; touch the loop to the third quadrant and spread the organism into the fourth quadrant using a continuous streak in a “tornado” pattern (Decrease the width of the streaks horizontally and increase the distance between the streaks vertically)
- I. Flame and cool the loop

Activity

- I. Complete the Streaking an Agar Plate Vocabulary
- II. Complete the Art of Streaking Laboratory Investigation

Assessment

Laboratory Investigation Rubric

Materials

- Streaking an Agar Plate Vocabulary Definitions
- Nonpathogenic *Escherichia coli* broth cultures
- MacConkey agar plates
- Inoculating loops
- Incubator @ 37 C
- Bunsen Burner
- Gloves
- Goggles
- Lab coats
- Surface disinfectant
- Paper towels

Burton, Gwendolyn R.W. and Engelkirk, Paul G. (2000). *Microbiology for the Health Sciences*, 6th ed. New York: Lippincott, Williams, & Wilkins.

Accommodations for Learning Differences

For reinforcement, the student will review the steps of the streaking technique and repeat the laboratory investigation.

For enrichment, the student will swab their own skin with 4 sterile swabs and inoculate a blood, chocolate, MacConkey and CNA plate and evaluate their streaking technique.

National and State Education Standards

HLC10.01 Health care workers will apply technical skill required for all career specialties. They will demonstrate skills and knowledge as appropriate.

TEKS

130.207(c)(1)(A) demonstrate safe practices during laboratory and field investigations;

130.207(c)(1)(B) demonstrate an understanding of the use and conservation of resources and the proper disposal or recycling of materials;

130.207(c)(2)(A) know the definition of science and understand that it has limitations, as specified in subsection (b)(2) of this section;

130.207(c)(2)(B) know that hypotheses are tentative and testable statements that must be capable of being supported or not supported by observational evidence. Hypotheses of durable explanatory power which have been tested over a wide variety of conditions are incorporated into theories;

130.207(c)(2)(C) know scientific theories are based on natural and physical phenomena and are capable of being tested by multiple independent researchers. Unlike hypotheses, scientific theories are well-established and highly-reliable explanations, but they may be subject to change as new areas of science and new technologies are developed;

130.207(c)(2)(D) distinguish between scientific hypotheses and scientific theories;

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, data-collecting probes, computers, standard laboratory glassware, microscopes, various prepared slides, stereoscopes, metric rulers, electronic 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)(2)(G) analyze, evaluate, make inferences, and predict trends from data;

130.207(c)(2)(H) communicate valid conclusions supported by the data through methods such as lab reports, labeled drawings, graphic organizers, journals, summaries, oral reports, and technology-based reports;

130.207(c)(3)(D) evaluate the impact of scientific research on society and the environment;

130.207(c)(4)(C) recognize the factors required for microbial reproduction and growth;

130.207(c)(4)(E) describe the morphology and characteristics of microorganisms using a variety of microbiological techniques;

130.207(c)(4)(F) discuss the results of laboratory procedures that are used to identify microorganisms.

Texas College and Career Readiness Standards

Mathematical Standards

IV. Measurement Reasoning D. Measurement involving statistics and probability 1. Compute and use measures of center and spread to describe data. 2. Apply probabilistic measures to practical situations to make an informed decision.

VI. Statistical Reasoning A. Data collection B. Describe data C. Read, analyze, interpret, and draw conclusions from data 1. Make predictions and draw inferences using summary statistics.

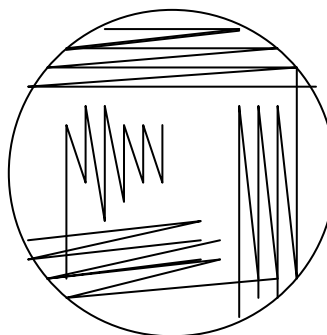
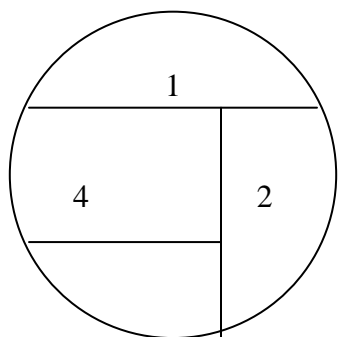
Science Standard

I. Nature of Science: Scientific Ways of Learning and Thinking A. Cognitive skills in science 4. Rely on reproducible observations of empirical evidence when constructing, analyzing, and evaluating explanations of natural events and processes. C. Collaborative and safe working practices 2. Understand and apply safe procedures in the laboratory and field, including chemical, electrical, and fire safety and safe handling of live or preserved organisms. 3. Demonstrate skill in the safe use of a variety of apparatuses, equipment, techniques and procedures.

Streaking an Agar Plate

PROCEDURE:

1. Hold the inoculating loop handle in one hand as you would a pencil. Hold the agar plate in the opposite hand.
2. Open the lid of the agar plate enough to insert the swab and spread the inoculum over the surface of one quadrant of the agar plate. Do not streak over previously streaked lines.
3. Flame and cool the inoculating loop.
4. Turn the plate so section two is in position. Streak the second quadrant of the plate by touching the loop into the first quadrant and streaking all the way across the second quadrant, making six to eight strokes.
5. Flame and cool the loop.
6. Streak the third quadrant by touching the loop into the second quadrant and streaking into the third quadrant, making six to eight strokes.
7. Flame and cool the loop.
8. Streak the fourth quadrant in a manner to produce isolated colonies. Touch the loop to the third quadrant and spread the organism into the fourth quadrant using a continuous streak in a “tornado” pattern. Decrease the width of the streaks horizontally and increase the distance between the streaks vertically.
9. Flame and cool the loop.



Streaking an Agar Plate Vocabulary

Agar	
Broth	
Colony	
Culture	
Culture medium	
Culture medium	
Differential media	
Enrichment media	
Inoculating loop	
Inoculum	
Pour plate	
Selective media	
Solid medium	
Spread plate	
Streak plate	

KEY -- Streaking an Agar Plate Vocabulary

Agar	a nutrient medium or culture medium that is commonly used to culture bacteria in the lab. Agar is also called AGAR-AGAR, a gelatin-like product made primarily from red algae and brown algae (e.g. kelps); Kingdom Protista).
Broth	liquid nutrient- rich medium used for cultivating microorganisms
Colony	population of cells arising from a single cell or spores or group of attached cells
Culture	microbes growing in/on culture medium
Culture medium	nutrients prepared for microbial growth
Culture medium	solution containing all of the nutrients and necessary physical growth parameters necessary for microbial growth
Differential media	are designed to separate organisms based on type of growth or end products of metabolic reactions
Enrichment media	used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium
Inoculating loop	made of platinum or wire used to streak, smear or take a sample from a culture
Inoculum	microbes introduced into a medium to initiate growth
Pour plate	bacteria are inoculated into a tube of melted agar cooled to a temperature of 45 degrees C to 47 degrees C, mixed, and poured into a sterile petri plate
Selective media	media that suppresses the growth of some microorganisms while allowing the growth of others
Solid medium	media containing agar or some other, mostly inert solidifying agent
Spread plate	liquid cultures are <i>spread</i> on the center of an agar surface using a bent sterile glass rod
Streak plate	petri dish is streaked in manner such that individual colonies may be isolated.

The Art of Streaking Laboratory Investigation

PURPOSE:

The student will learn how to streak an agar plate to isolate bacterial colonies.

BACKGROUND INFORMATION

MATERIALS

- Nonpathogenic *Escherichia coli* broth cultures
- MacConkey agar plates
- Inoculating loops
- Incubator @ 37 C
- Bunsen Burner
- Gloves
- Goggles
- Lab coats
- Surface disinfectant
- Paper towels

PROCEDURE

Read all instructions before beginning the procedure.

1. Wash hands and put on gloves and goggles.
2. Obtain all material and return to station.
3. Prepare work area.
4. Label agar plates with name of student, bacteria, and date.
5. Begin streaking the plates as follows:
SEE HANDOUT
6. To avoid contamination, use a different loop when streaking each plate.
7. Place in 37C incubator upside down for 24 hours.
8. Clean reusable equipment and returned to proper storage; put disposables in biohazard container.
9. Clean work area with surface disinfectant.
10. Remove gloves and wash hands with disinfectant.
11. Record results:

Record a yes or no in the appropriate boxes.

Data:

ISOLATED COLONIES RESULTS

PLATE	QUAD ONE	QUAD TWO	QUAD THREE	QUAD FOUR
1				
2				
3				

(Quad = quadrant of the agar plate)

CONCLUSIONS

1. Did you successfully isolate *Escherichia coli* colonies?
2. Explain in complete sentences the results of the chart above.
3. Why is it important to isolate colonies?
4. How can you determine if a colony on a plate is a contaminant or from the patient specimen.
5. Why does this streaking technique help to isolate colonies of bacteria?
6. How does isolation contribute to isolating bacteria in treating diseases?

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.					