

## Virtual Labs Implementation Guide and Instructor Notes

NOTE: At this time the IG only covers Aseptic Technique, Staining, and Isolation Methods simulations. The document will be added when we have the balance of topics covered.

### General Notes:

- This is a preliminary Implementation Guide that will be updated over time.
- Also, please make sure you have looked at the other help documents that are posted:
  - Helpful Information for Instructors
  - Helpful Information for Students
- Instructors can preview or skip through all simulation phases without completing steps by using the keyboard command *shift-control-k* (on Mac) or *ctrl+alt-K* (on PC).
- It is recommended that students complete the **Virtual Lab Tutorial** prior to attempting their first Virtual Lab simulation.
- Students may toggle between the Introduction and the Laboratory Simulation at any time.
- Student progress is saved with each phase they complete.
- The entire lab simulation or individual phases can be reset.
- Students will only receive check marks for steps completed and receive notification of steps not completed when they attempt to move onto the next phase.
- The timer will stop at the correct time for data measurement.

### Lab Data:

- Students are allowed to enter incorrect data in the Lab Data Table. A red box will appear at the top to indicate data is incorrect once student clicks outside of box. Students will be able to re-enter data during the phase.
- Students are allowed to continue to the next phase with incorrect data in Lab Data table with no impact on their simulation grade (see Grading section).
- Once data has been entered into the Lab data table and the student moves to the next phase, data from a previous phase cannot be changed unless the student resets the phase.
- If a question pops up during lab data entry, it has to be answered before you can correct lab data.

### Grading:

- The Connect Virtual Labs grade is based on completion of the simulation. A student who completes the simulation will receive 100% credit, with no deductions for resetting phases, selecting the wrong hypothesis, or requiring multiple attempts to correctly answer pop-up questions.
- Screenshots of pop-up questions are as of {INSERT DATE}. We make continual updates based on feedback from instructors and students. The Implementation Guide will be updated periodically to reflect the changes.

- If a student does not complete a simulation by the due date, the progress will autosubmit, and he or she will receive a grade that reflects the phases completed.
  - For example, completing 3 of 4 total phases will produce a grade of 75%.

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## Staining – Preparing a smear from a bacterial sample

### LEARNING OBJECTIVES:

- Recall the rationale and background of why smear preps are done
- Identify common microbiology laboratory equipment
- Practice the preparation of smear samples from bacteria grown in liquid media and agar plates
- Interpret the role of heat fixation
- Sequence the steps of smear preparation
- Recall basic microscopy technique
- Apply how correct smear preparation affects microscopic outcomes
- Predict how a stain is used in conjunction with a smear prep

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students have **prior completion of microscopy simulations**.
- As a fundamental skill repeated in most staining protocols, it is recommended to assign smear preparation before other staining simulations. In other staining simulations, smear preps are provided rather than student-completed, assuming working knowledge of the concepts herein.
- Upon completion, students may be prepared for Gram staining, capsule staining, acid-fast staining, etc.

### Phases in Virtual Lab = {8}

1. Identifying microbiology lab tools used in creating a smear
2. Function of microbiology lab tools
3. Preparation of bacterial smear from liquid culture
4. Preparation of bacterial smear from solid media
5. Simple staining and observation of smear preparations
6. Summarize steps of a bacterial smear
7. Apply what you have learned
8. Save Lab Data

Methods X

- ✓ **Phase 1: Identifying microbiology lab tools used in creating a smear**
  1. Label tools used in microbiology smear preparation
- ✓ **Phase 2: Function of microbiology lab tools**
  1. Match each object with its function
- ✓ **Phase 3: Preparation of bacterial smear from liquid culture**
  1. Label slide with marker
  2. Sterilize loop by placing into flame of Bunsen burner
  3. Pick up culture tube. Remove cap. Briefly heat mouth of tube in Bunsen burner flame to reduce contamination
  4. Insert loop into tube to acquire bacterial sample
  5. Briefly heat mouth of tube, replace cap, and return tube to rack
  6. Smear bacteria across center of slide
  7. Sterilize loop using the Bunsen burner. Return loop to holder. Allow bacterial smear to air dry for 5 minutes
  8. Using clothespin to hold slide, perform the heat fixation of sample by passing through the Bunsen burner for 3-5 seconds. Return slide to staining tray
- ✓ **Phase 4: Preparation of bacterial smear from solid media**
  1. Label slide with marker
  2. Sterilize loop by placing into flame of Bunsen burner
  3. Use loop to retrieve a drop of water from beaker. Add water drop to center of slide
  4. Reheat loop in Bunsen burner flame to reduce possible contamination. Acquire bacterial sample from plate
  5. Use loop to mix bacteria into water drop on slide. Spread sample material to make a thin smear
  6. Sterilize loop using the Bunsen burner. Return loop to holder. Allow bacterial smear to air dry for 5 minutes
  7. Using clothespin to hold slide, perform the heat fixation of sample by passing through the Bunsen burner for 3-5 seconds. Return slide to staining tray
- ✓ **Phase 5: Simple staining and observation of smear preparations**
  1. Move slide to microscope to observe sample
  2. Save image in Lab Data. Zoom out of microscope view
  3. Remove slide from microscope. Place onto slide staining tray
  4. Add methylene blue dye to slide smear. Wait 1 minute
  5. Rinse slide with distilled water
  6. Move slide to blotting paper and fold it over to remove excess stain and liquid from smear sample
  7. Move slide to microscope. Re-evaluate smear appearance.
  8. Save image in Lab Data
- ✓ **Phase 6: Summarize steps of a bacterial smear**
  1. Put the following steps in correct order for performing a smear preparation
- ✓ **Phase 7: Apply what you have learned**
  1. Select your answer to the question
- ✓ **Phase 8: Save Lab Data**
  1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

**Question** X

Why is aseptic technique important during laboratory activities like smear preparation?  
*Select all that apply.*

☒ A. Proper aseptic technique aids in keeping microbes from spreading to other surfaces, where they might be contacted by others in the lab.

☐ B. Aseptic technique aids in selecting a treatment, because we can observe what methods kill the infectious agent.

☒ C. Proper aseptic technique helps prevent contamination from being introduced into your sample during preparation.

☒ D. Aseptic technique helps prevent accidental infection of the handler.

**Correct**  
A. Correct  
C. Correct  
D. Correct

Continue

1. Phase 3 --

**Question** X

Creating a bacterial smear from solid media, such as an agar plate or slant, often feels more tangible than creating a smear from liquid media. Which of the following is appropriate when creating a smear in this fashion?

☒ Using the loop, you should disperse the bacterial sample material into a water drop on the slide.

☐ You can place a big lump of bacterial sample onto the slide to ensure the smear is as concentrated as possible.

☐ The tip of a gloved finger can be used to mix the bacteria and water on the surface of the slide.

**Correct**

Continue

2. Phase 4 --

Question

X

As you create your smear from a sample on solid media, it is important to use an appropriate amount of material and evenly disperse the organisms onto the surface of the slide. Which of the following can occur due to using excessive material as you create a smear preparation?  
*Select all that apply.*

☒ A. It will be difficult to remove excess dye stain in later steps of the smear procedure.

☐ B. It will be difficult to sterilize the slide after the procedure is complete

☒ C. It will be difficult to distinguish bacterial cell morphology and arrangements of cells on the smear sample.

☒ D. It will be difficult to apply stain correctly to the slide for visualization.

Correct

A. Correct

C. Correct

D. Correct

Continue

3. Phase 4 --

Information

X

In this simulation, the heat fixation was performed for you. Because each sample is unique, there is no "set" time for completing heat fixation. You should wait until the smear is totally air dry to prevent boiling your sample off. Then, gentle, gradual heating is needed to both fix to the slide and preserve the sample integrity. In a real lab experiment, students are often told to perform the fixation step by waving the slide back and forth through the flame several times and then to check the temperature of the slide by quickly touching the underside of the slide to the back of your hand. In general, if the slide is too hot to touch, the heat fixation has gone on too long and the cells may be damaged or lysed. Consult your lab instructor or lab protocols at your school for more information on this procedure.

4. Phase 4 --

Information

X

The slide provided for you here is a correctly-made smear prep from a bacterium on agar media, prepared in similar fashion to what you completed in previous phases. Because microscopy techniques are not the overall goal of this simulation, the focusing, magnification, and scanning usually done by the user will be provided for you as a best representative image.

5. Phase 5 --

6. Phase 5 --

**Question** X

This is how your smear preparation looks in the microscope immediately after completion.  
**True or False:** This image, as it appears, is able to be interpreted for information about the organism(s) on the slide.

☐ True

☒ False

**Correct**

Continue

7. Phase 5 --

**Question** X

This is how your smear preparation looks in the microscope after simple staining with methylene blue.  
**True or False:** This image, as it appears, is able to be interpreted for information about the organism(s) on the slide.

☒ True

☐ False

**Correct**

Continue

8. Phase 5 --

**Question** X

Now that you have seen the difference in the smear preparation after simple methylene blue staining, which of the following is correct regarding your smear now?  
 Select all that apply.

☒ A. The addition of methylene blue makes visualizing some bacterial cell characteristics more apparent, compared to no staining.

☒ B. The addition of methylene blue helps show the intact appearance and localization of the cells, compared with no staining.

☐ C. Addition of methylene blue highlights the bacterial cell nucleus and organelles that were difficult to discern without stain.

☒ D. The addition of methylene blue helps visualize the shape and arrangements of bacterial cells that were difficult to discern without stain.

**Correct**  
 A. Correct  
 B. Correct  
 D. Correct

Continue

### Ending Saved Lab Data and Report Materials:

- Image of smear prep slide without staining
- Image of smear prep slide with staining

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## Staining – Acid-fast staining

### LEARNING OBJECTIVES:

- Recall the rationale and background of smear preparation and acid-fast bacterial staining
- Recall the basics of acid-fast cell wall composition
- Predict why the acid-fast stain is used compared to other methodologies
- Contrast the differences of simple versus differential staining
- Identify common microbiology laboratory equipment
- Practice the technique of acid-fast staining
- Compare the role of heat fixation and mordant in the acid-fast stain versus other staining techniques
- Sequence the steps of acid-fast staining
- Recall basic microscopy technique
- Apply how correct acid-fast staining techniques affect microscopic outcomes
- Predict the visual outcome of an acid-fast stain
- Analyze possible errors or ineffective acid-fast protocols and interpret results

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have **prior completion of microscopy simulations and smear preparation simulations**. A pre-prepared smear is provided to students in this activity to focus them on the direct experimental protocol.
- This simulation is modeled after the Kinyoun staining (non-heated carbolfuchsin), but acknowledges the Ziehl-Neelsen method, which uses heated stain.
- Upon completion, students may be prepared for other staining simulations, such as Gram staining, capsule staining, spore staining, etc.

### Phases in Virtual Lab = {6}

1. Identifying microbiology lab tools used in an acid-fast stain
2. Explore how acid-fast staining works
3. Conduct an acid-fast stain
4. Apply what you have learned
5. Review
6. Save Lab Data

**Methods**

✓ **Phase 1: Identifying microbiology lab tools used in an acid-fast stain**

1. Match the labels to the tools used in an acid-fast preparation

✓ **Phase 2: Explore how acid-fast staining works**

1. Select your answer to the first question
2. Select your answer to the second question

✓ **Phase 3: Conduct an acid-fast stain**

1. Add carbolfuchsin to smear. Allow stain to penetrate for 5 minutes. Rinse smear with distilled water
2. Add acid alcohol decolorizer to smear. Wait 1 minute. Rinse smear with distilled water
3. Add methylene blue to smear. Wait 30 seconds. Rinse smear with distilled water
4. Blot stained smear with paper to remove excess stain and water
5. Move slide to microscope for observation
6. Observe slide on microscope. Save image in Lab Data

**Phase 4: Apply what you have learned**

1. Select your answer to the first question
2. Select your answer to the second question
3. Select your answer to the third question

**Phase 5: Review**

1. Correctly order steps of acid-fast staining

**Phase 6: Save Lab Data**

1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

1. Phase 3 --

**Question**

The primary stain, carbolfuchsin, binds to \_\_\_\_\_ in the sampled bacteria.

☒ cell wall lipids, such as mycolic acid

☐ carbohydrates

☐ membrane proteins

☐ peptidoglycan

**Correct!**

Continue



2. Phase 3 --

**Question** X

When staining a known acid fast organism, in the presence of acid alcohol decolorizer, carbolfuchsin dye should \_\_\_\_\_ in the sampled bacteria.

☐ change color

☒ be retained

☐ be neutralized

☐ be removed

**Correct!**

Continue

3. Phase 3 --

**Question** X

The rationale for using methylene blue as a counterstain in the acid-fast protocol is to \_\_\_\_\_

☐ wash non-acid-fast bacterial cells off the slide.

☐ neutralize the acid alcohol staining reagent before handling.

☒ provide contrasting color to non-acid-fast bacterial cells.

☐ kill the remaining acid-fast bacterial cells.

**Correct!**

Continue

4. Phase 3 --

**Question** X

Your acid-fast stain is complete and correct. Which of the following statements would apply to the image you see?

Select all that apply.

☒ A. You can observe non-acid-fast bacterial cells on this field.

☐ B. You cannot distinguish any bacteria on this field.

☒ C. You can observe acid-fast bacterial cells on this field.

☐ D. You can observe acid-neutral bacteria on this field.

**Correct**

A. Correct!

C. Correct!

Continue

### Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions (includes images)
- Correct sequenced order of steps to perform an acid-fast stain
- Collected Lab Data image of completed acid-fast stain

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## Staining – Capsule staining

### LEARNING OBJECTIVES:

- Recall the rationale and background of smear preparation and bacterial staining
- Recall the basics of bacterial cell and bacterial capsule composition
- Describe and interpret negative (background) staining
- Predict why the capsule stain is used compared to other methodologies
- Contrast the differences of simple versus differential staining
- Identify common microbiology laboratory equipment
- Practice the technique of capsule staining
- Compare the need for heat fixation in the capsule stain versus other staining techniques
- Sequence the steps of capsule staining
- Practice basic microscopy technique and interpretations
- Apply how correct capsule staining techniques affect microscopic outcomes
- Predict the visual outcome of a capsule stain
- Analyze possible errors or ineffective capsule stain protocols and interpret results

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, smear preparation, and basics of staining before attempting this simulation.
- Upon completion, students may be prepared for other staining simulations, such as Gram staining, acid-fast staining, spore staining, etc.

### Phases in Virtual Lab = {6}

1. Identifying microbiology lab tools used in a capsule stain
2. Mechanism of capsule staining
3. Capsule staining procedure
4. Perform a capsule stain
5. Apply what you have learned
6. Save Lab Data

Methods
X

**Phase 1: Identifying microbiology lab tools used in a capsule stain**

1. Match labels to tools used in capsule stain preparation

**Phase 2: Mechanism of capsule staining**

1. Match terms/images to correct positions on chart

**Phase 3: Capsule staining procedure**

1. Place basic steps of capsule staining in correct sequence

**Phase 4: Perform a capsule stain**

1. Turn on Bunsen burner
2. Pick up nigrosin stain and apply a drop to the clean slide on staining tray
3. Sterilize loop by placing into flame of Bunsen burner
4. Pick up bacterial culture tube. Remove cap. Briefly heat mouth of tube in Bunsen burner flame to reduce contamination
5. Insert loop into tube to acquire bacterial sample
6. Briefly heat mouth of tube, replace cap, and return tube to rack
7. Mix bacterial sample in loop into nigrosin on slide
8. Sterilize loop using Bunsen burner. Return loop to holder
9. Turn off Bunsen burner
10. Create thin smear by dragging a clean slide through sample. Allow sample to thoroughly air dry for 5 minutes
11. Apply crystal violet stain to smear. Wait 1 minute
12. Rinse slide with distilled water
13. Move slide to blotting paper to remove excess stain and water
14. Move slide to microscope for observation
15. Observe slide on microscope. Save image in Lab Data

**Phase 5: Apply what you have learned**

1. Select your answer to the first question
2. Select your answer to the second question
3. Select your answer to the third question

**Phase 6: Save Lab Data**

1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

1. Phase 2 --

Information
X

Special stains are used to emphasize certain cell parts that may not be revealed by conventional staining methods. Capsule staining allows the user to observe the microbial capsule, an unstructured protective layer surrounding the cells of some microorganisms. Because the capsule does not react with most stains, it is often negatively stained. The fact that not all microbes exhibit capsules is a useful feature for identifying pathogens. Based on what you understand from this and the simulation overview, can you complete the chart?

**Question** X

What is the purpose of skipping the heat fixation step in the capsule stain procedure?

☐ Capsules will appear too dark if heated.

☒ Capsules are fragile and can be destroyed with heating.

☐ Capsules are converted to slime layers if heated.

☐ Heat fixation is unnecessary because organisms with capsules naturally adhere to the slide.

☐ Heat fixation is unnecessary in capsule staining because the organisms are harmless.

**Correct!**

Continue

2. Phase 4 --

**Question** X

Your capsule stain is complete and correct. Which of the following statements would apply to the image you see?

Select all that apply.

☒ A. The bacterial cells are stained with crystal violet.

☒ B. The background is stained dark with nigrosin.

☐ C. The capsules have stained with the primary and secondary dye colors.

☐ D. No bacterial capsules can be visualized in this field.

☒ E. You can observe encapsulated organisms in this field.

**Correct**

A. Correct!

B. Correct!

E. Correct!

Continue

3. Phase 4 --

### Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions (includes images)
- Review of student-answered chart comparing positive and negative staining
- Correct sequenced order of steps to perform a capsule stain
- Collected Lab Data image of completed capsule stain

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## Staining – Gram staining

### LEARNING OBJECTIVES:

- Recall the rationale and background of smear preparation and Gram staining
- Recall the basics of bacterial cell wall composition
- Predict why the Gram stain is used compared to other methodologies
- Contrast the differences of simple versus differential staining
- Identify common microbiology laboratory equipment
- Practice the technique of Gram staining
- Compare the role of heat fixation and mordant in the Gram stain versus other staining techniques
- Sequence the steps of Gram staining
- Recall basic microscopy technique
- Apply how correct Gram staining techniques affect microscopic outcomes
- Predict the visual outcome of an Gram stain
- Analyze possible errors or ineffective Gram stain protocols and interpret results
- Predict how correct staining identification can assist in antibiotic therapy selection

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have **prior completion of microscopy simulations and smear preparation simulations**. A pre-prepared smear is provided to students in this activity to focus them on the direct experimental protocol.
- Upon completion, students may be prepared for other staining simulations, such as acid-fast staining, capsule staining, spore staining, etc.

### Phases in Virtual Lab = {6}

1. Identifying microbiology lab tools used in a Gram stain
2. Mechanism of Gram staining (Video example)
3. Performing a Gram stain
4. Apply what you have learned
5. Apply what you have learned, part 2
6. Save Lab Data

Methods

X

✓

**Phase 1: Identifying microbiology lab tools used in a Gram stain**

1. Label tools used in Gram staining

✓

**Phase 2: Mechanism of Gram staining**

1. Watch video to learn more about Gram stain

✓

**Phase 3: Performing a Gram stain**

1. Add crystal violet to smear. Wait 30 seconds. Rinse smear with water  
2. Add Gram's iodine to smear. Wait 1 minute. Rinse smear with water  
3. Add decolorizer to smear. Wait 5-10 seconds. Rinse smear with water  
4. Add safranin to smear. Wait 1 minute. Rinse smear with water  
5. Blot stained smear to remove excess stain and water  
6. Move slide to microscope for observation  
7. Observe slide on microscope  
8. Save image in Lab Data

✓

**Phase 4: Apply what you have learned**

1. Select your answer to the first question  
2. Select your answer to the second question

✓

**Phase 5: Apply what you have learned, part 2**

1. Select your answer to the first question  
2. Select your answer to the second question

✓


**Phase 6: Save Lab Data**

1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

**Question** X

Based on what you understood from the video, which of the following statements are correct?  
Select all that apply.



☒ A. This image likely depicts a Gram-stained bacterial smear.

☒ B. This image shows a cell morphology of cocci.

☐ C. This image shows a cell arrangement of staphylococci.

☐ D. This image suggests the Gram stain was done incorrectly.

☐ E. This image shows a cell morphology of bacilli.

☒ F. This image shows a cell arrangement of streptococci.

**Correct**  
A. Correct!  
B. Correct!  
F. Correct!

Continue

1. Phase 2 --

**Question** X

After correct Gram staining, the gram-negative cells will appear \_\_\_\_\_, whereas the gram-positive cells appear \_\_\_\_\_.

☐ unstained; green

☐ dark blue; light blue

☐ purple; red

☐ green; unstained

☒ red; purple

**Correct!**

Continue

2. Phase 2 --

Question

X

Your Gram stain is complete and correct. Which of the following statements would apply to the image you see? *Select all that apply*

☒ A. You can observe gram-negative bacilli on this field.

☒ B. You can observe gram-positive cocci on this field.

☐ C. You can observe gram-neutral bacteria on this field.

☐ D. You cannot distinguish any bacteria on this field.

Correct

A. Correct!

B. Correct!

Continue

3. Phase 3 --

### Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions (includes images)
- Collected Lab Data image of completed Gram stain

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## Staining – Spore staining

### LEARNING OBJECTIVES:

- Recall the rationale and background of smear preparation and bacterial staining
- Recall the basics of bacterial cell and bacterial spore composition
- Describe and interpret negative (background) staining
- Predict why the spore stain is used compared to other methodologies
- Contrast the differences of simple versus differential staining
- Identify common microbiology laboratory equipment
- Practice the technique of spore staining
- Compare the need for heat fixation in the spore stain versus other staining techniques
- Sequence the steps of spore staining
- Practice basic microscopy technique and interpretations
- Apply how correct spore staining techniques affect microscopic outcomes
- Predict the visual outcome of a spore stain
- Analyze possible errors or ineffective spore stain protocols and interpret results

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, smear preparation, and basics of staining before attempting this simulation.
- This simulation is modeled after the Schaeffer-Fulton method of spore staining.



- Upon completion, students may be prepared for other staining simulations, such as Gram staining, acid-fast staining, capsule staining, etc.

## Phases in Virtual Lab = {8}

1. Identifying microbiology lab tools used in a spore stain
2. Mechanism of spore staining (video example)
3. Spore staining procedure
4. Perform a spore stain
5. Apply what you have learned, part 1
6. Apply what you have learned, part 2
7. Common errors that can affect the outcome of the spore stain
8. Save Lab Data

**Methods** X

✓ **Phase 1: Identifying microbiology lab tools used in a spore stain**

1. Match the labels to the tools used in spore stain preparation

✓ **Phase 2: Mechanism of spore staining**

1. Watch video to learn more about the spore stain

✓ **Phase 3: Spore staining procedure**

1. Place the steps of the spore stain in the correct sequence

✓ **Phase 4: Perform a spore stain**

1. Add malachite green to prepared slide smear

2. Use tweezers to place paper strip onto slide

3. Place slide onto heating beaker for 5 minutes

4. Return slide to staining tray

5. Remove paper with tweezers and dispose in autoclave bag

6. Rinse slide with distilled water

7. Add safranin to slide for 30 seconds

8. Rinse slide with distilled water

9. Move slide onto the blotting paper to remove excess stain and water

10. Move slide to microscope for observation

11. Observe slide on microscope. Save image in Lab Data

✓ **Phase 5: Apply what you have learned, part 1**

1. Match the label to the representative structure

✓ **Phase 6: Apply what you have learned, part 2**

1. Select your answer to the question

✓ **Phase 7: Common errors that can affect the outcome of the spore stain**

1. Select your answer to the first question

2. Select your answer to the second question

✓ **Phase 8: Save Lab Data**


1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

**Question** X

Based on what you understood from the video, which of the following statements are correct?

Select all that apply.



☒ A. In this image, the endospores are green in color.

☒ B. This image shows primarily endospores.

☐ C. This image could be representative of a *Staphylococcus* species.

☒ D. This image could be representative of a *Bacillus* species.

☐ E. In this image, the endospores are red in color.

☐ F. This image shows primarily exospores.

Correct

4. Phase 2 --

**Question** X

What is the purpose of adding a strip of paper to the spore stain procedure?

Select all that apply.

☐ A. The paper helps hold stain away from the sample.

☒ B. The paper allows for more contact time between the dye and spores.

☒ C. The paper keeps the dye from evaporating too quickly during the heating step.

☐ D. The paper is sticky and removes live bacteria from the smear.

☐ E. The paper is protective to the scientist doing the stain.

Correct  
B. Correct!  
C. Correct!

Continue

5. Phase 2 --

**Question** X

Your spore stain is complete and correct. Which of the following statements would apply to the image you see?

Select all that apply.

☐ A. You can observe gram-positive and gram-negative bacteria on this field.

☒ B. You can observe spores on this field.

☒ C. You can observe vegetative bacteria on this field.

☐ D. You cannot distinguish any spores on this field.

Correct  
B. Correct!  
C. Correct!

Continue

6. Phase 4 --

### Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions (includes images)
- Correct sequenced order of steps to perform a spore stain
- Collected Lab Data image of completed spore stain

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## Aseptic Technique – Broth culture to sterile agar plate

### LEARNING OBJECTIVES:

- Recall the definition of aseptic technique
- Explain the importance and implications of aseptic technique in experiments
- Discuss what is meant by the term bacterial culture
- Compare the meaning of bacterial culture versus pure culture
- Discuss what microbiology equipment is necessary to perform an aseptic transfer between growth medias
- Order the steps of bacterial culturing
- Practice the correct steps of aseptic transfer of a bacterial broth culture to an agar plate
- Evaluate the success of a bacterial transfer between growth media
- Understand the roles of heat for sterilization during aseptic transfer
- Recall safe and correct plate labeling
- State and define several kinds of agar media used in microbiology

## Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, basics of bacterial growth, basic microbiology laboratory equipment, and the definitions of sterile, aseptic, and media.
- This simulation has students complete a basic transfer (without isolation) of bacteria grown in broth medium to a solid agar plate.
- Students are introduced to the simple aseptic technique of heating the mouth of a tube before and after opening as basic practice. Students will see this repeated throughout a great majority of simulations and expected to use this practice as fundamental to working with tubes and transfers.
- Upon completion, students may be prepared for other simulations in the Aseptic Technique series (broth-to-broth culturing, slant-to-slant culturing), as well as bacterial ubiquity and surface sampling for growth. Further application of these concepts can be through the Isolation Methods series (pour plating, quadrant streak, subculturing, colony counting, dilution methods, optical density) and Microbial Growth series (anaerobic jar growth, effects of osmotic pressure, oxygen requirements in FTM tubes, effects of temperature, and effects of pH).

## Phases in Virtual Lab = {4}

1. Transfer bacteria to sterile agar plate
2. Observe culture
3. Aseptic technique summary for agar plate inoculation
4. Save Lab Data

Methods

X

✓ **Phase 1: Transfer bacteria to sterile agar plate**

1. Label agar plate with marker
2. Turn on Bunsen burner
3. Sterilize loop by placing into flame of Bunsen burner
4. Pick up stock *E. coli* tube. Remove cap. Briefly heat mouth of tube in Bunsen burner flame to reduce contamination
5. Insert loop into stock *E. coli* tube to acquire bacterial sample
6. Briefly heat mouth of stock *E. coli* tube, replace cap, and return tube to rack
7. Use loop to inoculate sterile agar plate
8. Sterilize loop using Bunsen burner. Return loop to holder
9. Turn off Bunsen burner
10. Select Incubate to incubate tubes for 24 hours

✓ **Phase 2: Observe culture**

1. Save image to Lab Data
2. Select plate to observe and evaluate inoculation

✓ **Phase 3: Aseptic technique summary for agar plate inoculation**

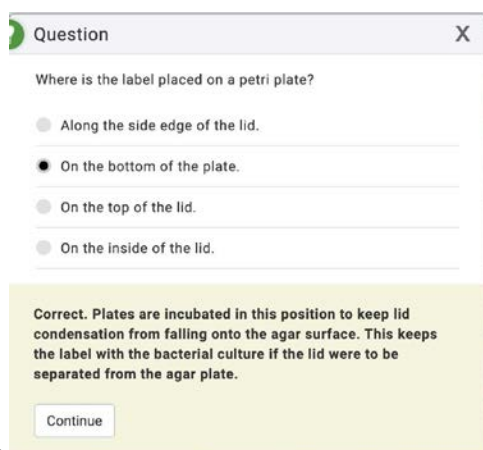
1. Place the following steps of the aseptic transfer technique into the correct order

**Phase 4: Save Lab Data**

1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

1. Phase 1 --



**Question** X

Where is the label placed on a petri plate?

☐ Along the side edge of the lid.

☒ On the bottom of the plate.

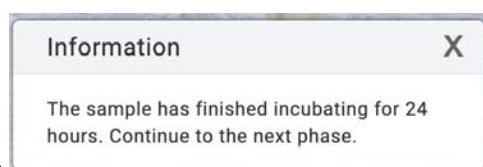
☐ On the top of the lid.

☐ On the inside of the lid.

Correct. Plates are incubated in this position to keep lid condensation from falling onto the agar surface. This keeps the label with the bacterial culture if the lid were to be separated from the agar plate.

Continue

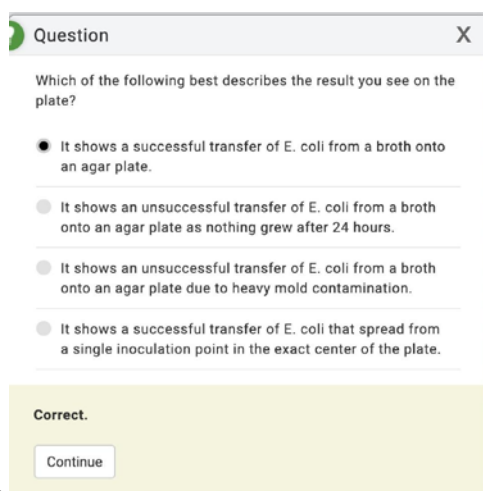
2. Phase 1 --



**Information** X

The sample has finished incubating for 24 hours. Continue to the next phase.

3. Phase 2 --



**Question** X

Which of the following best describes the result you see on the plate?

☒ It shows a successful transfer of E. coli from a broth onto an agar plate.

☐ It shows an unsuccessful transfer of E. coli from a broth onto an agar plate as nothing grew after 24 hours.

☐ It shows an unsuccessful transfer of E. coli from a broth onto an agar plate due to heavy mold contamination.

☐ It shows a successful transfer of E. coli that spread from a single inoculation point in the exact center of the plate.

Correct.

Continue

## Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions (includes images)
- Correct sequenced order of steps to perform a broth-to-plate transfer
- Collected Lab Data image of completed transfer colonies on agar plate

-----

## Aseptic Technique - Slant culture to sterile agar slant

### LEARNING OBJECTIVES:

- Recall the definition of aseptic technique
- Explain the importance and implications of aseptic technique in experiments
- Discuss what is meant by the term bacterial culture
- Compare the meaning of bacterial culture versus pure culture
- Discuss what microbiology equipment is necessary to perform an aseptic transfer between growth medias
- Order the steps of bacterial culturing
- Practice the correct steps of aseptic transfer of a bacterial agar slant to another agar slant
- Evaluate the success of a bacterial transfer between growth media
- Understand the roles of heat for sterilization during aseptic transfer
- Locate the regions of a slant tube
- Infer why an inoculation needle is used in this particular technique
- State and define several kinds of agar media used in microbiology

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, basics of bacterial growth, basic microbiology laboratory equipment, and the definitions of sterile, aseptic, and media.
- This simulation has students complete a basic transfer of bacteria grown on an agar slant to another solid agar slant using an inoculation needle. The classic “stab and run” technique is shown to students.
- Students are introduced to the simple aseptic technique of heating the mouth of a tube before and after opening as basic practice. Students will see this repeated throughout a great majority of simulations and expected to use this practice as fundamental to working with tubes and transfers.
- The video used in the simulation does NOT have sound attached. Instructors may wish to inform students of this prior to assigning the simulation. To avoid confusion or interpreting the lack of audio as a technical glitch, this is noted in the steps to students also.
- Upon completion, students may be prepared for other simulations in the Aseptic Technique series (broth-to-broth culturing, broth-to-plate culturing), as well as bacterial ubiquity and surface sampling for growth. Further application of these concepts can be through the Isolation Methods series (pour plating, quadrant streak, subculturing, colony counting, dilution methods, optical density) and Microbial Growth series (anaerobic jar growth, effects of osmotic pressure, oxygen requirements in FTM tubes, effects of temperature, and effects of pH).

### Phases in Virtual Lab = {5}

1. Video demonstration (video example)
2. Transfer bacteria to slant culture
3. Observe culture
4. Aseptic technique summary for agar slant inoculation
5. Save Lab Data

**Methods** X

✓ **Phase 1: Video demonstration**

1. Watch video that demonstrates how to transfer from a slant to a sterile slant. (This video does not have sound)

✓ **Phase 2: Transfer bacteria to slant culture**

1. Label sterile tube with marker
2. Turn on Bunsen burner
3. Sterilize loop by placing into flame of Bunsen burner
4. Pick up stock *E. coli* tube. Remove cap. Briefly heat mouth of tube in Bunsen burner flame to reduce contamination
5. Insert loop into stock *E. coli* tube to acquire bacterial sample
6. Briefly heat mouth of stock *E. coli* tube, replace cap, and return tube to rack
7. Pick up sterile tube. Remove cap. Briefly heat mouth of tube in Bunsen burner flame to reduce contamination
8. Streak agar in sterile tube
9. Briefly heat mouth of sterile tube, replace cap, and return tube to rack
10. Sterilize loop using the Bunsen burner. Return loop to holder
11. Turn off Bunsen burner
12. Select Incubate to incubate tubes for 24 hours

✓ **Phase 3: Observe culture**

1. Observe and evaluate inoculation. Record in Lab Data

✓ **Phase 4: Aseptic technique summary for agar slant inoculation**

1. Place the following steps of the aseptic transfer technique into the correct order

**Phase 5: Save Lab Data**

1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

### Pop-Up Questions and Topics for Students in Simulation:

1. Phase 2 --
 

**Question** X

Which of the following patterns is used to streak an agar slant?

☐ Pass the loop across the surface of the slant and cut several lines into the agar making a grid pattern.

☐ Pass the loop across the surface of the slant and push the loop completely to the bottom of the tube.

☒ Pass the loop across the surface of the slant.

**Correct.**

Continue
2. Phase 2 --
 

**Information** X

The sample has finished incubating for 24 hours. Continue to the next phase.

### Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions (includes images)

- Correct sequenced order of steps to perform a slant-to-slant transfer
- Collected Lab Data images of negative control slant and completed transfer of colonies onto a new agar slant

-----

## Aseptic Technique – Broth culture to sterile broth

### LEARNING OBJECTIVES:

- Recall the definition of aseptic technique
- Explain the importance and implications of aseptic technique in experiments
- Discuss what is meant by the term bacterial culture
- Compare the meaning of bacterial culture versus pure culture
- Discuss what microbiology equipment is necessary to perform an aseptic transfer between growth medias
- Order the steps of bacterial culturing
- Practice the steps of aseptic transfer of a bacterial broth culture to a new, sterile broth tube
- Evaluate the success of a bacterial transfer between growth media
- Understand the roles of heat for sterilization during aseptic transfer
- Recognize the use and importance of a negative control tube
- State and define several kinds of agar media used in microbiology

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, basics of bacterial growth, basic microbiology laboratory equipment, and the definitions of sterile, aseptic, and media.
- This simulation has students complete a basic transfer of bacteria grown in broth culture to another liquid broth using an inoculation loop.
- Students are introduced to the simple aseptic technique of heating the mouth of a tube before and after opening as basic practice. Students will see this repeated throughout a great majority of simulations and expected to use this practice as fundamental to working with tubes and transfers.
- The video used in the simulation does NOT have sound attached. Instructors may wish to inform students of this prior to assigning the simulation. To avoid confusion or interpreting the lack of audio as a technical glitch, this is noted in the steps to students also.
- Upon completion, students may be prepared for other simulations in the Aseptic Technique series (broth-to-plate culturing, slant-to-slant culturing), as well as bacterial ubiquity and surface sampling for growth. Further application of these concepts can be through the Isolation Methods series (pour plating, quadrant streak, subculturing, colony counting, dilution methods, optical density) and Microbial Growth series (anaerobic jar growth, effects of osmotic pressure, oxygen requirements in FTM tubes, effects of temperature, and effects of pH).



## Phases in Virtual Lab = {5}

1. Video demonstration (video example)
2. Transfer bacteria to sterile tube
3. Observe culture
4. Apply what you have learned
5. Save Lab Data

**Methods** X

**Phase 1: Video demonstration**

1. Watch this video that demonstrates transfer of broth culture to a sterile broth. (This video does not have sound)

**Phase 2: Transfer bacteria to sterile tube**

1. Label sterile tube with marker
2. Turn on Bunsen burner
3. Sterilize loop by placing into flame of Bunsen burner
4. Pick up stock *E. coli* tube. Remove cap. Briefly heat mouth of tube in Bunsen burner flame to reduce contamination
5. Insert loop into tube to acquire bacterial sample
6. Briefly heat mouth of tube, replace cap, and return tube to rack
7. Pick up sterile broth tube. Remove cap. Briefly heat mouth of tube in Bunsen burner flame to reduce contamination
8. Insert loop into tube
9. Briefly heat mouth of tube, replace cap, and return tube to rack
10. Sterilize loop using the Bunsen burner. Return loop to holder
11. Turn off Bunsen burner
12. Select Incubate to incubate tubes for 24 hours

**Phase 3: Observe culture**

1. Observe and evaluate inoculation. Record in Lab Data

**Phase 4: Apply what you have learned**

1. Place the following steps of the aseptic transfer technique into the correct order

**Phase 5: Save Lab Data**

1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

**Question** X

Which of the following are correct ways to label the organism found in a culture?  
Select all that apply.

☒ A. *E. coli*

☐ B. *Escheria c.*

☐ C. *Coli*

☒ D. *Escherichia coli*

Correct

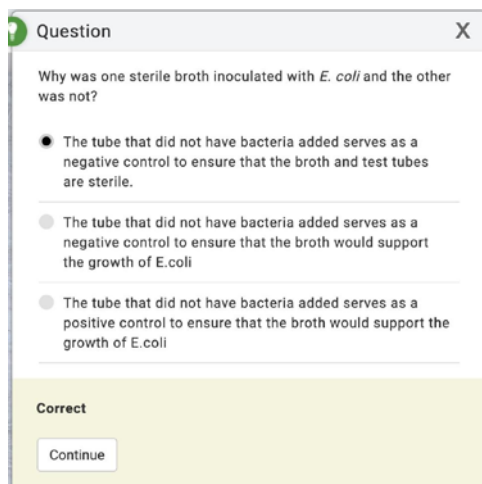
A. Correct

D. Correct

Continue

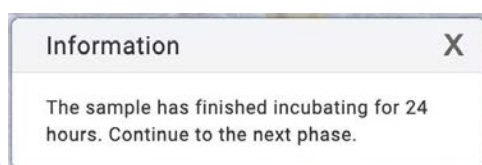
1. Phase 2 --

2. Phase 2 --



A screenshot of a 'Question' dialog box with a green question mark icon and a close button (X). The text asks: 'Why was one sterile broth inoculated with *E. coli* and the other was not?'. There are three radio button options. The first option is selected: 'The tube that did not have bacteria added serves as a negative control to ensure that the broth and test tubes are sterile.' The other two options are: 'The tube that did not have bacteria added serves as a negative control to ensure that the broth would support the growth of *E. coli*' and 'The tube that did not have bacteria added serves as a positive control to ensure that the broth would support the growth of *E. coli*'. Below the options, a yellow bar indicates 'Correct' and contains a 'Continue' button.

3. Phase 2 --



A screenshot of an 'Information' dialog box with a close button (X). The text states: 'The sample has finished incubating for 24 hours. Continue to the next phase.'

### Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions (includes images)
- Correct sequenced order of steps to perform a broth-to-broth transfer
- Collected Lab Data images of negative control broth and completed transfer with growth into a new broth tube

-----

## Ubiquity of microorganisms – Sampling surfaces for bacteria

### LEARNING OBJECTIVES:

- Define ubiquity and understand its importance to microbiology
- Recognize that bacteria are found in different amounts in different environments
- Define sterile
- Connect the importance of proper aseptic techniques to sterile environments
- List factors and conditions impacting microbial growth
- Investigate the amount of microbial life from different environmental samples
- Practice techniques used for sampling of surfaces and microbial growth
- Compare microbial growth amounts before and after disinfection or hand-washing

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, basics of bacterial growth, basic microbiology laboratory equipment, and the definitions of sterile, aseptic, and media.
- This simulation has students complete a basic sampling of surfaces for bacteria, followed by growth on agar plates or in broth, and basic analysis of amounts recovered.
- Students are introduced to the simple aseptic technique of heating the mouth of a tube before and after opening as basic practice. Students will see this repeated throughout a great majority of simulations and expected to use this practice as fundamental to working with tubes and transfers.
- Upon completion, students may be prepared for other simulations in the Aseptic Technique series (broth-to-plate culturing, slant-to-slant culturing), as well as bacterial ubiquity and surface sampling for growth. Further application of these concepts can be through the Isolation Methods series (pour plating, quadrant streak, subculturing, colony counting, dilution methods, optical density) and Microbial Growth series (anaerobic jar growth, effects of osmotic pressure, oxygen requirements in FTM tubes, effects of temperature, and effects of pH).

### Phases in Virtual Lab = {7}

1. Investigate bacterial presence
2. Investigating disinfection effectiveness
3. Investigating handwashing effectiveness
4. Examine bacterial colonies
5. Lab wrap-up
6. Apply what you have learned
7. Save Lab Data

**Methods**

**✓ Phase 1: Investigate bacterial presence**

1. Place agar plate on lab bench and label it with marker
2. Pick up applicator swab and dip in broth medium. Use applicator swab to sample money and spread it across agar plate
3. Dispose of applicator swab in biohazard container. Move plate to incubation tray
4. Place agar plate on lab bench and label it with marker
5. Pick up applicator swab and dip in broth medium. Use applicator swab to sample liquid from a sealed can and spread it across agar plate
6. Dispose of applicator swab in biohazard container. Move plate to incubation tray
7. Place agar plate on lab bench and label it with marker
8. Pick up applicator swab and dip in broth medium. Use applicator swab to sample banana and spread it across agar plate
9. Dispose of applicator swab in biohazard container. Move plate to incubation tray
10. Place agar plate on lab bench and label it with marker
11. Pick up applicator swab and dip in broth medium. Use applicator swab to sample computer keyboard and spread it across agar plate
12. Dispose of applicator swab in biohazard container. Move plate to incubation tray

**✓ Phase 2: Investigating disinfection effectiveness**

1. Place agar plate on lab bench and label it with marker
2. Pick up applicator swab and dip in broth medium. Use applicator swab to sample lab bench and spread it across agar plate
3. Dispose of applicator swab in biohazard container. Move plate to incubation tray
4. Sanitize lab bench with ethanol and dry with paper towel
5. Place agar plate on lab bench and label it with marker
6. Pick up applicator swab and dip in broth medium. Use applicator swab to sample lab bench and spread it across agar plate
7. Dispose of applicator swab in biohazard container. Move plate to incubation tray

**✓ Phase 3: Investigating handwashing effectiveness**

1. Place agar plate on lab bench and label it with marker
2. Pick up applicator swab and dip in broth medium. Use applicator swab to sample hand and spread it across agar plate
3. Dispose of applicator swab in biohazard container. Move plate to incubation tray
4. Select sink to begin washing hands using correct protocol
5. Place agar plate on lab bench and label it with marker
6. Pick up applicator swab and dip in broth medium. Use applicator swab to sample clean hand and spread it across agar plate
7. Dispose of applicator swab in biohazard container. Move plate to incubation tray
8. Select Incubate to incubate agar plates for 24 hours

**✓ Phase 4: Examine bacterial colonies**

1. Observe and record level of bacterial growth in Lab Data

**✓ Phase 5: Lab wrap-up**

1. Select your answer to the first question
2. Select your answer to the second question

**✓ Phase 6: Apply what you have learned**

1. Select your answer to the first question
2. Select your answer to the second question

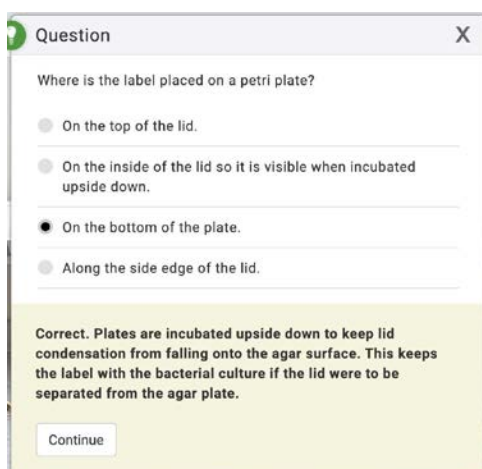
**Phase 7: Save Lab Data**

1. You can print this page for your personal reference.

To include your saved graph(s) in a lab report, take a screenshot of the graph(s) and paste into your report.

## Pop-Up Questions and Topics for Students in Simulation:

1. Phase #1 --



Question X

Where is the label placed on a petri plate?

☐ On the top of the lid.

☐ On the inside of the lid so it is visible when incubated upside down.

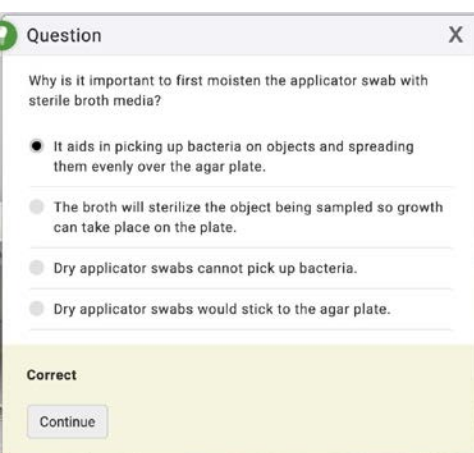
☒ On the bottom of the plate.

☐ Along the side edge of the lid.

Correct. Plates are incubated upside down to keep lid condensation from falling onto the agar surface. This keeps the label with the bacterial culture if the lid were to be separated from the agar plate.

Continue

2. Phase #1 --



Question X

Why is it important to first moisten the applicator swab with sterile broth media?

☒ It aids in picking up bacteria on objects and spreading them evenly over the agar plate.

☐ The broth will sterilize the object being sampled so growth can take place on the plate.

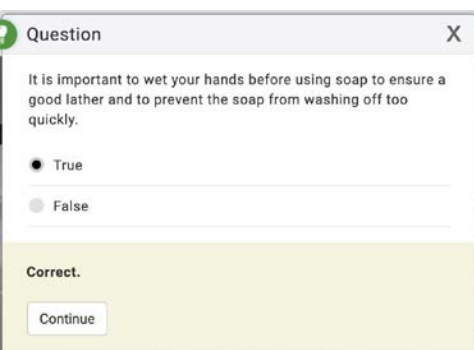
☐ Dry applicator swabs cannot pick up bacteria.

☐ Dry applicator swabs would stick to the agar plate.

Correct

Continue

3. Phase #3 --



Question X

It is important to wet your hands before using soap to ensure a good lather and to prevent the soap from washing off too quickly.

☒ True

☐ False

Correct.

Continue

## Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions
- Collected Lab Data table of plating results (amounts of bacteria) from various environments

-----

## Isolation Methods – Pour plating method

### LEARNING OBJECTIVES:

- Calculate a number of bacteria from a dilution method
- Predict how a dilution will change numbers of bacteria in the sample
- Recall that isolation methodology helps to select or identify a specific organism from a mixture
- Order the steps in a pour plate isolation
- Recognize the difference between surface and subsurface colonies
- Discuss how a pour plate isolation not only helps quantify bacteria, but also can be used for downstream applications
- Understand the use of liquid agar in a pour plate technique
- Define colony and colony forming unit (CFU)
- Practice separating organisms using the pour plate technique
- Explain the need for dilution techniques to accurately estimate bacteria in a sample

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, basics of bacterial growth, basic microbiology laboratory equipment, and the definitions of sterile, aseptic, and media.
- This simulation has students inoculate a 50°C molten-agar with bacteria, followed by dip-dilutions into three tubes and transfer to a solid agar medium for growth. Students are asked for a basic analysis of amounts recovered and types of colonies visualized.
- Students are expected to know and follow simple aseptic techniques of heating the mouth of a tube before and after opening. Students will see this basic aseptic technique practiced throughout the isolation methods series while working with tubes and transfers.
- Upon completion, students may be prepared for other simulations in the Aseptic Technique series (broth-to-plate culturing, slant-to-slant culturing), as well as bacterial ubiquity and surface sampling for growth. Further application of these concepts can be through the Isolation Methods series (quadrant streak, subculturing, colony counting, dilution methods, optical density) and Microbial Growth series (anaerobic jar growth, effects of osmotic pressure, oxygen requirements in FTM tubes, effects of temperature, and effects of pH).

### Phases in Virtual Lab = {6}

1. Identifying microbiology lab tools used in isolation techniques
2. Performing dilution preps for pour plating
3. Use inoculated agar to create pour plates for isolation
4. Evaluate isolation of bacterial colonies on pour plates

5. Apply what you have learned
6. Save Lab Data

**Methods** X

✓ **Phase 1: Identifying microbiology lab tools used in isolation techniques**

1. Label the tools used in isolation pour plating

✓ **Phase 2: Performing dilution preps for pour plating**

1. Turn on Bunsen burner
2. Sterilize loop by placing into flame of Bunsen burner
3. Pick up *E. coli* tube from rack and tube I from water bath in same hand
4. Remove caps from tubes. Heat mouths of tubes
5. Use loop to transfer sample from *E. coli* tube into tube I
6. Heat mouths of tubes. Replace caps. Heat loop
7. Click to shake tubes. Return *E. coli* tube to rack
8. While holding tube I, pick up tube II from water bath
9. Remove caps from tubes. Heat mouths of tubes
10. Use loop to transfer sample from tube I into tube II
11. Heat mouths of tubes. Replace caps. Heat loop
12. Shake tubes. Return tube I to water bath
13. While holding tube II, pick up tube III from water bath
14. Remove caps from tubes. Heat mouths of tubes
15. Use loop to transfer sample from tube II into tube III
16. Heat mouths of tubes. Replace caps. Heat loop. Return loop to holder
17. Shake tubes. Return tubes II and III to water bath

✓ **Phase 3: Use inoculated agar to create pour plates for isolation**

1. Turn on Bunsen burner
2. Lay out petri dishes on benchtop
3. Label petri dishes to match the pour plate tubes you created. Return marker to lab bench
4. In Lab Data, predict the growth for each plate
5. Pour melted, inoculated agar from tube I into appropriately labeled plate. Place tube in rack
6. Pour melted, inoculated agar from tube II into appropriately labeled plate. Place tube in rack
7. Pour melted, inoculated agar from tube III into appropriately labeled plate. Place tube in rack
8. Click petri dishes to gently swirl them to evenly distribute agar
9. Let agar cool and solidify at room temperature for 10 minutes
10. Select Incubate to incubate plates at 37°C for 24 hours
11. Record your observations and save image of plates in Lab Data

✓ **Phase 4: Evaluate isolation of bacterial colonies on pour plates**

1. Refer to Lab Data and select your answer to the first question
2. Select your answer to the second question

✓ **Phase 5: Apply what you have learned**

1. Select your answer to the first question
2. Select your answer to the second question

✓ **Phase 6: Save Lab Data**

1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

**Question** X

Which of the following statements is correct regarding the samples you created?

Select all that apply.

- ☒ A. Tube I contains the most bacteria in the series.
- ☒ B. The original, undiluted E.coli culture could be used in pour plating but would likely grow too many colonies to be analyzed.
- ☐ C. Tube II is not ready for pour plating until it is diluted back into Tube I.
- ☐ D. Tubes I, II, and III will likely grow identical numbers of surface and subsurface colonies.
- ☒ E. Tube III will be expected to contain the fewest number of CFUs (colony forming units).

**Correct**  
A. Correct!  
B. Correct!  
E. Correct!

Continue

1. Phase #2 --

**Question** X

The tubes are already inoculated, and we do not need the loop for this part of the protocol. However, some in-person microbiology lab procedures may recommend keeping your Bunsen flame on and working nearby it on the benchtop as you complete this experiment. Why might this recommendation be acceptable during an isolation methods protocol?

- ☒ A. The Bunsen flame augments our aseptic technique, as working near the flame reduces the chances that airborne microorganisms land on the open petri dishes when pour plates are being created.
- ☐ B. The Bunsen flame remains on to provide extra light for the scientist as plates are poured.
- ☐ C. The Bunsen flame will be used as the heat source for incubation.
- ☐ D. The Bunsen flame could be used to sterilize hands before creating pour plates.
- ☐ E. The Bunsen flame will need to be passed over the top of the melted agar, so as to sterilize the surface of the pour plate.

**Correct!**

Continue

2. Phase #3 --

## Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions (includes images)
- Correct sequenced order of key steps to perform a pour plate isolation



- Collected Lab Data images of three dilution pour plates (with surface and subsurface colonies), with student-predicted growth notes and analysis

-----

## Isolation Methods – Quadrant streak plate method

### LEARNING OBJECTIVES:

- Observe how a quadrant streak reduces a number of bacteria as a dilution method
- Predict how dilution will change numbers across the quadrants for the bacteria in the sample
- Recall that isolation methodology helps to select or identify a specific organism from a mixture
- Order the steps in a quadrant streak
- Explain why returning into a previous dilution is an error in the quadrant streak method
- Discuss how a quadrant streak plate isolation not only helps quantify bacteria, but also can be used for downstream applications
- Understand the use of agar plates in a quadrant streak technique
- Define colony and colony forming unit (CFU)
- Practice separating organisms using the quadrant streak technique
- Explain the need for dilution techniques to accurately estimate bacteria in a sample

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, basics of bacterial growth, basic microbiology laboratory equipment, and the definitions of sterile, aseptic, and media.
- This simulation has students perform a 4-quadrant streak for isolation of single colonies. Students are asked for a basic analysis of amounts recovered and types of colonies visualized.
- Students are expected to know and follow simple aseptic techniques, including heating their inoculation loop in the bunsen burner between streaks. Students will see this basic aseptic technique practiced throughout the isolation methods series while working with plates, tubes, and transfers.
- Upon completion, students may be prepared for other simulations in the Aseptic Technique series (broth-to-plate culturing, slant-to-slant culturing), as well as bacterial ubiquity and surface sampling for growth. Further application of these concepts can be through the Isolation Methods series (pour plating, subculturing, colony counting, dilution methods, optical density) and Microbial Growth series (anaerobic jar growth, effects of osmotic pressure, oxygen requirements in FTM tubes, effects of temperature, and effects of pH).

### Phases in Virtual Lab = {6}

1. Mechanism of Quadrant Streak Plating for Isolation
2. Quadrant streaking basics

3. Preparing initial inoculum of quadrant streak plating
4. Isolation phases of quadrant streak plating
5. Apply what you have learned
6. Save Lab Data

**Methods** X

✓ **Phase 1: Mechanism of Quadrant Streak Plating for Isolation**

1. Watch the video to see a quadrant streak plating procedure

✓ **Phase 2: Quadrant streaking basics**

1. Select your answer to the first question
2. Select your answer to the second question

✓ **Phase 3: Preparing initial inoculum of quadrant streak plating**

1. Label agar plate with marker
2. Turn on Bunsen burner
3. Sterilize loop by placing into flame of Bunsen burner
4. Pick up bacterial culture tube. Remove cap. Briefly heat mouth of tube in Bunsen burner flame to reduce contamination
5. Insert loop into tube to acquire bacterial sample
6. Briefly heat mouth of tube, replace cap, and return tube to rack
7. Use loop to streak bacterial sample onto one quadrant of plate
8. Sterilize loop using Bunsen burner

✓ **Phase 4: Isolation phases of quadrant streak plating**

1. Select red arrow to turn agar plate to streak next quadrant
2. Sterilize inoculating loop
3. Streak bacterial sample from first quadrant onto second quadrant of plate
4. Turn agar plate
5. Sterilize inoculating loop
6. Streak bacterial sample from second quadrant onto third quadrant of plate
7. Turn agar plate
8. Sterilize inoculating loop
9. Streak bacterial sample from third quadrant onto final quadrant of plate
10. Sterilize loop using Bunsen burner
11. Select Incubate to incubate plate for 24 hours at 37°C
12. Observe your quadrant streak plate results for isolated colony formation. Record plate image, results, and interpretation in Lab Data

✓ **Phase 5: Apply what you have learned**

1. Select your answer to the first question
2. Select your answer to the second question

✓ **Phase 6: Save Lab Data**

1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

3. Phase #3 --

**Question** X

If you stopped the procedure here and analyzed your quadrant streak plate after incubation, which of the following would correctly describe the outcome?

- ☒ Heavy growth visible, appears only in one quadrant, no isolated colonies present
- ☐ Isolated colonies would be present in one quadrant
- ☐ Scanty growth visible, appears in multiple quadrants, intermediate number of isolated colonies present
- ☐ Intermediate growth visible, appears in only two quadrants, high number of isolated colonies present

**Correct!**

Continue

4. Phase #4 --

**Information** X

This plate has been inoculated in the first quadrant with the bacterial sample, identical to the phase you just performed. You will now complete the quadrant streak plate technique by performing the dilution streaks into the remaining three quadrants. Notice the tracked lines of the loop across the agar surface, showing you the initial inoculum area.

5. Phase #4 --

**Information** X

The sample has finished incubating for 24 hours.

6. Phase #4 --

**Question** X

If your completed quadrant streak plate showed two different and distinct colony appearances, what could you conclude?

Select all that apply.

- ☐ A. Your Bunsen burner flame was too hot and caused growth of bacteria too early during the streaking.
- ☒ B. Your plate was inoculated with a single species, but break in aseptic technique could have allowed contaminant bacteria to also grow.
- ☒ C. Your initial bacterial culture inoculum contained two unique bacteria species.
- ☐ D. Your technique was poor, as correct quadrant streak plate protocol would have eliminated all but one species in the sample.

**Correct**  
B. Correct!  
C. Correct!

Continue

### Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions (includes images)
- Image of quadrant streak diagram/pattern and key steps to perform a quadrant streak
- Collected Lab Data images of quadrant streak performed, with student-predicted growth notes and analysis

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## Isolation Methods – Subculturing of bacteria

### LEARNING OBJECTIVES:

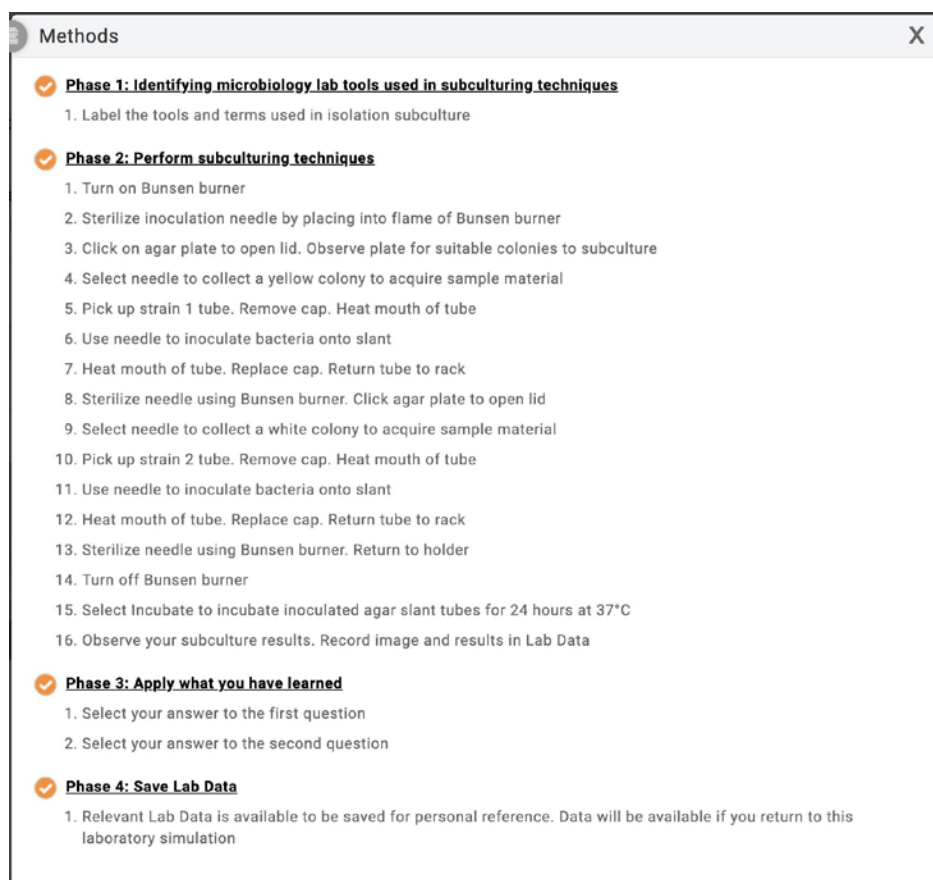
- Observe how subculturing of bacteria is used for both isolation and dilution
- Predict how dilution will change numbers across the growth of the bacteria in the sample
- Recall that isolation methodology helps to select or identify a specific organism from a mixture
- Order the steps in a subculture
- Explain why growth and subculture are necessary microbiological techniques
- Discuss how a subculture not only helps identify bacteria, but also can be used for downstream applications
- Understand the use of agar plates in a quadrant streak technique
- Define colony and colony forming unit (CFU)
- Recall colony morphology and how that knowledge is applied in subculturing
- Practice separating organisms using the subculture technique
- Explain the need for isolation and dilution techniques to accurately identify bacteria in a sample

### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, basics of bacterial growth, basic microbiology laboratory equipment, and the definitions of sterile, aseptic, and media.
- This simulation has students perform a subculture from a mixed population on an agar plate to two different agar slants for isolation. The inoculation is automated for students, but instructors may want to emphasize the 'stab-and-run' technique commonly used with slant media. This can be visualized in the introduction materials. Students are asked for a basic analysis of amounts recovered and types of colonies visualized.
- Students are expected to know and follow simple aseptic techniques, including heating tube mouths and their inoculation needle in the bunsen burner between streaks. Students will see this basic aseptic technique practiced throughout the isolation methods series while working with plates, tubes, and transfers.
- Upon completion, students may be prepared for other simulations in the Aseptic Technique series (broth-to-plate culturing, slant-to-slant culturing), as well as bacterial ubiquity and surface sampling for growth. Further application of these concepts can be through the Isolation Methods series (pour plating, quadrant streak, colony counting, dilution methods, optical density) and Microbial Growth series (anaerobic jar growth, effects of osmotic pressure, oxygen requirements in FTM tubes, effects of temperature, and effects of pH).

## Phases in Virtual Lab = {4}

1. Identifying microbiology lab tools used in subculturing techniques
2. Perform subculturing techniques
3. Apply what you have learned
4. Save Lab Data

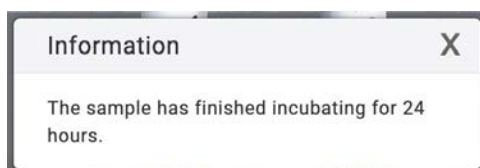


**Methods**

- ✓ **Phase 1: Identifying microbiology lab tools used in subculturing techniques**
  1. Label the tools and terms used in isolation subculture
- ✓ **Phase 2: Perform subculturing techniques**
  1. Turn on Bunsen burner
  2. Sterilize inoculation needle by placing into flame of Bunsen burner
  3. Click on agar plate to open lid. Observe plate for suitable colonies to subculture
  4. Select needle to collect a yellow colony to acquire sample material
  5. Pick up strain 1 tube. Remove cap. Heat mouth of tube
  6. Use needle to inoculate bacteria onto slant
  7. Heat mouth of tube. Replace cap. Return tube to rack
  8. Sterilize needle using Bunsen burner. Click agar plate to open lid
  9. Select needle to collect a white colony to acquire sample material
  10. Pick up strain 2 tube. Remove cap. Heat mouth of tube
  11. Use needle to inoculate bacteria onto slant
  12. Heat mouth of tube. Replace cap. Return tube to rack
  13. Sterilize needle using Bunsen burner. Return to holder
  14. Turn off Bunsen burner
  15. Select Incubate to incubate inoculated agar slant tubes for 24 hours at 37°C
  16. Observe your subculture results. Record image and results in Lab Data
- ✓ **Phase 3: Apply what you have learned**
  1. Select your answer to the first question
  2. Select your answer to the second question
- ✓ **Phase 4: Save Lab Data**
  1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

## Pop-Up Questions and Topics for Students in Simulation:

1. Phase #2 --



**Information**

The sample has finished incubating for 24 hours.

### Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions
- Collected Lab Data images of 2 subculture tubes performed, with student-predicted growth notes and analysis

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### Isolation Methods – Quantification by colony counting

\*This lab has randomization.

#### LEARNING OBJECTIVES:

- Observe how subculturing of bacteria is used for both isolation and dilution
- Recall that colony counting directly enumerates organism amounts
- Apply how a colony count and dilution can be used to mathematically estimate numbers of bacteria in the original culture
- Consider the impact of plating a volume of less than 1.0mL to require a correction factor for accurate estimates.
- Explain why not every plate grown is usable in a colony count
- Discuss how a colony count not only helps identify bacteria, but also can be used for downstream applications
- Understand the use of agar plates in a colony count technique
- Define colony and colony forming unit (CFU)
- Recall colony morphology and how that knowledge is applied in colony counting
- Practice counting organisms using the colony count technique
- Explain the need for isolation and dilution techniques to accurately identify bacteria in a sample

#### Related Virtual Labs and Suggested Sequencing/Assignment:

- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, basics of bacterial growth, basic microbiology laboratory equipment, and the definitions of sterile, aseptic, and media.
- This is a free-standing simulation to teach colony counting and is not a continuation of *Isolation Methods: Serial Dilutions* with matching plates or labels.
- This simulation begins with students asked to select a countable plate from a pre-prepared set of serially diluted agar plates. Once identified, students perform a manual colony count with a digital colony counter. Students have options on the colony magnifier to set light on/off and grid on/off to their comfort of viewing colonies. Tick marks for counted colonies cannot be duplicated, nor placed/counted where no colony exists, so counts will be accurate.
- The number of colonies shown on the plate is randomized for each attempt and will change if the counting phase is reset. Students are asked for a basic mathematical analysis of amounts recovered and types of colonies visualized. Instructors may wish to note to students that the dilution calculation explanation is listed in the lab data tab under “How to Calculate Bacterial

Concentration in Original Sample.” The volume of the diluted sample (1.0 mL or 0.1 mL) is also randomized for each attempt and may change if the phase is reset.

- Students are expected to know and follow simple aseptic techniques, including handling agar plates without opening them, until necessary. Students will see this basic aseptic technique practiced throughout the isolation methods series while working with plates, tubes, and transfers.
- Upon completion, students may be prepared for other simulations in the Aseptic Technique series (broth-to-plate culturing, slant-to-slant culturing), as well as bacterial ubiquity and surface sampling for growth. Further application of these concepts can be through the Isolation Methods series (pour plating, quadrant streak, subculturing, dilution methods, optical density) and Microbial Growth series (anaerobic jar growth, effects of osmotic pressure, oxygen requirements in FTM tubes, effects of temperature, and effects of pH).

### Phases in Virtual Lab = {4}

1. Selecting samples for colony counting quantification
2. Performing colony counting quantification
3. Apply what you have learned
4. Save Lab Data

**Methods** X

✓ **Phase 1: Selecting samples for colony counting quantification**

1. Click Zoom In to view plates. Pick up each plate to view dilution factor label for each plate. Record observations and save image in Lab Data

✓ **Phase 2: Performing colony counting quantification**

1. Place plate on counter surface grid. If desired, zoom in to count

2. Adjust backlight and surface grid on/off until comfortable with colony appearance to count

3. Click on a colony to digitally count a CFU. If necessary, zoom out to see current count

4. Continue counting the total number of colonies on the plate. If necessary, zoom out to view count when finished. Record count in Lab Data

✓ **Phase 3: Apply what you have learned**

1. Refer to your Lab Data. Select your answer to the first question

2. Select your answer to the second question

3. Select your answer to the third question

✓ **Phase 4: Save Lab Data**

1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

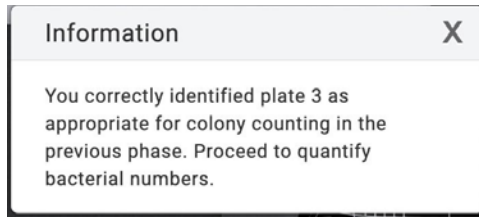
### Pop-Up Questions and Topics for Students in Simulation:

1. Phase #1 --

**Information** X

These four plates have been inoculated with serial dilutions of a bacterial culture, isolated from a patient. In this phase, you are tasked with identifying an appropriate plate for counting.

2. Phase #2 --



### Ending Saved Lab Data and Report Materials:

- Review of all student-answered questions
- Answer to calculation of CFU in original culture, based on dilution of plate and colonies counted
- Collected Lab Data images of original dilution plates, student-selected plate for counting, dilution information, and final colony count. Additional information includes student-predicted growth notes and analysis

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