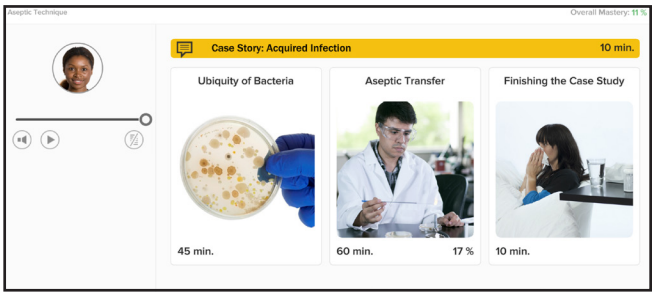
LearnSmart Labs® Microbiology
Aseptic Technique

General Lab Outline

I. Case Study Introduction: Acquired Infection
II. Ubiquity of Bacteria Exercise
III. Aseptic Transfer Exercises
- **a. Broth to Broth**
- **b. Slant to Slant**
- **c. Broth to Agar Plate**

IV. Finishing the Case Study



Assessed Learning Outcomes

Ubiquity of Bacteria Exercise

A. Core Concepts
1. Understand the ubiquitous presence of microorganisms
2. Explain that there are many places without bacteria
3. Understand what factors will influence the growth of microorganisms

B. Working with Agar Plates
1. Recall the correct labeling of a plate
2. Recall the correct position for incubation of plates
3. Recall the common incubation temperature used in the microbiology lab

C. Simulator: Environmental Exposure of Agar Plates
1. Handwashing
   a. Remember to use a towel to turn off water
   b. Remember to make hands wet before using soap
2. Bacterial Exposure
   a. Wet the cotton stick before sampling
   b. Change the cotton stick between sampling
   c. Switch plate between sampling
   d. Sample four different everyday environments
   e. Investigate the effect of handwashing on bacterial abundance
   f. Investigate the effect of lab bench disinfection on bacterial abundance
   g. Analyze the result of the bacterial sampling
   h. Label all plates correctly

D. Ubiquity of Bacteria Post-lab
1. Understand what indicates growth or absence of growth
2. Contrast different environments in terms of microbial growth

3. Recall what characteristics can be determined from observation of macroscopic growth
4. Understand that different environments have different bacteria, but most sampling reveal growth
5. Identify the length of incubation that is best for the observation of bacterial growth

Aseptic Transfer of Bacterial Cultures Exercises

A. Core Concepts: Aseptic Transfer
   1. Understand the importance of aseptic technique in transferring organisms and maintaining pure cultures
   2. Recall the definition of a pure culture
   3. Recall the definition of contamination

B. General Aseptic Practices
   1. Summarize the appropriate disinfection of the lab bench
   2. Recall the definition of culture medium
   3. Recall the tools used to transfer bacteria from one medium to another
   4. Summarize correct sterilization of transfer tools
   5. Explain the correct handling of medium containers during transfers

C. Transfer from a Broth Culture to a Sterile Broth
   1. Review: Transfer from a Broth Culture to Sterile Broth
      a. Understand the characteristics of broths
      b. Recall the steps of aseptic transfer from broth culture to sterile broth tube
   2. Simulator: Transfer from a Broth Culture to a Sterile Broth
      a. Heat tool before taking out bacterial sample from broth tube
      b. Heat mouth after removing the cap and before replacing the cap on a tube
      c. Transfer a loopful of broth culture to the sterile broth
      d. Avoid contaminating the sterile broth
      e. Remember to sterilize the loop after ending the experiment
      f. Recall the correct labeling procedure for organism names
      g. Analyze whether the aseptic transfer was successful
      h. Avoid inoculation or contamination of negative control
   3. Post-Lab Review: Transfer from a Broth Culture to a Sterile Broth
      a. Analyze negative result from aseptic transfer experiments
      b. Analyze positive result from aseptic transfer experiments

D. Transfer from a Slant Culture to a Sterile Slant
   1. Review: Transfer from a Slant Culture to a Sterile Slant
      a. Understand the characteristics of slants
      b. Recall the steps of aseptic transfer from slant culture to sterile slant
   2. Simulator: Transfer from a Slant Culture to a Sterile Slant
      a. Heat tool before sampling
      b. Heat mouth of tube before sampling
      c. Heat mouth of tube after sampling
      d. Remove a loopful of bacteria from the tube
      e. Replace cap on tube after sampling
      f. Insert loop with bacteria in sterile tube
      g. Gently apply inoculums to the surface with the loop
      h. Heat tool before replacing needle or loop in receptacle
      i. Analyze whether the aseptic transfer was successful
      j. Understand how to streak an agar slant
   3. Post-Lab Review: Transfer from Slant Culture to a Sterile Slant
      a. Recognize correct post-incubation results in a slant
      b. Recognize errors in aseptic transfer

E. Transfer from a Broth Culture to a Sterile Agar Plate
   1. Review: Transfer from a Broth Culture to a Sterile Agar Plate

a. Understand the use of plate media
b. Recall the steps of aseptic transfer of a broth culture to a sterile agar plate

2. Simulator: Transfer from a Broth Culture to a Sterile Agar Plate
   a. Heat tool before sampling
   b. Heat mouth of bacterial broth culture before sampling
   c. Heat mouth of bacterial broth culture after sampling
   d. Remove loopful of bacteria from the tube
   e. Replace cap on tube after sampling
   f. Streak bacteria onto agar plate
   g. Heat tool before replacing it in the rack
   h. Evaluate whether the bacterial transfer was successful
   i. Correctly label plate before incubation
3. Post-lab review: Transfer from a Broth Culture to a Sterile Agar Plate
   a. Recognize correct post-incubation results on a plate
   b. Recognize errors in aseptic transfer

F. Final Summary Questions: Aseptic Technique
1. Recognize correct sterilization/use of the transfer tool
2. Recognize errors in working with medium containers

Finishing the Case Study

A. Understand what the appropriate techniques are to prevent lab-acquired infections in the laboratory setting
B. Recognize errors committed by lab technicians in case study
C. Understand the purpose of vaccination of heath care workers

Student Instructions for Simulators

Ubiquity of Bacteria:

Tasks

- Investigate the bacterial presence in four everyday environments (banana, soda can, keyboard, cash).
- Investigate how hand washing affects bacterial quantity.
- Investigate the impact of ethanol disinfection on lab bench bacterial load.

Follow these steps.

- Place a sterile agar plate on the table and label it.
- Dip the cotton tip in the bacterial medium tube (standing in the rack).
- Sample the environment and streak the plate.
- Move the plate to the white plastic tray.
- When all samples are collected, press incubate in the lower right corner.

Aseptic Transfer:

Broth Culture to Sterile Broth

Task: Transfer bacteria from the *E. coli* tube to the sterile tube.

Follow these steps.

- Label the sterile tube.
- Use the loop to obtain a sample from the *E. coli* broth culture.

- Inoculate the sample into the sterile broth.
- Incubate the new broth culture for 24 hours and evaluate whether the inoculation was successful.

Remember good aseptic technique. . .
- Sterilize the loop before obtaining sample and before leaving the lab.
- Heat the mouth of the tube after removing the cap and before replacing it.

Slant Culture to Sterile Agar Slant

Task: Aseptically transfer bacteria from a slant culture to a sterile slant.

Follow these steps.
1. Pick the test tube and label it.
   - Use the loop to obtain a sample from the *E. coli* slant culture.
   - Inoculate the sterile slant culture with this sample.
   - Incubate the new slant culture for 24 hours and evaluate whether the inoculation was successful.

Remember good aseptic technique. . .
- Sterilize the loop before obtaining sample and before leaving the lab.
- Heat the mouth of the tube after removing the cap and before replacing it.

Broth Culture to Sterile Agar Plate

Task: Transfer bacteria from the *E. coli* broth culture to the sterile agar plate.

Follow these steps.
- Label the sterile agar plate.
- Use the loop to obtain a sample from the *E. coli* broth culture.
- Inoculate the bacteria on the sterile agar plate.
- Incubate the agar plate for 24 hours and evaluate whether the inoculation was successful.

Remember good aseptic technique. . .
- Sterilize the loop before obtaining sample and before leaving the lab.
- Heat the mouth of the tube after removing the cap and before replacing it.

INSTRUCTOR NOTE: Students are not expected to learn and use an isolation method to inoculate this plate. The bacterial growth pattern will appear very random after incubation. Isolation methods (streak plating, etc.) are taught in another separate LearnSmart Labs® module.