Edvo-Kit # 160

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# Identification and Characterization of Bacteria

## Experiment Objective:

Gram staining is one of the most popular, important, and beautiful procedures in microbiology! In this experiment students will use this staining technique to examine the size, shape, arrangement, and gram status (+/-) of *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus luteus*.

**See page 3 for storage instructions.**

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**Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets**


# Experiment Components

| Component | Storage | Check (✓) |
|---|---|---|
| • *Escherichia coli* BactoBeads™ | 4° C (with desiccant) | ❑ |
| • *Micrococcus luteus* BactoBeads™ | 4° C (with desiccant) | ❑ |
| • *Bacillus subtilis* BactoBeads™ | 4° C (with desiccant) | ❑ |

**This experiment is designed for 8 lab groups.**

***Store all components below at room temperature.***

**Reagents and Supplies**

- Bottle of ReadyPour™ Luria Broth Agar ❑
- Large Petri Plates ❑
- 10 mL Sterile Pipet ❑
- Inoculating Loops ❑
- Toothpicks ❑
- Transfer Pipets ❑
- Microscope slides ❑
- Gram's Crystal Violet ❑
- Gram's Iodine ❑
- Gram's Safranin ❑
- Sterile water ❑
- Microcentrifuge tubes ❑

# Requirements *(Not included with this kit)*

- Microwave
- Pipet pump
- Incubator (recommended)
- Timers
- Slide holders or clothpins
- Bunsen burner or alcohol lamp
- Lab marker
- Beakers, flasks, disposable cups, or other liquid containers (25 mL or bigger)
- Small tray or beaker for trash
- Squeeze bottles, large beakers (100 mL or bigger), or sink access for all groups
- 95% ethanol
- Distilled Water
- Bleach or other lab disinfectant
- Paper towels or blotting paper
- Gloves, Goggles, Lab coat
- Microscopes (total magnification of 100x or higher )
- Microscope cover slips and oil (if required)

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

# Background Information

Bacteria may be Earth's most successful organisms. Best estimates guess that there are around one nonillion ($1x10^{30}$) bacteria on our planet! For reference, the biomass of one nonillion bacteria is more than all other living animals and plants on the planet. These bacteria are found in soil, water, hot springs, mountain tops, deep ocean vents, and even radioactive waste. Bacteria also live in symbiotic (beneficial) and parasitic (detrimental) relationships within plants and animals - including us!

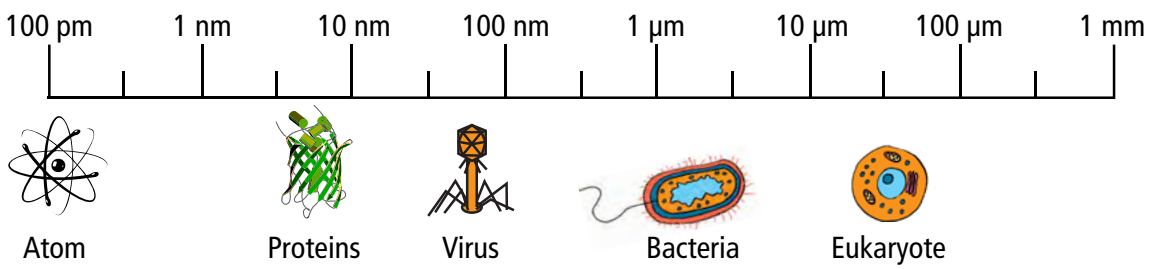



**Figure 1:** Average bacteria size.

Who are these prolific and ubiquitous beings? Bacteria are a large and highly diverse group of single-celled organisms that lack membrane-bound organelles (like mitochondria, chloroplasts, Golgi apparatus, etc.) To efficiently take in nutrients and excrete waste without these organelles, bacteria remain small (Figure 1). Bacteria also lack a nucleus. However, they do contain DNA in a centralized threadlike mass known as the nucleoid (Figure 2) and sometimes as additional circular plasmids. In addition to these structures, bacteria also have ribosomes for protein assembly and most have cell walls. Some bacteria cells also have capsules or "slime layers" that help prevent viral infections, phagocytosis, and desiccation. Others have external extensions called flagella or pilli that allow them to move or attach to other cells.

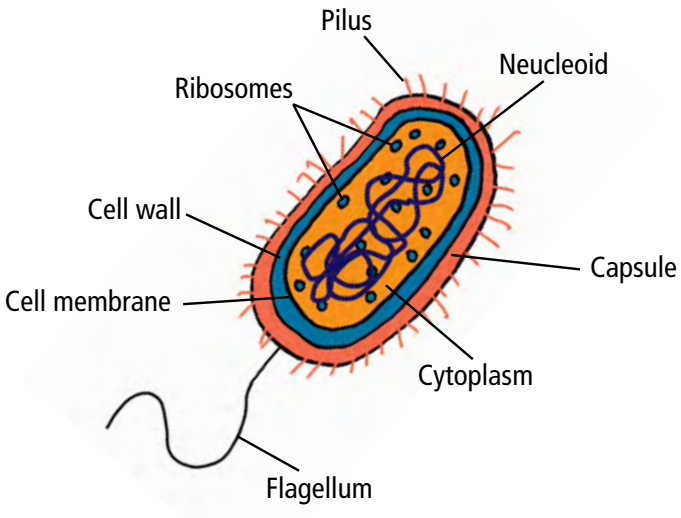



**Figure 2:** A bacteria cell.

Bacteria's success may be a result of their cellular simplicity, small size, and short generation time. It may also be due to evolutionary strategies, such as horizontal gene exchange, that allow for fast genetic changes. However, these traits - along with the immense diversity that they have created - also make studying bacteria a challenge. This is particularly true of classification. Classification is the practice of naming and organizing a group of organisms based on similar traits. This practice enables scientists to better understand and describe the diversity of a group and to quickly identify particular species.
In bacteriology, different classification schemes are used for different tasks. For example, biologists studying the evolution of bacteria primarily use DNA sequences to describe the relationship between multiple species. Sci-

entists that monitor the safety of drinking water and food often use more accessible but less precise tests that change color when specific bacterial proteins are present. In this setting, bacteria are grouped by their ability to produce certain proteins. Clinicians and clinical biologists that need to quickly identify pathogenic species (or beneficial species) use microscopes to observe key morphological traits and then classify bacteria based on these observations. Gram staining is one of these microscopic techniques and is now a cornerstone of bacteriology.

## GRAM STAINING

Gram staining is a quick, effective, and inexpensive identification test that has become one of the most essential tools in bacterial classification. It was originally developed by the Danish physician HC Gram (1853-1938). Gram was searching for a way to stain bacteria cells in tissue samples without also staining the human cells. However, what he found was even more useful - a way to quickly sort bacteria into two major groups based on their ability to absorb and retain crystal violet stain. Today these groups are known as gram-positive and gram-negative.

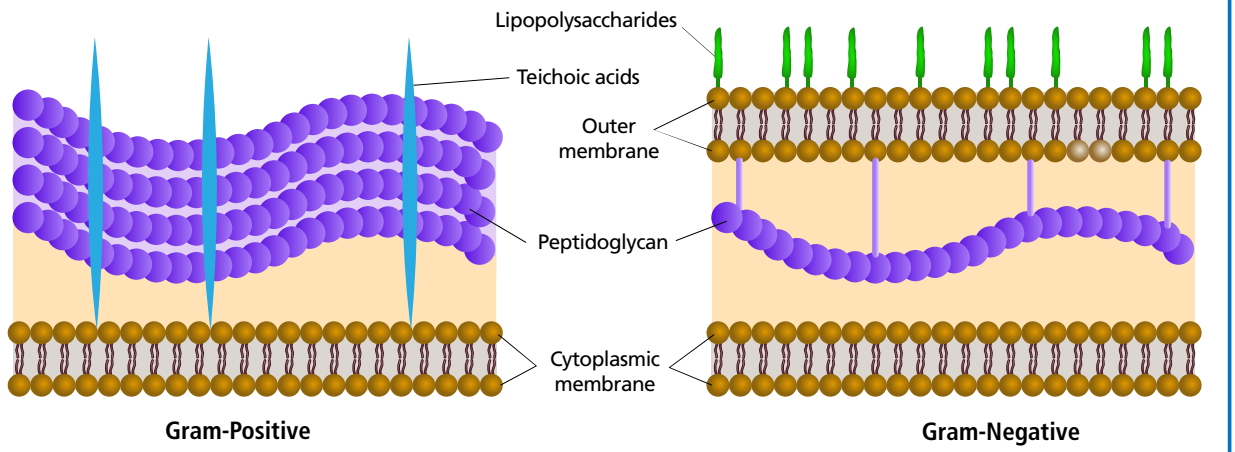



**Figure 3:** Gram-positive and gram-negative cells.

One reason that Gram staining has withstood the test of time is that the division of bacteria into positive or negative groups is based on large - and medically significant - differences in the cell walls of these two groups (Figure 3). Gram-positive bacteria have thick cell walls (15-80 nanometers) compromised mostly of a molecule called peptidoglycan. These molecules form net-like sheets which are then tightly cross-linked to each other. Gram-positive bacteria also have a group of molecules known collectively as teichoic acids that run perpendicular to the peptidoglycan sheets. Gram-negative bacteria have thinner cell walls (10-12 nanometers), a single layer of peptidoglycan, and no teichoic acids. However, they do have a highly permeable outer membrane which contains proteins, phospholipids, and the endotoxin lipopolysaccharide.

Gram staining allows scientists to quickly visualize the different cell walls of gram-positive and negative cells by turning the former purple and the later pink in a six-step process (Figure 4). The procedure begins by fixing the bacteria to the slide which can be accomplished either by exposing the cells to an organic solvent or by gently heating the cells and slide. This both preserves the cells and ensures that they do not get swept away during any of the subsequent steps.

Once the bacteria are firmly attached to the slide the crystal violet stain is added. On the slide, crystal violet dissociates into positive and negative ions (CV+ and Cl-) which quickly permeate the outer cell membranes of most cells. However, it is the positively charged CV+ ions that are responsible for dyeing cells a purple-blue. At this point in the procedure all cells - gram-positive, gram-negative, and even nonbacterial - are this color. This step is completed by washing off excess crystal violet either by briefly dipping the slide in a beaker of distilled water or by gently running water down the slide.

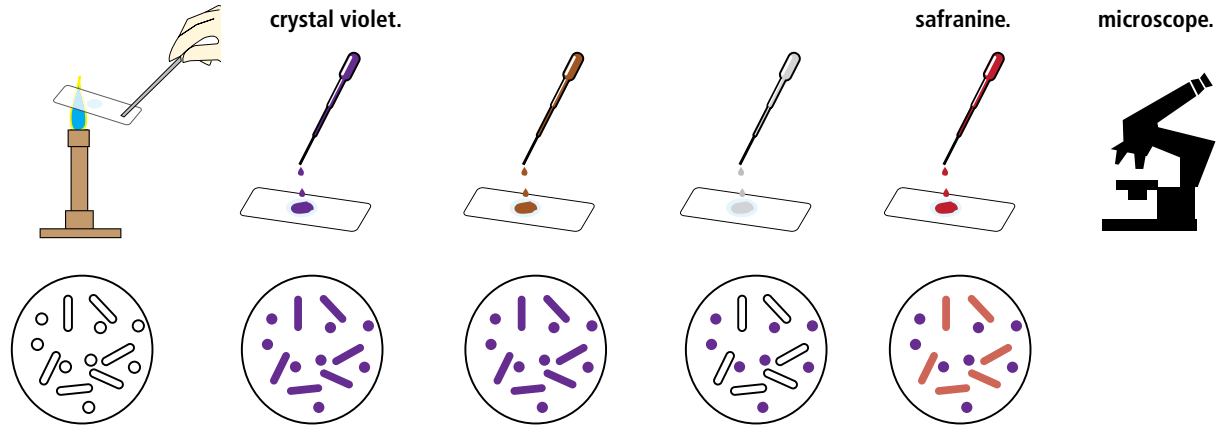



**Figure 4:** Gram staining.

Following staining, an iodine solution is added to trap the stain within a cell's wall. Iodine accomplishes this by binding to the CV+ ions and creating a large crystal violet-iodine complex. As with the addition of crystal violet, this step also includes washing away any excess iodine with water.

Next, a high percentage alcohol is added. This solution interacts with the cell wall of the two bacteria types very differently. In gram-negative cells, the alcohol strips away the outer membrane and then degrades the single layer of peptidoglycan. This makes gram-negative cells "leaky". In contrast, alcohol causes the multiple peptidoglycan layers in gram-positive cells to become more tightly crosslinked which further entraps the colorful crystal violet-iodine complexes. When the slide is washed again gram-negative bacteria - as well as any eukaryotic cells - lose most crystal violet stain while gram-positive cells maintain the stain. Because of its effect on gram-negative and non-bacteria cells, this step is called a "decolorization" step.

After decolorization, another stain is added to the slides so that the gram-negative cells can also be seen under the microscope. The stain often used in this step is safranin which stains bacteria cells a red-pink. The use of safranin is partly tradition but also because this stain is dark enough to make the gram-negative cells easy to see but light enough to not change the distinct blue-purple coloring of the gram-positive cells.

Finally, the cells are examined under a microscope and their color recorded. During this step, microbiologists will also observe and record the shape, arrangement, and sizes of the cells. These three additional characteristics help to further classify a bacteria sample - sometimes down to species. The four most common shapes that bacterial cells take are spherical, rod-like, spiral, and comma-shaped. These correspond to the bacterial groups cocci, bacilli, spirilla, and vibrios (Figure 5, top). Different species of bacteria also tend to have characteristic cell arrangements - some exist alone while others grow together in pairs, clusters, or long chains (Figure 5, bottom). Many species can be quickly identified based on the combined observation of their gram stain color, shape and arrangement. In other cases, additional information - such as cell size or whether or not the cells require oxygen to survive - may also be needed to rule out similar species.

There are also cases where alternative testing is needed. A few bacteria, such as those species belonging to the group Mycoplasma, lack cell walls. These species do not properly absorb and maintain the crystal violet or safranin stains and so are called gram indeterminate. There are also a small number of gram variable bacteria. In these species, the number of peptidoglycan layers changes rapidly as the cell grows which causes the cell to sometimes appear pink and other times appear purple. Sometimes an indeterminate or variable classification can still help narrow down a bacteria's identity. However, because these results can also be due to experimental mistakes, additional testing is still needed.

## MEDICAL APPLICATIONS OF GRAM STAINING

In medicine, gram staining is often used to both confirm a bacterial infection as well as to identify the offending bacteria or bacterial group. Many health conditions can be caused by either a virus, a bacteria, a larger parasite, or a non-infectious trigger. When the presenting symptoms are not enough to determine a treatment, doctors use gram staining as a first-round diagnostic test. For example, meningitis is a potentially fatal condition that occurs when the protective membrane around an individual's brain and spinal cord becomes swollen. This swelling is usually caused by a viral or bacterial infection but can also be triggered by some fungal infections, an adverse reaction to certain drugs, or cancer. When a patient comes in with meningitis symptoms (headache, stiff neck, and fever) doctors order a lumbar puncture. While multiple tests are performed on the recovered cerebrospinal fluid, gram staining is fast and accurate and so often guides initial treatment decisions.

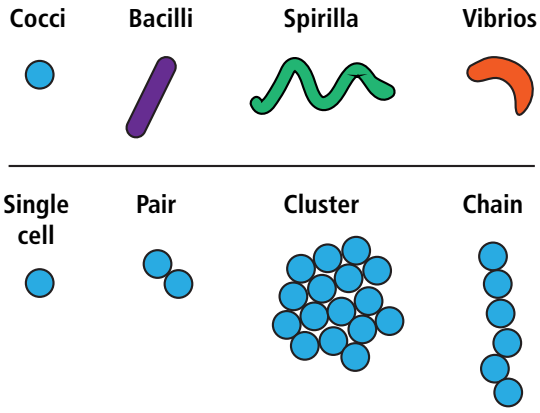



**Figure 5:**
Common shapes and cellular arrangements.

Staining quickly reveals whether or not bacteria are present (Figure 6). If present a doctor will prescribe antibiotics. Gram staining also helps doctors decide which antibiotic to use. Gram-negative bacteria have an outer membrane and additional enzymes that protect these cells from many popular antibiotics. Furthermore, the outer membrane also contains dangerous endotoxins that can cause sepsis in a patient. Consequently, when treating gram-negative infections doctors will prescribe specialized antibiotics that combat these defenses and will also monitor the patient for sepsis shock. Gram-positive bacteria tend to be easier to treat with most antibiotics. However, some species are known to form spores which can lead to reinfection unless addressed in the initial treatment.

In this lab, you will identify three nonpathogenic bacteria species based on their gram stain, shape, and size. In addition, your class may also observe the different colony morphology of each species when grown on agar plates.

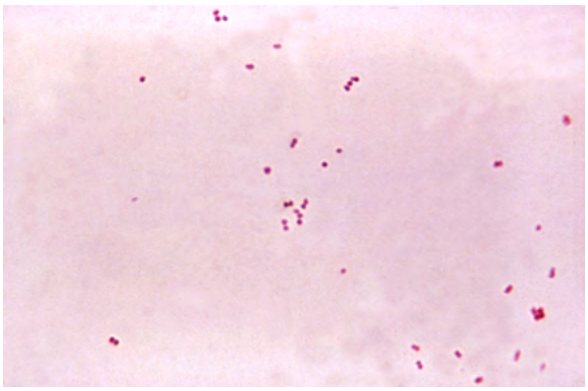


**Figure 6:** Stained slide of the gram negative and meningitis causing bacterium *Neisseria meningitidis.*

*Image provided courtesy of CDC/Dr. Brodsky, 1966. Image ID#6423, public domain.*

# Experiment Overview

## EXPERIMENT OBJECTIVE:

Gram staining is one of the most popular, important, and beautiful procedures in microbiology! In this experiment students will use this staining technique to examine the size, shape, arrangement, and gram status (+/-) of *Escherichia coli, Bacillus subtilis,* and *Micrococcus luteus*.

## LABORATORY SAFETY

Wear gloves and safety goggles

1. Exercise caution when working with the open flame used to heat your slides.
2. The bacteria used in this experiment are not considered pathogenic, but it is still important to follow simple safety guidelines. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant before and after the experiment, wash hands thoroughly with soap and water after working in the laboratory, and disinfect material that has come in contact with the bacteria before disposing them.
3. Wear safety goggles and gloves.

## LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

**Before starting the Experiment:**

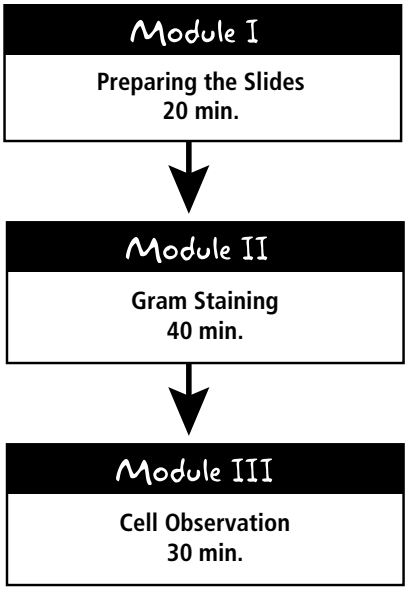
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

**During the Experiment:**

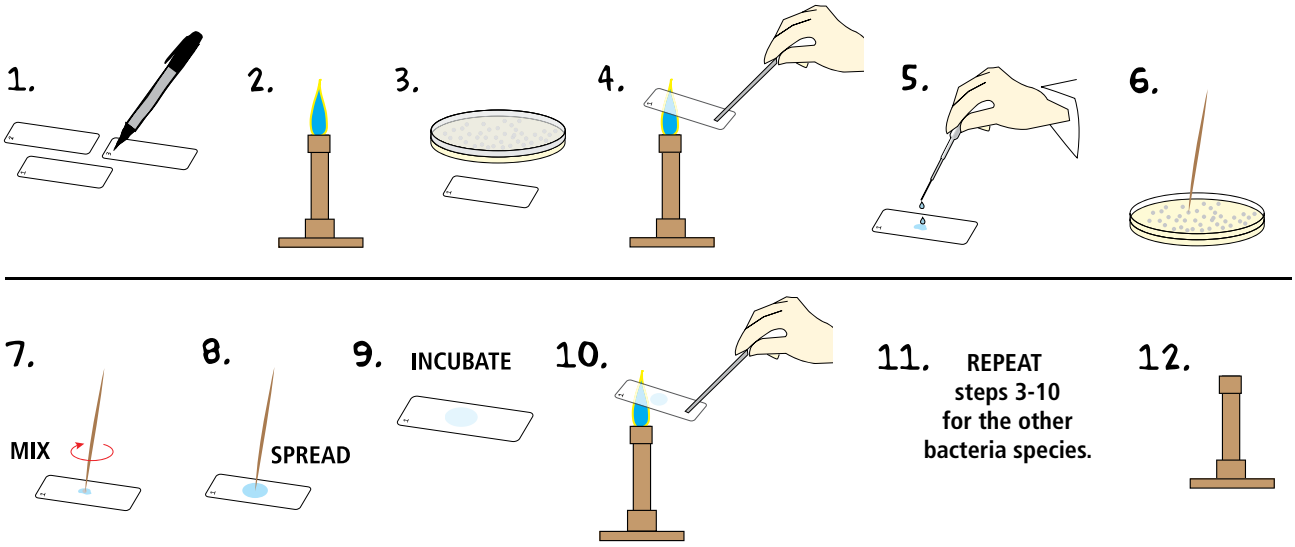
- Record your observations.

**After the Experiment:**

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.



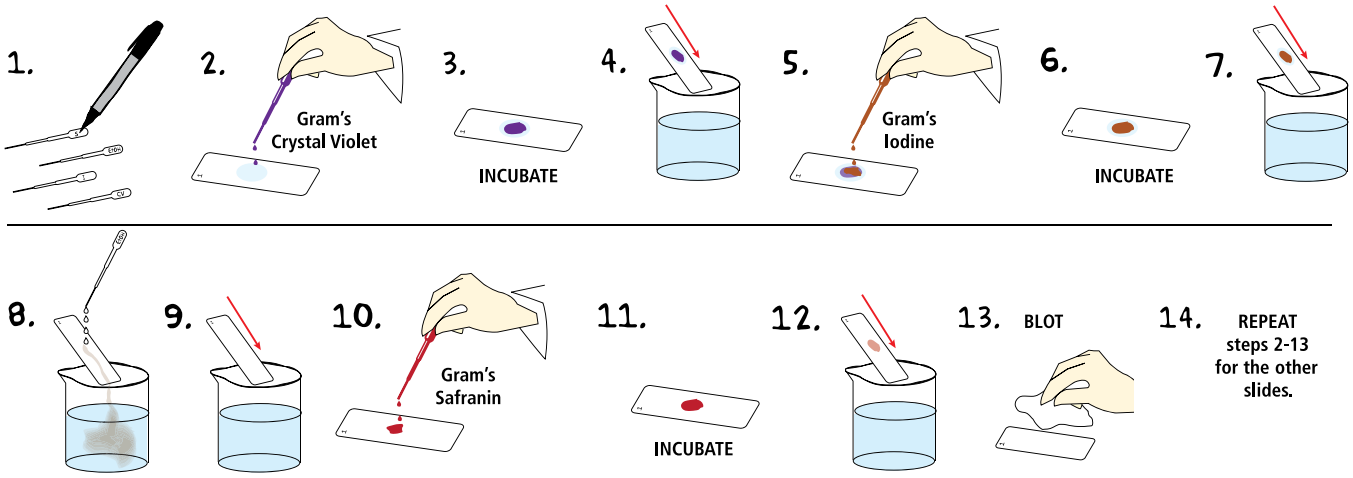
# Module I: Preparing the Slides




1. **WRITE** your initials or group's ID as well as the numbers 1, 2, or 3 on the edge of three slides. **LAY** these marked slides on a paper towel to prevent scratching.
2. Carefully **TURN ON** your Bunsen burner or light your alcohol lamp.
3. **COLLECT** an agar plate. Then **SELECT** the slide corresponding the plate's number.
4. Using a slide holder or clothespin, quickly **PASS** this slide through a flame two times. Pass the slide horizontally and so that only the middle of the slide comes in contact with the flame. The flame should touch the central section of the slide for one second during each pass (2 seconds total) .
5. While still hot, **ADD** a single drop (20 - 50 µL) of water to the center of the slide. If more than 50 µL of water is added, extend the incubation period in step 9.
6. **REMOVE** the lid of the agar plate and gently TOUCH a single bacterial colony with a toothpick to collect the bacteria. N***OTE: Although you will not be able to see them simply touching a colony with the tip of a toothpick will collect more than enough bacteria for gram staining.***
7. Using the same toothpick, **MIX** the bacteria with the water on the slide. The solution may appear slightly cloudy but make sure that no clumps are present.
8. With the same toothpick, use a circular motion to **SPREAD** the mixture so that it becomes a dime sized film in the center of the slide.
9. **INCUBATE** the slide at room temperature for 1 minute or until the water has dried completely.
10. **PASS** the slide through the flame two more times (like in step 4). Have the cells facing up so that they do not come into direct contact with the flame.
11. **PLACE** the slide aside, **EXCHANGE** your source plate with another group that has a different number, and **REPEAT** steps 3-10 *twice* for the two other bacteria species.
12. **TURN OFF** the Bunsen burner or extinguish the flame of the alcohol lamp.


**OPTIONAL STOPPING POINT:**
Slides can be stored in the dark at room temperature for multiple days.

# Module II: Gram Staining




1. **LABEL** four transfer pipets CV (Crystal Violet), I (Iodine), EtOH (Ethanol), and S (Safranin).
2. Using a transfer pipet, **ADD** 2 drops of Gram's Crystal Violet to the center of one slide - on top of the dime sized film of bacterial cells.
3. **INCUBATE** at room temperature for 30 seconds.
4. **REMOVE** excess dye by either rinsing with slow running water or by repeatedly dipping the slide into a beaker of distilled water. **SHAKE** off any excess water.
5. Using a transfer pipet, **ADD** 4 drops of Gram's Iodine to the center of the slide.
6. **INCUBATE** at room temperature for 30 seconds.
7. **REMOVE** excess iodine by either rinsing with slow running water or by repeatedly dipping the slide into a beaker of distilled water. **SHAKE** off any excess water.
8. **HOLD** the slide at a 45 degree angle over a sink or beaker. Gently **FLUSH** the cells with 95% ethanol by creating a steady stream of drops that hit the center of the slide and then flow down the slide. Continue until the slide appears clear - between 5 to 10 seconds.
9. **REMOVE** excess ethanol by either rinsing with slow running water or by repeatedly dipping the slide into a beaker of distilled water. **SHAKE** off any excess water.
10. Using a transfer pipet, **ADD** 4 drops of Gram's Safranin to the center of the slide.
11. **INCUBATE** at room temperature for 30 seconds.
12. **REMOVE** excess iodine by either rinsing with slow running water or by repeatedly dipping the slide into a beaker of distilled water. **SHAKE** off any excess water.
13. **DRY** the slide by gently blotting it with a paper towel or with filter paper. **DO NOT** rub dry and **DO NOT** blot directly on top of the cells. (To wick away water from the central cell spot, place paper on the side of any large droplets.)
14. **REPEAT** steps 2-13 for the two other prepared slides.


**OPTIONAL STOPPING POINT:**
Slides can be stored in the dark at room temperature for multiple days.

# Module III: Cell Observation

1. **PLACE** a slide under a microscope and **FASTEN** it to the platform.
2. **LOCATE** the cells using the weakest magnification. What color do the bacteria appear to be?
3. **SWITCH** to stronger magnifications. Remember to bring the stained cells into focus following each shift. If you are using an oil immersion microscope add a cover slip and a drop of microscope oil to the slide when appropriate.
4. **OBSERVE** the color, shape, and arrangement of the bacteria on the slide. **RECORD** this information in your lab book or in the table below. You may also want to **DRAW** a sketch of the slide under the microscope.
5. **REPEAT** steps 1-4 for the other two slides.
6. Use Table 1 to **IDENTIFY** the species growing on each agar plate.

| Slide # | Color | Shape | Arrangement |
|---|---|---|---|
| | | | |
| | | | |
| | | | |

## BACTERIA IDENTIFICATION INFORMATION

| Species | Gram Stain | Shape | Additional Descriptions | Size |
|---|---|---|---|---|
| *Micrococcus luteus* | Gram positive (Purple) | Cocci | Spherical, occurring in pairs, tetrads, or irregular clusters, but not in chains | 0.5 micrometers by 2.0 micrometers |
| *Bacillus subtillis* | Gram positive (Purple) | Bacilli | Rods with either round or square ends. Occurring singly or in chains. | 0.5-1.2 micrometers by 2.5-10.0 micrometers |
| *Escherichia coli* | Gram negative (Pink) | Bacili | Straight rods. Occurring singly or in irregular clusters. | 1.1-1.5 micrometers by 2.0-6.0 micrometers |

# Study Questions

1. Organelles are specialized subunits within a cell that have a specific function. Name three organelles that bacteria have and their functions. How do these organelles differ from most organelles found in eukaryotes?
2. Name at least two ways to classify bacteria. Why might there be several different ways to classify bacteria?
3. How are gram-positive and gram-negative bacteria different?
4. What is the purpose of adding 95% ethanol (or similar high percentage alcohol) during gram staining?

# Instructor's Guide

**NOTE TO TEACHERS:**

This experiment is intended for 8 student groups. It includes BactoBeads™ for three species (a gram-positive cocci, a gram-positive bacilli, and a gram-negative bacilli) as well as reagents for nine agar plates and 24 stained slides. We suggest preparing three source plates of each species in the prelab and having students prepare a slide for all three species during the experiment. This will require groups to exchange plates. Alternatively, groups can prepare a single slide and then share observations.

To expand this experiment, you can choose to have students pour their own agar plate and/or prepare their own source plates. Appendix A and B also describe the optional activities of observing a mixed species slide and identify the three species based on colony growth.

## PreLab Preparations

1. Up to two weeks before Module I, pour agar plates (page 14).
   The day before Module I, prepare source plates (page 15).
2. Aliquot 0.5 mL of distilled water into eight microcentrifuge tubes.
3. Aliquot 5 mL of Gram's Crystal Violet into eight labeled flasks. Cover to prevent evaporation.
4. Aliquot 5 mL of Gram's Iodine into eight labeled flasks. Cover to prevent evaporation.
5. Aliquot 20 mL of 95% ethanol into eight labeled flasks. Cover to prevent evaporation.
6. Aliquot 5 mL of Gram's Safranin into eight labeled flasks. Cover to prevent evaporation.
7. For slide washing, either prepare eight beakers or eight squeeze bottles with water.
8. Distribute solutions, slides, plastics, and equipment to each each student group.

**FOR THIS EXPERIMENT, EACH GROUP WILL NEED:**

**Module I**

- 3 slides
- Marker or slide labels
- 1 Source Plate*
- 3 Toothpicks
- Distilled water
- 1 Small transfer pipet
- Paper towels
- Bunsen burner or alcohol lamp
- Slide holder or clothespin

* *Student groups will need to exchange source plates in order to prepare slides of all three species.*

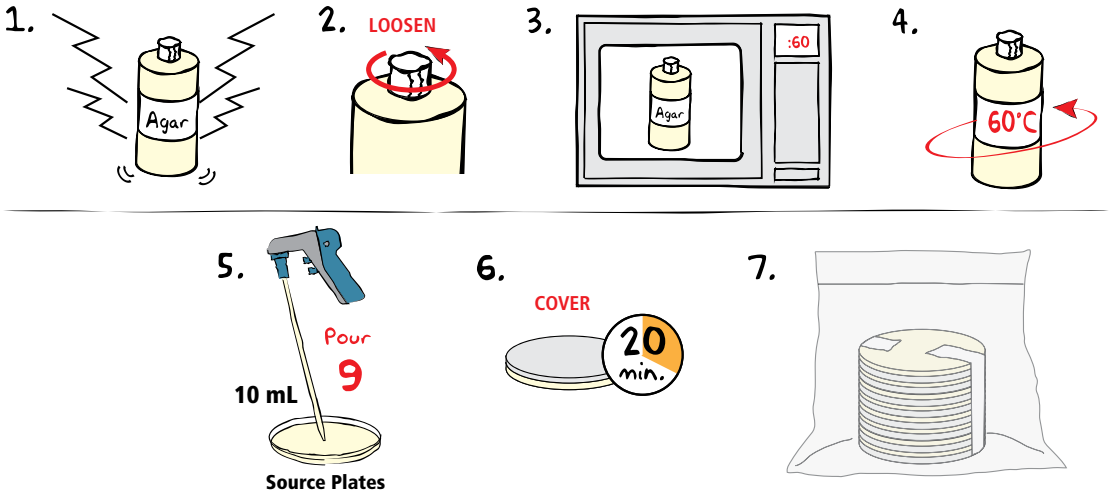
**Module II**

- 5 mL Gram's Crystal Violet
- 5 mL Gram's Iodine
- 20 mL 95% Ethanol
- 5 mL Gram's Safranin
- 4 Large transfer pipettes
- Bunsen burner or alcohol lamp
- Squeeze bottles filled with water and access to a sink OR Beakers filled with distilled water
- Slide holder or clothespin
- Paper towels or Blotting Paper

**Module III**

- Microscope

## Pouring Agar Plates




1. **BREAK** solid ReadyPour™ Agar into small chunks by vigorously squeezing and shaking the plastic bottle.

2. **LOOSEN**, but DO NOT REMOVE, the cap on the ReadyPour™ Agar bottle. This allows the steam to vent during heating. ***CAUTION: Failure to loosen the cap prior to heating may cause the bottle to break or explode.***

3. **MICROWAVE** the ReadyPour™ Agar on high for 60 seconds to melt the agar. Carefully remove the bottle from the microwave and mix by swirling the bottle. Continue to heat the solution in 30-second intervals until the agar is completely dissolved (the amber-colored solution should be clear and free of small particles).

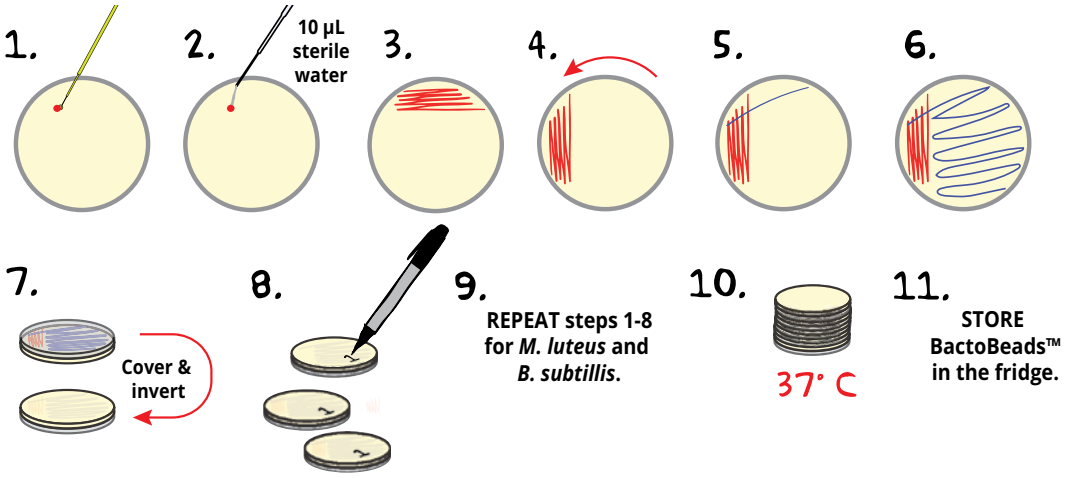
4. **COOL** the ReadyPour™ Agar to 60° C with careful swirling to promote even dissipation of heat.

5. **POUR** 10 mL of the cooled ReadyPour™ Agar into each of the nine large petri dishes (source plates) using a 10-mL pipet and pipet pump.

6. **COVER** and wait at least twenty minutes for the LB-agar plates to solidify. For optimal results, leave plates at room temperature overnight.

7. **STORE** plates at room temperature for no more than two days. Plates should be inverted and placed in a sealable plastic bag to ensure that they do not dry out. Inverted and bagged plates can also be stored in the refrigerator for up to two weeks.

## Preparing the Source Plates

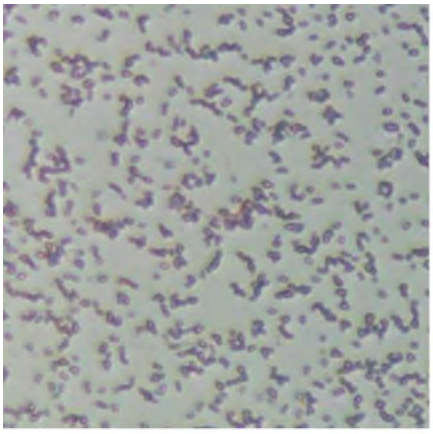



1. Remove a single *E.coli* BactoBead™ from the vial using a sterile inoculating loop and transfer to the top edge of a large LB petri plate.
2. Dissolve the bead by adding 10 µL of sterile water.
3. Move the flat end of the loop back and forth through the dissolved bead to make a primary streak of tight zig zag lines that is approximately 1.5 cm wide. At the end of this step, the top segment of the plate should have a dense solution of bacteria spread over it.
4. Rotate the plate 90 degrees to the left. Turn the loop so that the unused side is now facing the agar.
5. Pass the new side of the loop through the entire width of the primary streak. The location of this intersect should be near the top. Keeping the loop on the agar, continue to the end of the petri plate.
6. Keeping the loop on the agar and WITHOUT touching the primary streak again, move the loop back and forth and down the plate. Unlike step 3, these zig zag line should be loose. We recommend 7 – 10 back and forth movements. ***Reminder: During this step keep the loop between the edge of the previous streak and the edge of the plate. DO NOT let the loop pick up bacterial from the first streak.***
7. Cover the plate and wait at least 5 minutes before inverting.
8. Repeat steps 1-7 with a second and third *E.coli* BactoBead™. Label all three plates "1".
9. Repeat steps 1-8 for *M. luteus* and *B. subtillis* labeling plates "2" or "3".
10. Invert and incubate plates at 37° C overnight. If you do not have access to an incubator, incubate plates at room temperature for 24-48 hours.
11. Store remaining BactoBeads™ in the fridge with the rubber lid tightly on.

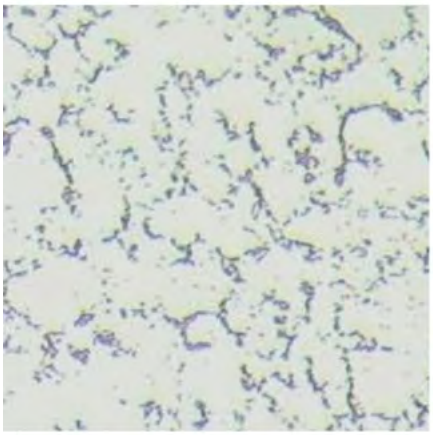
**NOTE:** Isolated colonies create great slides by limiting the number of bacteria that get transferred to the slide (too many bacteria and the shape and arrangements can be hard to see). In addition, isolated colonies are required for the classification activity described in Appendix B. However, gram staining can be performed without isolated colonies. If one or more source plates have only continuous growth encourage students to collect bacteria from the thinnest streak growing on the plate.

# Experiment Results and Analysis

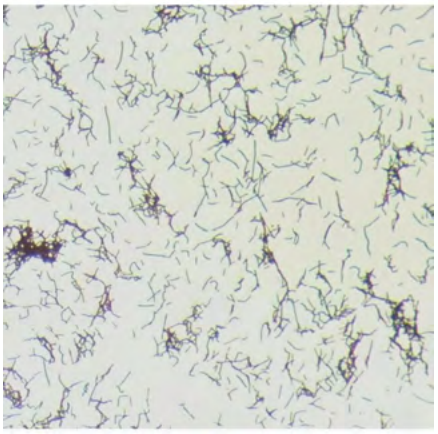
Slide #1: *Escherichia coli*



Slide #2: *Micrococcus luteus*



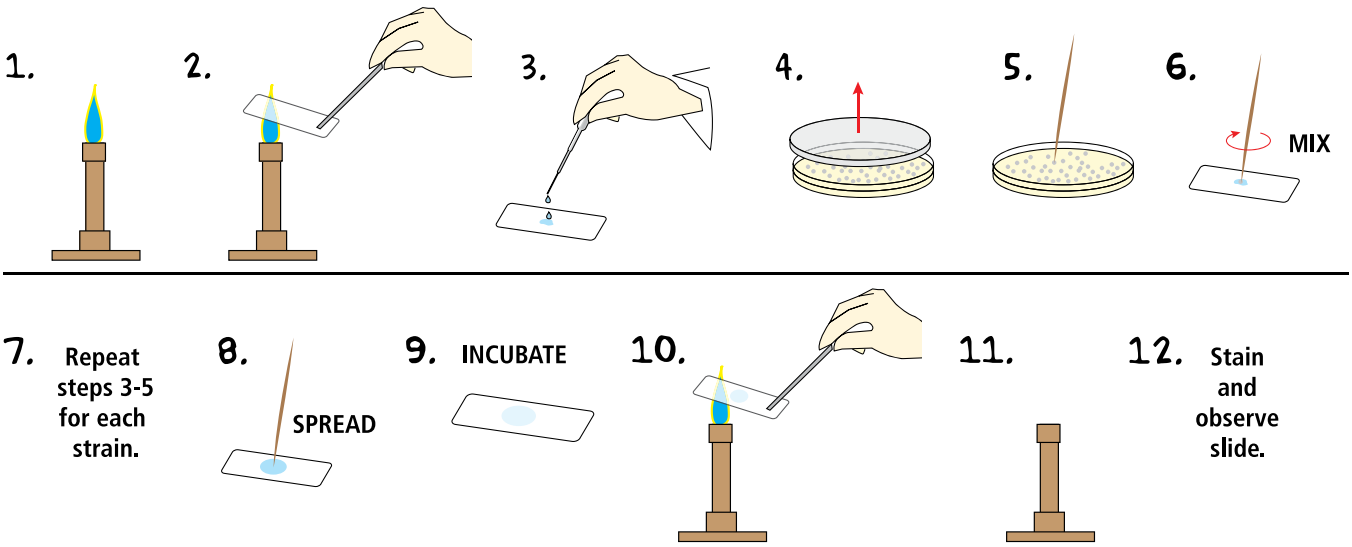
Slide #3: *Bacillus subtillis*



**Please refer to the kit insert for the Answers to Study Questions**

# Appendix A

## Creating Mixed Species Slides




To directly observe the contrast between gram negative and gram positive bacteria you may wish to create a mixed slide containing two or more of the provided species using the following modified slide preparation procedure. As these steps can *easily* lead to plate contamination we suggest that mixed species slides be prepared as part of the prelab rather than the experiment itself. This activity will require access to additional blank slides and additional toothpicks.

1. **TURN ON** a Bunsen burner or light the flame of an alcohol lamp.
2. Using a slide holder or clothespin, **PASS** this slide through the open flame two times.
3. While still hot, **ADD** two drops of water to the slide.
4. **REMOVE** the lid of an agar plate.
5. **TOUCH** a single colony with a toothpick to collect the bacteria.
6. Use the toothpick to **MIX** the bacteria and water.
7. **REPEAT** steps 3-6 for each strain. Make sure to switch toothpicks!
8. Using any toothpick, **SPREAD** the mixture so that it becomes a small circular film about the size of a dime. The liquid should be slightly cloudy but no "clumps" should be present.
9. **INCUBATE** at room temperature for 1 minute or until the water has dried completely.
10. **PASS** the slide through the flame two times, smear side up.
11. **TURN OFF** the Bunsen burner or extinguish the flame of the alcohol lamp.
12. Have students **STAIN** and **OBSERVE** the slide as outlined in Modules II and III.

# Appendix B

## Bacteria Classification Based on Colony Morphology

Many bacteria reproduce rapidly on agar. The color, shape, and texture of this growth is another way to distinguish between *different* species. This is particularly true of colony morphology - a colony is a group of identical bacteria that originate from a single plated bacteria cell. Examine each plate (1, 2, and 3) BEFORE gram staining to form a hypothesis about the possible species identity.

1. **LOCATE** any distinct colonies on the plate. For the most part these will appear as small, round (or semi round), and slightly raised growths.
2. **DESCRIBE** the colony. What color is it? What is its diameter? What is its approximate height? How is it expanding? What does the border between the colony and the agar look like? Is it shiny or dull? Are there any other notable features such as concentric circles?
3. Carefully **LIFT** the plate lid half an inch and then slowly **FAN** your hand towards your nose. What does the plate smell like? (If you do not smell anything record no odor, do not put the plate nearer to your face!)
4. **RECORD** your observations in your lab book or in the table below.
5. Once you have described the plate and recorded your observations, **EXCHANGE** plates with another group and repeat steps 1-4. Continue switching plates until you have observations for all three unique species.
6. Use the descriptions below to see if you can **IDENTIFY** the species on each plate based on colony morphology alone.

| Plate # | Color | Size | Shape | Border (wavy serrated, smooth, etc.) | Texture | Odor |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |
| | | | | | | |

### SPECIES COLONY DESCRIPTIONS

| Strain/Species | Color | Size | Shape | Border (wavy serrated, smooth, etc.) | Texture | Odor |
|---|---|---|---|---|---|---|
| *Micrococcus luteus* | Bright yellow | 1-2 mm | Round & domed | Smooth | Smooth | Yes |
| *Bacillus subtillis* | White or light yellow | 2-4 mm | Round & flat | Jagged | Slightly fuzzy | Yes |
| *Escherichia coli* | Off white or beige | 1-4 mm | Round & slightly raised | Smooth | Smooth and semi-translucent | Yes |