

Mystery Growth Laboratory Practical

Course

Medical
Microbiology

Unit VII

Laboratory
Investigations &
Identification of
Causative
Agents

Essential Question

How can we
differentiate
unknown patient
samples based
on basic
bacterial
growth?

TEKS

130.207(c)
1A
2EFGH
4BCEF

Prior Student Learning

Bacterial Growth
Characteristics
Urease Testing
Gram Stain

Estimated time

Set up – 24h
growth and up to
60 mins of
placing
Class – 1-2 60
min classes

Rationale

Recognizing reactions to media is important in identifying unknown microorganisms.

Objectives

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

Identify unknown bacteria by analyzing typical biochemical reactions on growth media

Engage

Today's lab is one that requires some prior knowledge to be used to solve some unknown mysteries. In the clinical microbiology lab all of our patient samples begin as mysteries. Today you will evaluate colony growth on selective medias to identify the type of bacteria present or the biochemical reaction going on. This would help you get closer to a specific identification if you were handling unknown patient samples of current infections that needed the proper antibiotic to be selected. (Notes from the labs mentioned below are encouraged to be used.)

Key Points

*Teacher Note

Students should have successfully completed the Bacteria Growth Characteristics Laboratory Investigation before attempting the Laboratory Practical. Completing the Urease and Gram Stain labs would also be advised. Some review and orientation of special characteristics of some of the species used in the lab would be helpful to allow students to select the proper answer.

I. Types of Media

A. Agar commonly used for initial culture

1. Blood
2. Chocolate
3. MacConkey
4. Citrate nutrient agar – for stool cultures helping to identify *Salmonella* and *Shigella* species with a pH indicator that turns yellow/orange when acidic and blue when pH is greater than 7.6. Only organisms that can use citrate as their sole source of carbon will grow.

B. Broths commonly used for initial culture

1. Gram-negative broth
2. Trypticase soy broth

- C. Most commonly used agars are blood and chocolate. Both of these types of media will contain nutrients for both Gram-positive and Gram-negative bacteria.
- D. List media/broths that will be used in the student laboratory and the importance of each:
1. MSA – Mannitol Salt Agar – will develop a yellow halo around colonies of *Staphylococcus aureus* when they break down the mannitol and turn the pH indicator from red to yellow. The high levels of salt keep most growth to gram positive species.
 2. MAC – MacConkey Agar – contains bile salts and crystal violet dye preventing gram positive growth. Colonies will appear pink when lactose fermentation is present. Other characteristics can be noticed with specific species; for instance *Proteus vulgaris* will show swarming while *Pseudomonas aeruginosa* will develop a green metallic sheen.
 3. BLD – Blood (usually sheep blood) – encourages most species to grow; great for initial culture. Show hemolysis that can be seen as a clear ring around a colony producing beta hemolysis and a greenish ring around alpha hemolysis.
 4. EMB – Eosin Methylene Blue – will show blue colonies with growth from *Escherichia coli* because of their ability to digest lactose lowering the pH and allowing those colonies to absorb the eosin and methylene blue stain appearing dark blue-purple. Non-lactose fermenters keep the pH elevated and will stay uncolored as they will not be able to absorb the dye.
 5. CNA – Colistin-nalidixic acid Agar – allows for gram positive growth only due to the addition of colistin and nalidixic acid.
 6. PEA – Phenylethyl Alcohol Agar -- not used in this lab but can be used for selective growth for anaerobic bacteria.
 7. TSA - Trypticase Soy Agar – general purpose media that most bacteria can grow on, allows for colony color and morphology to not be affected by the agar allowing some natural colors to show through like the red growth of *Serratia marcescens*.
 8. CHOC – Chocolate – an enrichment broth that encourages slow or poor growers like *Neisseria sp.* and *Haemophilus sp.*
 9. TSB – Trypticase Soy Broth – not used in this lab, also a multipurpose growth media, excellent for some liquid samples and wound cultures very similar to TSA but in liquid form.
 10. UREA BROTH – contains large amounts of ammonia to raise the pH and a pH indicator that will show when a species can break ammonia down by turning the media from orange/yellow to pink.
 11. GN BROTH – not used in this lab; used for the selective enrichment of *Salmonella* and *Shigella* species.

Activity

Complete the Mystery Growth Laboratory Practical.

Assessment

Successfully complete the Mystery Growth Laboratory Practical

Materials

Prepare 24-48hr growth of the following combinations:

E. coli on MAC agar, and EMB agar

Neisseria sp. or *Haemophilus sp.* on Chocolate agar

S. aureus on MSA, (Optional on CNA and BLD also)

S. pyogenes (or other beta hemolytic colony) on BLD (opt on CNA)

P. aeruginosa on MAC

P. vulgaris on MAC, and in Urea broth,

S. marcescens on TSA

Slide of one GN and one GP

Available from your local hospitals (a great resource -- use their outdated supplies)

Mystery Growth Laboratory Practical Student Page

Accommodations for Learning Differences

For reinforcement, the student will create a poster depicting different biochemical reactions of the organisms to the media.

For enrichment, the student will predict the type of media to use for the following specimens: wound, cerebral spinal fluid (CSF), and gastrointestinal.

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)(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)(4)(B) Identify chemical processes of microorganisms

130.207(c)(4)(C) Recognize the factors required for microbial growth

130.207(c)(2)(E) describe the morphology and characteristics of microorganisms using a variety of microbiological techniques; and

130.207(c)(2)(F) discuss the results of laboratory procedures that are used to identify microorganisms.

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

MYSTERY GROWTH LABORATORY PRACTICAL

NAME: _____ DATE: _____ CLASS: _____

Please answer the following questions.

STATION 1

1. This organism is a:
 - a Lactose fermenter
 - b Non-lactose fermenter
2. This is commonly used for:
 - a Gram-positive bacteria
 - b Gram-negative bacteria

STATION 2

3. This media is:
 - a enrichment media
 - b differential media
 - c selective media
 - d a and b
 - e b and c

STATION 3

4. This media is used for growth of:
 - a *Neisseria* and *Hemophilus*
 - b *Shigella* and *Salmonella*
 - c *Escherichia coli*
 - d None of the above

STATION 4

5. This selective media is used for growth of
 - a Gram-positive bacteria
 - b Gram-negative bacteria

STATION 5

6. Describe the colonial morphology you see on this media.

7. The type of hemolysis seen on this media is:
 - a Alpha hemolysis
 - b Beta hemolysis
 - c Delta hemolysis
 - d Gamma hemolysis

8. This media is commonly used to differentiate between species of:
 - a *Hemophilus*
 - b *Neisseria*
 - c *Streptococcus*
 - d *Staphylococcus*

STATION 6

9. The metallic green sheen on this media usually indicates:
 - a *Escherichia*
 - b *Pseudomonas*
 - c *Proteus*
 - d *Serratia*

STATION 7

10. The colony "swarming" on this media indicates:
 - a *Escherichia*
 - b *Pseudomonas*
 - c *Proteus*
 - d *Serratia*

STATION 8

11. The bacteria that causes the reaction occurring on this media is:
- a *Staphylococcus aureus*
 - b *Staphylococcus epidermidis*
 - c *Enterococcus faecalis*
 - d *Micrococcus luteus*

STATION 9

12. The red color of this colony on a TSA plate is indicates:
- a *Escherichia*
 - b *Pseudomonas*
 - c *Proteus*
 - d *Serratia*

STATION 10

13. The bacteria in this test tube have changed the broth from a clear color to a pink color. Explain the reaction that has occurred.

STATION 11

14. Describe the microorganisms seen on this microscope slide.

STATION 12

15. Describe the microorganisms seen on this microscope slide.

MEDICAL MICROBIOLOGY
MYSTERY GROWTH LABORATORY PRACTICAL
ANSWER KEY

The twelve stations for the laboratory practical should be placed far enough away from each other so students may feel comfortable during the test time. Each station should be labeled with the number corresponding to the test questions. You may choose to set a time limit at each station with students rotating around the room at the same time.

The stations are as follows:

1. **Station 1:** Gram-negative *Escherichia coli* on a MacConkey agar plate. The colonies will be purple in color, indicating a lactose fermenter.
2. **Station 2:** Gram-negative *Escherichia coli* on an Eosin Methylene Blue (EMB) agar plate. The colonies will be colored on the EMB agar.
3. **Station 3:** Chocolate agar plate for growth of *Neisseria sp.* or *Hemophilus sp.*
4. **Station 4:** CNA plate for Gram-positive bacteria like any *Staphylococcus* or *Streptococcus*.
5. **Station 5:** Sheep blood agar plate with growth of a *Streptococcus pyogenes* (group A strep) or *Staphylococcus aureus*. Beta hemolysis visible with the clear ring around colony growth.
6. **Station 6:** Gram-negative *Pseudomonas aeruginosa* grown on MacConkey agar plate. The bacteria will display a metallic green sheen after 24 hours of growth.
7. **Station 7:** Gram-negative *Proteus vulgaris* on MacConkey agar plate. The bacteria will display a “swarming” growth, covering the whole plate after 24 hours.
8. **Station 8:** Gram-positive *Staphylococcus aureus* grown on Mannitol Salt agar plate. A yellow halo will appear around the colony.
9. **Station 9:** Gram-negative *Serratia marcescens* grown on Trypticase Soy agar plate. The colonies will appear red in color after 24 hours.
10. **Station 10:** Gram-negative *Proteus vulgaris* in urea broth. The *Proteus* produces the enzyme urease which breaks down urea to ammonia, water and carbon dioxide. The pH is changed to alkaline from the byproducts and the indicator phenol red changes from yellow to reddish-purple.
11. **Station 11:** Microscope with slide of Gram-positive bacilli or cocci of your choice.
12. **Station 12:** Microscope with slide of Gram-negative bacilli or cocci of your choice.