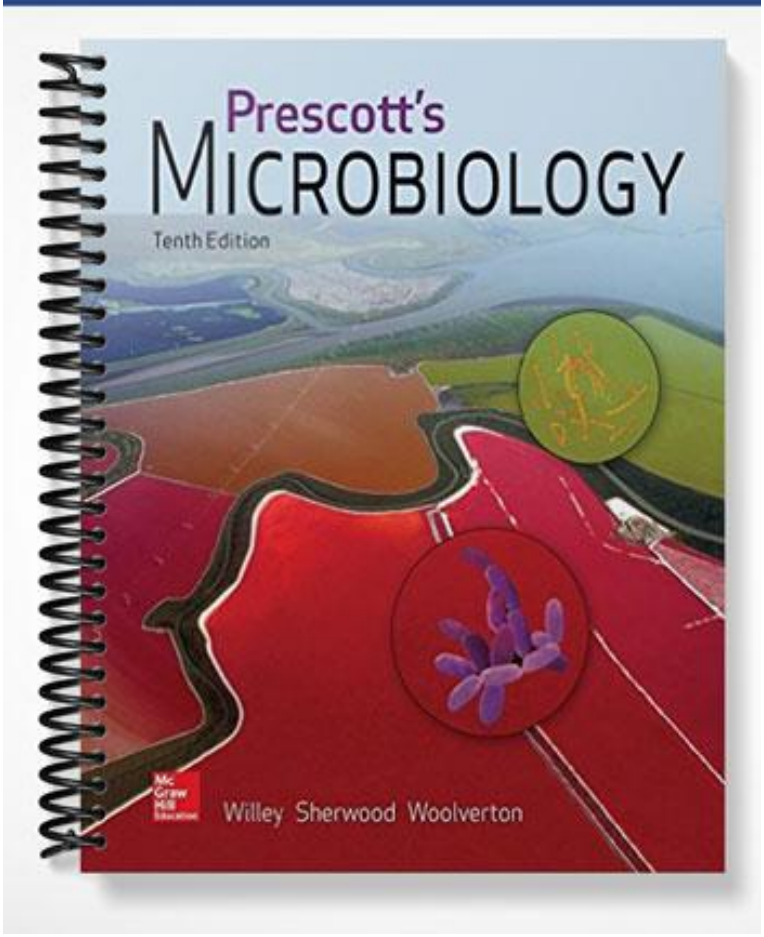
SOLUTIONS MANUAL



1 The Evolution of Microorganisms and Microbiology

CHAPTER OVERVIEW

This chapter introduces the field of microbiology and discusses the importance of microorganisms not only as causative agents of disease, but also as important contributors to food production, antibiotic manufacture, vaccine development, and environmental management. It presents a brief history of the science of microbiology and an overview of the microbial world. The origin of life and microbial evolution is put in the context of microbial phylogenies.

LEARNING OUTCOMES

After reading this chapter students should be able to:

- differentiate the biological entities studied by microbiologists from those studied by other biologists.
- explain Carl Woese's contributions in establishing the three domain system for classifying cellular life
- provide an example of the importance to humans of each of the major types of microbes.
- determine the type of microbe (e.g. bacterium, fungus, etc) when given a description of a newly discovered microbe
- propose a time line of the origin and history of microbial life and integrate supporting evidence into it
- design a set of experiments that could be used to place a newly discovered cellular microbe on a phylogenetic tree based on small subunit (SSU) rRNA sequences
- compare and contrast the definitions of plant and animal species, microbial species and microbial strains
- evaluate the importance of the contributions to microbiology made by Hooke, Leewenhoek, Pasteur, Koch, Cohn, Beijerinck, von Behring, Kitasato, Metchnikoff and Winogradsky
- outline a set of experiments that might be used to decide if a particular microbe is the causative agent of a disease
- predict the difficulties that might arise when using Koch's postulates to determine if a microbe causes a disease unique to humans
- construct a concept map, table or drawing that illustrates the diverse nature of microbiology and how it has improved human conditions
- support the belief held by many microbiologists that microbiology is experiencing its second golden age

GUIDELINES FOR ANSWERING THE RETRIEVE, INFER, APPLY QUESTIONS

As the name of this section implies, these questions range from first-order retrieval of information from the preceding text to application of the content to open ended questions or scenarios. As such the answers are either easily obtained from the text or may have many different answers that can be considered valid. For this reason, we do not include these questions in the instructor's manual.

GUIDELINES FOR ANSWERING THE MICRO INQUIRY QUESTIONS

Figure 1.1 *How would you alter this concept map so that it also distinguishes the cellular organisms from each other?*
Modify this hierarchical concept map by adding a dichotomy between prokaryotes and eukaryotes under the *cellular* classification. Bacteria (eubacteria) and archaea would be under prokaryotes and fungi and protists under eukaryotes.

Figure 1.2 *How many of the taxa listed in the figure include microbes?*
All of them.

Figure 1.6 *Why are the probionts pictured above not considered cellular life?*
All cells contain DNA, RNA, and proteins, and can self replicate.

Figure 1.8 *Why does the branch length indicate amount of evolutionary change but not the time it took for that change to occur?*
The lines on a phylogenetic tree indicate a measure of relatedness because they represent genetic sequence divergence. They are not a measure of time because it is not known how much time was needed for organisms to diverge because divergence is influenced by multiple factors.

GUIDELINES FOR ANSWERING THE COMPARE, HYPOTHESIZE, INVENT QUESTIONS

1. Microscopic organisms such as rotifers are not studied by microbiologists. Why is this so?
See Key Concepts 1.1 a. While near-microscopic in size, rotifers are complex animals, with multiple organ systems, and often over 1000 cells in their adult body.

2. Why aren't viruses, viroids, satellites, and prions included in the three domain system?
Only cellular organisms are in taxonomic systems, because only they are descended from each other with vertical gene transmission. Viruses do not reproduce, they are assembled (like machines). Viroids and satellites are RNA, not organisms. Prions are proteins, not organisms.

3. Why was the belief in spontaneous generation an obstacle to the development of microbiology as a scientific discipline?
If true, spontaneous generation of microorganisms would make them impossible to classify and would prevent generalizations between microorganisms and larger organisms. If fact, prokaryotes are very similar at the molecular and cellular level to eukaryotes, and most of what we understand about biology at that level is from studying model bacteria, especially *E. coli*. Research surrounding the debate over spontaneous generation was foundational in the field of microbiology.

4. Would microbiology have developed more slowly if Fanny Hesse had not suggested the use of agar? Give your reasoning.
This is opinion, so various answers are possible. Just look for logical and factual support of their argument. Relevant issues include the intrinsic resistance of agar to degradation as compared to gelatin, and the necessity of solid media to obtain pure cultures by physical separation, which is much more difficult in liquid media (dilution to extinction).

5. Some individuals can be infected by a pathogen yet not develop disease. In fact, some become chronic carriers of the pathogen. How does this observation affect Koch's postulates? How might the postulates be modified to account for the existence of chronic carriers?
In the carrier state, a host carries the pathogen without clinical symptoms. For example, approximately 20% of college students carry *Staphylococcus aureus* in their nasal passages, with no ill effects. However, it can cause disease (damage to the host) if they become immunocompromised. They can also transmit the bacteria to others. Technically, this does not relate to Koch's postulates because they are designed to isolate the causative agent of a disease, and the carrier state is not disease. The bottom line is that while Koch's postulates certainly do not apply in every case and they have limitations, they were of tremendous historical importance in demonstrating experimentally the germ theory of disease, which was a tremendous advance in modern medicine.

6. Develop a list of justifications for the usefulness of microorganisms as experimental models.
-easy to culture and manipulate, easy to store in frozen state
-genetic manipulation (adding DNA or removing DNA) is straightforward because single-celled and haploid
-large numbers easy to grow, very short generation times, inexpensive media
-all basic central cell processes (replication, transcription, translation) is conserved across all life
-no limitations based on animal safety and use rules

7. History is full of examples in which one group of people lost a struggle against another.
a. Choose an example of a battle or other human activity such as exploration of new territory and determine the impact of microorganisms, either indigenous or transported to the region, on that activity.
Tetanus and World War II, malaria and the building of the Panama canal, world trade travel and the bubonic plague spreading across Europe in the 14th century, the down-turn of Napoleon's march into Russia; the truncation of the conquests of Alexander the Great and Attila the Hun, the success of relatively few Spanish conquistadors aided by smallpox, the casualties of the Civil War in the United States and WWI due to sepsis, and ergotism and the Salem witch trials are just some examples.
b. Discuss the effect that the microbe(s) had on the outcome in your example.
In most of those cases, a microbe new to an area was brought in by human activity (emerging infectious disease). For example, the Spaniards were already relatively resistant to smallpox, and they brought this virus to a naive population in Central America, thus it spread extremely rapidly.
c. Suggest whether the advent of antibiotics, food storage and preparation technology, or sterilization technology would have made a difference in the outcome.
Vaccines and antibiotics would have helped with the spread of tetanus, smallpox, and the plague. Antimalarial prophylaxis would have helped in the success of building the Panama Canal. Correct food storage would have reduced rats that spread the plague and ergotism. Knowledge of mold contamination would have reduced cases of ergotism.

8. Antony van Leeuwenhoek is oft en referred to as the father of microbiology. However, many historians feel that Louis Pasteur, Robert Koch, or perhaps both, deserve that honor. Decide who should be considered the father of microbiology and justify your decision.
This is purely opinion, so judge based on inclusion of appropriate facts and making a logical argument. Leeuwenhoek's major contribution is the microscope and careful observations of microorganisms and cellular structures. Pasteur has numerous contributions related to vaccines, development of germ theory, disproval of spontaneous generation, development of sterilization procedures, and understanding of fermentation. Koch also had numerous contributions related to experimental demonstration of germ theory, identification of pathogens, and methods of bacterial pure culture.

9. Consider the discoveries described in sections 1.3 and 1.4. Which do you think were the most important to the development of microbiology? Why?
Numerous possible answers. Be sure they are factual, and make a logical argument.

10. Support this statement: "Vaccinations against various childhood diseases have contributed to the entry of women, particularly mothers, into the full-time workplace."
-children spent less time being sick, thus mothers spend less time away from work
-because childhood mortality has been lowered and thus more children survive to adulthood, this may contribute to fewer children on average to each family, thus less childcare burden on the mother
-A number of sites including NORC (National Opinion Research Center) have compiled statistics comparing vaccination with employment statistics.

11. Scientists are very interested in understanding when cyanobacteria first emerged because, as the first organisms capable of oxygenic photosynthesis, it is thought that they triggered a sharp rise in atmospheric oxygen. For many years, certain lipid biomarkers have served as "molecular fossils" to date the first appearance of cyanobacteria. However, a 2010 study questioned whether these lipids provide accurate information in light of a 2007 discovery that they also are produced by an anoxygenic phototrophic bacterium—a bacterium that does not produce oxygen as it uses light energy. The authors of the 2010 study identified genes in extant bacteria involved in synthesis of the lipid biomarkers and then constructed phylogenetic trees based on comparisons of these genes. They also identified the phyla to which the bacteria belonged (based on SSU rRNA analysis) and noted the habitats and metabolic capabilities of the bacteria used in the study. Discuss the specific challenges encountered in the study of microbial evolution. What results from the phylogenetic analysis would support their claim that 2-methylhopanoids are not reliable biomarkers? Why were habitat and metabolic characteristics also part of their analysis?
Read the original paper:

Proc. Natl. Acad. Sci.
Microbial evolution is difficult to study because events took place billions of years ago and the microbial fossil record is very sparse. Furthermore, there is the problem of contamination from nonindigenous markers in samples such as is seen with hydrocarbon biomarkers. In comparison, the use of molecular markers such as SSU rRNA nucleotide sequences in current bacterial samples are compared with relative ease. The greater the difference in sequence the greater the evolutionary divergence.

12. *It is possible to artificially create bacterial cells that completely lack a cell wall. The resulting cells, called L-forms, can be maintained and cultured. L-forms have been known for many years and have been of interest because of their unusual physiological characteristics. One of their most unusual features is that they reproduce by becoming deformed and then "falling apart," producing two or more additional L-forms. The progeny L-forms then grow larger and reproduce by a similar method. This is done without the aid of the normal division machinery. In 2013 scientists in the United Kingdom reported their attempts to understand how L-forms "divide." They generated mutant strains of* Bacillus subtilis *L-forms that promoted L-form proliferation. The only mutations found with this effect were those that increased production of membrane lipids. They argued that these lipids were inserted into the L-form membrane and that this increased the surface area of the L-forms without increasing their volume. The increased surface-to-volume ratio caused the cells to assume unusual shapes and experience torsional stress. The torsional stress served as the biophysical force causing the L-forms to split apart. They also suggested that the study of L-form division provided a good model for how primitive cells divided before the evolution of the complex division machinery that exists in modern cells. Why did they suggest this? Do you agree? Explain your answer.*

Read the original paper: Mercier, R., et al. 2013. Excess membrane synthesis drives a primitive mode of cell proliferation. Cell 152:997. http://www.cell.com/cms/attachment/2007944888/2030464522/mmc9.pdf
The use of L-form bacteria as a model for primitive cell division is based on at least two essential facts: early cells did not have cell walls (e.g., peptidoglycan) and their division was most likely driven by purely physical forces, as observed in this experimental system. The remainer of the question asks the student to express an opinion and defend it. Students may consider the notion that if early cells divided much like L-forms, replication would not require chromosome replication or protein synthesis.

2 Microscopy

CHAPTER OVERVIEW

This chapter provides a relatively detailed description of the bright-field microscope and its use. Other common types of light microscopes are also described. Following this, various procedures for the preparation and staining of specimens are introduced. The chapter continues with a description of the two major types of electron microscopes and the procedures associated with their use. It concludes with descriptions of recent advances in microscopy: electron cryotomography and scanning probe microscopy.

LEARNING OUTCOMES

After reading this chapter students should be able to:

- relate the refractive indices of glass and air to the path light takes when it passes through a prism or convex lens
- correlate lens strength and focal length
- evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope
- predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen
- create a table that compares and contrasts the various types of light microscopes in terms of their uses, how images are created, and the quality of images produced
- recommend a fixation process to use when the microbe is a bacterium or archaeon and when the microbe is a protist
- plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible
- compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure
- create a concept map, illustration or table that compares transmission electron microscopes (TEM) to light microscopes
- decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography
- distinguish scanning tunneling from atomic force microscopes in terms of how they create images and their uses
- evaluate light microscopy, electron microscopy, and scanning probe microscopy in terms of their uses, resolution and the quality of the image created

GUIDELINES FOR ANSWERING THE RETRIEVE, INFER, APPLY QUESTIONS

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GUIDELINES FOR ANSWERING THE MICRO INQUIRY QUESTIONS

Figure 2.2 *How would the focal length change if the lens shown here were thicker?*
As you increase the lens thickness, then the focal length would decrease, which in turn means the magnification would be greater. Note that this is true of the convex lenses used in microscopes, not for all lenses.

Figure 2.9 *What is the purpose of the annular stop in a phase-contrast microscope? Is it found in any other kinds of light microscopes?*
Using an annular stop and an objective phase plate, the microscope can be aligned to superimpose illuminating light rays passed through the annulus onto the objective phase ring to achieve phase-contrast illumination. Dark field microscopy uses a dark field stop similar to the annular ring to produce a hollow cone of light and differential interference contrast microscopy uses prisms to generate the two beams of light.

Figure 2.13 *How might the fluorescently labeled antibody used in figure 2.13b be used to diagnose strep throat?*
Fluorescent microscopy can be performed on a throat swab taken from the patient. Because the antibody is specific for the pathogen, an image should only be obtained with *S. pyogenes*. Fluorescently tagged antibodies are commonly used to diagnose certain bacterial infections (e.g., syphilis, chlamydial infections).

Figure 2.16 *How does the light source differ between a confocal light microscope and other light microscopes?*
Confocal microscopes require a laser light source rather than just a bulb. Traditional light microscopes collect light from all areas of the sample to be viewed not just the plane of focus whereas the confocal microscope uses a laser beam for specimen illumination and any stray light is eliminated by the use of an aperture placed above the objective lens.

Figure 2.18 *Why is the decolorization step considered the most critical in the Gram-staining procedure?*
The difference between Gram-positive staining cells and Gram-negative staining cells is not absolute, but temporal. Thus, over-destaining (too long) causes all cells to appear Gram-negative (because with enough time, even Gram-positive cell walls will lose the crystal violet-iodine complex), and under-destaining (too short) causes all cells to falsely appear Gram-positive. So the destain step is technically critical. This is the step where the Gram-positive and Gram-negative cells are first visually different as well.

Figure 2.24 *Why are all electron micrographs black and white (although they are sometimes artificially colorized after printing)?*

Color in light comes from different wavelengths of the photons, while the electrons used do not have colors. The images are artificially colorized to make them easier to view (see Figure 2.27).

Figure 2.29 *Compare the magnification of this image with that shown in figures 2.24 (TEM) and 2.27 (SEM). Which has the highest magnification and which has the lowest?*
Scanning Tunneling Microscopy has the highest magnification of the image (x2,000,000) followed by Transmission Electron Microscopy (fig 2.24b, x 42,750), while the Scanning Electron Micrograph (fig 2.27, X 15,549) has the lowest magnification.

GUIDELINES FOR ANSWERING THE COMPARE, HYPOTHESIZE, INVENT QUESTIONS

1. *If you prepared a sample of a specimen for light microscopy, stained it with the Gram stain, and failed to see anything when you looked through your light microscope, list the things that you may have done incorrectly.*
Students consider the many steps that have to be performed successfully with the Gram stain. There could be issues with: insufficient heat fixation, over-vigorous washing, improper staining or destaining, over-decolorization, focusing, etc.

2. *In a journal article, find an example of a light micrograph, a scanning or transmission electron micrograph, or a confocal image. Discuss why the figure was included in the article and why that particular type of microscopy was the method of choice for the research. What other figures would you like to see used in this study? Outline the steps that the investigators would take to obtain such photographs or figures.*
The student's understanding of why investigators choose particular systems and methods can be developed with this exercise. Discuss the theory behind each method and discuss why an understanding of the theory extends the interpretation of what is represented in the figure. Understand what aspects of a sample are revealed by each type of microscopy. The students can also develop critical thinking through experimental design.

3. *How are freeze etching and electron cryotomography similar? How do they differ? What is the advantage of electron cryotomography?*
Both methods are based on rapid deep-freezing to preserve cellular structures, and thus avoid fixation artifacts. While freeze etching makes an image along one surface of the specimen, cryotomography forms a 3D image of the specimen by reconstructing multiple views from a tilt series.

4. *STED microscopes were first developed about 10 years ago. They are modifications of confocal microscopes that use two highly focused beams of light rather than one as in typical confocal microscopy. The first beam uses a wavelength that excites the fluorophore with which a specimen is labeled. This creates a fluorescent spot where the fluorophore is located. The second beam is a hollow cone. It strikes the outermost edges of the fluorescent spot illuminated by the first beam. The wavelength used by the second beam returns the fluorophore to its ground state, thus only the center of the fluorescent spot is visible. The result is that the resolution of STED microscopes is greater and objects smaller than 200 nm can be resolved. In 2010 a STED microscope was used to observe the mechanism by which human immunodeficiency virus (HIV) particles are transferred from dendritic cells to T cells. These are important immune system cells and play critical roles in the development of HIV infection. Why was STED*

microscopy used rather than TEM or electron cryotomography?
Read the original paper: Felts, R. L., et al. 2010. 3D visualization of HIV transfer at the virological synapse between dendritic cells and T cells. *Proc. Nat. Acad. Sci., USA.* 107:13336. http://www.pnas.org/content/107/30/13336.full.pdf+html

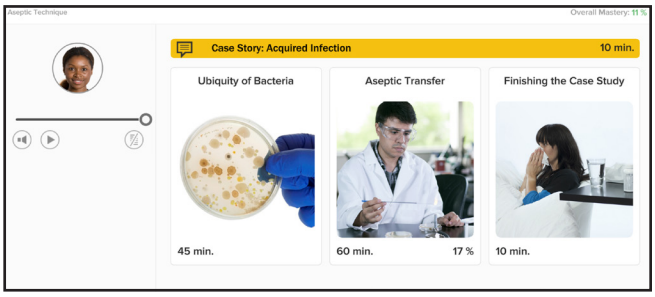
A 3D image was needed, thus TEM was not appropriate. While electron cryotomography can generate a 3D image, STED allows fluorescent tagging of specific viral or cellular proteins in the image, thus marking specific structures and allowing much greater detail in visualizing the viral-cellular interactions.

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.