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Don't drink that water: A microbiological investigation of fecal water contamination.

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Abstract

Residents in your college town are falling ill and there is concern that fecal contamination of the water supply is to blame. Your municipal council is hiring your students to investigate and identify the source of contamination. This article deconstructs a multi-week freshman-level biology lab sequence focused on a case study/scenario-based investigation. A suite of microbiological techniques including microscopy, simple and Gram staining of bacteria, growth phenotype on selective and differential media, and presumptive water testing are employed to identify the source of contamination. Students learn and practice techniques needed to identify the presence of fecal coliform bacteria in water samples containing mixed bacterial cultures. Novel strategies for economical media usage and high-success Gram staining are described. Finally, methods for simple modification of this lab scenario are described to tailor this experience to any campus location to leverage the link between student engagement and personal experience.

Keywords: microbiology, bacteriology, inquiry-based learning, case study, techniques, multi-week, microscopy, Gram staining

Link to Supplemental Materials:

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Supplemental Materials

Student Outlines: Week 2 – Week 5

Materials: Week 2 – Week 5

Instructor Notes: Week 2 – Week 5

Student Outlines: Week 2 – Week 5

Bacteriology Part II

Learning Objectives: This week's lab builds on basic lab techniques by introducing simple staining of bacterial cultures and introduces the use of differential and selective media to learn about the biochemical properties of bacteria. A scenario is presented that serves as the basis for the final investigative project.
• Demonstrate how to prepare a heat fixed smear of a bacterial culture to stain for observation. • Describe the difference between selective and differential growth media for culturing bacteria. • Use differential and selective media to characterize strains of bacteria. • Demonstrate an understanding of how to obtain single colonies by making a streak plate from a bacterial culture.

Outline:

- **Procedures and introduction**
- **Exercise #1 – Simple Staining**
- **Exercise #2 – Selective and Differential Media**
- **Exercise #3 – Isolation of single colonies – Streak Plates**

Important Note: Be very deliberate in recording results from this week's experiments because they may be an important source of images for your final presentation.

Looking Ahead. Assignments for the Microbiology labs:

- Microbiology Part II assignment – **due week 3** (20 points)
- Microbiology Part III assignment – **due week 4** (20 points)
- Oral Final Presentation in person – **Week 6** to be scheduled (50 points)

General Notes: This week's lab is set up in a "station" format in which your group will rotate between different areas of the lab to complete separate activities.

Bacteria grow quickly. In most cases, the cultures you start will be ready to observe after 24 hours of growth. For the next three lab sessions, **you will be required to return to lab the next day** to collect results and document your experiments. Your plates and tubes from lab WILL be kept at room temperature until the next lab meeting, but **timely documentation of results is expected**. Close-up photos with a cell phone camera are an excellent way to document your experiments and will facilitate easy incorporation in a notebook, lab report, or presentation as needed.

This week we will be using five strains of bacteria; *Escherichia coli (E. coli), Serratia marcescens, Bacillus cereus, Staphylococcus epidermidis,* and *Enterobacter aerogenes*. These bacteria are considered Biosafety Level 1 (BSL-1) organisms and do not typically cause disease in healthy individuals. These non-pathogenic strains *could* cause health issues for people with open wounds or compromised health, so gloves and goggles will be required. **No food or drink will be allowed in the lab at any time.** Failure to adhere to this rule will forfeit any remaining participation points for the semester and possible loss of credit on the final lab. This is for your protection and the protection of those you may come in contact with.

Sanitation: Before working in the lab each week, you will be required to clean your benches with a sanitizing agent called BDD disinfectant. Spray some BDD on your bench and wipe with paper towels, ensuring that the whole bench surface comes in contact with the disinfectant. Your bench will be sanitized (but not sterile) when the disinfectant has dried. You will repeat this process before leaving the lab for the day.

Exercise #1 – Simple Staining

Visualization of microorganisms in the living state is quite difficult, not only because they are minute, but also because they are transparent and practically colorless. To study bacterial morphology and to help

divide microorganisms into specific groups, staining procedures and light microscopy are important tools in microbiology.

A stain is defined as an ionic compound containing a benzene ring with a colored chemical group called a chromogen. Basic stains are cationic (have a positive charge) and therefore have a strong affinity for the negative constituents of the cell.

Nucleic acids and the negatively charged outer surface of bacteria will readily bind to and accept the color of the cationic basic stain. Structurally, **methylene blue, crystal violet, and safranin** are basic stains. Prior to staining, bacteria need to be prepared on a glass slide. Preparation for staining requires making a heat-fixed smear of bacteria on a slide, so staining is only possible on dried and dead cells. Heat fixing serves to a) kill the bacteria and b) denatures membrane proteins which cause the cells to adhere to the slide. Cells that are not properly heat-fixed will simply wash off the slide during the staining procedure. Cells that are over heated will burn and lose their shape. Heat fixing is a delicate process, so follow the directions below carefully!

Materials: per lab group:

- Box of slides (4-6 slides needed per group)
- Bunsen burner / lighter
- Distilled water in squirt bottle
- Clothes-pin slide holder
- Methylene blue in 50 mL conical tube
- Safranin in 50 mL conical tube
- Crystal Violet in 50 mL conical tube
- Microscope

Each person will make **two smears** of different bacteria; *Escherichia coli*, *Bacillus cereus,* or *Staphylococcus epidermidis*. Stain each with a different stain; crystal violet, methylene blue, or safranin (it doesn't matter which bacterium you pair with which stain).

Protocol for Exercise #1 – Simple Staining:

Organisms cultured on solid media produce thick, dense surface growth and cannot be directly transferred to a slide for staining. Plate grown cultures must be diluted by resuspension in water on the center of a slide.

Bacterial Smear:

1) Using a pipette or dropper bottle, place a **small** drop of water (more than 10 µL is TOO MUCH) on a clean slide.

2) Transfer a small sample of bacteria using a sterile inoculating loop or sterile toothpick. **Only the tip of the loop or toothpick should touch the culture to prevent transfer of too many cells.**

3) Spread the cells in a circular motion in the drop of water with the loop or needle. The finished smear should occupy an area about the size of a nickel or quarter and should appear as a translucent, or semitransparent, confluent whitish film (see example at right).

4) Allow the smear to air dry completely. Do **NOT** blow on slide or wave it in the air because it could aerosolize the bacteria.

5) Heat fix the bacteria by passing the dry slide over a Bunsen burner once or twice, (don't cook the bacteria!), holding with slide with a clothes pin.

Simple Staining:

All staining will be carried out in 50 mL conical tubes containing stains that are located near the sinks at the side of each lab-bench.

1) Using a clothespin as a slide holder, place a slide with a fixed bacterial smear into a tube of the indicated stains using the appropriate exposure time for each:
    a. Safranin, 2 minutes
    b. Crystal violet, 60 seconds
    c. Methylene blue 2 minutes.

2) Gently rinse the smear with distilled water to remove excess stain. Wash the slide from above the smear and let the water cascade over the bacteria. Do not spray water directly on the smear to avoid the loss of bacteria from the slide. Be gentle yet generous with the water as you wash the slide. Continue rinsing until the wash water runs clear.

3) Place slide between sheets of bibulous paper to blot it dry, but do not wipe the slide.

4) Repeat this procedure with the remaining bacteria, using a different stain for each.

5) Examine all stained slides under the microscope when air dried.

Make sure to record your results, by taking a picture through the microscope eyepiece with your phone. Tips for labeling what you see under the microscope:

a) Always write the magnification at which the observations were made (100X - 600X)

b) Label the drawings with any observable features, including the color of objects (see figure 1 for a great example).

6) Answer the questions in the Exercise #1 section of your lab assignment.

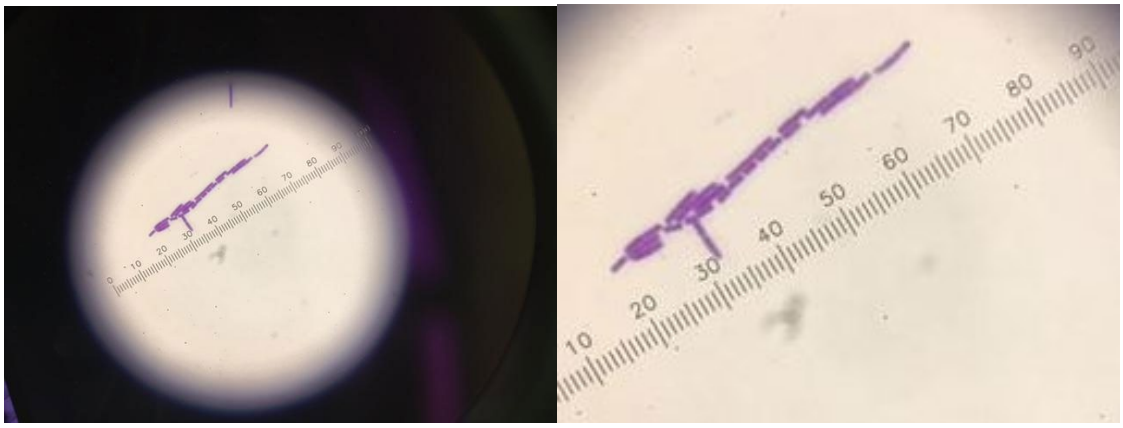


Figure 1. Proper formatting for a microscope photograph. Left: The original photo is too small to visualize and has distracting and unnecessary borders. Right: Cropped and zoomed photo is professional looking and clear. *B. cereus* (2-4 µM*)* chained bacilliform, 1000x magnification, crystal violet stain. (Photo courtesy of Sara Treacy, SUNY Geneseo, Biol 349)

Exercise #2 – Selective and Differential Media

The identification of bacteria from the environment or a clinical sample can be aided by growing the isolate on diagnostic media. Diagnostic media can be ***selective***, ***differential***, or both. Selective media is growth media in which compounds have been added to inhibit the growth of certain microbes but not others. Differential media contains an indicator (often a pH sensitive dye) that changes color if certain chemical reactions take place during the growth of a strain. For example, Mannitol Salt Agar (MSA), which is not used in this lab, contains a high concentration of sodium chloride (>7.5%) that inhibits the growth of most bacteria but allows salt-tolerant *Staphylococcus spp.* to grow. MSA is also differential because it contains the fermentable sugar mannitol and the pH sensitive dye phenol red. Microbes that are able to ferment mannitol as a carbon source produce acid as a by-product which changes the color of the dye from red to yellow (Figure 2).

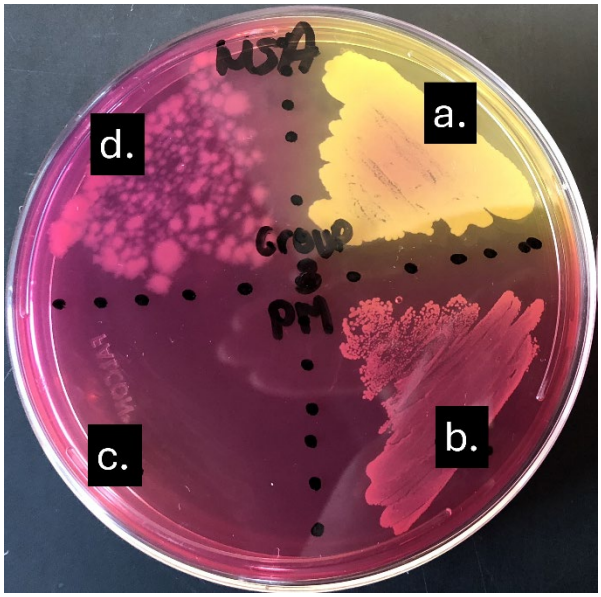


Figure 2. Mannitol Salt Agar (MSA) is a selective and differential media used to differentiate *Staphylococcus spp*. *S. aureus* (a.) is salt tolerant and can ferment mannitol, resulting in growth and a yellow color change, while *S. epidermidis* (b.) is salt tolerant but does not ferment mannitol, illustrating the differential nature of MSA. *E. coli* (c.) is selected against because it cannot tolerate high salt and does not grow. An unknown strain (d.) appears to be tolerant of high salt but does not ferment mannitol. (Photo courtesy of Jessica Staggers, SUNY Geneseo, Biol 349)

In this experiment, you will investigate and determine the selective and differential properties of a diagnostic media called **Eosin Methylene Blue (EMB)** agar. The American Society for Microbiology has an extensive set of laboratory protocols that provide information that will be very helpful for this activity (https://asm.org/Protocols/Eosin-Methylene-Blue-Agar-Plates-Protocol).

Materials: per lab **group**:

- 1 TSA plate
- 1 Eosin Methylene Blue (EMB) plate
- Disposable inoculating loops
- Marking pen
- Indicator strains of bacteria (on petri plates);
    - *Escherichia coli*
    - *Serratia marcescens*
    - *Staphylococcus epidermidis*
    - *Bacillus cereus*
    - *Enterobacter aerogenes*

Protocol for Exercise #2 - Differential and selective media:

1. Divide the EMB plate into 5 pie-shaped sections by drawing with a sharpie on the **back** of the plate. Label with the media name, your group number, section, and incubation temperature.

2. Label each section with the organism you're going to inoculate

3. Using aseptic technique, gently touch a sterile loop to a colony from one of the provided plates of indicator bacteria.

    a. Do NOT scoop up a chunk of bacteria. Simply touching a colony will pick up sufficient bacteria to inoculate your plate section. Inoculating too much bacteria will not produce clear results.

4. Transfer the sample of the corresponding bacterial culture for each quadrant and inoculate using a zigzag motion to spread the culture within the quadrant. **Remember to use your (by now amazing) aseptic technique so you don't contaminate the stocks. Try not to overlap the different bacteria on the plate**

5. Repeat steps 1-4 with the TSA plate. This plate serves as a control for the experiment.

6. Once all strains are inoculated, incubate your plates at 37 °C for 24-48 hours and answer the Exercise #2 questions in the lab assignment.

Exercise #3. Using aseptic technique to inoculate a culture

A key microbiology skill is the ability to transfer microbes from one location to another. For example, you may need to transfer your microbe from a tube to a plate, or a smaller liquid culture to a larger liquid culture. However, microbes are everywhere (bench surfaces, your hands, surfaces of plates, tubes, etc). So, it's very important to be able to transfer ONLY the microbe you're interested in to your culture medium, without also getting contamination from the surrounding environment. Every technique in microbiology requires the use of sterile media, tools, and growth containers. Results are only trustworthy if there is a guarantee that no contamination has occurred.

The primary tool for microbiology is an inoculation loop which consists of a handle and a wire twisted into a small loop. The inoculation loop is typically sterilized with flame or another heat source until it glows red hot, burning away any living organism and rendering the loop completely sterile. After flame sterilization, the loop must be cooled so that the next cells the loop touches are not killed by the hot metal.

Isolation of single colonies via streak plating: A key skill in microbiology is to be able to isolate single colonies. If you dilute a sample of bacteria enough, we can assume that each colony that grows on a plate (each colony is composed of several million cells), originated from one, single cell. Keep in mind that this is an assumption we make about each colony, which is that it came from a single cell. In order to isolate single colonies, we perform streak plates from a mixed culture of *E. coli* and *Serratia marcescens* on a petri dish. Streak plates are extremely useful to microbiologists because they allow you to go from a concentrated suspension to single colonies, all in one plate. This technique will also allow us to separate different species of bacteria growing together to isolate a pure culture.

<u>Materials:</u>

- 1 Trypticase Soy Agar (TSA) Plate **per person**
- Sterile disposable loops
- Marking Pen

1. Label your plate with:
    a. Media type (TSA)
    b. Bacteria on the plate (*E. coli and S. marcescens*)
    c. Date, lab section, group number, name

2. Being careful not to contaminate the tip, remove one disposable loop from the package on your bench. As long as nothing is returned to the package, we can assume that the remaining loops are still sterile.

3. Carefully touch your loop to a sample of bacteria from the provided petri dish. Do NOT use the loop to scoop up a chunk or mass of bacteria… remember, less is more in microbiology.

4. Place the loop coated with culture onto the TSA plate, toward the edge. Use your loop to spread the culture over a small area near the edge of the plate.

5. Acquire a new loop. **This is important!** Otherwise, you're not really diluting your culture, you're just spreading around the initial, very concentrated culture. Pass the sterile loop through the edge of area one, **once or twice only**, and spread it around in a zigzag fashion (see figure 3). It's important that you don't pick up a lot of the initial, concentrated culture from area 1.

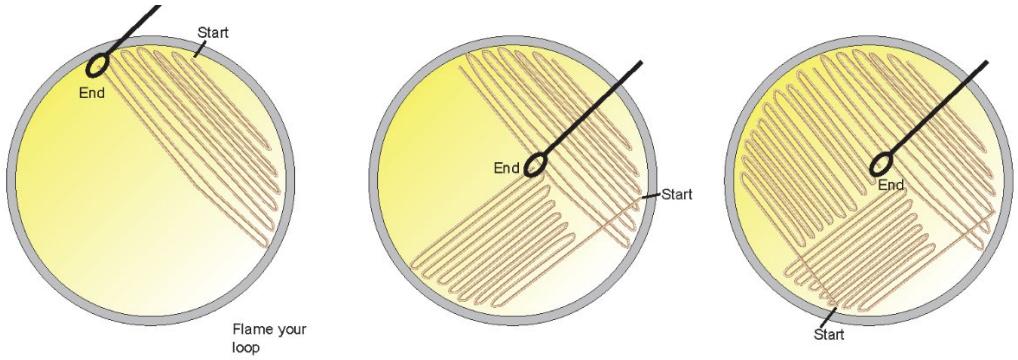


Figure 3. Streak plate method. Using the entire plate, repeat the streaking process one or two more times to ensure better dilution across the entire petri dish. (Image courtesy of Collin Feissner, SUNY Buffalo)

6. **Incubate your plates at 37 °C for 24 hours.**

7. After 24-48 hours, photograph your plates and answer the Exercise #3 questions in the lab assignment.

Lab Section _____ Group # _____

Names: _____

Microbiology Assignment #2 – **due week 3**
(20 points)

Directions
Results from microscopy, inoculations, and presumptive tests. Answer the in-lab questions and insert your pictures and photos directly into this document. One person from your group must save this document and submit the Word file to your Lab Instructor before lab starts on week 3.

Exercise #1 – Simple Staining (8 pts.)

1) Insert labeled photos of at least four slides from your group that represent different cell sizes, morphologies, and stains. Each figure must have a legend indicating specimen name, magnification, and stain.

(Replace this text with your labeled pictures)

Exercise #2 – Selective and Differential Media (6 pts.)

1) Photograph your plates after incubation (one at a time is best). Insert the photos below and label each plate with the media type, incubation temperature, and bacteria growing on each plate.

(Replace this text with your labeled pictures)

2) List the ingredients in EMB that enable the media to be differential and selective and explain why each works.

3) For each strain on your EMB plates, characterize the colonies that grew. Do you see different types of colonies? Describe the appearance of each of the test strains on your plates and explain what the growth/color indicates about the type of bacteria present.

Exercise #3 – Streak Plating (6 pts.)

1) Photograph your plates after incubation (one at a time is best). Insert the photos below and label each plate with the media type, incubation temperature, and bacteria growing on each plate.

(Replace this text with your labeled pictures)

2) Look at the streak plates and record your results. If you have single, well separated colonies – great! If not, what do you think went wrong?

Below are photographs of four attempts at performing a triple-streak of *Serratia marcescens*. Note, these plates were incubated at 25 °C instead of the standard 37 °C.

3) Look at the following four examples of triple-streaked plates. Critique the technique used on each plates and explain if the streak plate worked. If not, what did the microbiologist do wrong?

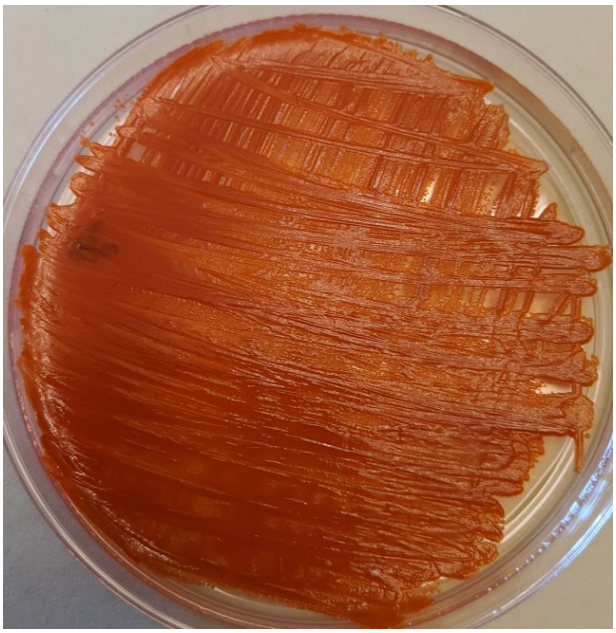


Plate a)

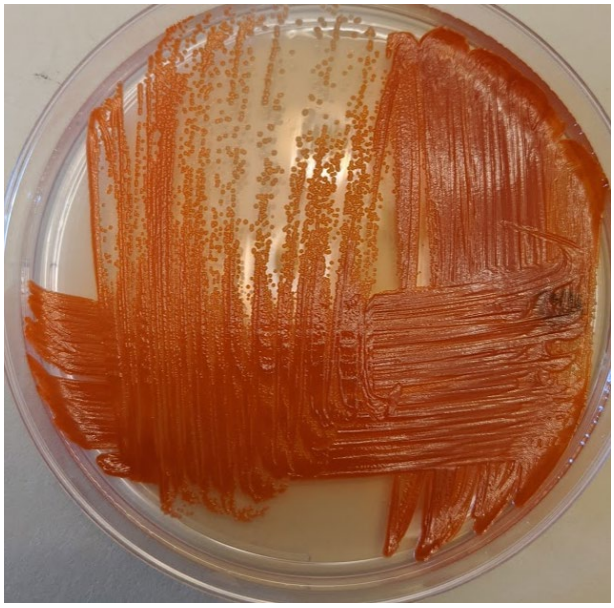


Plate b)

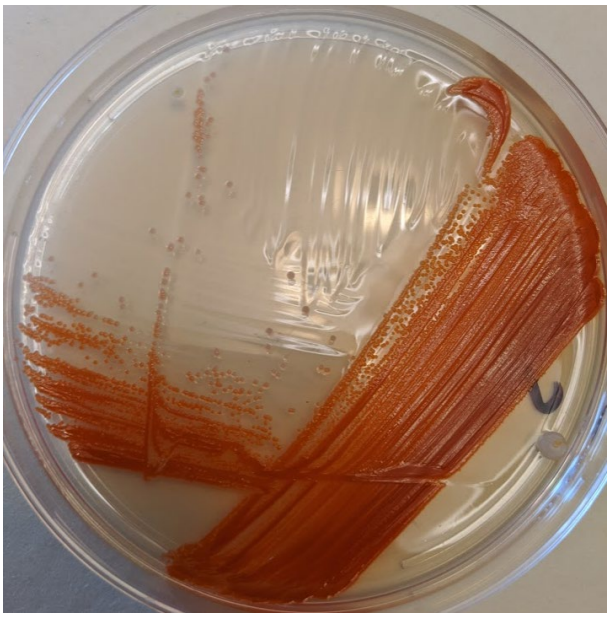


Plate c)

Plate d)

Bacteriology Part III

Learning Objectives: This week's lab builds on basic lab techniques by introducing Gram staining of bacterial cultures. Water testing, a practical application of microbiological techniques in described, and a scenario is presented that serves as the basis for the final investigative project.

- Demonstrate how to prepare and evaluate a Gram stain.
- Understand the relationship between Gram negative, enteric, coliform, and fecal coliform bacteria with respect to water safety
- Describe how water sources can be quickly screened for the presence of coliform bacteria.
- Understand the purpose of and begin initial water testing experiments.

Outline:

- **Exercise #1 – Gram Staining**
- **Introduction to the Case Study, "The Great Flood of 2024" Water Testing for Fecal Coliform Bacteria**
- **Exercise #2 – Presumptive tests for fecal coliform bacteria**

Important Note: Be very deliberate in recording results from this week's experiments because they WILL be an important source of images for your final presentation.

Experiment #1 – Gram Staining

The differential media (EMB) we used last week allowed us to observe differences in bacterial metabolism with different colors that indicate lactose fermentation, differential staining causes bacteria to stain differently based on some difference. The Gram stain is a differential staining procedure that differentiates between bacterial cell wall morphologies.

Differential staining uses two or more stains in sequence. The first stain is called the **primary stain** and is used to color all cells. A **mordant,** or fixative is added to bind to the primary stain to increase its color and increase retention. After rinsing of the primary stain, a **decolorizing agent** is used to wash away the color from some cells based on the presence or absence of cell structures. Finally, the cells are stained with a **counterstain** to re-color any cells that did not retain the primary stain.

For Gram staining, the primary stain is **crystal violet (CV)** and stains all cells purple**. Iodine** serves as the mordant to bind to the CV to intensify the color and retain the dye in the thick cell wall of Gram + bacteria. After decolorizing with 95% ethanol, Gram – cells lose their CV and become colorless, while Gram + cells remain dark purple. To make the Gram – bacteria visible, they are counterstained with **safranin**. At the end of the staining procedure, Gram + cells appear purple and Gram – bacteria appear pink

Gram staining is useful to help categorize unknown bacterial isolates, and is also part of the confirmation test for identifying the presence of *E. coli* in a potentially contaminated water sample.

Materials: per lab group:

- Box of slides (4-8 per group)
- Staining rack (over sink on bench)
- Bunsen burner / lighter
- Distilled water in squirt bottle
- Clothes-pin slide holder
- Crystal Violet (in 50 mL tube)
- Gram's Iodine (in 50 mL tube)
- Safranin (in 50 mL tube)
- Decolorizer (95% EtOH)
- Pipettes and tips
- Microscope

Each person will make **two slides** with **two** of the lab cultures each; one gram positive and one gram negative on the same slide. Lab strains include *Escherichia coli (Gram negative), Enterobacter aerogenes (Gram negative), Bacillus cereus (Gram positive), and Staphylococcus epidermidis (Gram positive).* Each slide will be Gram stained using the protocol below.

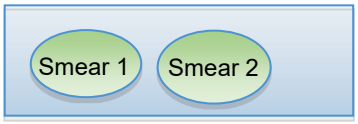
Procedure – Gram Staining:
Last week's smears may have been too dense to get a good look at individual bacteria. The Gram stain protocol works best with a single layer of bacteria on a slide (as opposed to clumps of bacteria). Organisms cultured on solid media produce thick, dense surface growth and cannot be directly transferred to a slide for staining. Plate grown cultures must be diluted by resuspension in water on the center of a slide.

Bacterial Smear:

6) Using a pipette or dropper bottle, place a 10 µl drop of water **1/3 of the way down** a clean slide.
7) Transfer a small sample of bacteria using a sterile inoculating loop. **Only the tip of the loop or needle should touch the culture to prevent the transfer of too many cells.**

8) Spread the cells in a circular motion in the drop of water with the loop or needle. The finished smear should occupy an area about the size of a nickel and should appear as a translucent, or semitransparent, confluent whitish film. If you can't read the print in this lab through your smear, it is much too dense. You should be able to see a haze on the slide, yet still be able to see through it.

9) Repeat for a 2nd strain of bacteria a little above the first smear. Each slide should have one Gram-negative and one Gram-positive strain. This will make comparison of the staining differences easy to observe.



10) Allow the smear to air dry completely. Do **NOT** blow on slide or wave it in the air because it could aerosolize the bacteria.

11) Heat fix the bacteria by passing the dry slide over a Bunsen burner once or twice, (don't cook the bacteria!), holding with slide with a clothes pin.

Gram Staining:
All staining will be carried out in 50 mL conical tubes containing stains. This will help to minimize stain waste.

7) **Primary Stain:** Place a slide with a heat-fixed smear into a tube of crystal violet so that the smear is submerged in stain. Let stand for 1 minute.

8) Remove the slide and gently wash the smear with distilled water to remove excess stain. Wash the slide from above the smear and let the water cascade over the bacteria. Do not spray water directly on the smear to avoid the loss of bacteria from the slide.

9) **Mordant:** Place the slide into a tube of Gram's Iodine so that the smear is submerged in stain. Let stand for 1 minute.

10) Rinse the slide with water, as before, to remove excess iodine

11) **Decolorize**: This step is crucial. Over-use of the decolorizer will wash the all stain away from all cells. Under-decolorizing will not wash the stain from any cells. Using a dropper this time, slowly drip decolorizer down the smear until the excess runs clear.

    a. After decolorizing, the ***B. cereus*** *and* ***S. epidermidis*** smears should look visibly purplish, while the ***E. coli*** and ***E. aerogenes*** smears will be very pale purple or colorless.

12) Rinse the slide with water, as before, to remove excess decolorizer.

13) Counterstain: Place the slide into a tube of safranin so that the smear is submerged in stain. Let stand for 1 minute.

14) Rinse the slide with water, as before, to remove excess safranin.

15) Place slide between sheets of bibulous paper to blot it dry, but do not wipe the slide.

16) Examine all stained slides under the microscope when dried.

Make sure to record your results by taking a picture through the microscope eyepiece with your phone. Tips for labeling what you see under the microscope:

c) Always write the magnification at which the observations were made (100X - 600X)

d) Label the drawings with any observable features, including the color of objects.

e) These images may serve as good positive and negative controls for comparing to the bacteria in your water sample in the next experiment (next week).

The Enterobacteriaceae and Water Quality

Enteric bacteria, or members of the phylogenetic group of bacteria called ***Enterobacteriaceae*** represent some of the most medically and environmentally important bacteria. The word "enteric" is defined as related to or occurring in the intestines, and is used to describe bacteria such as *Citrobacter, Serratia, and E. coli* that are most often found in the large intestine of healthy people and warm-blooded animals. Many members of the *Enterobacteriaceae,* including *Salmonella spp., Klebsiella pneumoniae*, and *E. coli* can also cause diseases that affect millions of people each year. Therefore, characterization of enteric bacteria is very important.

Enteric bacteria are all Gram-negative rod-shaped bacteria that can grow in the presence OR absence of oxygen and can ferment glucose to generate energy. A smaller group of enteric bacteria called **coliform** or **total coliform** bacteria are enteric bacteria that have the additional ability to ferment the disaccharide lactose resulting in the production of acid and gas. *Serratia marcescens* is an example of an enteric bacteria that is a non-coliform because it cannot ferment lactose. Many coliform bacteria are naturally found in the soil. Subdividing the coliform group further leads to a smaller group of bacteria called **fecal coliform bacteria.** Fecal coliform bacteria are a group of bacteria that are passed through the fecal excrement of humans, livestock and wildlife and aid in the digestion of food. While some fecal coliforms can be natural inhabitants of the soil, their presence is mostly due to fecal contamination. Ideally, we would like to be able to distinguish the sub-type of coliforms that are specifically of fecal origin from other coliforms that may come from non-fecal sources because it is the fecal coliforms that are real indicators of potential fecal contamination. For instance, the fecal coliform bacterium *E. coli* is not usually found in water, so if *E. coli* are found in a water sample, it is likely that intestinal waste has made it into the water somehow. Therefore, we can create a final subgroup within the fecal coliform bacteria simply represented by *E. coli* (Figure 1).

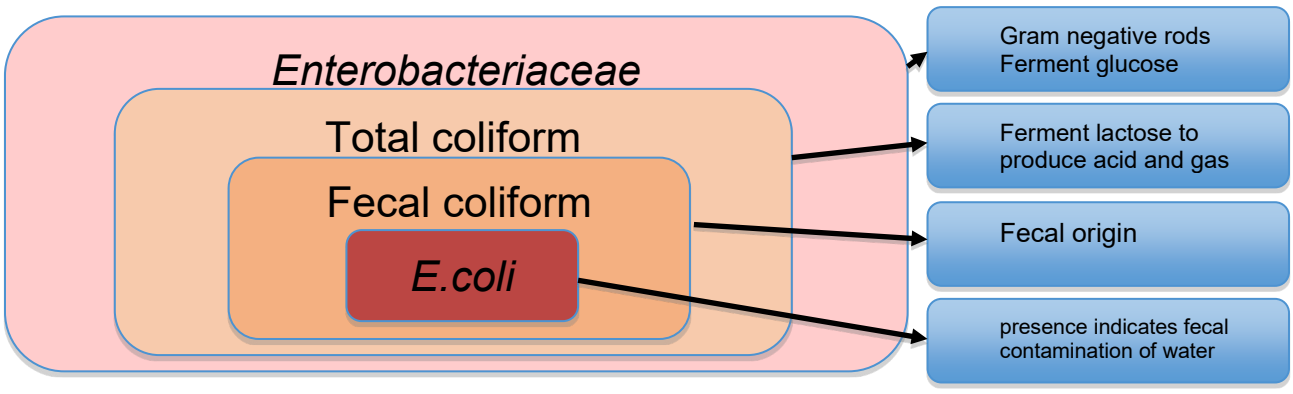


Figure 1. Inclusive sub-groupings of enteric bacteria.

The presence of Fecal Coliform in well water may indicate recent contamination of the groundwater by human sewage or animal droppings which could contain other bacteria, viruses, or disease causing organisms. This is why coliform bacteria are considered "indicator organisms"; their presence warns of the potential presence of disease causing organisms and should alert the person responsible for the water to take precautionary action.

Fecal coliform bacteria can enter rivers through direct discharge of waste from mammals and birds, from agricultural and storm runoff, and from untreated human sewage. Individual home septic tanks can become overloaded during the rainy season and allow untreated human wastes to flow into drainage ditches and nearby waters. Agricultural practices such as allowing animal wastes to wash into nearby streams during the rainy season, spreading manure and fertilizer on fields during rainy periods, and allowing livestock watering in streams can all contribute fecal coliform contamination.

At the time this occurs, the source water may be contaminated by pathogens or disease producing bacteria or viruses, which can also exist in fecal material. Some waterborne pathogenic diseases include ear infections, dysentery, typhoid fever, viral and bacterial gastroenteritis, and hepatitis A. The presence of fecal coliform tends to affect humans more than it does aquatic creatures. While many coliform bacteria do not directly cause disease, high quantities of fecal coliform bacteria can indicate the presence of disease-causing agents. The presence of fecal contamination is an indicator that a potential health risk exists for individuals exposed to this water. During high rainfall periods, the sewer can become overloaded and overflow, bypassing treatment. As it discharges to a nearby stream or river, untreated sewage enters the river system. Runoff from roads, parking lots, and yards can carry animal waste to streams through storm sewers.

Fecal coliform concentrations are reported in units of the number of bacterial colonies per 100 mL of sample water (#/100 mL). The current United States Environmental Protection Agency recommendations for body-contact recreation is fewer than 200 colonies/100 mL; for fishing and boating, fewer than 1000 colonies/100 mL; and for domestic water supply, for treatment, fewer than 2000 colonies/100 mL. The drinking water standard is less than 1 colony/ 100ml.

By growing and counting colonies of fecal coliform bacteria from a sample of stream water, we can determine approximately how many bacteria were originally present.

Case Study and Final Project:

The Spring of 2024 was a very wet season with unusually high rainfall. During one very wet week in February, consistent precipitation resulted in complete flooding of the low elevation areas in the Genesee River floodplain west of Geneseo. During high rain events, when storm water enters the sewers, the capacity of the sewer system may be exceeded, and the excess water gets discharged directly to nearby rivers and streams. Many residents in Piffard that rely on well-water have fallen ill with gastroenteritis. It is suspected that fecal coliform contaminated water is to blame.

Although the flood waters have receded since the storm, officials at the Department of environmental Conservation suspect that there may be a persistent source of fecal contamination that jeopardizes the water supply of Piffard well-water users. You have been hired to determine where the water contamination originated, to determine what bacteria are present, and to quantify bacterial numbers at the location of the leak. You have identified four potential locations that fecal coliform bacteria may have been released into the water supply **(Figure 2**). Your job is to perform a preliminary screening of water samples in the Genesee River between Geneseo and Piffard. Money for water sampling and testing is always in short supply so you have limited resources at your disposal, but they should be sufficient to do the job.

When you have completed your investigation, your research group will inform the public of your findings at a Town of Geneseo Board meeting that is open to the public. You will be allotted less than 10 minutes to describe the problem, explain your findings and the rationale behind your work, and provide a conclusion

that includes a recommendation for cleanup of the site of contamination. In short, you will present an oral version of a complete research project to an audience that does not have extensive biological knowledge.

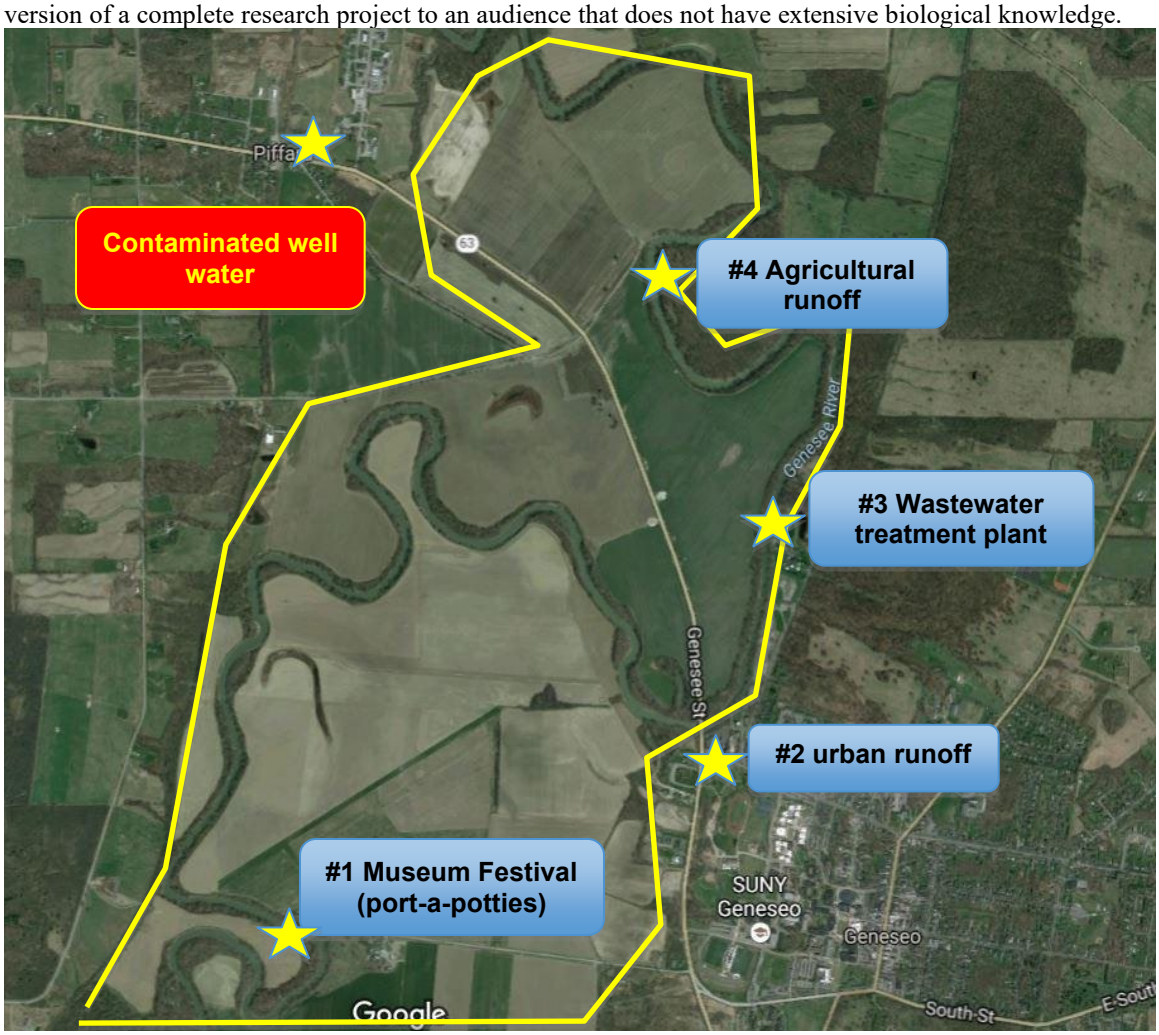


Figure 2. Extent of flooding in the Genesee River floodplain in Geneseo, NY, Spring 2023. The yellow outline indicates the region that was completely submerged by floodwaters in February, 2024. High-probability locations for fecal coliform water contamination include the National Warplane Museum port-a-potties (location #1), sewer overflow from the Town of Geneseo (location #2), Leakage from the Geneseo Wastewater Treatment Plant (Location #3), and agricultural runoff from fields with grazing cattle (Location #4).

Protocol:

Begin thinking about how you can apply the techniques you are learning about to answer the following questions;

- How can fecal coliform bacteria be distinguished from other coliform bacteria and the bacteria normally found in the environment?
- What test can be used to identify the presence of fecal coliform bacteria?
- How can bacterial numbers be determined from a sample of water?

The following is an informative resources that provides valuable background information; Indicator Organisms and Water Safety (https://www.hach.com/asset-get.download.jsa?id=7639982206)

Exercise #2 – Presumptive test for coliforms in water samples

A series of tests will be conducted to determine if total coliforms are present in water. The first test is called a presumptive test. A presumptive test is used to analyze a sample and establish one of the following; the sample definitely *is not* a certain substance, or the sample *probably is* the substance. In this case, the presumptive test will help tell us if a water sample definitely does not contain a fecal coliform or that it probably does. For this test, we will use **Phenol Red Lactose Broth** and EMB plates.

As described above, coliform bacteria are distinguished by their ability to ferment lactose to produce acid and gas as byproducts. A simple test to determine if *any* coliform bacteria are present in water is to use Phenol Red Lactose Broth. Phenol Red fermentation medium contains peptone, phenol red (a pH indicator) and lactose. Phenol red is yellow at a pH < 6.8 and red at a pH of > 7.4, therefore if a bacterium ferments lactose to acid, a yellow color will develop. Additionally, phenol red tubes contain an inverted Durham tube (a small upside-down glass tube). If a bacterium produces a gas during fermentation the Durham tube will trap the gas and a bubble will collect in the Durham tube. Sometimes an orange color will develop in the tube, this represents a pH > 6.8 and should not be considered positive. Examples of results from a series of phenyl red lactose test are shown below (Figure 3)

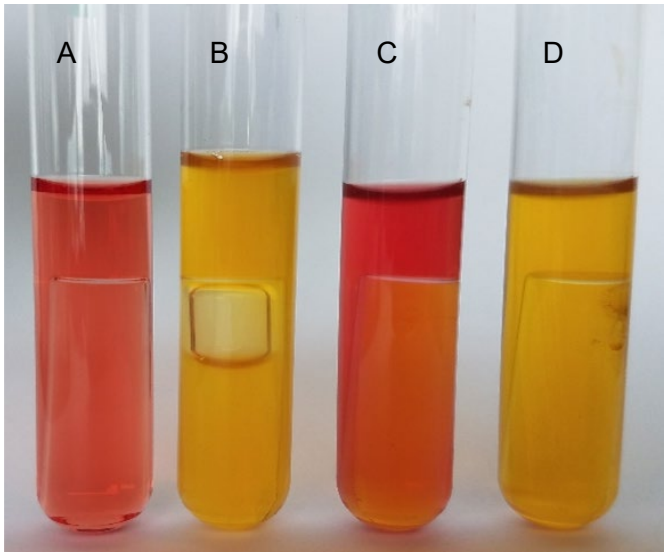


Figure 3. The phenyl red lactose test illustrates how bacteria metabolize lactose. A, un-inoculated tube; B, Lactose fermentation with acid and gas production; C, No lactose fermentation (note the slight orange color); D, lactose fermentation with acid and no gas.

Materials: per lab group:

- 4 Phenol Red Lactose Broth tubes
- 1 TSA plate
- 1 Eosin Methylene Blue (EMB) plate
- Disposable inoculating loops
- Marking pen
- Water samples collected from locations #1-#4 (each group will receive a set of samples)

Protocol for Exercise #2 – Presumptive tests, Parts A and B
Part A: Phenol Red Lactose broth

1) Label each phenol red lactose broth tube with your group number and the Location # (e.g. Group 2 – Loc #3).

2) Using sterile technique, use a micropipettor and sterile tips to inoculate the tube labeled Location #1 with **50 µL of water from location #1.**

3) Repeat for Locations #2 - #4

4) Put your tubes in the class test-tube rack for incubation at 37ºC for 24 hours.

5) After 24 hours, photograph your tubes and answer the notebook questions for Exercise #2

Part B: EMB

1. Divide the EMB and TSA plates into four sections each by drawing lines through the center with a sharpie on the **back** of the plate. Label with the media name, your group number, section, and incubation temperature.

2. Label each quarter with the number of a water sample location.

3. Using aseptic technique, gently collect a sterile loopfull of water from one of the water samples.

4. Transfer the sample to corresponding quadrant of a TSA plate and inoculate using a zigzag motion to spread the culture. **Remember to use your (by now amazing) aseptic technique so you don't contaminate the stocks. DO NOT allow the water to overlap or come in contact the water from a neighboring section on the plate**

5. Repeat steps 2-4 with the remaining water samples on both TSA and EMB plates.

6. If the plates are visibly wet after inoculation, allow them to dry on the bench before covering

7. Once all strains are inoculated, incubate your plates at 37 °C for 24-48 hours .

Lab Section _____ Group # _____

Names: _____

Microbiology Assignment #3 – **due week 4**
(20 points)

Results from Gram stains, microscopy, and presumptive tests. Answer the in-lab questions and insert your pictures and photos directly into this document. One person from your group must save this document and submit the Word file to your Lab Instructor before lab starts on week 4.

Exercise #1 – Gram Staining (10 pts.)

1) Insert labeled photos of gram stained bacteria that includes all of the strains used in lab this week. Each figure should have a legend indicating specimen name, magnification, and Gram classification.

(Replace this text with your labeled pictures)

Exercise #2 – Presumptive Water Tests (10 pts.)

1) Photograph your phenyl red lactose broth tubes after incubation. Insert the photo(s) below and label each tube with the media type, incubation temperature, and water source.

(Replace this text with your labeled pictures)

2) Photograph your plates after incubation (one at a time is best). Insert the photos below and label each plate with the media type, incubation temperature, and water source for the bacteria growing on each plate.

(Replace this text with your labeled pictures)

3) Each water source contains more than one species of bacteria. For each water source tested determine the likelihood of fecal coliform contamination. Support your answer with evidence from the tests you did in Exercise 2 (Remember that evidence from two tests provides stronger support than just one.)

Bacteriology Part IV

Learning Objectives: This week's lab completes experimentation on contaminated water samples. A final technique, the Gram stain, is introduced to further characterize the properties of bacteria. This scenario presented last lab serves as the basis for the final investigative project that will be completed this week.

- Complete tests necessary to confirm the presence of, and calculate the number of bacteria present in a contaminated water sample
- Prepare an oral presentation of the results and methodology of your contaminated water investigation that could be presented at a Town Council meeting in lab.

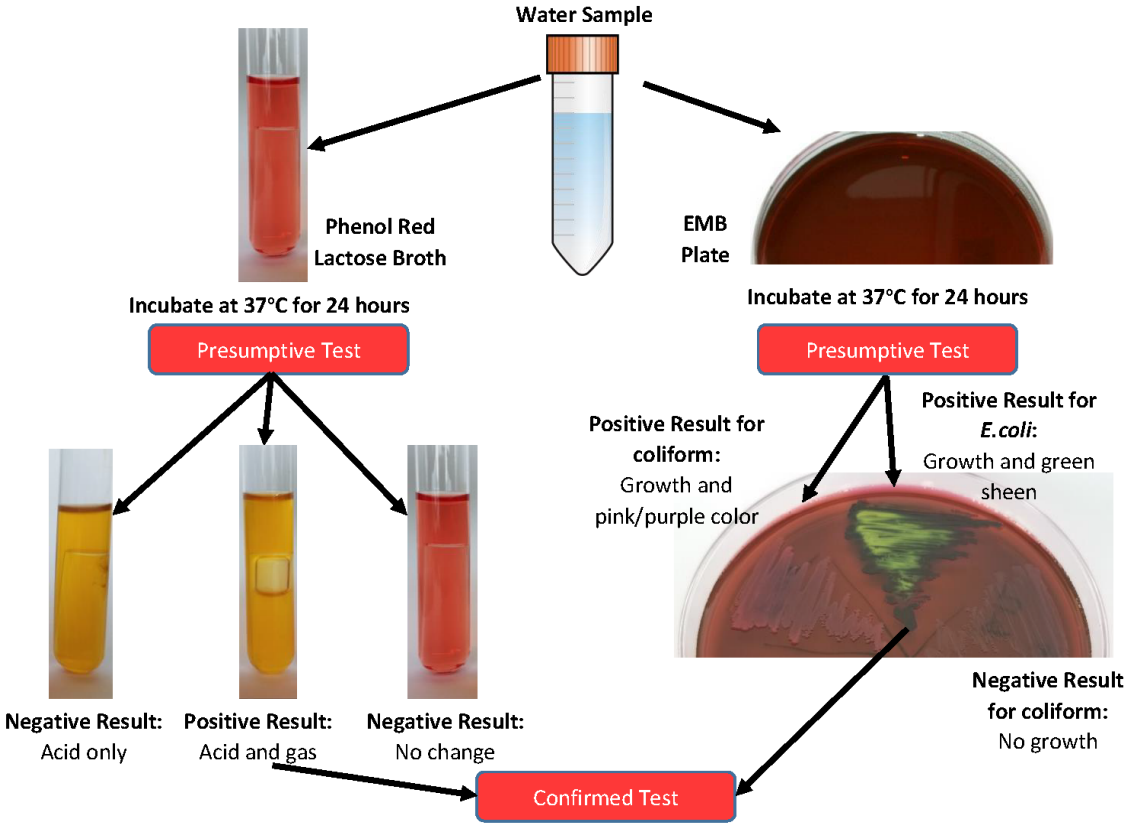
Outline:

- **Introduction**
- **Completed tests for fecal coliform bacteria:**
  - **Gram Stain of plate colonies**
  - **Enumeration**
  - **Verification of PRLB and EMB results**
- **Presentation requirements**

Introduction: Interpreting Presumptive Tests

The presumptive tests you completed during the last lab session provide a wealth of information about the microbes present in the water in different locations around Geneseo. Recall that the residents of Piffard became ill when their well water was contaminated with flood water. The exact cause of the illness is not known, but it is likely that the infectious agent was of fecal origin. If the agent of disease itself is not known, how can scientists establish the source of the contamination? The answer lies in indicator organisms. If an indicator that is always present in feces and not normally in water happens to be present, then it can be presumed that its presence is due to fecal contamination. The presumptive tests are a first step in detecting indicator species.

You carried out two experiments to detect the presence of fecal coliform bacteria; The Phenol Red Lactose broth test and growth on EMB media. Both tests use growth media that are selective for and enrich the growth of total coliform bacteria. Positive results from both tests allows us to presume that coliforms are likely present. Negative results or only a single positive allow us to presume that coliforms are absent. The flowchart on the next page (Figure 1) will help you evaluate the results of your presumptive tests and allow you to narrow down the source of fecal contamination to one or two water sources.



Water samples with a positive Phenol Red Lactose result AND are positive for coliforms on EMB are positive for the confirmed test (see example below)

Water Sample:	Location #1	Location #2	Location #3	Location #4
Lactose:	+	+	+	-
EMB:	+, pink	+, green	-	-
Confirmed?	YES	YES	no	no
Likely:	Lactose fermenting total coliform	Lactose fermenting *E. coli*	Lactose fermenting non-coliform	

Completed Test

Gram Stain: Pink bacteria + **Phenol Red Lactose Broth:** Yellow, gas + **EMB:** Growth and green sheen + **Bacterial count:** Dilution plating and paddle test

Figure 1. The presence of indicators of fecal water contamination requires three levels of tests. This flowchart is useful for interpreting the results of presumptive tests, the results of which confirm the presence of coliform bacteria. Completed tests are performed on confirmed water samples to eliminate false positives and verify the presence of a fecal coliform (gram-negative rod that produces acid and gas from lactose fermentation and is clearly of fecal, not environmental origin, e.g. *E. coli*). The panel of tests includes all four components, so lactose broth and EMB are tested again.

Completed tests:
Completed tests for fecal coliforms usually focus on verifying the presence of *E. coli* because it is not normally found in water, but is always present in feces. Because funding for water screening is tight, you only have enough resources to confirm the presence of *E. coli* in water samples that have passed the presumptive test. Re-evaluate your results from last week (**Question #2, Lab assignment #2**). From only

the water samples that passed the presumptive test you will carry out the completed tests, all of which must be positive to confirm the presence of the indicator species;

1) **Phenol Red Lactose Test**: reconfirm that an acid and gas producing bacterium is present. This is not enough to confirm fecal contamination because there are non-coliform bacteria that can live naturally in the environment that also ferment lactose.
2) **EMB media**: EMB is selective and differential for gramnegative bacteria that utilize lactose, characteristics of coliform bacteria. *E. coli* has a distinctive green sheen on EMB that can be used to indicate its presence. Coliforms of both fecal and environmental origin may grow on EMB, so this test alone is not sufficient to confirm the presence of the indicator species.
3) **Gram Staining**: The gram stain is useful for confirming the shape and cell wall structure of the bacteria in the water. Coliforms are all gram-negative rods. If the bacteria are non-rods or gram-positive, fecal contamination cannot be confirmed.
4) **Enumeration**: The bacterial load, or amount of bacteria present in the water is necessary to establish whether the contamination is within the safe limits for different uses. This can be done using dilution plate counting.

Final Experiment – Enumeration of Bacteria and Completed Tests
As you recall, the EPA has established levels of acceptable contamination for different activities. The bacterial load, or the amount of bacteria present per unit volume is important to determine. The typical measure is colony forming units per mL of water, whereas the EPA lists acceptable bacterial load in CFU/ 100 mL. Dilution plating is the best way to establish bacterial numbers, but it is time consuming and requires a lot of growth media. Some manufacturers produce inexpensive and quick sampling tools that can provide a non-exact estimate of bacterial load. These testers are designed to be dipped directly into a water sample, shaken off, and incubated. The colonies that grow can be compared to standards.

Procedure – Completed Tests:
Perform a dilution of your water sample to get a good count of the average number of bacteria in your water sample. The dilution is necessary to guarantee countable colonies on plates. For the completed tests, you will do total plate counts on TSA and EMB and re-inoculate a Phenol Red Lactose Broth tube. Carefully consider how you will do this BEFORE starting the tests. An example of one possible workflow for inoculation and plate spreading can be found in Figure 2. You are encouraged to design your own experiment based on your presumptive results.

Materials: (Per group)
- Distilled water in squirt bottle
- Pipettors (P1000, P200, P20, shared in lab)
- Pipette tips
- 3 TSA plates
- 1-2 EMB plates
- 1 Phenol Red Lactose Broth tube
- Alcohol and spreader
- Bunsen burner

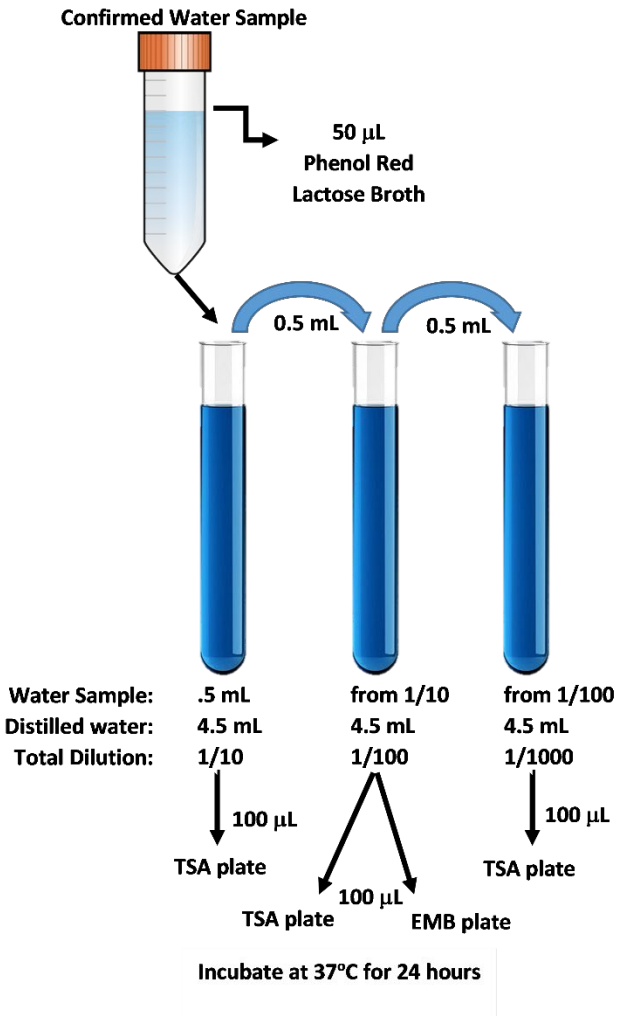


Figure 2. Example Enumeration and Completed test workflow.

Protocol: Serial dilution for plate counting

1) You will be testing undiluted water and three serial dilutions (1/10 dilutions will work well here).

2) Using the diagram in Figure 2, carry out three 1:10 serial dilutions in distilled water.

3) Plate 100 µL of each dilution on TSA plates so you can make an accurate bacterial count in your water sample.

4) Incubate plates upside down at 37^{O}C for 24-48 hours. Refer to weeks 1 and 2 to calculate the original bacterial count (CFU/mL) from your dilutions and colony counts.

Protocol: Phenyl Red Lactose Broth Confirmation

1) Label one phenol red lactose broth tube with your group number and the Location # from your positive confirmed test tube (e.g. Group 2 – Loc #3).

2) Using sterile technique, use a micropipettor and sterile tips to inoculate the tube with 50 µL of water.

3) Put your tube in the class test-tube rack for incubation at 37ºC for 24 hours.

4) After 24 hours, photograph your tube and collect results.

Protocol: Gram Stain

Materials: per lab group:

- Box of slides (2-4 per group)
- Bunsen burner / lighter
- Distilled water in squirt bottle
- Clothes-pin slide holder
- Crystal Violet (in 50 mL tube)
- Gram's Iodine (in 50 mL tube)
- Safranin (in 50 mL tube)
- Decolorizer (95% EtOH)
- Pipettes and tips
- Microscope

Each group will make **two slides** with **two** of strains on each; a colony of your lactose-fermenting water-borne bacteria and one control. The controls will be one gram-positive strain and one gram-negative strain. Lab strains include *Escherichia coli (Gram negative), Bacillus cereus (Gram positive), and Staphylococcus epidermidis (Gram positive)*. Each slide will be Gram stained using Week 3 protocol.

Bacteriology Final Assignment - Persuasive Oral Presentation

You and your lab group will deliver an oral presentation with visual aids (PowerPoint) to the Geneseo Town Board (in reality, your lab instructors). Your presentation will include most of the components of a lab report. The difference is that you need to be as concise (short, direct, to the point) as possible. Your presentation should have all the elements of a complete lab report: Introduction, Methods, Results (including tables and figures), and a Conclusion

You may refer to any of a number of scientific writing guides for guidance in preparing your presentation. You will be limited to 7 minutes of presentation time with an additional 3 minutes available for questions from the Town council. The 7 minute limit will be strictly enforced, so make sure you are able to present the info you need within that time-frame. You do not have a limit to the number of slides you can prepare, but too many slides can get you into trouble as you will be cut off at the 7 minute mark. Every member of your group is required to present a portion of the report and should be ready to answer questions at the end.

The grading rubric for this presentation will be similar to the rubrics from your lab reports with a greater emphasis on content and explanation. Your "audience" (the Geneseo Town Board) will consist of educated professionals that are not scientists. You should be ready to present your research and conclusions with enough explanation and detail to inform a lay audience. For instance, don't expect your audience to know what a fecal coliform bacteria is, but you can be confident that they know what a petri dish is used for. Your audience may not need to know what the different steps of the gram stain procedure do, but as the experts, you should be able to describe them if you are asked!

The content is up to you, but must provide enough information for the council to believe that your analysis is comprehensive and can be trusted. The town has limited funds to clean up one and only one location and the residents of Piffard are relying on you to make the right assessment.

Be sure to include the following in your Presentation:

- Your **introduction** should be brief but should set the stage for the question you asked with your study. Your audience does not necessarily know what you are investigating or why it matters. At the end of the introduction you should have a statement that tells the reader the purpose of your experiment.

- Your **methods** will not be a section of their own, but will be integrated into the presentation when necessary. If it is more concise for you to present methods in outline, bullet point, or flowchart form, you may do that. Your audience does NOT need a description of all of the steps in your methods, but should be able to understand why you performed a given test. Pictures convey a great deal of information in a visually interesting manner.

- Your **results** should include both a brief text description of your results and relevant tables and figures. Figures are **most important** in a presentation, so make sure they are clear and visible. All figures must be clearly labeled so they could be understood on their own. Descriptive titles that contains an answer to a question (each figure was the result of a particular question) are very helpful. Remember, it might not be the best use of space to include a table of raw data in your presentation – figures that summarize the important results of your data are usually best. **You must use figures from your own experiments in this presentation. Do not use stock photos or images found from the internet as your own.**

- Your **discussion** should relate your results to the problem at hand – this is the significance of your study. You must be able to justify your conclusion with data and be able to convince the town council that they should spend money on the problem.

This presentation will be presented to and graded by your faculty supervisor. Each of the presenters should be prepared to answer questions about any part of the experiment and the presentation.

There is a lot to discuss in a short 7 minutes. Prepare early and practice, practice, practice. The groups that expect to do well will have a polished presentation that flows smoothly and is well organized.

Some questions to consider during your public presentation. (This list is NOT comprehensive)

- What did you find at each location?
- Why is *E. coli* used as an indicator organism for fecal contamination?
- Can you distinguish between environmental total coliform and fecal coliforms?
- What tests did you complete, what do they test for, and what did you learn about the water from each location?
- About how much fecal coliform bacteria are present in the water (CFU/mL)
- What is a safe level of fecal coliform in drinking water?
- How do you know there isn't fecal contamination of the other water samples?
- What location(s) would you tell the council needs to be remediated?
- How can you be sure other locations are NOT contaminated?

Materials: Week 2 – Week 5

MATERIALS

Described here, are the materials needed for a 24-student laboratory consisting of six 4-student groups. All materials, excluding live cultures, may be prepared up to 6 weeks in advance and all autoclaved media will be stable for at least 3 months at 4°C.

Strains

The strains of bacteria used for this lab are common type strains and are considered BSL1, and do not typically cause disease in healthy individuals. Of note, it is essential that the *E. coli* strain used in the lab is positive for lactose fermentation (many research strains are Lac-). All strains grown on solid media in petri dishes are incubated at 37°C overnight in a standard microbiological incubator. Liquid stocks for preparatory and lab use are grown overnight in 150x16 glass tubes in a shaking incubator at 37°C.

Strain	note	supplier
Escherichia coli	Gram -, Lac+	https://www.atcc.org/products/25922
Enterobacter aerogenes	Gram -, re-named *Klebsiella aerogenes*	https://www.atcc.org/products/51697
Serratia marcescens	Gram -	https://www.atcc.org/products/13880
Staphylococcus epidermidis	Gram +	https://www.atcc.org/products/14990
Bacillus cereus	Gram +	https://www.atcc.org/products/14579

Media & Reagents

All media should be prepared following the manufacturer's directions.

Culture Media	Supplier	Product number
BD Bacto™ Tryptic Soy Broth (Soybean-Casein Digest Medium),	Fisher Scientific	DF0370-17-3
BD Difco™ Dehydrated Culture Media: Tryptic Soy Agar (Soybean-Casein Digest Agar)	Fisher Scientific	DF0369-17-6
BD BBL™ Dehydrated Culture Media: Eosin Methylene Blue Agar, Levine	Fisher Scientific	B11221
BD BBL™ Dehydrated Culture Media: Phenol Red Lactose Base	Fisher Scientific	B11519

Materials & Equipment

Standard microbiology-related equipment is needed for this sequence of labs.

Equipment	notes
Stationary incubator (37°C)	Stock preparation and lab media incubation (size dependent on class size and number)
Shaking incubator (37°C)	Strain preparation
Compound microscopes	At least one per student group, preferably more. 10x, 20x, 40x or 60x recommended magnification
Bunsen burners & lighters	2-6 sets
Test Tube racks	For 13x100 tubes, 6x
Test Tube Caps	For 13x100 and 16x150 glass test tubes, bulk
Spring clothespins	slide holders for staining, 24-48x
Micropipettors and sterile tips	p20, p200, and p1000, 2 sets or more

Consumable Materials	Supplier	Product number
Methylene Blue (Certified Biological Stain)	Fisher Scientific	M291-25
Durham tubes: Fisherbrand™ Expansion Short Style Glass Shell Vials without Closures	Fisher Scientific	03-339-30A
Fisher Science Education™ Gram Stain Kit (contains crystal violet, Gram's	Fisher Scientific	S25344

iodine, safranin, and destain)		
Decon™ BDD™ Bacdown™ Detergent Disinfectant	Fisher Scientific	18-800-101
Stage Micrometer	Ward's Science	470175-914
Bellco Glass, Inc. Test Tubes. 13x100mm 8 mL	Fisher Scientific	50-233-0591
Thomas Scientific Culture Tube, Borosilicate Glass, 16 x 150mm, 15mL	Fisher Scientific	50-146-1434
Research Products International Corp Disposable Petri Dishes, Sterile, 100 x 15 mm	Fisher Scientific	50-476-472
Fisherbrand™ 50mL Easy Reader™ Plastic Centrifuge Tubes	Fisher Scientific	06-443-18
Inoculating Loop and Needle, Flexible 10 μL	Fisher Scientific	22-363-597
Fisherbrand™ L-Shaped Cell Spreaders	Fisher Scientific	14-665-230
Fisherbrand™ Economy Plain Glass Microscope Slides	Fisher Scientific	12-550-A3
Fisherbrand™ Bibulous Paper	Fisher Scientific	12-587-112

If available and when time allows, reusable bacteriological loops and spreaders can be utilized to save on plastic waste and cost. If unavailable, sterile toothpicks can be substituted for loops for the purpose of transferring bacteria for sectored plates and staining. Test tube sizes are recommendations but can be substituted for anything available.

Materials by week (one 24-student lab)
Week 2 Advance preparation (24 hours prior to lab)

Item	Total	notes
E. coli & *S. marcescens* mixed culture plate (TSA)	1	Inoculate both strains as a lawn on the plate. For triple-streak technique.
E. coli, S, marcescens, B. cereus, E. aerogenes, S. epidermidis culture plates (TSA)	2-6 plates of each	Inoculate strains as a lawn on the plate. For staining and inoculation. Plates can be shared between groups to minimize the number of plates. These may be reused for multiple sections each day if not cross-contaminated .

Week 2 For Lab

Item	Per group	Per student	Total	notes
Microscope setup	1	-	6 (min.)	Microscope. Use more microscopes if available.
Staining setup	6	-	6	Box of glass slides, water rinse bottle, clothespins (x4-8), bibulous paper, Bunsen burner and lighter
p20 & tips	1	-	6	for slide preparation
0.3% methylene blue stock	1	-	6	40 mL in 50 mL conical tubes for staining
Safranin (from gram stain kit)	1	-	6	40 mL in 50 mL conical tubes for staining
Crystal violet (from gram stain kit)	1	-	6	40 mL in 50 mL conical tubes for staining
TSA petri dishes	1 + 4	-	30	
EMB petri dishes	1	-	6	
Disposable Loops	30	-	180	72 for triple streaking, remainder are for transfers

Week 3 Advance preparation (24 hours prior to lab)

Item	Total	notes
E. coli, S, marcescens, B. cereus, E. aerogenes culture tubes (TSB)	1 ea	For preparing water samples
E. coli, S, marcescens, B. cereus, E. aerogenes, S. epidermidis culture plates (TSA)	2-6 plates of each	Inoculate strains as a lawn on the plate. For staining and inoculation. Plates can be shared between groups to minimize the number of plates. These may be reused for multiple sections each day if not cross-contaminated .
Water samples from scenario locations in labeled 50mL conical tubes	6 sets	One set of water from each location per groups. Instructions are found in Supplemental Materials.

Week 3 For Lab

Item	Per group	Per student	Total	notes
Microscope setup	1	-	6 (min.)	Microscope. Use more microscopes if available.
Staining setup	6	-	6	Box of glass slides, water rinse bottle, clothespins (x4-8), bibulous paper, Bunsen burner and lighter
p20, p100, and tips	1	-	6	for slide preparation and inoculation
Gram Stain Reagents	1	-	6	Crystal Violet, Safranin, and Gram's Iodine (40 mL in 50 mL conical tubes), water squirt bottle, destain (or 95% ethanol) in squirt bottle.
TSA petri dishes	1	-	6	
EMB petri dishes	1	-	6	
Phenol Red Lactose Broth tubes (13x100 w/durham tubes)	3-4	-	18-24	Number is dependent on number of unknown locations in scenario
Disposable Loops	8	-	48	for transfers

Week 4 Advance preparation (24 hours prior to lab)

Item	Total	notes
E. coli, S, marcescens, B. cereus, E. aerogenes culture tubes (TSB)	1 ea	For preparing water samples
E. coli, S, marcescens, B. cereus, E. aerogenes, S. epidermidis culture plates (TSA)	2-6 plates of each	Inoculate strains as a lawn on the plate. For staining and inoculation. Plates can be shared between groups to minimize the number of plates. These may be reused for multiple sections each day if not cross-contaminated .
Water samples from scenario locations in labeled 50mL conical tubes	6 sets	One set of water from each location per groups. Instructions are found in Supplemental Materials.

Week 4 For Lab

Item	Per group	Per student	Total	notes
Microscope setup	1	-	6 (min.)	Microscope. Use more microscopes if available.
Staining setup	6	-	6	Box of glass slides, water rinse bottle, clothespins (x4-8), bibulous paper, Bunsen burner and lighter
Spreader setup	-	-	2-4	Bunsen burner and glass/metal spreaders, ethanol bath OR disposable spreaders, plating turntables can be helpful if available.
p20, ps00, p1000 & sterile tips	1	-	6	for slide preparation and dilutions
Gram Stain Reagents	1	-	6	Crystal Violet, Safranin, and Gram's Iodine (40 mL in 50 mL conical tubes), water squirt bottle, destain (or 95% ethanol) in squirt bottle.
TSA petri dishes	3	-	18	
EMB petri dishes	1	-	6	
Phenol Red Lactose Broth tubes (13x100 w/durham tubes)	1	-	6	Number is dependent on number of unknown locations in scenario
13x100 glass tube with 1.8 mL sterile water. Morton caps	3	-	18	Serial dilution

Weekly Teaching Notes: Week2 – Week 5

Teaching Notes - Bacteriology Lab #2

Learning Objectives: This week's lab builds on basic lab techniques by introducing simple staining of bacterial cultures and introduces the use of differential and selective media to learn about the biochemical properties of bacteria. A scenario is presented that serves as the basis for the final investigative project.

- Demonstrate how to prepare a heat fixed smear of a bacterial culture to stain for observation.
- Describe the difference between selective and differential growth media for culturing bacteria.
- Use differential and selective media to characterize strains of bacteria.
- Demonstrate an understanding of how to obtain single colonies by making a streak plate from a bacterial culture.

Lab materials are described for a 24-student lab with 6 groups of four students each. Numbers and volumes can be scaled appropriately for different configurations.

This lab has been designed to be taught in a station format. Materials for each technique are distributed throughout the lab space so students can spread out. Another benefit to this approach is that bacteria and flames (sterilization) can be localized to one area for closer supervision). Other configurations will certainly work.

Safety

- **Absolutely NO food or drink in the laboratory.**
- The strains of bacteria used for this lab are considered **BSL-1**, and do not typically cause disease in healthy individuals, but we are to treat them as if they were pathogenic.
- All benches must be wiped down with BDD disinfectant before lab starts, and after lab ends
- All students must wear gloves and goggles during lab when using bacteria or open flame.
- We will be using open flame. This week it will be limited to the front benches to keep it in a small area. Next week it will be used at all benches. Re-acquaint yourselves with
    - **Fire blanket**
    - **Fire extinguisher**
    - **Gas port shutoffs**
    - **Emergency gas shutoffs**
    - **Safety showers and eyewash stations**
    - **Phone locations and emergency contact procedures**

The stations should be set-up when students arrive in lab. Reset each station to the way you found it before leaving for the day if multiple lab sections are taught during the week.

Resources and waste

- Bulk materials needed for this lab
    - Microscope slides
    - Stains for simple staining
        - Methylene blue, Safranin, and Crystal violet
    - Clothes pins for slide holders
    - TSA Plates (30 per lab, 1 per person for triple-streak, 1 per group for Exercise 3)
    - EMB Plates (6 per lab, 1 per group)

- Plates of bacterial cultures for simple staining AND differential and Selective media testing – The cell wall architecture (Gram + or -) is for your benefit, don't share with students yet.
  - ***Escherichia coli*, *Serratia marcescens*, *Enterobacter aerogenes* (Gram -)**
  - ***Bacillus cereus*, *Staphylococcus epidermidis* (Gram +)**
- Disposable loops and/or sterile toothpicks for bacterial transfer
- Microscopes (12x)
- Gloves
- Bunsen burners and strikers
- 3 micropipettes (20 μL only this week)

- **Waste: Manage waste following protocols for your institution. The following are template guidelines used by the authors.**
  - **Loops and tips** all get disposed of in the bags in the white plastic 1.5 gallon containers on the benches. NO GLASSS, Paper towels, or gloves go in this container!
  - **Paper towels** used for sanitizing benches and **inverted gloves** go in the trash.
  - At the end of lab each day, empty the buckets into the autoclave waste box in the hood for autoclaving.
  - Any bacterial spills should be wiped up with paper towels and then sanitized with BDD

Before Lab starts:

- **Wipe down front bench.**
- **Collect the bacterial cultures needed for lab. Each lab will need TSA culture plates (1 of each strain) which will prepped the day prior and will be in a 37 degree incubator. These can be taken out of the incubator in the morning and will be fine for the whole day. Each lab will have 1 plate of each bacteria to be shared amongst all students.**

Setup:

Microscopy (2 per bench)

- Microscopes
- Student prepared simple stained slides

Heat-fixing station - (Two or more stations may be used)

- Bunsen burners (1 per bench)
- Clothes pins

We try to limit the number of open flames in the lab and use only two or four (out of six) benches for heat fixing. You may increase this to best suit your lab situation.

Everything else can be done at the group's benches when materials are ready! Groups with burners will need to be patient as people pop-over to heat fix their slides which takes only a few seconds each.

The stations should be set-up when you get to lab. PLEASE reset each station to the way you found it before leaving for the day.

For Lab: Get to know students a little each week!

There will be a few demonstrations before you set your students loose to the stations.

1) Have students look at the pictures of the plates from last week (should be in their completed Week 1 assignment).
    a. How did the dilution plates look? Lawns on all plates indicate;
        i. Dilution errors
        ii. Pipetting errors
        iii. Plating errors
    b. Colonies on High-dilution (low colony number) plates can be counted to estimate the number of bacteria in the original culture! Tell them to keep this in mind because they might be required to do this next week.
2) Go over safety rules again including the loss of all future participation for eating or drinking in lab. All food must be left in the safe area for the entire lab. This includes YOU!
3) Refresh pipetting technique if necessary. Only p20 pipettes will be out this week
4) Go over the theory of streaking for single colonies.
5) Emphasize that media may be limiting, so continue with your experiments even if a mistake is made.
6) Gloves and goggles are needed at stations with live bacteria… not at microscopes.
7) Demonstrate proper smear & heat fix technique
8) Have students describe the purpose of differential and selective media to you
    a. Experiment #3 is an investigation to determine how different bacteria behave on EMB, a differential AND selective media. TSA is used as a control to show that even if a strain does not grow on EMB, it is still living and CAN grow on rich media.
    b. Plates for this section get sectored into 5 parts, one for each strain. One pair of plates per group.
    c. The inoculum should be so small as to be invisible… single colonies would be best, but we are not triple streaking.

Before leaving:

1) Take all of your section's plates to the 37 degree incubator where there is space. They should stay together in the incubator (Sterlite plastic shoe-sized boxes work great to keep lab section plates together).
    a. The color of the *S. marcescens* will start to turn red after sitting on the side benches for more than 24 hours. You will be able to see two different colony colors after the weekend if left out.

2) Reset all stations
    a. Make sure there are enough pipette tips available for the next class
    b. Return all micropipettes to the front bench.
    c. Return your section's unused plates to the refrigerator in your lab and bacterial stocks to the front bench
    d. If any waste was generated, make sure it goes to the appropriate location
    e. BDD the side and front benches.

3) After 24 hours, return to the incubator to get your plates and bring them to the lab bench. Make sure doors will be open for your students to come in and document their results.

Teaching Notes - Bacteriology Lab #3

Learning Objectives: This week's lab builds on basic lab techniques by introducing Gram staining of bacterial cultures. Water testing, a practical application of microbiological techniques in described, and a scenario is presented that serves as the basis for the final investigative project.

- Demonstrate how to prepare and evaluate a Gram stain.
- Understand the relationship between Gram negative, enteric, coliform, and fecal coliform bacteria with respect to water safety
- Describe how water sources can be quickly screened for the presence of coliform bacteria.
- Understand the purpose of and begin initial water testing experiments.

Lab materials are described for a 24-student lab with 6 groups of four students each. Numbers and volumes can be scaled appropriately for different configurations.

This lab has been designed to be taught in a station format. Materials for each technique are distributed throughout the lab space so students can spread out. Another benefit to this approach is that bacteria and flames (sterilization) can be localized to one area for closer supervision). Other configurations will certainly work.

Safety

- **Absolutely NO food or drink in the laboratory.**
- The strains of bacteria used for this lab are considered **BSL-1**, and do not typically cause disease in healthy individuals, but we are to treat them as if they were pathogenic.
- All benches must be wiped down with BDD disinfectant before lab starts, and after lab ends
- All students must wear gloves and goggles during lab when using bacteria or open flame.
- We will be using open flame. This week it will be limited to the front benches to keep it in a small area. Next week it will be used at all benches. Re-acquaint yourselves with
    - **Fire blanket**
    - **Fire extinguisher**
    - **Gas port shutoffs**
    - **Emergency gas shutoffs**
    - **Safety showers and eyewash stations**
    - **Phone locations and emergency contact procedures**

Resources and waste

- Bulk materials needed for this lab
    - Microscope slides
    - Stains for gram staining
        - Safranin
        - Crystal violet
        - Iodine
        - De-stain (95% ethanol) in small squirt bottles
    - Clothes pins for slide holders
    - TSA Plates (1 per group)
    - EMB Plates (1 per group)
    - Phenol Red Lactose Broth (4 per group)
    - TSA Plates of bacterial cultures for Gram staining
        - *Escherichia coli (Gram -)*
        - *Bacillus cereus (Gram +)*

        - *Staphylococcus epidermidis (Gram +)*
        - *Enterobacter aerogenes (Gram -)*
    - Disposable loops
    - Sterile Toothpicks
    - Microscopes (on benches)
    - Gloves
    - Bunsen burners and strikers (on benches)
    - 3 micropipettes (200 and 20 μL this week)
    - Water samples PER GROUP (4 locations), In Fridge – REUSE THESE FOR ALL LAB SECTIONS! DO NOT DISPOSE! THESE CAN BE REUSED BY ALL LABS FOR THE WEEK!

- **Waste: Manage waste following protocols for your institution. The following are template guidelines used by the authors.**
    - **Loops and tips** all get disposed of in the bags in the white plastic 1.5 gallon containers on the benches. NO GLASSS, Paper towels, or gloves go in this container!
    - **Paper towels** used for sanitizing benches and **inverted gloves** go in the trash.
    - At the end of lab each day, empty the buckets into the autoclave waste box in the hood for autoclaving.
    - Any bacterial spills should be wiped up with paper towels and then sanitized with BDD

Before Lab starts:

- **Wipe down teaching benches with BDD.**
- **Prep the bacterial cultures needed for lab. Each lab will need one 5 mL culture tube from a 37°C shaker that will be used to make 4 small tubes of bacteria to be shared amongst all students. These belong at Station #3 – bacterial dilution and spread plating.**
- **Prepare water samples using the Water Prep Guide.**

Setup:

Microscopy

- Microscopes (12) Two per bench
- Student prepared gram-stained slides

Heat-fixing station preparation - (4x)

- Bunsen burners (1 per bench)
- Clothes pins

We try to limit the number of open flames in the lab and use only two or four (out of six) benches for heat fixing. You may increase this to best suit your lab situation.

Everything else can be done at the group's benches when materials are ready! Groups with burners will need to be patient as people pop-over to heat fix their slides which takes only a few seconds each.

The stations should be set-up when you get to lab. PLEASE reset the lab to the way you found it before leaving for the day.

For Lab:

There will be a few demonstrations before you set your students loose to do staining.

9) Have students look at pictures of plates from last week.
    a. What grew on the EMB plates? What colors were the colonies? What does that mean about their identity and biochemistry? (growth = Gram negative, Gram Positives were inhibited and selected against. Color change is an indication of the level of Lactose fermentation. Dark = fermentation, light = low or no fermentation)
10) Go over safety rules again including the loss of all future participation for eating or drinking in lab. All food must be left in the safe area for the entire lab. This includes YOU!
11) Refresh pipetting technique if necessary.
12) Go over the theory of streaking for single colonies and have students re-evaluate their technique. Did things work well last week? What would you do differently?
13) Emphasize that we have limited media, so continue with your experiments even if a mistake is made.
14) Gloves and goggles are needed at stations with live bacteria.
15) Re-explain proper smear technique and Gram stain procedures.
    a. Have students smear TWO cultures per slide (one Gram +, one Gram -), being careful to minimize contamination.
    b. Air Dry, heat fix.
    c. Stain. Rinse with H_2O between steps, but don't dry.
    d. Keep de-stain AWAY from Bunsen burners.
    e. DON'T trust the color of the smear, look at individual cells under the microscope. Smears lie!
16) After staining seems to be wrapping up, remind students about the scenario in the lab. the whole story may be presented IN LAB, or prior to lab as part of a pre-lab video or in-person lecture. It would be beneficial to have students explain it to you to make sure they understand what they are doing. Reading the lab is key!
17) Phenol Red lactose broth tubes should be treated gently to avoid introducing bubbles. Inoculate these with 50 μL of water from each location. The tubes do NOT need to be mixed or stirred.
    a. Tubes should be labeled so that they can be identified easily after incubation e.g. "Sec 01, Grp 1, Location A" etc.
    b. NO TAPE OR LABELS ON CAPS. All labels must be written on the tube itself.
    c. Tubes should not be tipped or shaken. If a large bubble is present in the Durham tube prior to incubation, replace.
    d. 1 loop of each water sample will get streaked on a quartered EMB and TSA plate. Loopfulls of water are approximately 10 μl of water. The loop must be completely filled with a meniscus of water, NOT a tiny film from a broken meniscus
        i. An alternative is to pipette 10 μl of water to the edge of the plate and streak through a quadrant of the plate with a sterile loop. Failure to transfer enough water may lead to a false negative result.
    e. DO NOT CONTAMINATE WATER SAMPLES. These are going to get used again in the afternoon. Replace with a new preparation if contamination is suspected.
18) Lab assignment #3 for this lab will require photo documentation of results. Make sure students are working on their photo documentation skills.

Before leaving:

4) Return Water samples to the refrigerator.
    a. Prep new water samples if contamination is suspected. Water samples are stable in the refrigerator overnight.
5) Reset the lab
    a. Make sure there are enough pipette tips available for the next class
    b. Return your section's unused tubes and plates and bacterial stocks to the lab fridge

c. If any waste was generated, make sure it goes to the appropriate location.
d. BDD the side and front benches.

6) Take all of your section's plates and tubes to a 37 degree incubator where there is space. They should stay in your bin in the incubator. Phenol Red lactose tubes need to all go in one rack, separated by group, and put at 37 degrees also.

7) After 24 hours, return to the incubator to get your plates and bring them to the lab bench. Lab doors should be open for your students to come in and document their results.

8) The EMB plates from each group must be saved in the fridge for use in the week 4 lab.

Teaching Notes - Bacteriology Lab #4 – Completed Tests

Learning Objectives: This week's lab completes experimentation on contaminated water samples. A final technique, the Gram stain, is introduced to further characterize the properties of bacteria. This scenario presented last lab serves as the basis for the final investigative project that will be completed this week.

- Complete tests necessary to confirm the presence of, and calculate the number of bacteria present in a contaminated water sample
- Prepare an Oral presentation of the results and methodology of your contaminated water investigation that could be presented at a Town Council meeting in lab.

Lab materials are described for a 24-student lab with 6 groups of four students each. Numbers and volumes can be scaled appropriately for different configurations.

This lab has been designed to be taught in a station format. Materials for each technique are distributed throughout the lab space so students can spread out. Another benefit to this approach is that bacteria and flames (sterilization) can be localized to one area for closer supervision). Other configurations will certainly work.

Please dedicate some time to each group to help them interpret their results from last week's lab

Safety

- **Absolutely NO food or drink in the laboratory.**
- The strains of bacteria used for this lab are considered **BSL-1**, and do not typically cause disease in healthy individuals, but we are to treat them as if they were pathogenic.
- All benches must be wiped down with BDD disinfectant before lab starts, and after lab ends
- All students must wear gloves and goggles during lab when using bacteria or open flame.
- We will be using open flame. This week it will be limited to the front benches to keep it in a small area. Next week it will be used at all benches. Re-acquaint yourselves with
    - **Fire blanket**
    - **Fire extinguisher**
    - **Gas port shutoffs**
    - **Emergency gas shutoffs**
    - **Safety showers and eyewash stations**
    - **Phone locations and emergency contact procedures**

The stations should be set-up when students arrive in lab. Reset each station to the way you found it before leaving for the day if multiple lab sections are taught during the week.

Resources and waste

- Bulk materials needed for this lab
    - Microscope slides
    - Materials for gram staining
        - Safranin
        - Crystal violet
        - Iodine
        - Ethanol (de-stain)
    - Clothes pins for slide holders
    - TSA Plates (3 per group)

- EMB Plates (1 per group)
- Phenol Red Lactose Broth (1 per group)
- Student unknown bacteria samples for gram staining. These sample will come from an EMB plate streaked from Water-sample EMB colonies from last week's plates.
- Plates of bacterial cultures for gram staining controls
  - *Escherichia coli (gram - control)*
  - *Bacillus cereus (gram + controls)*
  - *Staphylococcus epidermidis*
- Disposable loops or sterile toothpicks
- Microscopes
- Gloves
- Bunsen burners and strikers
- micropipettes (p1000, p200, and p20 this week)
- Alcohol, hockey sticks, turntables.
- Water samples PER GROUP (as needed – see below)

- **Waste: Manage waste following protocols for your institution. The following are template guidelines used by the authors.**
  - **Loops and tips** all get disposed of in the bags in the white plastic 1.5 gallon containers on the benches. NO GLASSS, Paper towels, or gloves go in this container!
  - **Paper towels** used for sanitizing benches and **inverted gloves** go in the trash.
  - At the end of lab each day, empty the buckets into the autoclave waste box in the hood for autoclaving.
  - Any bacterial spills should be wiped up with paper towels and then sanitized with BDD

Before Lab starts:

- **Wipe down front bench.**
- **Collect the bacterial cultures needed for lab. Each lab will need TSA culture plates (1 of each strain) which will prepped the day prior and will be in a 37 degree incubator. These can be taken out of the incubator in the morning and will be fine for the whole day. Each lab will have 1 plate of each bacteria to be shared amongst all students.**

Setup:

Station #1: Microscopy

- Microscopes
- Student prepared gram stained slides

Station #2 – Heat-fixing station preparation

- Bunsen burners (1 per bench)
- Clothes pins

Station #3 – Spread plates

- Bunsen burners (1 per bench)
- Hockey sticks
- Alcohol in beakers

The stations should be set-up when you get to lab. PLEASE reset each station to the way you found it before leaving for the day.

For Lab Week 4:

There will be a discussion and two demonstrations before you set your students loose to the stations.

19) Have students look at their photos of their EMB plates from last week. Each group will need to choose ONE (1) water sample to follow up on. The town in your scenario does not have enough money to follow up on all locations!
    a. Things to discuss… The presumptive tests of the 4 water samples should tell students which ONE of their locations is most likely to be contaminated with fecal coliform bacteria.
    b. Have students use the flowchart on page 2 of the lab to identify which water sample they wish to concentrate on. Walk around to make sure groups are interpreting their results correctly. There will be some ambiguity and some failed experiments.
    c. There are a number of things to take into consideration when making a choice.
        i. Colony color changes over time. After 24 hours, *E. coli* will be dark purple or green on EMB and the non-fecal coliform bacteria in the water will appear pinkish. Beyond 24 hours, non-fecal coliform bacteria will slowly continue fermenting lactose and will get darker and darker, while *E. coli* will get lighter and lighter. After 3 or 4 days, *S. marcescens* will look exactly like *E. coli* did after 24 hours and *E.coli* might have faded to be indistinguishable from other coliform bacteria.
            1. Pictures from 24 hours are the best in determining a positive test for *E.coli* on the EMB plates.
            2. Plates that have been out for a week might need supporting evidence to make a conclusion.

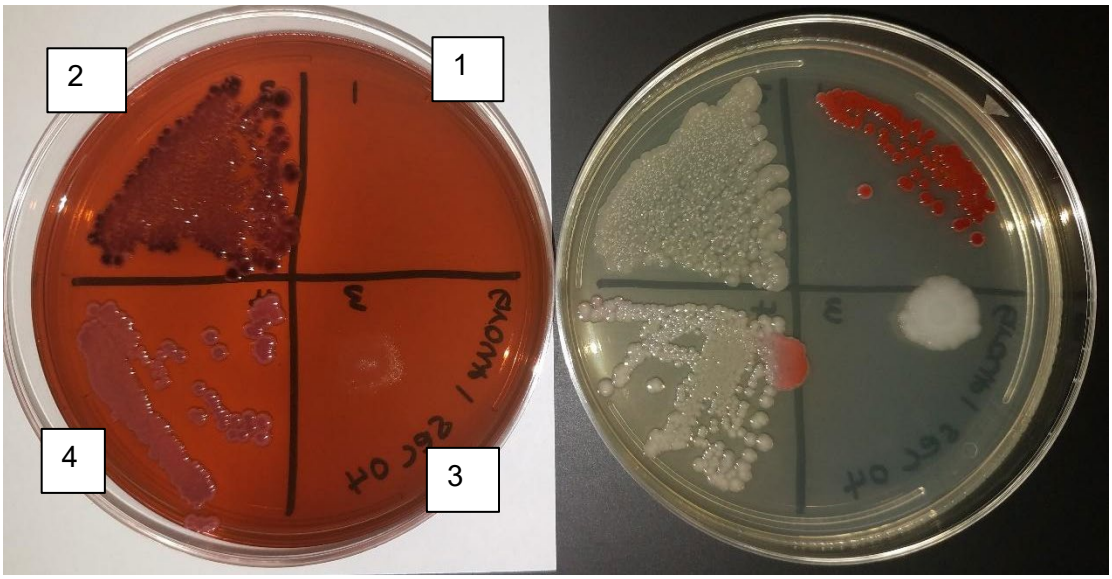


Figure 1. Clear example of fecal coliform contamination on EMB plates results in dark purple colonies with or without a greenish sheen. Location #2 provides support for the presence of *E. coli* while the other locations do not.

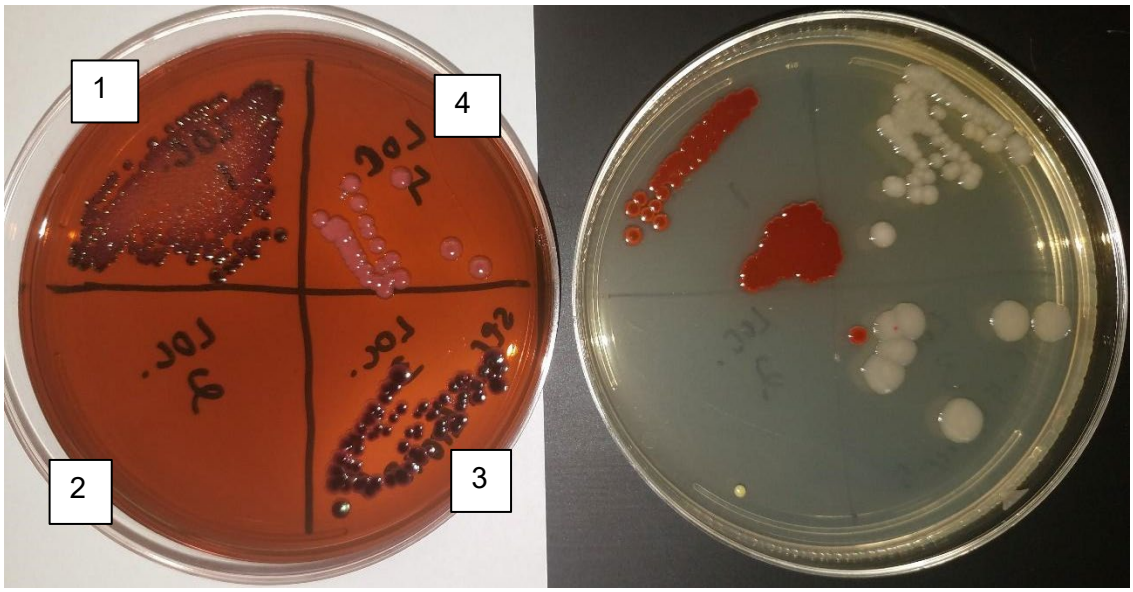


Figure 2. Ambiguous results on EMB plates with multiple dark purple colonies in multiple locations. Locations #1 and #3 are both dark in color. After 3 days, it is likely that the colonies in one of the two quadrants was originally light colored and grew darker over time as lactose continued to ferment. The results from this plate need support from the phenol red lactose experiment to determine the source of fecal contamination (i.e. *E. coli*).

i. Phenol red lactose test results are often very clear-cut after 24 hours. Like EMB, the color will continue to change over time and gas will continue to be produced slowly.
ii. Pictures from 24 hours are the best in determining a positive test for *E.coli* in phenol red lactose broth.

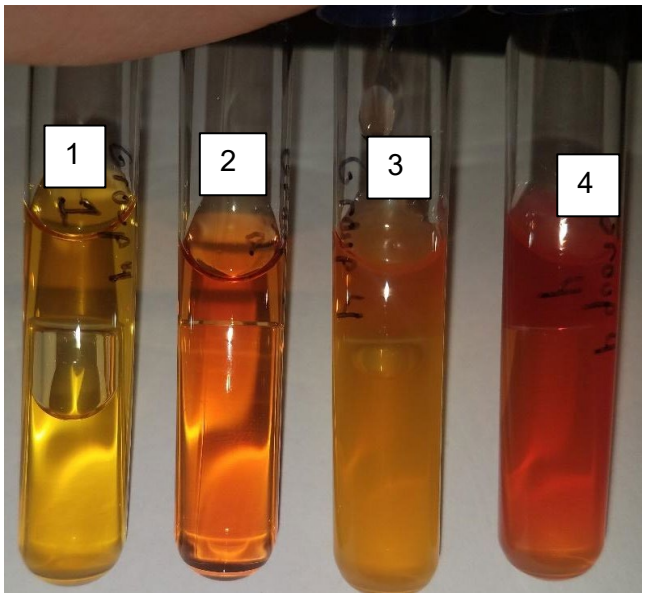


Figure 3. Positive phenol red lactose broth results are yellow (acid) with a bubble (gas) after 24 hours. Locations #1 is the only tube to show lactose fermentation with acid and gas, representative of *E. coli*, and therefore, fecal contamination. These tubes are the same locations as the plate in figure 2. The small bubble in tube 3 was likely not present after 24 hours.

From the information in Figures 2 and 3, we can conclude that the example location 1 is positive for the presumptive test because it has both dark colonies on EMB and fermented lactose with acid and gas (yellow/bubble) in phenol red lactose broth. This would be the water location to test in lab this week.

- If students have no growth on their plates, use the phenol red tubes as the strongest evidence.
- If students have purple colonies in multiple quadrants, pick the sector that matches the phenol red tubes.
- You may need to use the process of elimination to rule out samples that have no positive results.
- The table below has the correct contaminated sample for each group

Here is the Key for the correct water samples using the water setup guide in the Supplemental Materials):

Group	Positive sample location
1	2
2	3
3	4
4	1
5	3
6	1

20) Using the results from EMB/Phenol Red Lactose broth to choose a location make up the confirmed test.

Why do we need two tests? To eliminate false positive results that can occur on each type of media.

- *Staphalococcus aureus* is a gram +, non-coliform bacteria that produces acid and gas in lactose and could give a false positive.
- *Citrobacter* is a non-coliform genera of bacteria that will ferment lactose and turn green on EMB which would be a false positive.
- Neither of these will be positive for BOTH tests, while only *E.coli* will.

21) Gram Staining – this is to be performed from a dark purple/green colony from last week's EMB plate. This is a stain of the actual contaminant. Does the Gram- lactose fermenting growth from their positive water sample look like a Gram (–) rod?
    a. Procedure will be the same from last week
    b. **IDEA TO CONSIDER:** Examine the bacteria from the EMB plate on two slides with controls.
        i. make two side-by-side smears on the same slide, one Gram + and one of the unknown water sample to see the difference in staining
        ii. make a second slide with two side-by-side smears on the same slide, one Gram negative and one of the unknown water sample to see the similar staining.
            1. Gram + = *S. epidermidis or B. cereus*
            2. Gram - = *E. coli*
22) The completed tests are a set of experiments used to;
    a. re-verify the results from the presumptive and confirmed tests using bacteria isolated and grown from contaminated water
    b. enumerate the bacteria in the contaminated water to establish the extent of the contamination.

Students will get 1 (ONE) tube of water this week that will be handed out when they make their choice. These will be prepped in advance for all of the correct locations.

IF there is uncertainty on which sample is the contaminated sample after the confirmed test is evaluated, you may give a group two tubes of water, the "CONTAMINATED" water should be delivered in the proper location and the "CLEAN" water should be delivered in the other location. ONLY the CONTAMINATED water will have *E. coli* in it to ensure reportable results. This will double the number of resources each group needs, so please only allow this if there is a completely unclear issue from 2 weeks ago.

In ANTHING needs to be reset, or if water gets dumped by accident, you can refill from stocks. Prep 2 flasks of water with one labeled CONTAMINATED and one labeled CLEAN.. When students are ready, they will ask you for their sample. Simply pour 20ish mL of the appropriate water in the tube labeled with their group and location.

Completed tests:

1) **Gram stain their bacteria from last week's EMB plate** (will be dark purple or greenish by now). The presence of gram – rods is evidence of a possible coliform. The gram stain will be completed from the student's own 4-quarter EMB plates.

2) **Phenol Red lactose broth – 50 μL of their new water sample** should be used to inoculate one fresh phenol red lactose broth tube to confirm acid/gas production. Evidence of a possible coliform
3) **Reconfirm purple/green growth on EMB** – This will be combined as part of the enumeration test
4) **Enumeration** – How much total bacteria is present (some of which might be harmless) and how much fecal coliform bacteria is present?

Enumeration is done in two ways… the quick estimate method using commercial testers and the much more precise spread plates from serial dilution counting method. **Only the serial dilution / spread plate techniques will be used this week.** The procedure in the lab will be demonstrated, but briefly;

a) **Plate enumeration**: The water has too much bacteria to plate and count directly, so we will need to dilute a few times to guarantee at least one dilution is countable. 1/10 dilutions will work well this week. They should be done serially using Figure 4 in the lab as an example.

    a. Planning and teamwork will make this process run smoothly
        i. Prepare plates (3x TSA and 1 EMB) before diluting samples.
        ii. For the three DILUTIONS, use the spread plate technique to spread 100 μL on TSA to count.
        iii. From tube #2, plate 100 μL on EMB to reconfirm the presence of purple/green colonies.

Everything gets incubated at 37 degrees overnight. Return the next day to retrieve your plates.

Please read and go over the expectations for the final presentation. Details are on the last 3 pages of this week's lab. Presentations are scheduled in the syllabus to be delivered to us in an upcoming lab meeting. The following format works very well and allows the groups to have two graders for every presentation.

Create a schedule with 15 minute blocks for the duration of the 3-hour lab. Students should share their presentation with you in advance so you can have it open and ready on your computer hooked up to a projector. Set up one presentation room and have groups come to the room to present to you (not the whole class). Students waiting to present may assemble and practice in a nearby classroom. Presentations should be 7 minutes with 3 minutes for questions and discussion. The remaining 5 minutes is used for graders to finalize the rubrics and to bring in the next group.

Before leaving:

Reset all stations
- a. Make sure there are enough pipette tips available for the next section
- b. Return your section's unused tubes and plates and bacterial stocks.
- c. If any waste was generated, make sure it goes to the appropriate location.
- d. BDD the side and front benches.

Teaching Notes - Bacteriology Lab #5 – Presentation Preparation and Discussion

This lab period focuses on:
1. Demonstrating good vs. poor presentations
2. Creating a model slide to practice in front of class
3. Establishing presentation procedure for next week
4.Giving students some dedicated time to work on their presentations

Please read and go over the expectations for the final presentation. Details are on the last 3 pages of last week's lab. Presentations are scheduled in the syllabus to be delivered during lab #6.

Long lab (3-hour) Format:
- Instructors two labs that meet simultaneously serve as the town council for all lab groups in both concurrent labs.
- Up to 12 presentations can be held in a 3-hour period. Presentations are made to only the council, no classmates.
- One lab is used for staging and practice, the other lab is used for presentations and grading

This is not possible during a 2-hour lab (not enough time for 12 presentations!)

Short lab (2-hour) Format:
- Town council is Faculty and student lab instructors and assistants, plus a small audience of student groups (but not the whole class)
- Student observers can ask questions, but will not directly contribute to grading
- Split lab into two 1-hour timeslots for half of the class to attend at a time. (E.g. groups 1-3 meet at 8:30-9:30, groups 4-6 at 9:30-10:20)
    - Benefit… students can see some other presentations and provide feedback.
    - Benefit… students don't have to sit through 6 mostly-identical presentations (stamina and focus have diminishing returns).
    - Downside… No lab available for staging and prep.
    - Downside… nervousness of presenting in front of other students.

Student Expectations: 10-15 minutes for each group to set-up, present, and answer questions
a) Presentations should be designed to be completed in 7-8 minutes.
b) 2-3 minutes should be reserved for questions
c) Groups should evaluate one or two other student presentations, but not more than that.

Create a schedule with 15 minute blocks for the duration of the 2-hour lab. Assign groups to those timeslots and arrange for attendance as you choose. Students should share their presentation with you in advance so you can have it open and ready on your computer which will get hooked up to the projector. Use your lab room as the presentation room and have groups come to the room for their assigned time. Students waiting to present may assemble and practice in the atrium or hallway. Presentations should be 7 minutes with 3 minutes for questions and discussion. The remaining 5 minutes is used to finalize the rubrics and to allow the next group to setup.

Combine the faculty/student assistant rubrics and average them for a final assignment grade. You may return these to students after grades have been entered in the gradebook. Use the anonymous student evaluations to provide comments for students but not a grade contribution.

For lab:

A) Instructors present a demonstration presentation. You may use the provided powerpoint or use one of your own.
    a. Make this a poor or mediocre presentation so that your students can (constructively) rip you apart.
    b. Provide a grading rubric to your students to grade you. This will the same rubric we will use for their presentations!
    c. Use the feedback to discuss places that need improvement and suggest ways to make the presentation stronger.
B) Assign a single slide/presentation element to each lab group and have them create, then present their topic to the class.
    a. E.g.: Introduction, Methods\outline, methods\gram stain, methods\media, methods\enumeration, results, discussion\conclusions, etc.
    b. Give students at least 20-minutes as a group to prepare a single powerpoint slide.
    c. Each presentation should be appx 2 minutes
    d. Any and all feedback is welcome

Before leaving lab this week:

    e. Email your students with a presentation schedule! Have fun listening to presentations next week!

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit https://www.ableweb.org/.

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