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%.

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

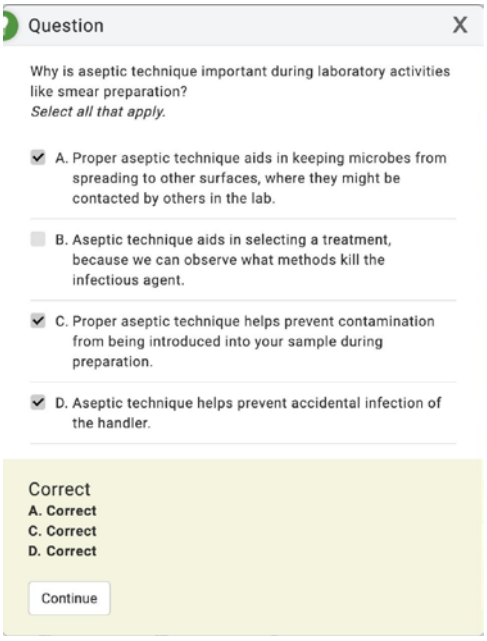
1. Select your answer to the question

Phase 8: Save Lab Data

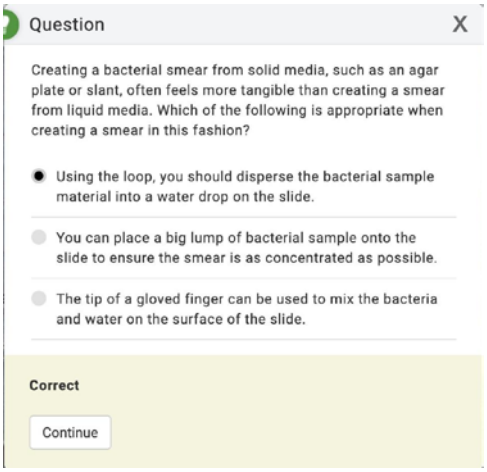
1. Relevant Lab Data is available to be saved for personal reference. Data will be available if you return to this laboratory simulation

Pop-Up Questions and Topics for Students in Simulation:

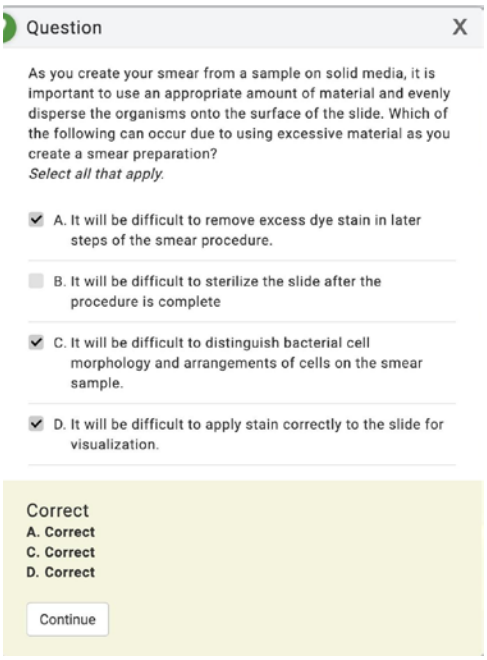
1. Phase 3 --



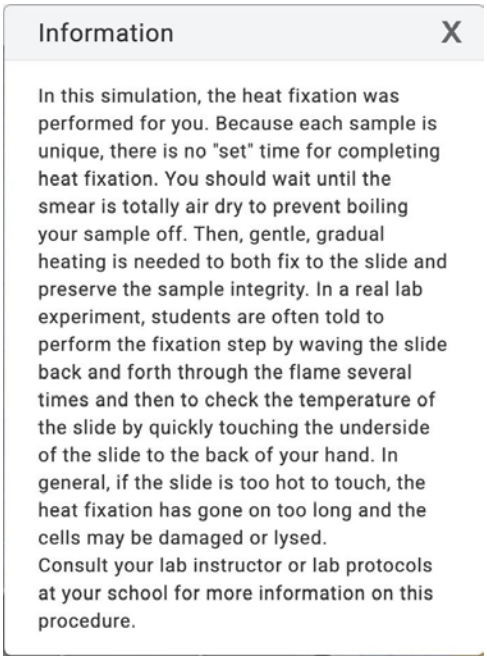
2. Phase 4 --



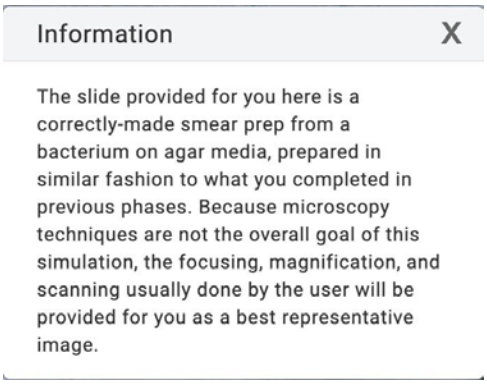
3. Phase 4 --



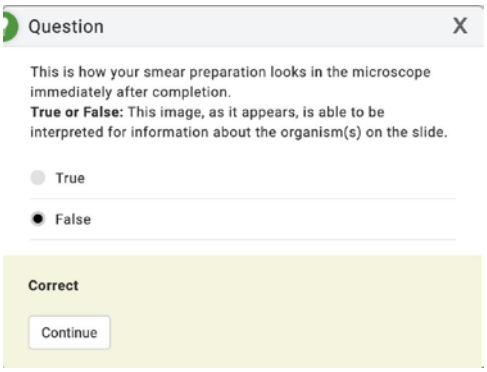
4. Phase 4 --



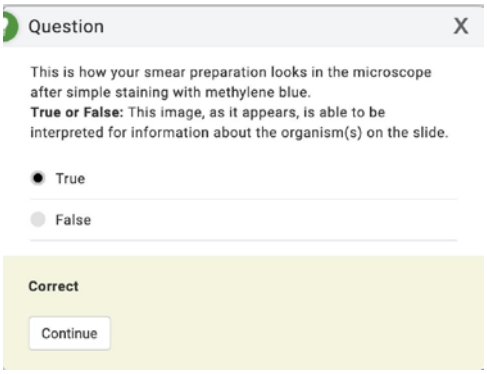
5. Phase 5 --



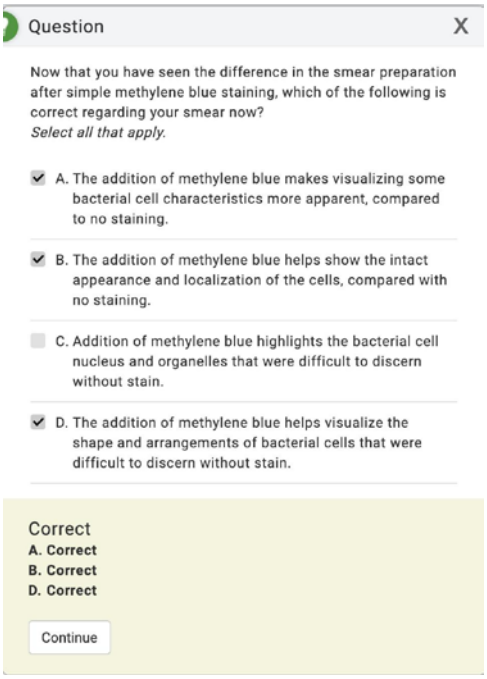
6. Phase 5 --



7. Phase 5 --



8. Phase 5 --



Ending Saved Lab Data and Report Materials:

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

Staining – Acid-fast staining

LEARNING OBJECTIVES:

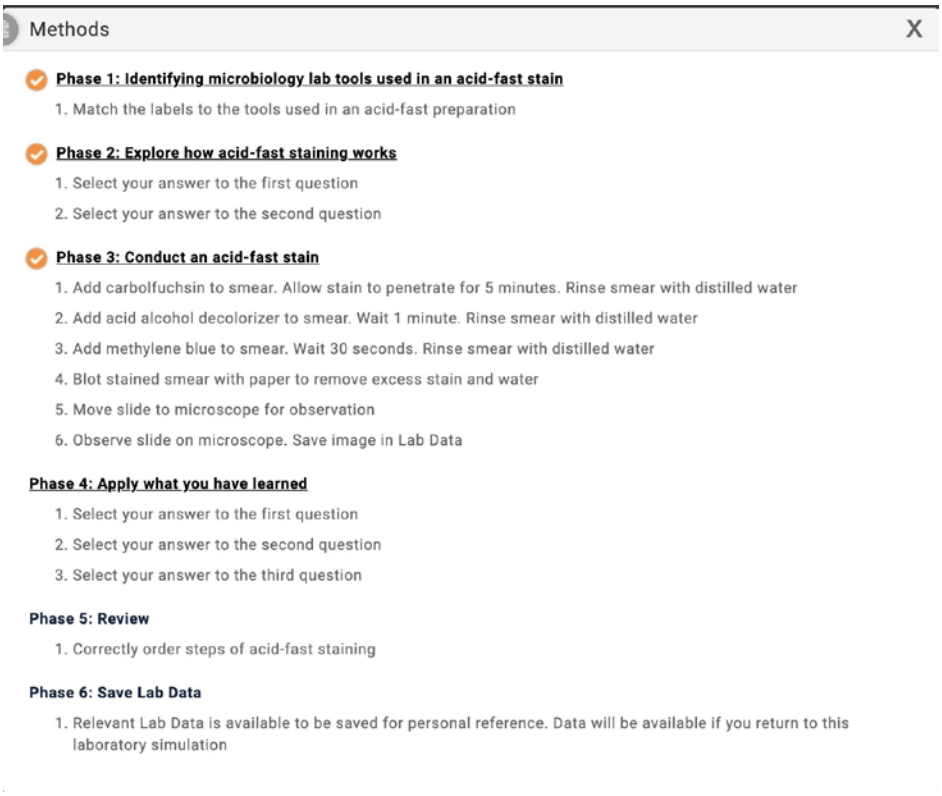
- Recall the rationale and background of smear preparation and acid-fast bacterial staining
- Recall the basics of acid-fast cell wall composition
- Predict why the acid-fast stain is used compared to other methodologies
- Contrast the differences of simple versus differential staining
- Identify common 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.

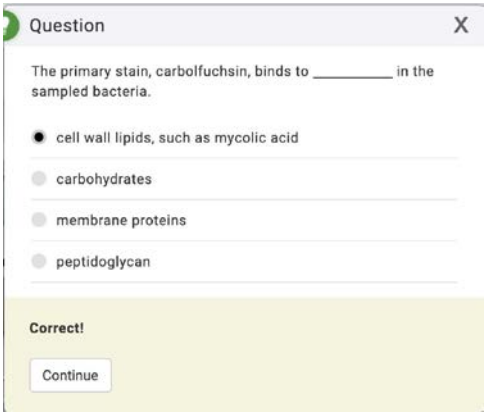
Phases in Virtual Lab = {6}

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

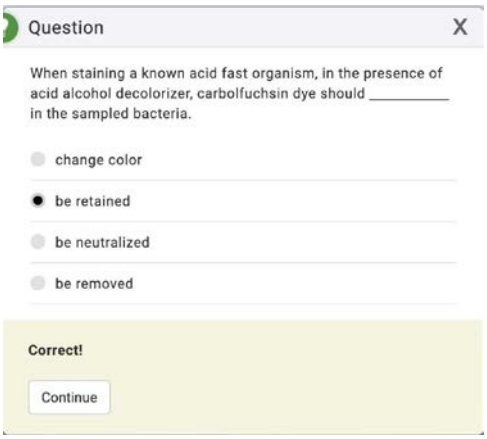


Pop-Up Questions and Topics for Students in Simulation:

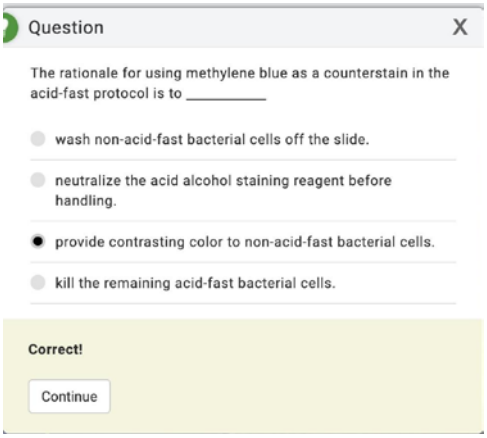
1. Phase 3 --



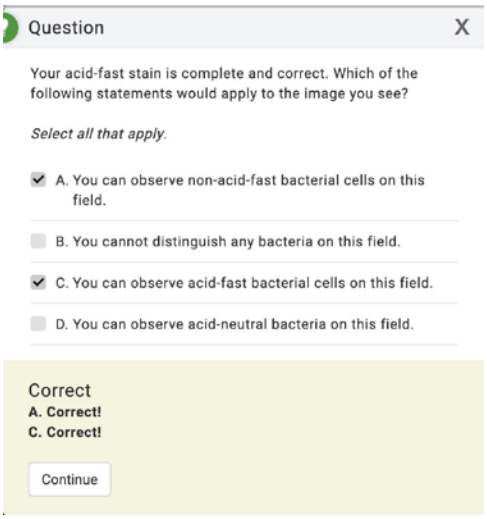
2. Phase 3 --



3. Phase 3 --



4. Phase 3 --



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

Staining – Capsule staining

LEARNING OBJECTIVES:

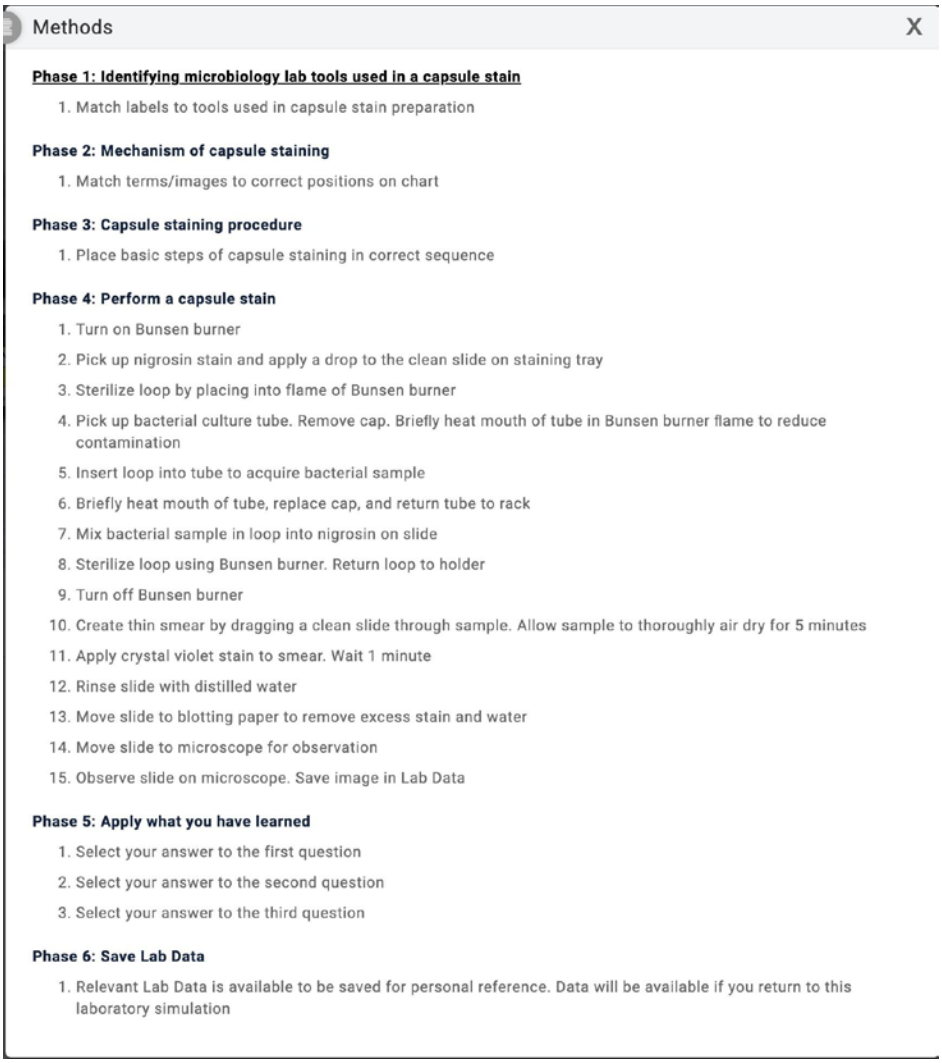
- Recall the rationale and background of smear preparation and bacterial staining
- Recall the basics of bacterial cell and bacterial capsule composition
- Describe and interpret negative (background) staining
- Predict why the capsule stain is used compared to other methodologies
- Contrast the differences 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.

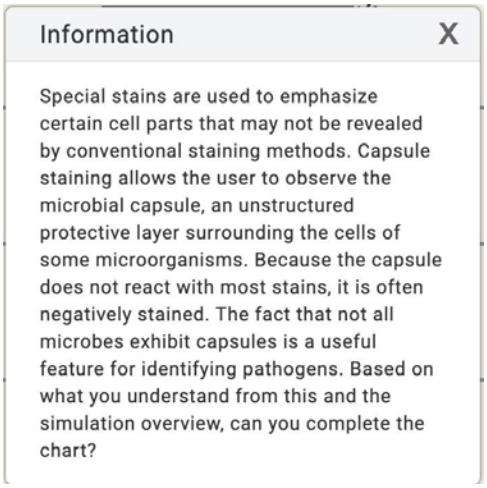
Phases in Virtual Lab = {6}

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

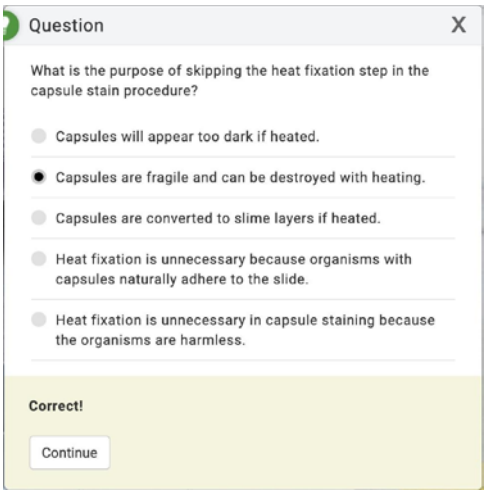


Pop-Up Questions and Topics for Students in Simulation:

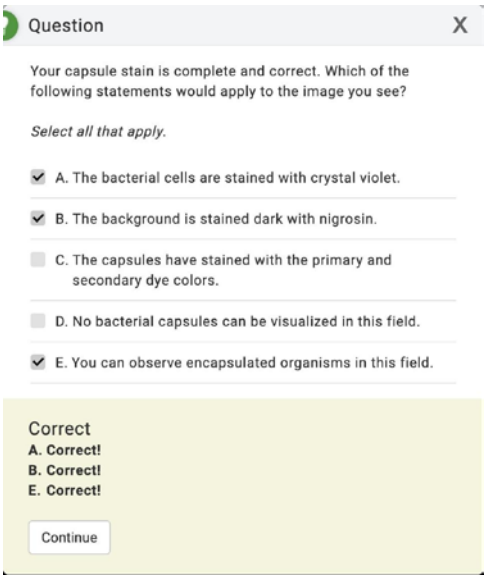
1. Phase 2 --



2. Phase 4 --



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

Staining – Gram staining

LEARNING OBJECTIVES:

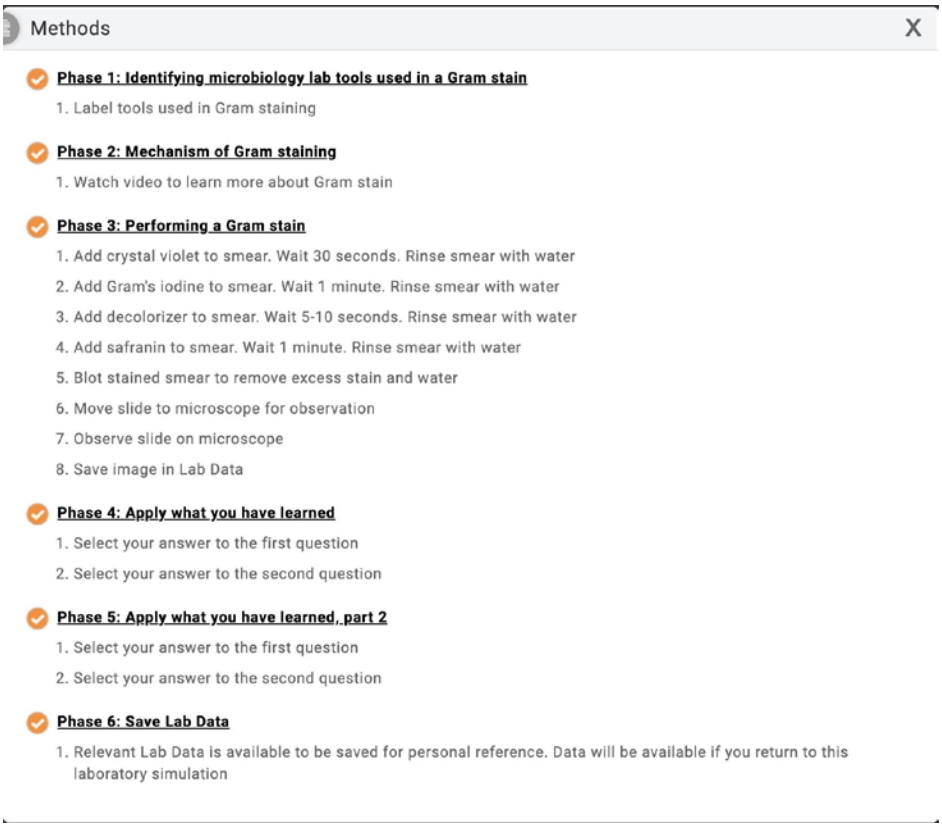
- Recall the rationale and background of smear preparation and Gram staining
- Recall the basics of bacterial cell wall composition
- Predict why the Gram stain is used compared to other methodologies
- Contrast the differences of simple versus differential staining
- Identify common microbiology laboratory 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.

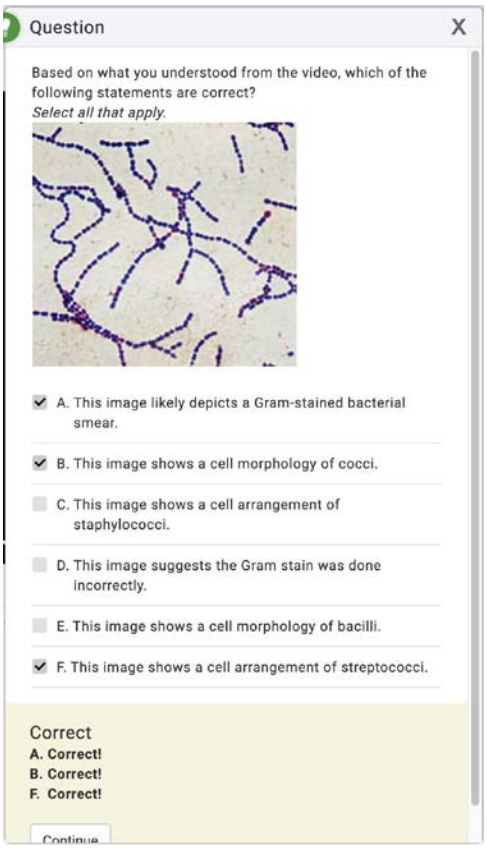
Phases in Virtual Lab = {6}

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

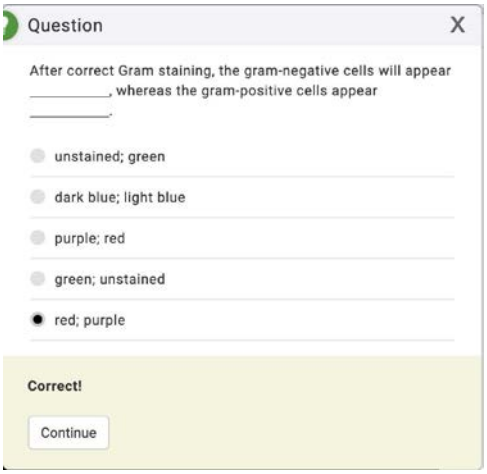


Pop-Up Questions and Topics for Students in Simulation:

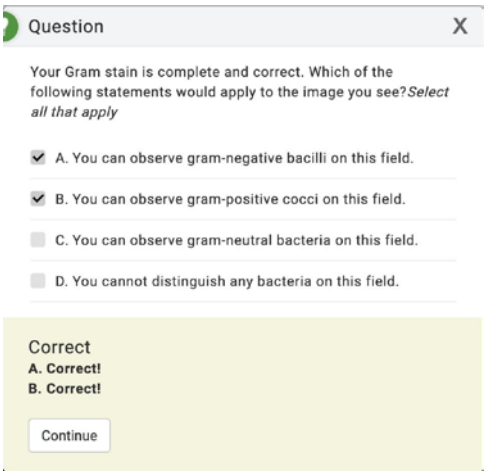
1. Phase 2 --



2. Phase 2 --



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

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.

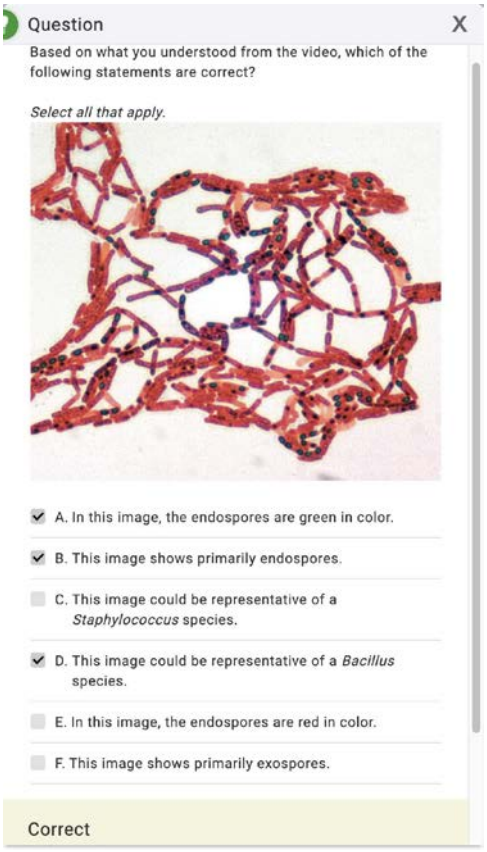
- Upon completion, students may be prepared for other staining simulations, such as Gram staining, acid-fast staining, capsule staining, etc.

Phases in Virtual Lab = {8}

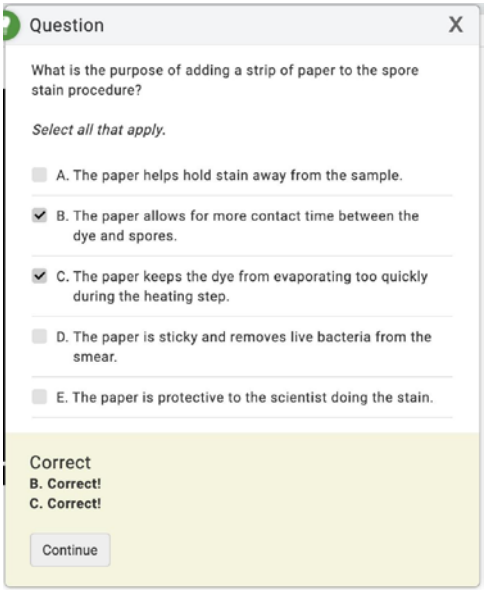
1. Identifying microbiology lab tools used in a spore stain
2. Mechanism of spore staining (video example)
3. Spore staining procedure
4. Perform a spore stain
5. Apply what you have learned, part 1
6. Apply what you have learned, part 2
7. Common errors that can affect the outcome of the spore stain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

Pop-Up Questions and Topics for Students in Simulation:

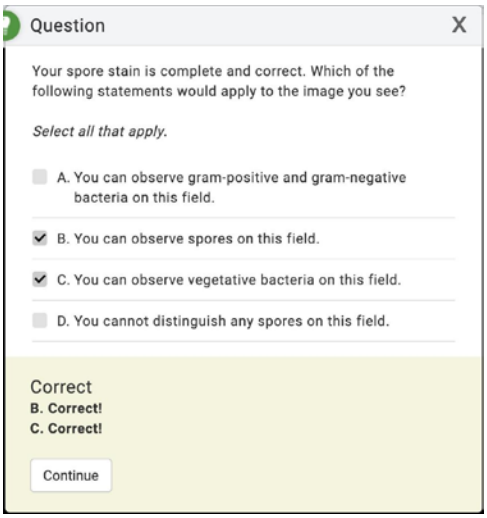
4. Phase 2 --



5. Phase 2 --



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

Aseptic Technique – Broth culture to sterile agar plate

LEARNING OBJECTIVES:

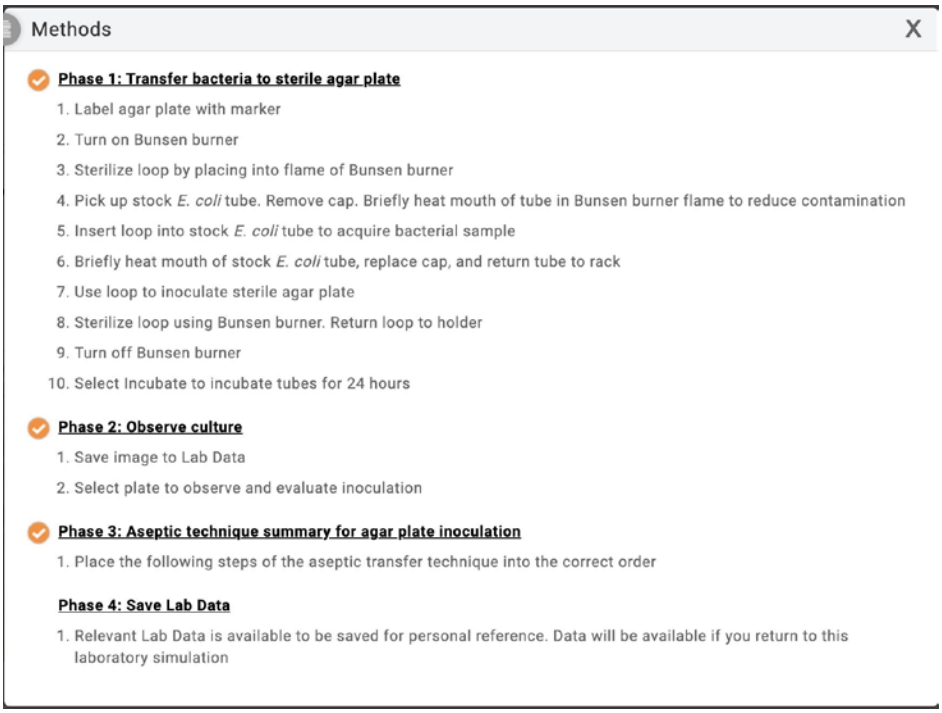
- Recall the definition of aseptic technique
- Explain the importance and implications of aseptic technique in experiments
- Discuss what is meant by the term bacterial culture
- Compare the meaning of bacterial culture versus pure culture
- Discuss what microbiology equipment is necessary to perform 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).

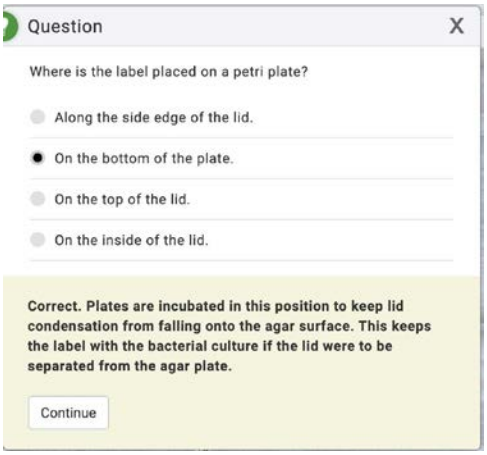
Phases in Virtual Lab = {4}

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

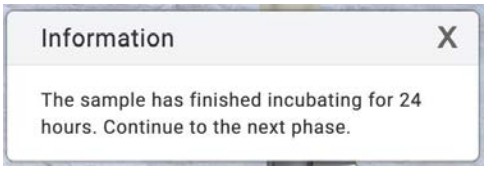


Pop-Up Questions and Topics for Students in Simulation:

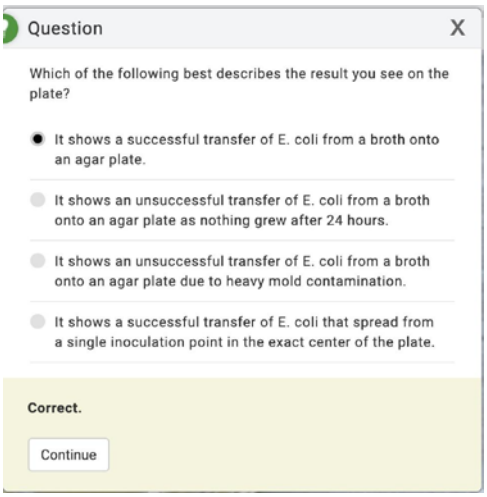
1. Phase 1 --



2. Phase 1 --



3. Phase 2 --



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

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Aseptic Technique - Slant culture to sterile agar slant

LEARNING OBJECTIVES:

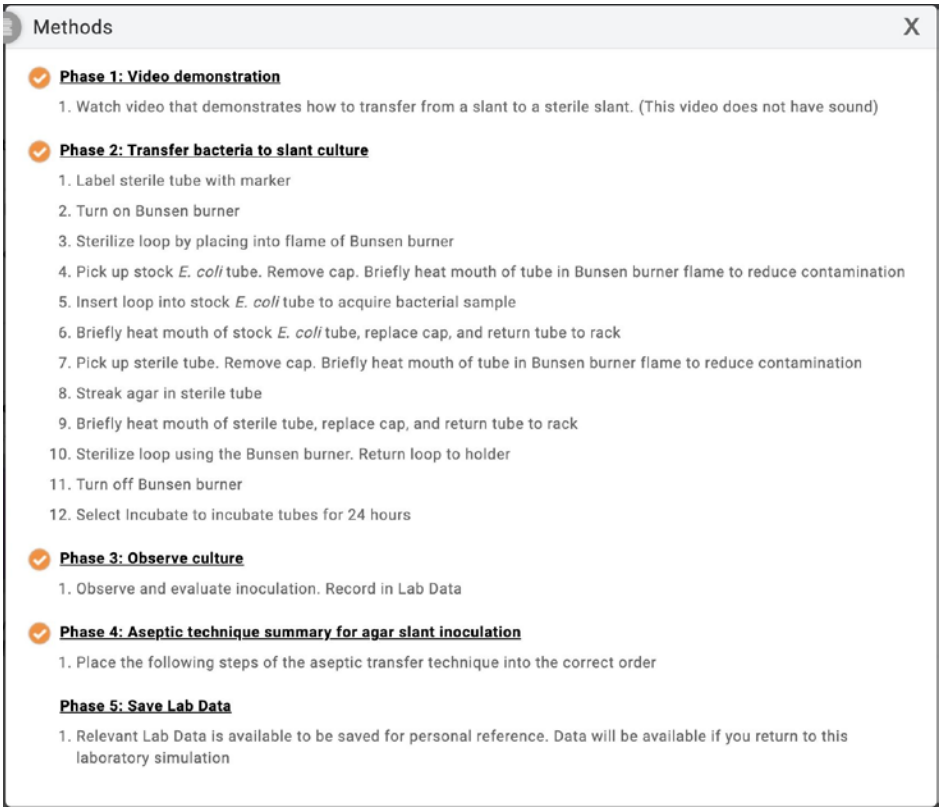
- Recall the definition of aseptic technique
- Explain the importance and implications of aseptic technique in experiments
- Discuss what is meant by the term bacterial culture
- Compare the meaning of bacterial culture versus pure culture
- Discuss what microbiology equipment is necessary to perform 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).

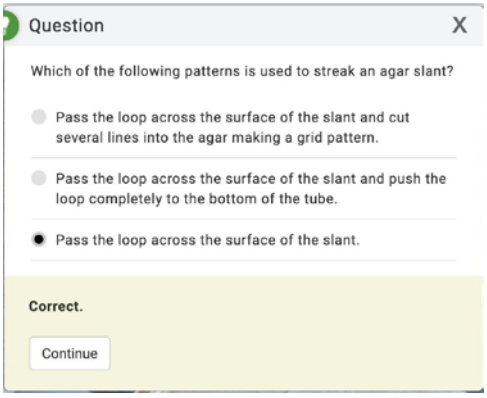
Phases in Virtual Lab = {5}

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

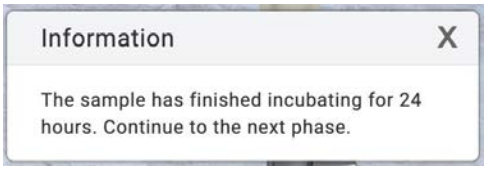


Pop-Up Questions and Topics for Students in Simulation:

1. Phase 2 --



2. Phase 2 --



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:

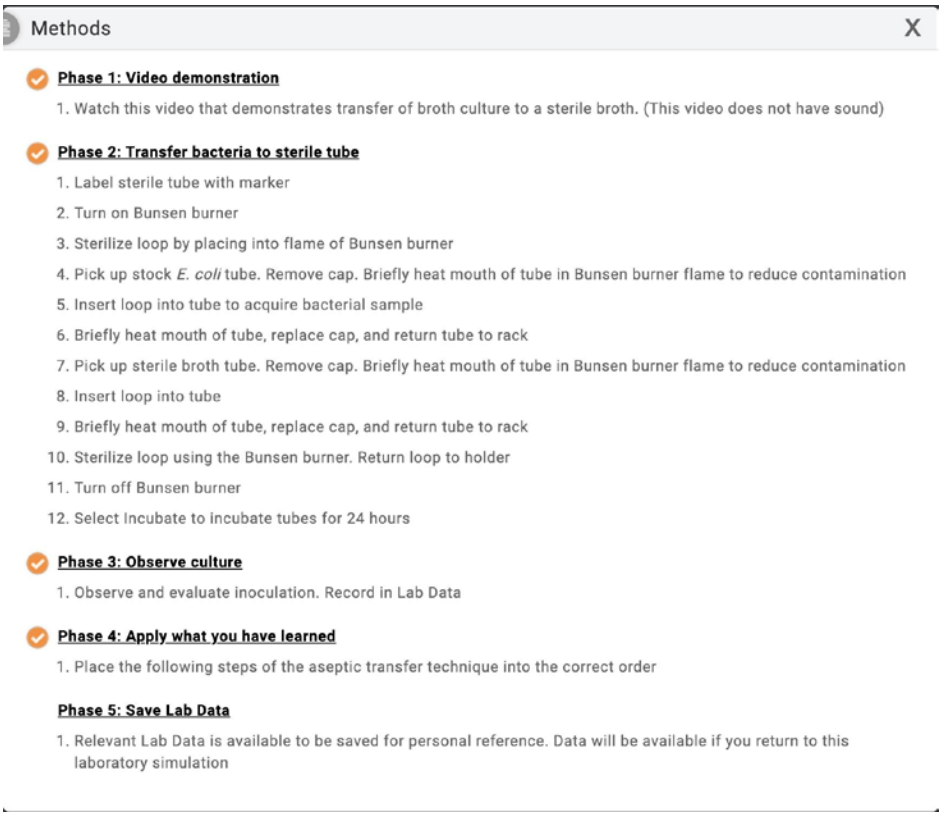
- Recall the definition of aseptic technique
- Explain the importance and implications of aseptic technique in experiments
- Discuss what is meant by the term bacterial culture
- Compare the meaning of bacterial culture versus pure culture
- Discuss what microbiology equipment is necessary to perform 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).

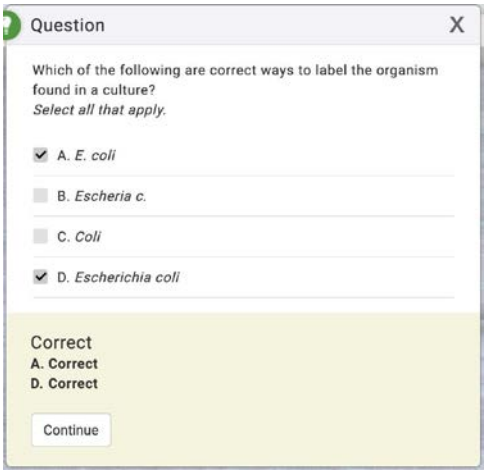
Phases in Virtual Lab = {5}

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

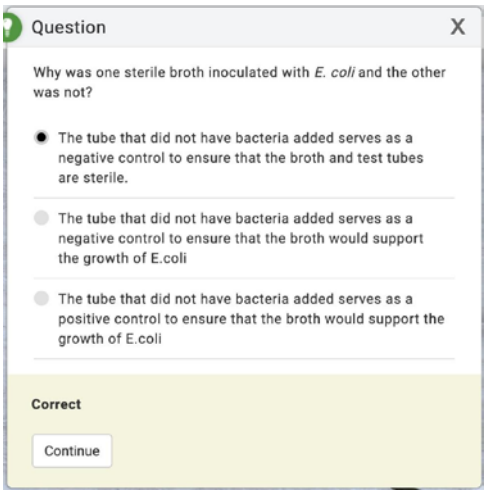


Pop-Up Questions and Topics for Students in Simulation:

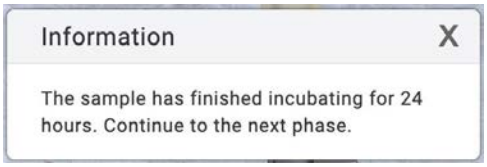
1. Phase 2 --



2. Phase 2 --



3. Phase 2 --



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:

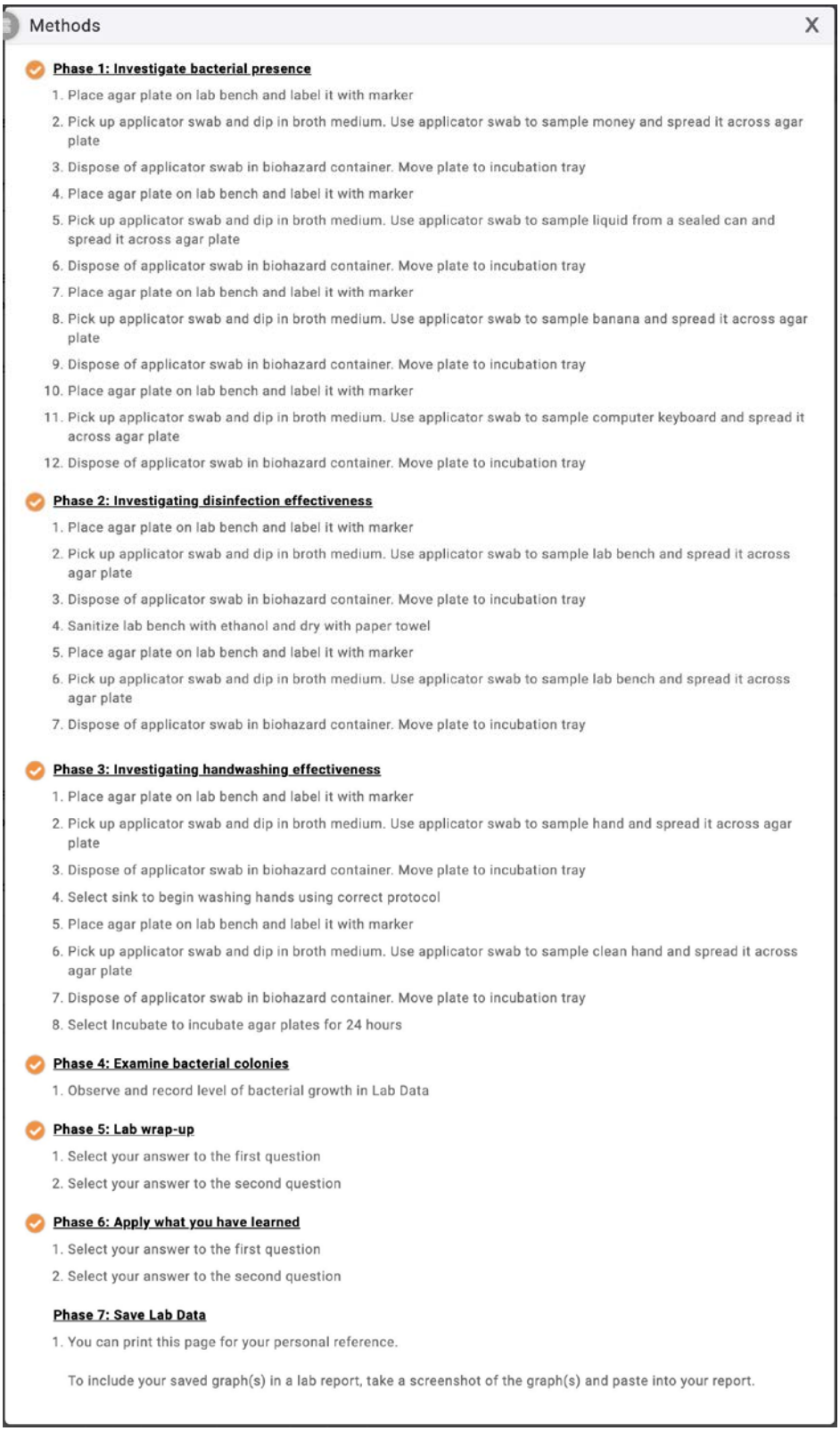
- Define ubiquity and understand its importance to microbiology
- Recognize that bacteria are found in different amounts in different environments
- Define sterile
- Connect the importance of proper aseptic techniques to sterile environments
- List factors and conditions impacting microbial growth
- 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

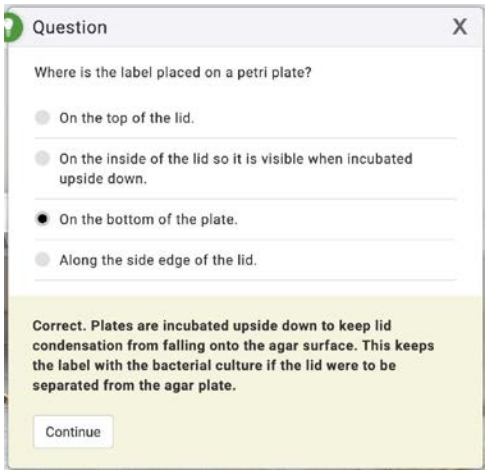
Phase 7: Save Lab Data

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

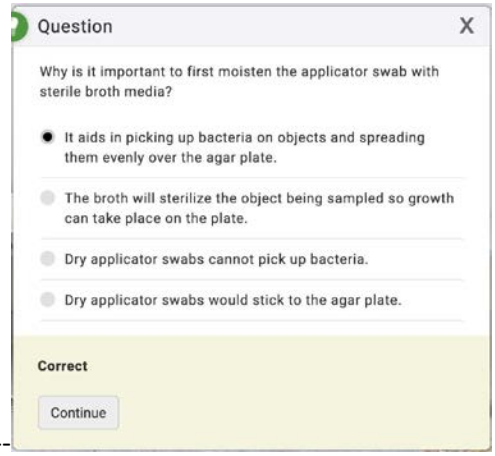
To include your saved graph(s) in a lab report, take a screenshot of the graph(s) and paste into your report.

Pop-Up Questions and Topics for Students in Simulation:

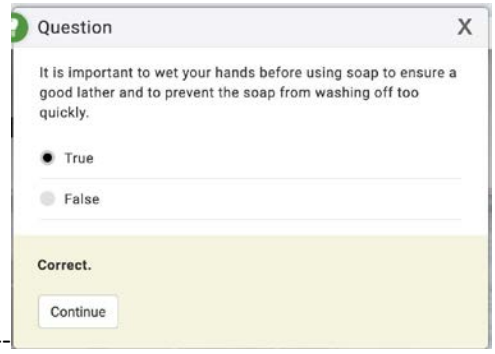
1. Phase #1 --



2. Phase #1 --



3. Phase #3 --



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

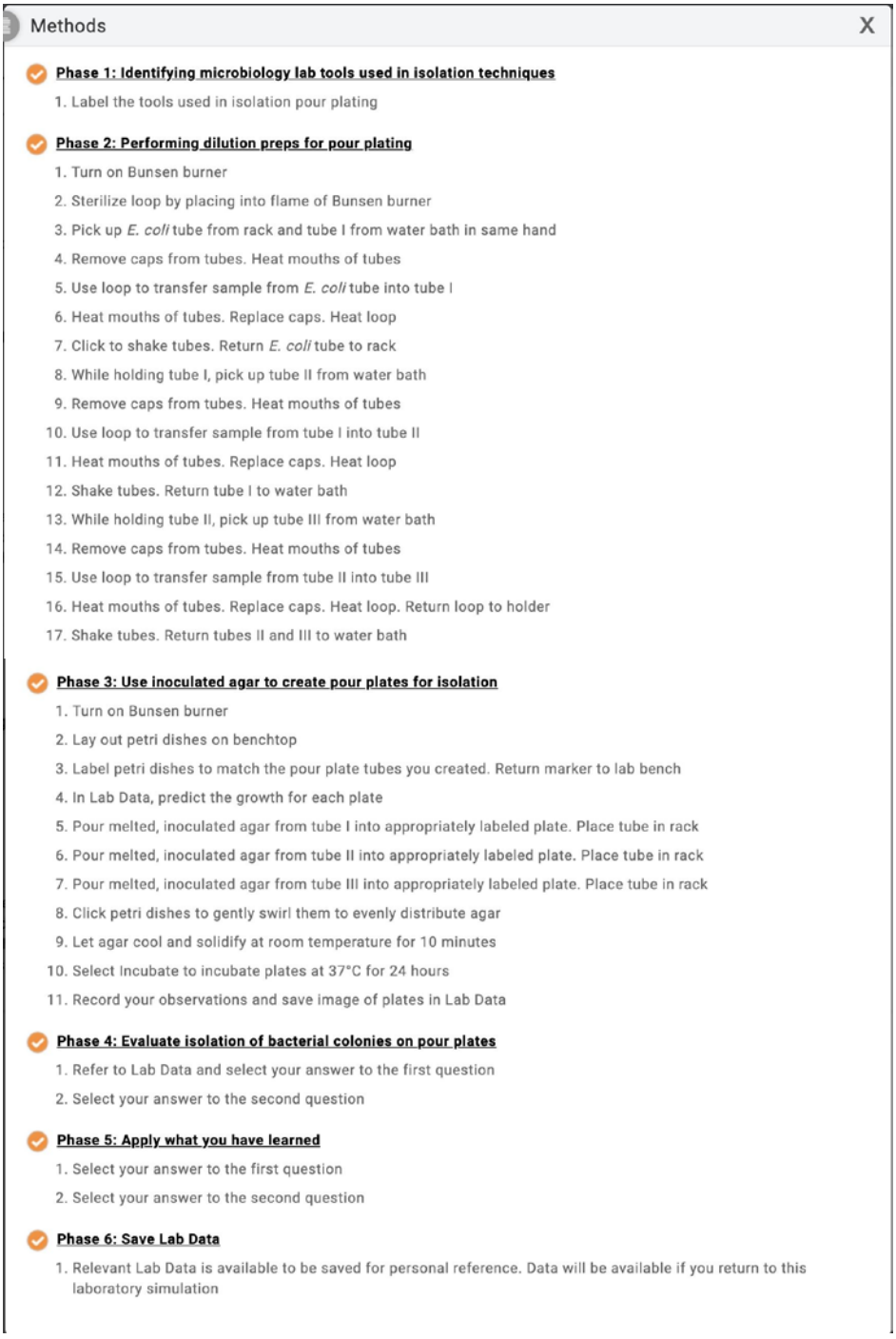
Related Virtual Labs and Suggested Sequencing/Assignment:

- 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}

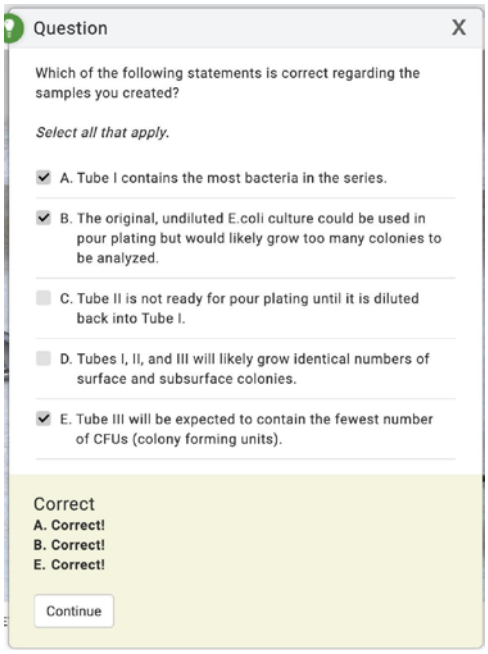
1. Identifying microbiology lab tools used in isolation techniques
2. Performing dilution preps for pour plating
3. Use inoculated agar to create pour plates for isolation
4. Evaluate isolation of bacterial colonies on pour plates

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

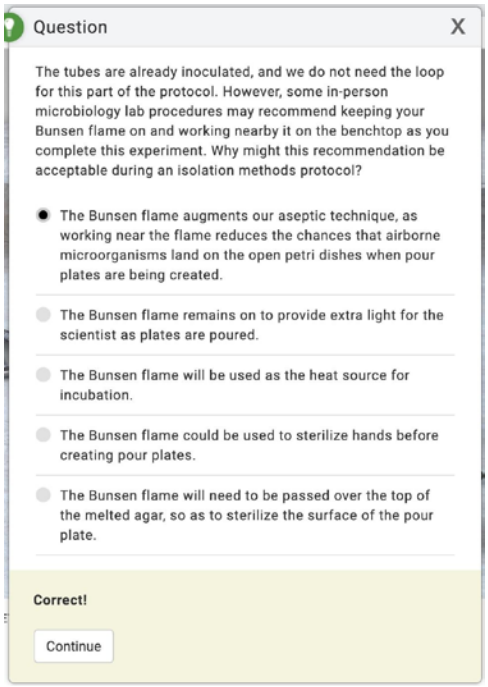


Pop-Up Questions and Topics for Students in Simulation:

1. Phase #2 --



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

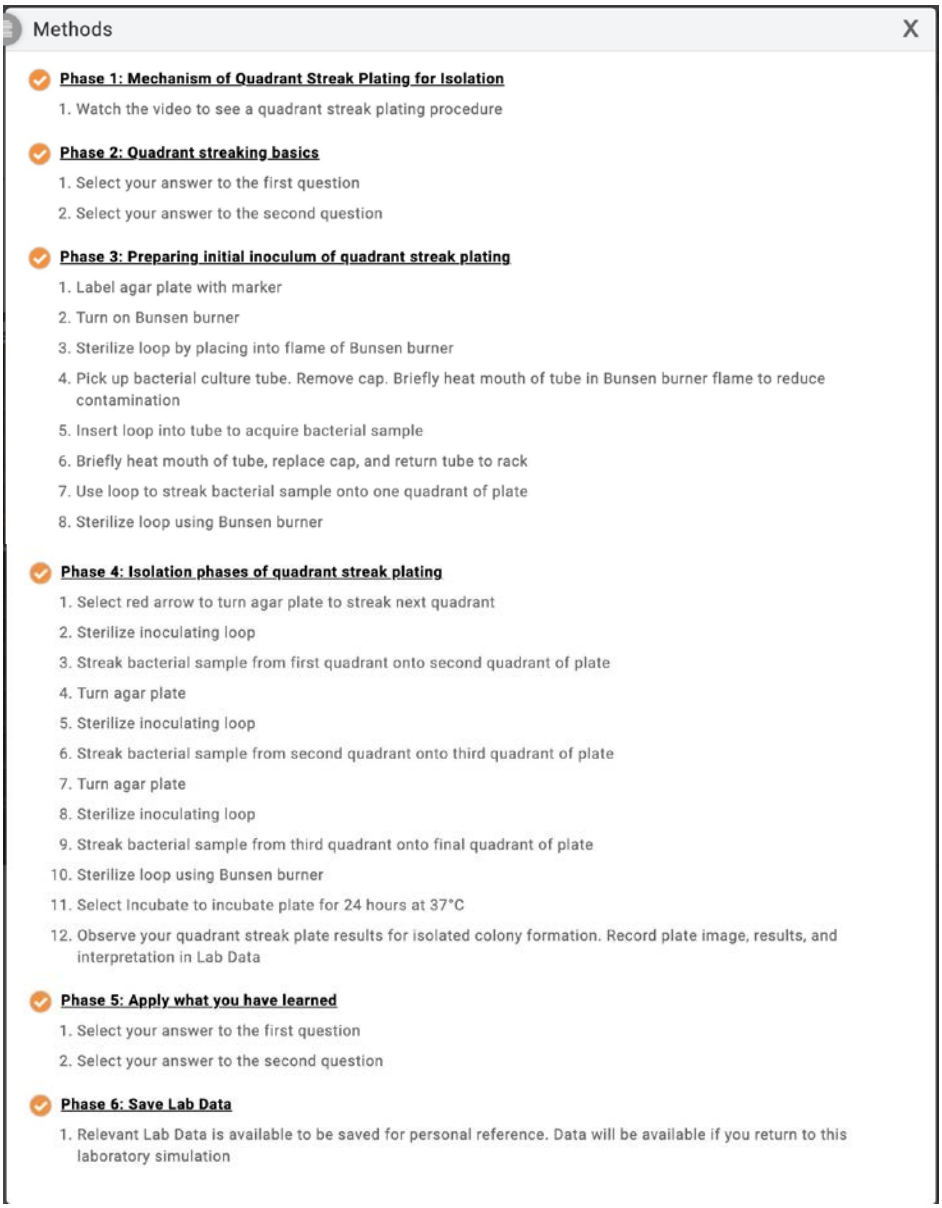
Related Virtual Labs and Suggested Sequencing/Assignment:

- 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}

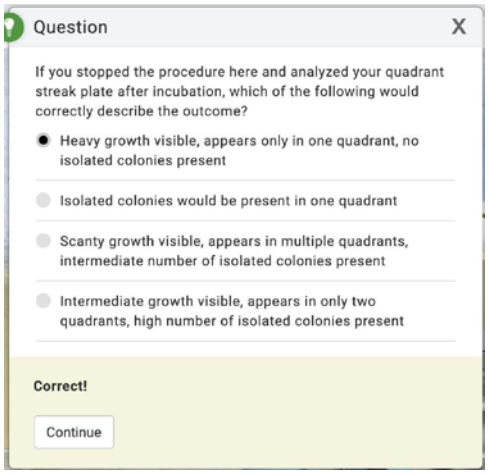
1. Mechanism of Quadrant Streak Plating for Isolation
2. Quadrant streaking basics

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

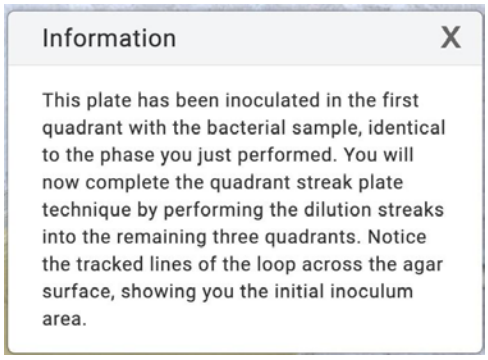


Pop-Up Questions and Topics for Students in Simulation:

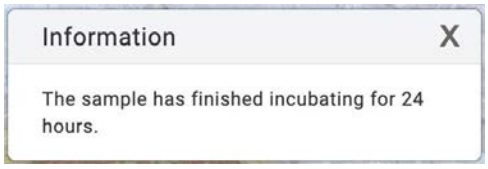
3. Phase #3 --



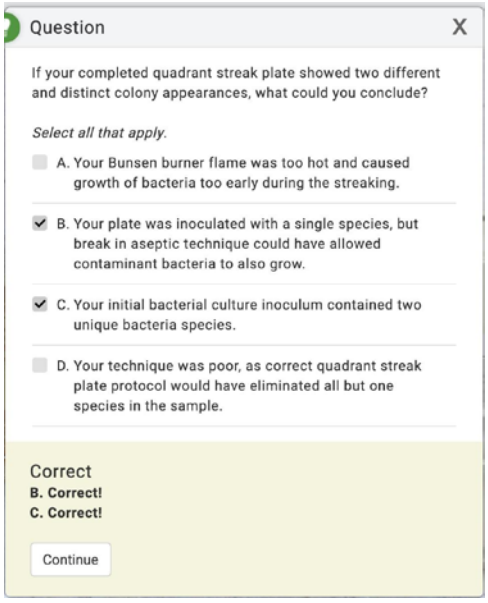
4. Phase #4 --



5. Phase #4 --



6. Phase #4 --



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

---

Isolation Methods – Subculturing of bacteria

LEARNING OBJECTIVES:

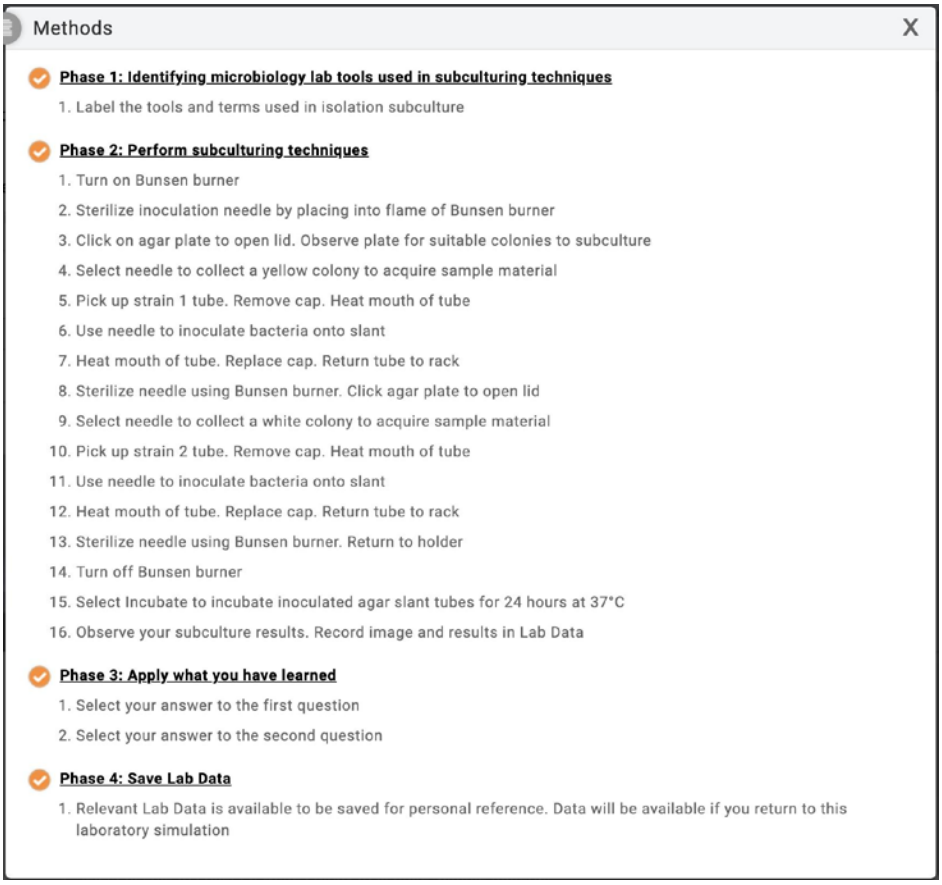
- Observe how subculturing of bacteria is used for both isolation and dilution
- Predict how dilution will change numbers across the growth of the bacteria in the sample
- Recall that isolation methodology helps to select or identify a specific organism from a mixture
- Order the steps in a subculture
- 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).

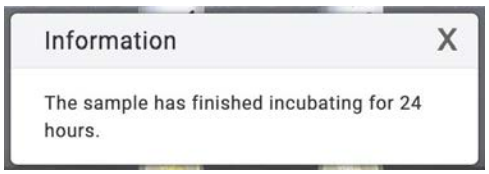
Phases in Virtual Lab = {4}

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



Pop-Up Questions and Topics for Students in Simulation:

1. Phase #2 --



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

---

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:

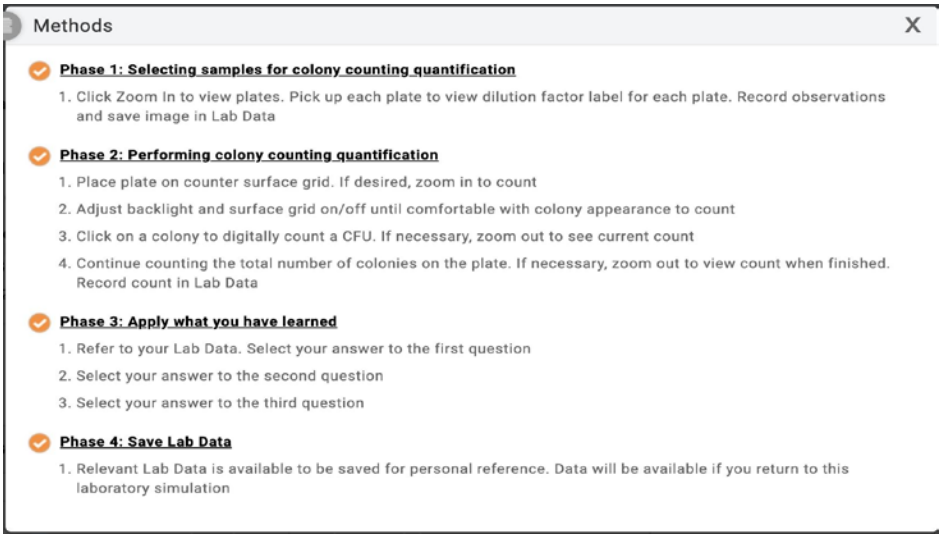
- It is recommended that students understand or have completed lecture material or lab simulations related to bacterial structure and function, basics of bacterial growth, basic microbiology laboratory equipment, and the definitions of sterile, aseptic, and media.
- This is a free-standing simulation 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).

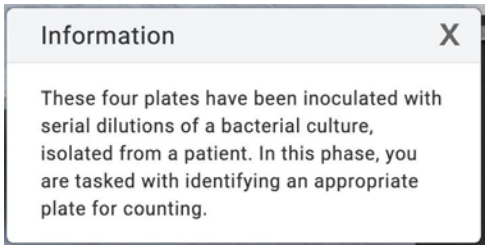
Phases in Virtual Lab = {4}

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

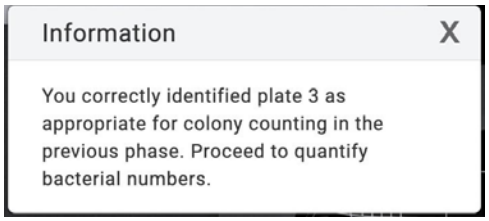


Pop-Up Questions and Topics for Students in Simulation:

1. Phase #1 --



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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