Solutions for Laboratory Applications in Microbiology A Case Study Approach 4th Edition by Chess

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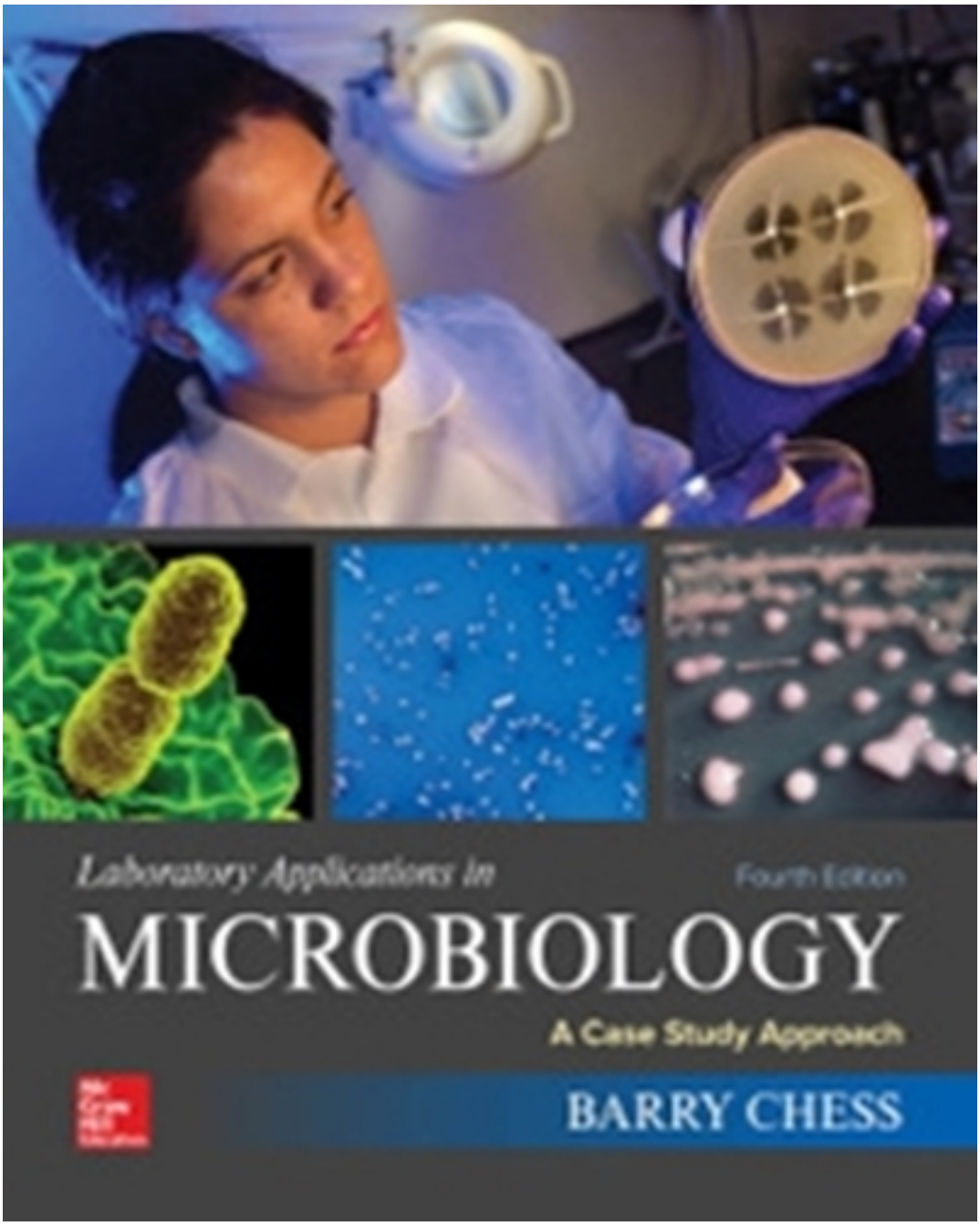


Solutions

Exercise 1
Safety Considerations in the Microbiology Lab

Laboratory Objectives: This exercise serves as an introduction to the safety and organizational concerns in the microbiology laboratory.

Time required: 30 minutes

Instructor preparation

No laboratory manipulations are performed in this lab, but students are asked to identify the location of safety equipment in the lab. It is advisable for the instructor to spend a few minutes comparing the exercise with the physical setup of the actual laboratory, noting any differences in procedures or equipment so that these may be pointed out to students.

Answers to Questions:

Pre-Lab

1. B
2. D
3. B
4. B
5. C
6. C
7. C
8. C

Review Questions

1. *Marburg virus* BSL-4
   M. tuberculosis BSL-3
   B. subtilis BSL-1
   C. tetani BSL-2

2. Negative airflow prevents contamination of the surrounding environment in the event of a laboratory accident.

   Gloves, safety glasses and a lab coat protect the user against spills, splashes, and related accidents, either their own or those of someone else in the lab.

   Foot petal activation of sinks reduces the chance of sink handles acting as common vehicles, passing infectious agents from person to person.

   Prohibitions on eating and drinking in the lab keep potentially contaminated food and fingers away from the mouth.

3. Petri dishes should be taped closed and placed in the biohazard bag. Marks need not be removed as plastic dishes are not reused.

   A glass culture tube should be cleansed of marks or tape on the outside only, with no attempt made at decontamination. The tube should be placed in a rack or container for autoclaving.

   A spill containing broken glass and a bacterial culture should be reported to the instructor prior to cleaning. Cover the spill with paper towels and saturate with disinfectant for twenty minutes. Finish by carefully cleaning the spill, disposing of the broken glass in the sharps container and paper towels in the biohazardous trash.

Case Study

The first case involves three outbreaks of *Salmonella* Typhimurium infection that began in a college teaching laboratories and eventually affected 109 patients across 38 states. A study by the CDC found that students in labs where illnesses occurred were less likely to have biosafety training than students in labs where no illnesses occurred. The second case involves a plague researcher who becomes infected with an attenuated strain of *Yersinia pestis*. The hobbled strain should have been non-infectious as it requires additional iron to grow, but the researcher, unbeknownst to himself, suffered from hemochomatosis and had higher than normal levels of iron in his blood. The final case centers on a research assistant at UCLA who suffered fatal burns during a laboratory accident. A combination of inadequate training, along with lack of proper safety equipment allowed a minor situation to become far worse than it should have been. Both UCLA and the head of the laboratory were faulted in court.

Case Study Questions

1. Although it is impossible to determine how the initial *Salmonella* infection occurred, the fact that, in some cases, family members of students were infected indicates that proper primary protection was not used and items used (and contaminated) in the lab were then taken home. As much as is practical, students should have a set of items used exclusively in the lab (pens, pencils, etc.) but clearly there will be times when items used in the lab (calculators, lab books) may be taken from lab.

   The researcher in this case, although a seasoned laboratory veteran, was known to forgo gloves and skimp on some laboratory precautions as he was under the impression that the bacterium he was working with was incapable of causing infection.

   The key in the burn “accident” is that it may not have been preventable. Accidents will after all occur, even to the most skilled hands. However, the outcome could have been far less tragic had the researcher been wearing a lab coat over her highly flammable sweater and working behind a shield. It is also unclear that she knew where fire extinguishers and safety showers were located in

the lab. All of this points toward inadequate training and a lack of understanding of the true dangers inherent in any laboratory.

2. Any condition that results in lowered immunity would be acceptable. This includes open wounds, pregnancy in some laboratories (especially those that may work with *Listeria monocytogenes*), having an immunodeficiency disease or undergoing chemotherapy.

Exercise 2
Microscopy and Measurement of Microscopic Specimens

Laboratory Objectives: This exercise introduces the proper care and use of the brightfield microscope, the underlying theory of microscopy, and the procedure for using ocular and stage micrometers to measure specimens. Darkfield and phase contrast microscopes are briefly described.

Time required: 45 minutes.

Instructor preparation

Each student should have access to:
Lens tissue*
Prepared slides of:

- Bacteria*
- *Vorticella* or *Spirogyra**
- Paramecium*

Stage and ocular micrometers (If microscopic measurements will be attempted). It is generally easiest to have a few microscopes set up in the front of the lab that have already been equipped with stage and ocular micrometers. This limits the swapping of oculars on the scopes, which is a common cause of dirt entering the microscope.
*www.wardsci.com

Preparation for this lab is minimal. Learning to correctly work with a light microscope can be a frustrating experience for some students, especially those who feel that simply by turning knobs and sliding levers they will eventually happen upon an adequate image. It is important that students develop a hierarchy of procedures and manipulations, even an informal one, which allows them to craft an adequate image of their specimen. Once students are used to such a hierarchy, they will be able to properly adjust light sources, clean lenses, and otherwise optimize their microscopes in the future.

Answers to Questions:

Pre-Lab

1. B
2. C
3. D
4. B
5. A
6. A
7. A
8. A
9. C

10.

Total Magnification	Ocular Magnification	Objective Magnification
100x		
450x		
1000x		
		75x
	5x	

Resolution	Wavelength	Numerical Aperture
763 nm		
1068 nm		
244 nm		

Review Questions

1. Both *parcentric* and *parfocal* refer to a set of objective lenses that have been matched to one another. Parcentric means that when a specimen is in the center of the microscopic field, it will remain centered when different objective lenses are moved into the light path. Parfocal refers to the fact that when a specimen is in focus it will remain in focus as the objective lenses are changed

2. A 100x objective would increase magnification but, because resolution is limited to about two-tenths of a micrometer regardless of magnification, the image produced would lack clarity.

3. Total magnification is 675x.

4. The condenser should be at its uppermost limit and the diaphragm should be almost completely open.

5. Use only optical tissue or cotton swabs, and if needed, a small amount of ethyl alcohol.

6.
   a. 5.88 µm /unit
   b. 2.35 µm /unit
   c. 0.52 µm /unit
   d. 0.24 µm /unit

Case Study

The case study contains short excerpts of letters from Anton van Leeuwenhoek to the Royal Society of London for the Improvement of Natural Knowledge, describing the microscopic organisms he was able to see when collected rainwater and dental plaque were observed microscopically. The study goes on to identify these organisms based on their descriptions, as well as further examination of his specimens with modern instruments.

Case Study Question

1. Student drawings will vary but relative sizes should approximate those shown on the web site.

Exercise 3
A Survey of Protists

Laboratory Objectives: This exercise introduces the protists, using the most up-to-date scheme of classification. The traditional method of classification that relies on photosynthetic pigments, chemical makeup of the cell wall, cellular morphology, and primary food storage molecules to partition the algae into different hierarchical levels is still included.

Time required: 1-2 hours.

Instructor preparation

Each student should have access to:
Microscope slides and cover slips*
Pond water samples or cultures of protists*
Forceps and Pasteur pipettes*
Lens tissue*
* www.wardsci.com

Pond water samples are easily obtained from ponds, streams, etc. Pure or mixed cultures of various protists may also be obtained. In any case, each sample should be equipped with forceps and Pasteur pipettes.

Instead of mixed samples of protists, some instructors prefer to use pure cultures of different protest species. This setup simplifies matters for the students and can be used if little time is to be devoted to the lab. In such a case, having students look at several identified species followed by examination of a few 'unknown' cultures can be helpful.

Answers to Questions:

Pre-Lab

1. A
2. A, D
3. C
4. A
5. C
6. A
7. D
8. D
9. B
10. B
11.
    a. Chlorophyll-green
    b. Phycoerythrin-red
    c. Caratonoids-orange

d. Xanthophylls-yellow
e. Fucoxanthin-brown

12. Definitions

Flagellum:	Cellular structure used to propel organisms through a fluid. Found in some bacteria, protozoa, and algal species.
Cilia:	Cellular structure similar to a flagella that propels a protozoan through the environment.
Pseudopod:	Protozoan appendage responsible for motility.
Photosynthetic Pigment:	Chemical pigments that absorb light for photosynthesis. The color of photosynthetic pigments can be used to partially identify many algal species.

Review Questions

1. A trophozoite is the active, motile, feeding stage of a protest while the cyst stage is an inactive, protective stage. Cysts are generally more infective as they are to pass through the acidic environment of the stomach and initiate an infection in the gastrointestinal tract.

2.

a. Green algae
b. *Plasmodium*
c. Brown algae
d. Ciliophora
e. Stramenopiles (diatoms)
f. Tubulinids
g. Dinoflagellates
h. *Euglena sp.*

Case Study

This case study concerns two infections with *Naegleria fowleri*, which occurred within a few months of one another in Louisiana. While the organism itself is quite commonly found in warm water lakes, rivers, and springs, infection is exceedingly rare, with only a few dozen cases seen in the last decade. Because the disease caused by infection—primary amebic encephalitis—is nearly always fatal, two cases in close proximity to one another, both in location and time, attracted the attention of public health officials. After a five-month investigation it was determined that both patients had become infected through the use of neti pots, which are used to rinse the sinuses with warm water. These two cases represented the first time that *N. fowleri* had been associated with municipal tap water. The CDC and FDA released recommendations that included boiling or

filtering tap water, or using distilled water, in conjunction with sinus rinsing, and that the neti pot be washed and allowed to dry completely between uses.

Case Study Questions

1. A thermophilic organism is one that favors warm (or hot) water. *N. fowleri* are commonly found living in warm water lakes and rivers, especially as water temperatures rise toward the end of summer.
2. Prohibitions on swimming are excessive, as the organism is very common, yet the infection is quite rare. The CDCs recommendations were based on the fact that *N. fowleri* initiates infection via the nose (and then moves along the olfactory nerve to the brain). They recommended that nose clips or other devices be used when swimming in warm water springs, and that diving or ducking under water be avoided.
3. In the case of *Entamoeba histolytica* infection, the protozoan must survive passage through the low pH of the stomach favoring the cyst form of the organism. The eyeball provides a much less hostile environment, and because of this, *Acanthomoeba* infections can be initiated by either form of the organism.

There's More to the Story...

The Phytoplankton Monitoring Network offers opportunities for training as a volunteer screener. Current details of the program can be found searching PMN at www.chbr.noaa.gov.

Exercise 4
A Survey of Fungi

Laboratory Objectives: This exercise presents the student with an introduction to the fungi and the information needed to identify and classify fungi on an introductory level.

Time required: 1 hour.

Instructor preparation

Each student should have access to:
Microscope slides and cover slips*
Prepared slides of:

- *Aspergillus sp.**
- *Rhizopus sp.**
- *Penicillium sp.**

Lens tissue

A common workstation should be equipped with:
Sealed agar plate cultures of

- *Aspergillus sp.**
- *Rhizopus sp.**
- *Penicillium sp.**

These plates should be sealed with parafilm or tape. Prior to inoculating the Petri dishes, coat the inside surface of the lid of the dish with anti-fog solution (used for scuba diving and found at most sporting goods stores). This will prevent fogging of the sealed plates.

Agar plate culture of *Saccharomyces cerevisiae** (1 plate per four students is generally adequate)
Small bottles of methylene blue, iodine, or lactophenol cotton blue (1 bottle per four students)
* www.wardsci.com

Answers to Questions:

Pre-Lab

1. B
2. D
3. A
4. A
5. B
6. B
7. D
8. A
9. C
10. D

Review Questions

1. A hypha is the tubular thread that makes up the structure of a filamentous fungus (mold). A web of branched and intertwining hyphae is known as a mycelium. A pseudohyphae is a chain of easily separated yeast cells separated by partitions rather than septa.

2. Sketches will vary. See figure 4.4.

3. A mycosis is any disease caused by a fungus.

4. Yeasts consist of round to oval cells, which usually appear singly but may be seen in short chains known as pseudohyphae. Most yeast multiply by budding, a process in which new, smaller cells appear from older ones. Molds are filamentous fungi consisting of individual strands, called hyphae, which mesh together to form mycelia. Septate hyphae are partitioned into smaller sections by crosswalls, or septa, while non-septate hyphae are continuous. In contrast to yeasts, reproduction in molds is via the production of spores. Spores borne within a sporangium are termed sporangiospores, while those borne from specialized sexual hyphae are called conidia. Drawings will vary.

5. Sexual reproduction allows for more variable offspring, increasing the odds that some offspring will be well suited to future environmental conditions. Unfortunately, sexual reproduction requires more specific environmental cues, as well as the presence of a fungus of the opposite mating type, for reproduction to occur.

6.
    a. Plants photosynthesize, fungi are heterotrophs.
    b. Animals ingest their food prior to digestion; fungi must digest their food prior to ingesting it.
    c. Members of the deuteromycota reproduce exclusively through asexual reproduction.

Case Study

This case concerns an outbreak of histoplasmosis among a group of day camp counselors in Nebraska in 2012. The counselors most likely to have fallen ill were those who participated in camp preparation, which included cleaning bird and bat guano from picnic tables and digging fire pits. *Histoplasma capsulatum* is commonly found associated with soil, bird, and bat droppings in parts of the United States, Mexico, and Central America. The camp was relocated to a different site and the counselors were provided training on how to discourage bat roosting and how to recognize and properly deal with contaminated sites.

Case Study Questions

1. Bats, and bat guano, are commonly found in caves. As histoplasmosis is caused by inhaling spores found in bat guano, exploring caves is a risk factor for contracting the disease. Ringworm is a fungal infection of the skin and activities that encourage a great deal of skin-to-skin contact increase the risk of infection.

2. Bacterial cells are prokaryotic, while both human and fungal cells are eukaryotic. Bacterial infections may be treated by targeting structures or organelles found only in prokaryotic cells (cell walls, 70S ribosomes, and some metabolic pathways), reducing side effects. Because both human and fungal cells are eukaryotic, many of the targeted structures or pathways in fungi are present in human cells as well. Treatment tends to be quicker as bacterial metabolism is faster; fifty bacterial generations may be covered in less than a day (1 generation=~30 minutes) as compared to the much slower metabolism of fungi (1 generation=~3 hours).

3.

Flucytosine	d.
Amphoteracin	a
Imidazole	a.
Nystatin	a.
Griseofulvin	c.
Echinocandins	b.
Terbinafine	a.

Exercise 5
A Survey of Parasitic Worms

Laboratory Objectives: This exercise introduces the parasitic worms, using a traditional system of classification that relies on their physical appearance.

Time required: 1 hour.

Instructor preparation

Each student should have access to:
Prepared slides of *Taenia*, *Ascaris*, *Necator*, *Trichinella*, *Fasciola*, *Planaria*, *Trichuris* eggs*
Living cultures of *Planaria**
Dissecting microscope
* www.wardsci.com

Answers to Questions:

Pre-Lab

1. C
2. C
3. B
4. A
5. A
6. B
7. D
8. B
9. B
10. D

Review Questions

1. Pigs which roam free commonly engage in cannibalism or eat other dead animals that are often infected with *Trichinella* worms. Confined animals rarely have access to dead, infected animals.

2. Pinworms are spread through inadequate handwashing. Toddlers don't display the same inhibitions about scratching an itch that adults do and so will routinely scratch their anus in response to pinworm infection. They also will not hesitate to put their fingers into their own, or others, mouths after doing this.

Case Study

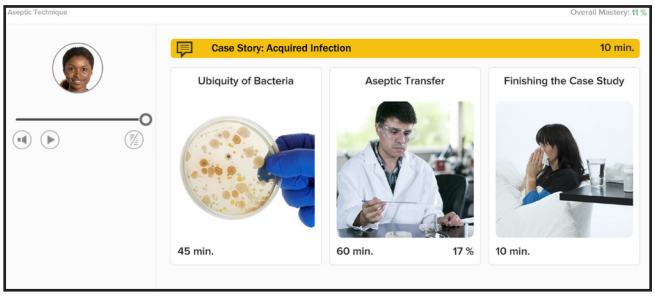
This lab contains two case studies, each involving a different worm species. The first case deals with trichinellosis spread among nine people at a barbecue. Included in the BBQ were sausages made with pork obtained from wild boar killed on a hunting trip. Trichinella spiralis larvae were found in the sausages.

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.