

Ubiquity of Bacteria (continued)

3. Collect data from other students in your class to complete the table and answer the questions that follow.
- a. Using the number of colonies as an indicator, which habitat sampled by the class appears to contain the most bacteria?
Anywhere on the body, for instance; The lips
 - b. Why do you suppose this habitat contains such a high microbial count?
Because they are really hard to get all off. (The warm (temperature) and wet environment)
 - c. Describe the colonies seen on your plates, using terms from the introduction (elevation, texture, border, etc.).
 - d. Were any plates lacking in colonies? Do you think that the habitat sampled was really sterile? If your answer is *no*, then how can you account for the lack of growth on the plate? If your answer is *yes*, defend it.

B. Short-Answer Questions

- 1. Why is the level of contamination measured as the number of colonies rather than the size of colonies?
A single cell means the number of colonies because bacterial are continuously replicating.
- 2. Should one be concerned to find bacteria on the skin? Explain.
Not normal bacteria, but molds are contaminant which is concerning.
- 3. After conducting additional research, how can microbial levels be controlled on the skin? On surfaces in the environment? In the air?
By hand washing, use disinfectants (bleach) for surfaces & HEPA with filters the air.
- 4. Based on what you observed in this lab exercise, compare the use of and the general growth seen in broths and agar plates. Why might you choose one over the other type of medium in future lab exercises?

Laboratory Report

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Student: _____

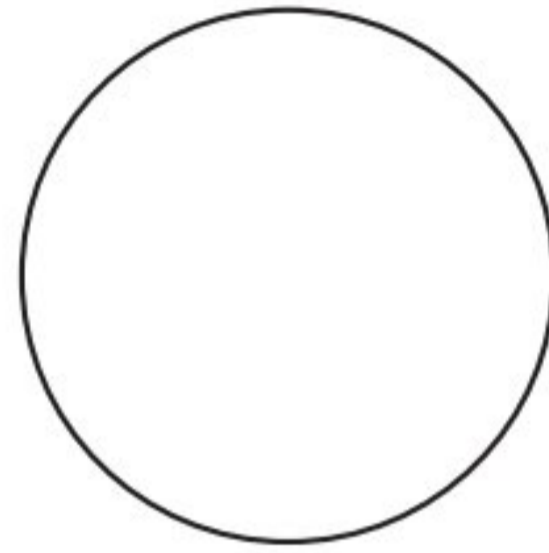
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7 The Fungi: Molds and Yeasts

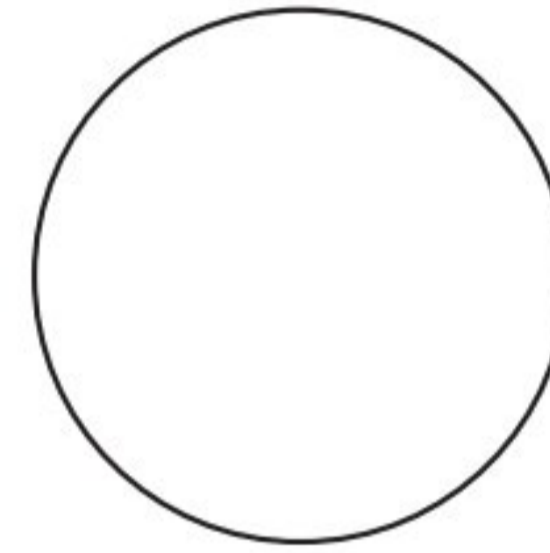
A. Results

1. Yeast Study

Draw a few representative cells of *Saccharomyces cerevisiae* in the appropriate circles below. Blastospores (buds) and ascospores, if seen, should be shown and labeled.



Prepared Slide



Living Cells

2. Mold Study

In the following table, list the genera of molds identified in this exercise. Under colony description, give the approximate diameter of the colony, its topside color, and backside (bottom) color. For microscopic appearance, make a sketch of the organism as it appears on the slide preparation.

GENUS	COLONY DESCRIPTION	MICROSCOPIC APPEARANCE (DRAWING)

B. Short-Answer Questions

1. What does the term “coenocytic” mean?

2. What criteria are the basis for traditional classification schemes? What modern approach to classification has shown that traditional schemes do not apply?

3. What unique compound is found in the cell walls of fungi but is absent in plant cell walls?

The Fungi: Molds and Yeasts (continued)

4. Name one fungus that is responsible for infections of skin and nails.

5. What does the term “dimorphic” refer to? Give an example of an organism that is dimorphic and what disease it causes.

6. How do zygospores differ from conidiospores?

7. What foods are produced by fungi?

8. What is considered to be the difference between mushrooms and toadstools?

9. Why might fungi have been theorized to be involved in the Salem witch trials?

10. What are mycotoxins? In what popular food might they be found?

11. What are the ectomycorrhizae?

12. What components of wood must be degraded for its turnover by the wood-rotting fungi?

Laboratory Report

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Student: _____

Date: _____ Section: _____

8 Aseptic Technique

A. Results

1. Were all your transfers successful? _____
2. How do you know your transfer to a broth was successful? How do you know your transfers to agar slants were successful?

3. If any of your transfers were unsuccessful, suggest possible errors that may have been made in the transfer process.

B. Short-Answer Questions

1. Provide three reasons why the use of aseptic technique is essential when handling microbial cultures in the laboratory.

2. Provide two examples of how heat is used during inoculation of a tube culture.

3. How is air contamination prevented when an inoculating loop is used to introduce or take a bacterial sample to/from an agar plate?

4. Where should a label be written on an agar plate?

5. How should agar plates be incubated? Why?

Aseptic Technique (continued)

6. Disinfectants are effective against which types of organisms? Which types of organisms may remain on the lab bench even after disinfection? What disinfectant(s) is used in your laboratory?

7. Compare and contrast the growth of bacteria in different physical types of media (broths, slants, and agar plates). What might be the advantages and disadvantages of using each type?

C. Multiple Choice

Circle the answer that best completes the following statements.

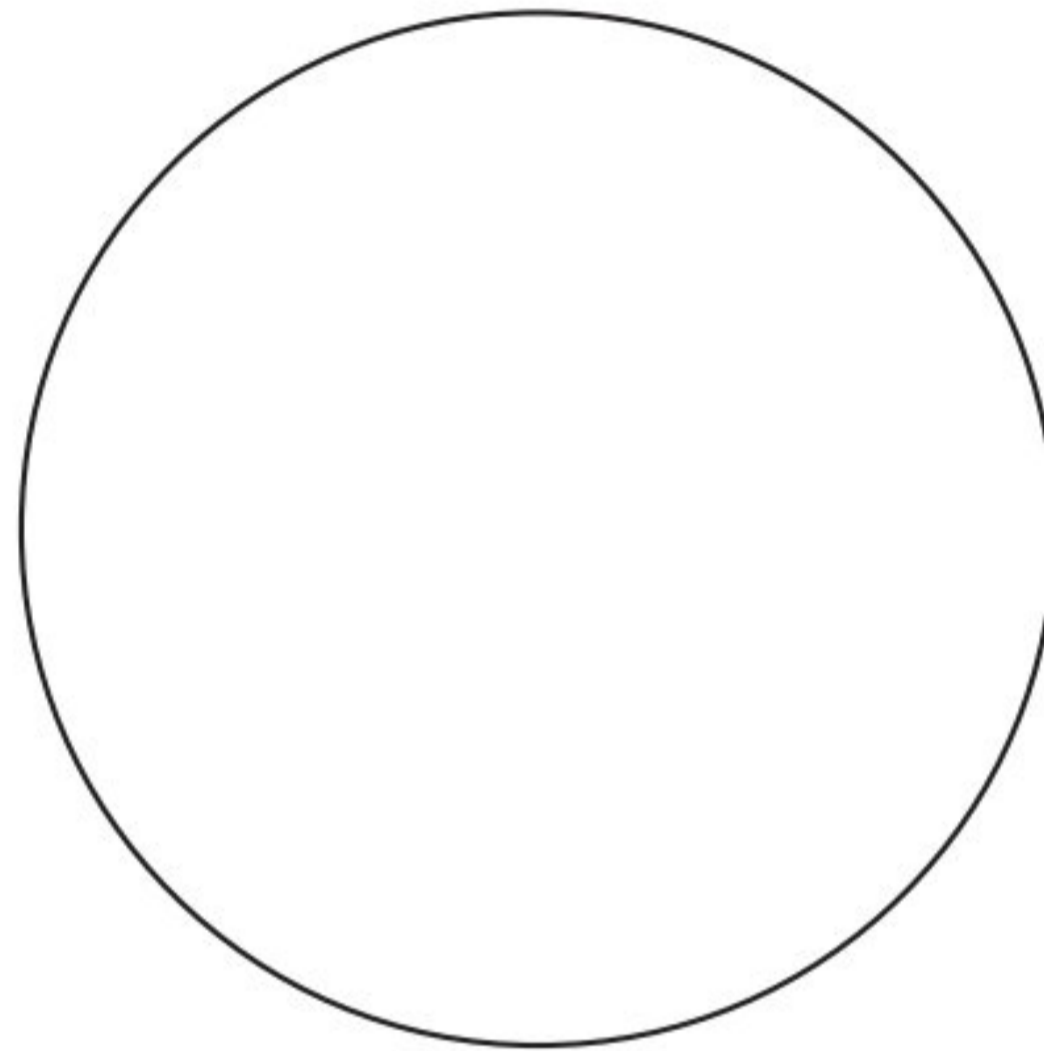
1. A disinfectant is used on your work surface
 - a. before the beginning of laboratory procedures.
 - b. after all work is complete.
 - c. after any spill of live microorganisms.
 - d. Both (b) and (c) are correct.
 - e. All of the above are correct.
2. To retrieve a sample from a culture tube with an inoculating loop, the cap of the tube is
 - a. removed and held in one's teeth.
 - b. removed and held with the fingers of the loop hand.
 - c. removed with the fingers of the loop hand and placed in the fingers of the tube hand.
 - d. removed with the fingers of the loop hand and placed on the laboratory bench.
 - e. Any of these methods can be used.
3. An inoculating loop or needle is sterilized using heat
 - a. by one brief passage.
 - b. for exactly 5 minutes.
 - c. until the entire wire is bright red.
 - d. until the handle is bright red.
 - e. until just the tip is bright red.
4. Which of the following would be a correctly labeled agar plate?
 - a. *Staph* on the bottom
 - b. *S. aureus* on the bottom
 - c. *S. aureus* on the lid
 - d. *Staphylococcus aureus* on the lid
5. Noah wanted to transfer *Staphylococcus aureus* from a broth to an agar plate. He picked up the broth culture, removed the cap, and flamed the mouth of the tube. He inserted an inoculating loop to obtain a bacterial sample. Then, he flamed the mouth of the tube and replaced the cap. Noah opened the lid of a labeled agar plate diagonally and used the loop to streak the surface of the agar. After closing the lid, he flamed the loop in an incinerator and put it back in its container. The plate was incubated upside down for 24–48 hours. What did Noah do wrong in this transfer?
 - a. He did not use the transfer tool correctly.
 - b. He did not handle the culture tube correctly.
 - c. He did not handle the agar plate correctly.
 - d. He used the wrong tool in transfer.

9 Pure Culture Techniques

A. Results

1. Evaluation of Streak Plate

Show within the circle the distribution of the colonies on your streak plate. To identify the colonies, use red for *Serratia marcescens* and yellow for *Micrococcus luteus*. (Use purple for *Chromobacterium violaceum* and yellow for *E. coli* if these species were included in your mixed culture.) If time permits, your instructor may inspect your plate and enter a grade where indicated.



Grade _____

2. Evaluation of Pour Plates

Show the distribution of colonies on plates II and III, using only the quadrant section for plate II. If plate III has too many colonies, follow the same procedure. Use colors to illustrate the different bacterial species.

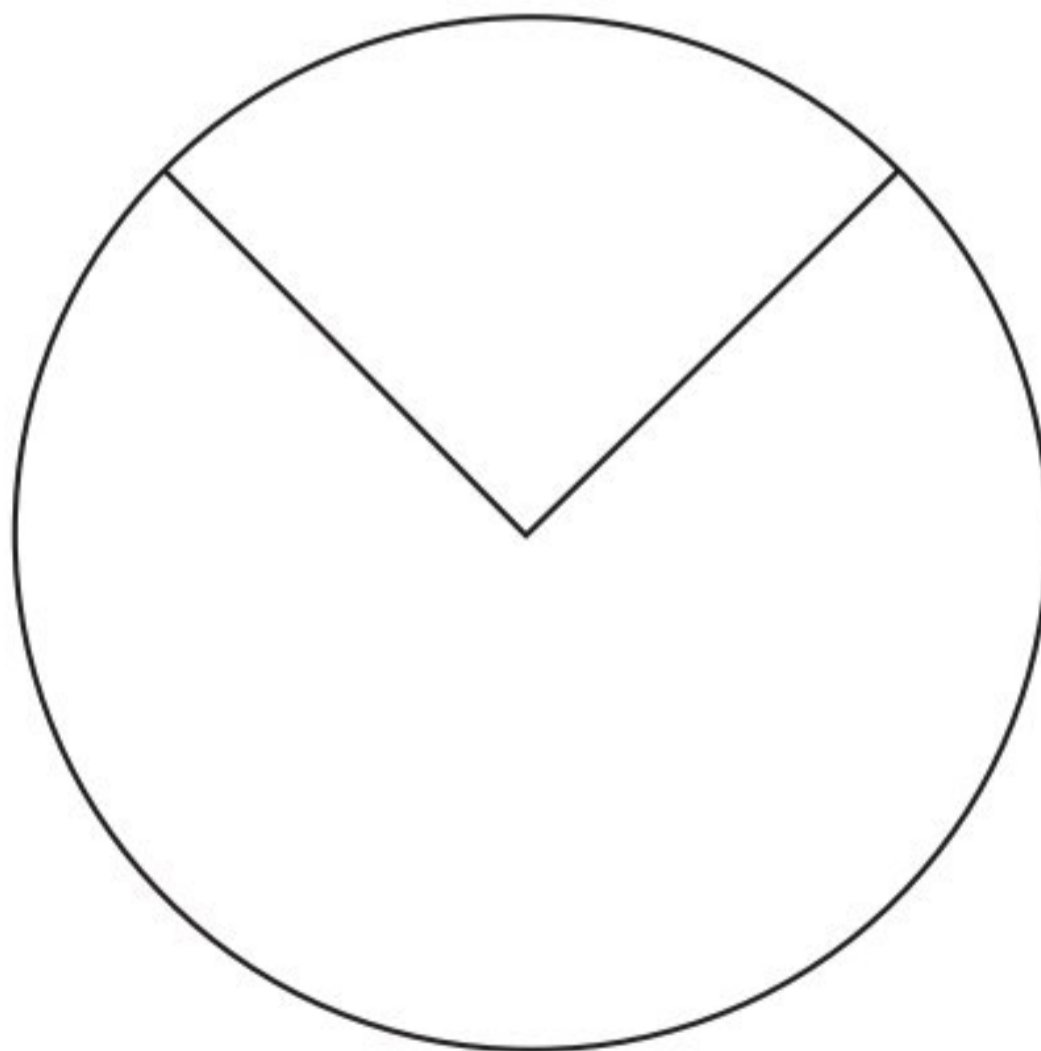


plate II

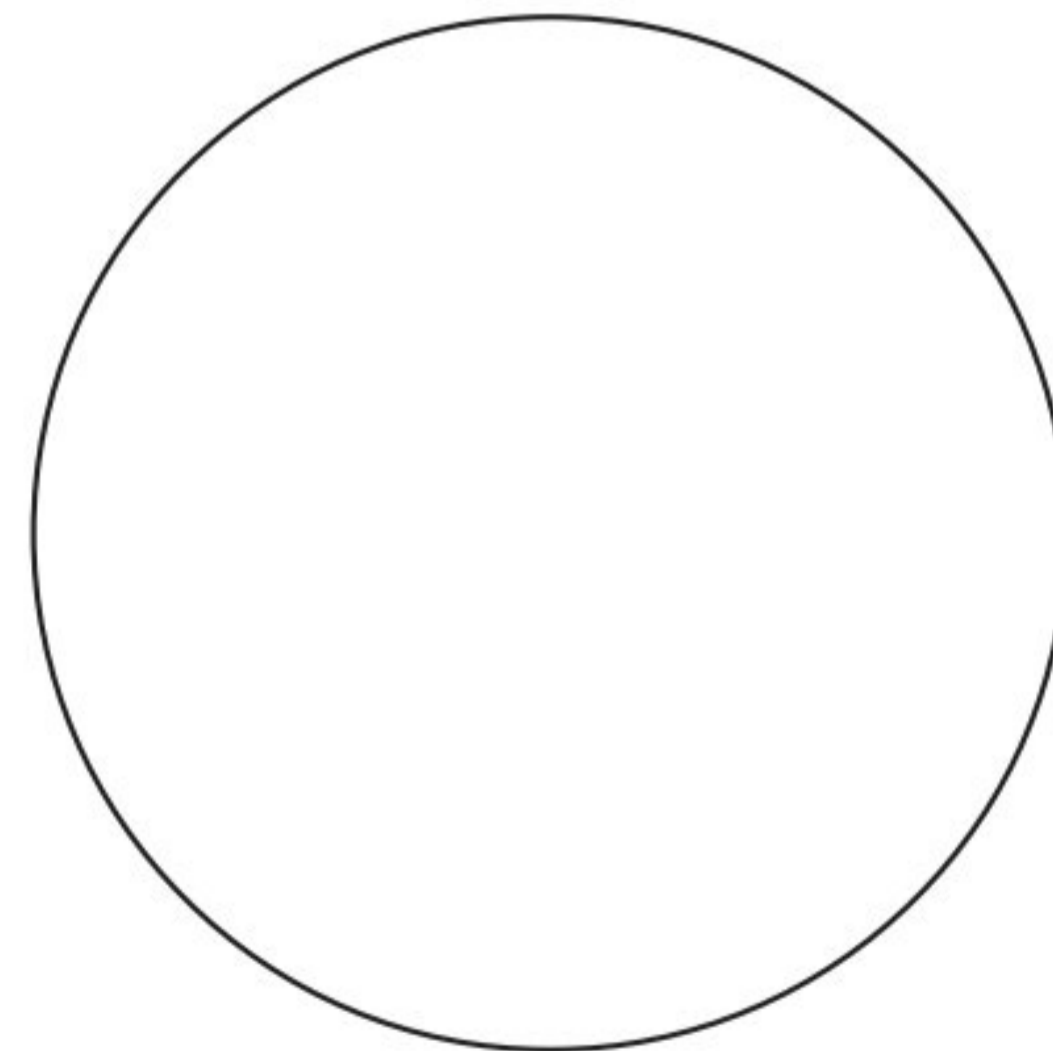


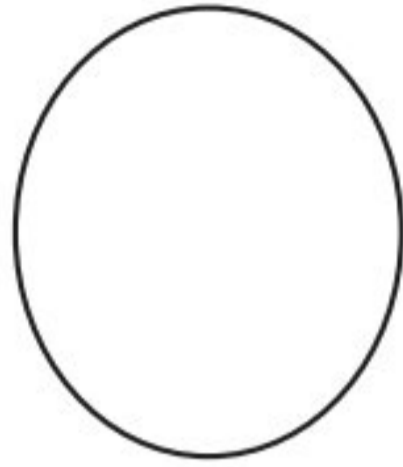
plate III

3. **Subculture Evaluation**

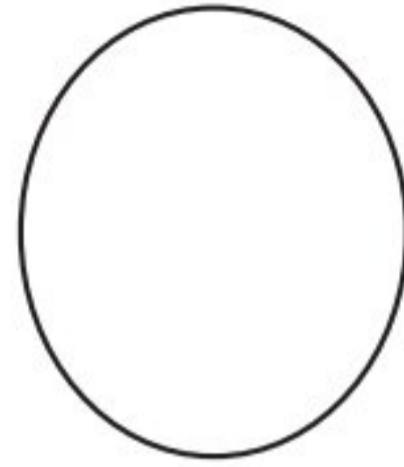
With colored pencils, sketch the appearance of the growth on the slant diagrams below. If you prepared and observed Gram-stained slides of the subcultured bacteria, draw a few cells of each organism as revealed by Gram staining in the adjacent circle.



Serratia marcescens
(or *Escherichia coli*)



Micrococcus luteus
(or *Chromobacterium violaceum*)



4. Compare the results of your streak and pour plates. Which method achieved the best separation of species?

5. Do your slants contain pure cultures? How would you confirm their purities?

B. Short-Answer Questions

1. Define the term “colony” as it relates to bacterial growth on solid media.

2. What colony characteristics can be used for the differentiation of bacterial species? As an example, compare the properties of colonies of *Serratia marcescens* and *Micrococcus luteus* on your streak plate. Use figure 35.4 in Exercise 35 for terms to use in your comparison.

3. Why is dilution a necessary part of pure culture preparation?

4. What advantage(s) does the streak plate method have over the pour plate method?

Pure Culture Techniques (continued)

5. What advantage(s) does the pour plate method have over the streak plate method?

6. Why is the loop flamed before it is placed in a culture tube? Why is it flamed after completing the inoculation?

7. Before inoculating and pouring liquified nutrient agar into a plate, why must the agar first be cooled to 50°C?

8. Explain why plates should be inverted during incubation.

9. Describe the difference between the appearance of surface and subsurface colonies in a pour plate. If this is the same bacterial species, why do these differences in colonial growth occur?

Laboratory Report

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Student: _____

Date: _____ Section: _____

10 Smear Preparation

Short-Answer Questions

1. How does smear preparation of cells from a liquid medium differ from the preparation of cells from a solid medium?

2. Why is it important to limit the quantity of cells used to prepare a smear?

3. Describe the potential consequences of making a smear that is too thick.

4. For the preparation of a smear on a slide, what is the purpose of heat fixation? What problems can arise when the slide is heated in a flame?

Laboratory Report

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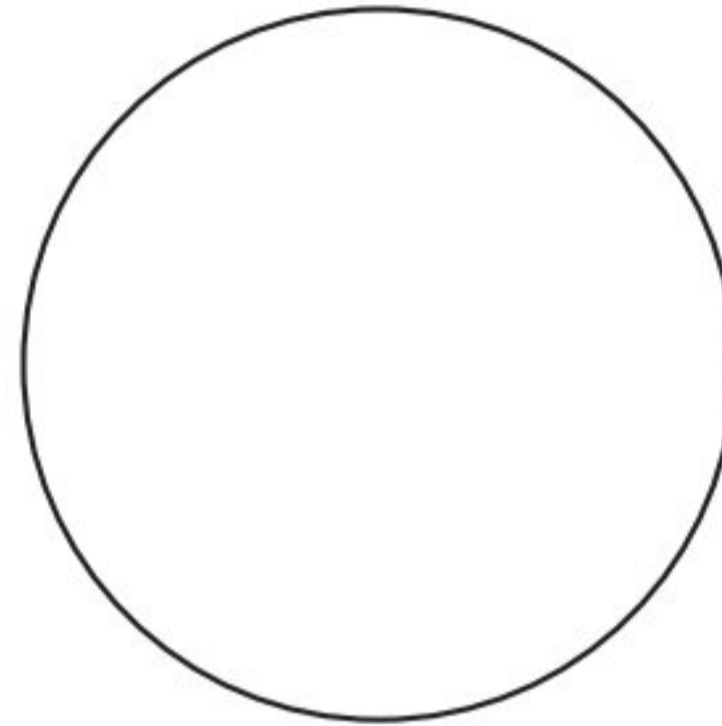
Student: _____

Date: _____ Section: _____

11 Simple Staining (Observing Bacterial Cell Morphology)

A. Results

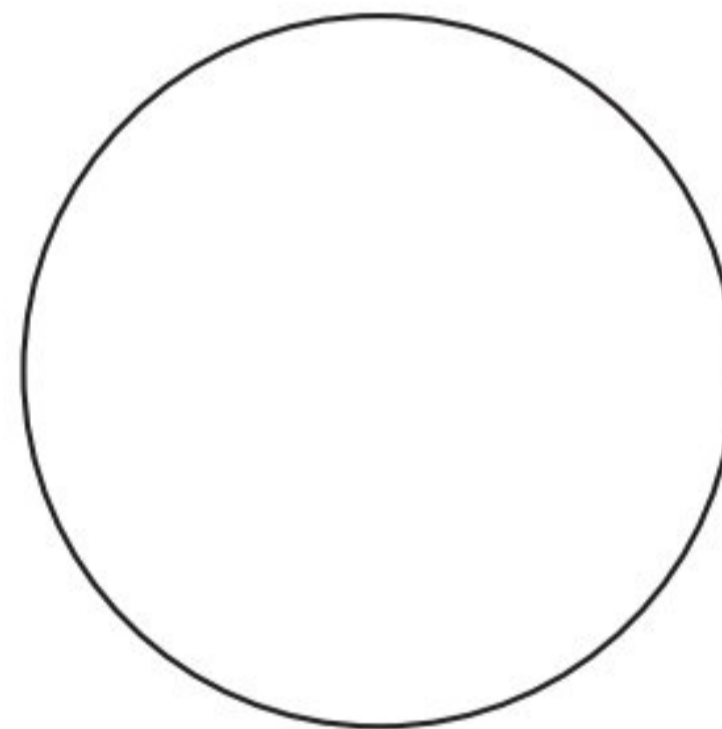
1. What two noteworthy physical characteristics of *Corynebacterium xerosis* are visible after performing a simple stain? Draw cells from your slide to demonstrate these characteristics.



Corynebacterium xerosis

Total Magnification _____

2. Describe the arrangement and cell morphology of staphylococci. Draw cells from your slide to demonstrate these characteristics.

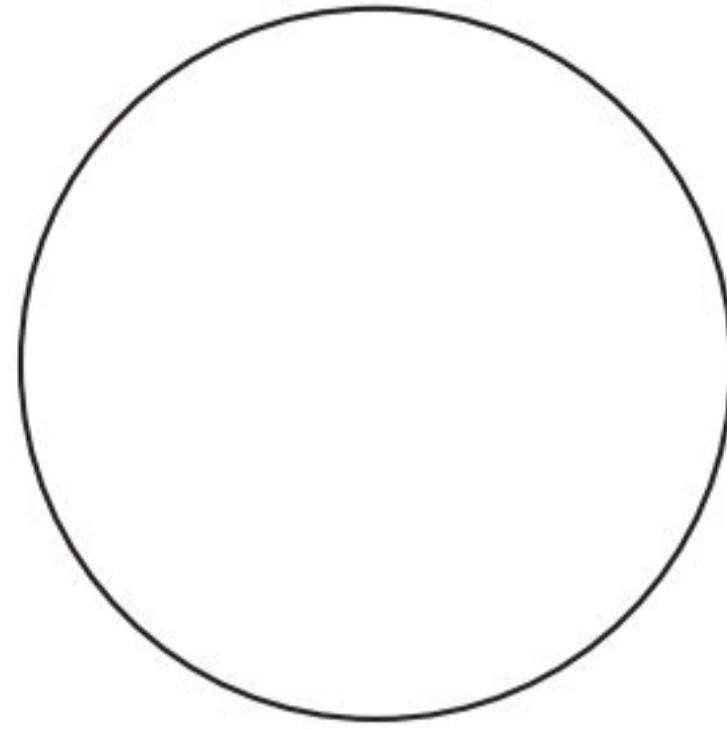


Staphylococcus epidermidis

Total Magnification _____

Simple Staining (Observing Bacterial Cell Morphology) (continued)

3. Describe the arrangement and cell morphology of members of the genus *Bacillus*. Draw cells from your slide to demonstrate these characteristics.



Bacillus megaterium

Total Magnification _____

B. Short-Answer Questions

1. What are chromophores?

2. What is the difference between basic and acidic dyes?

3. Why do acidic dyes leave bacterial cells unstained?

4. Crystal violet is an example of what type of stain?

5. What is meant by the palisade arrangement of cells?

6. What shape does *Vibrio cholerae* have?

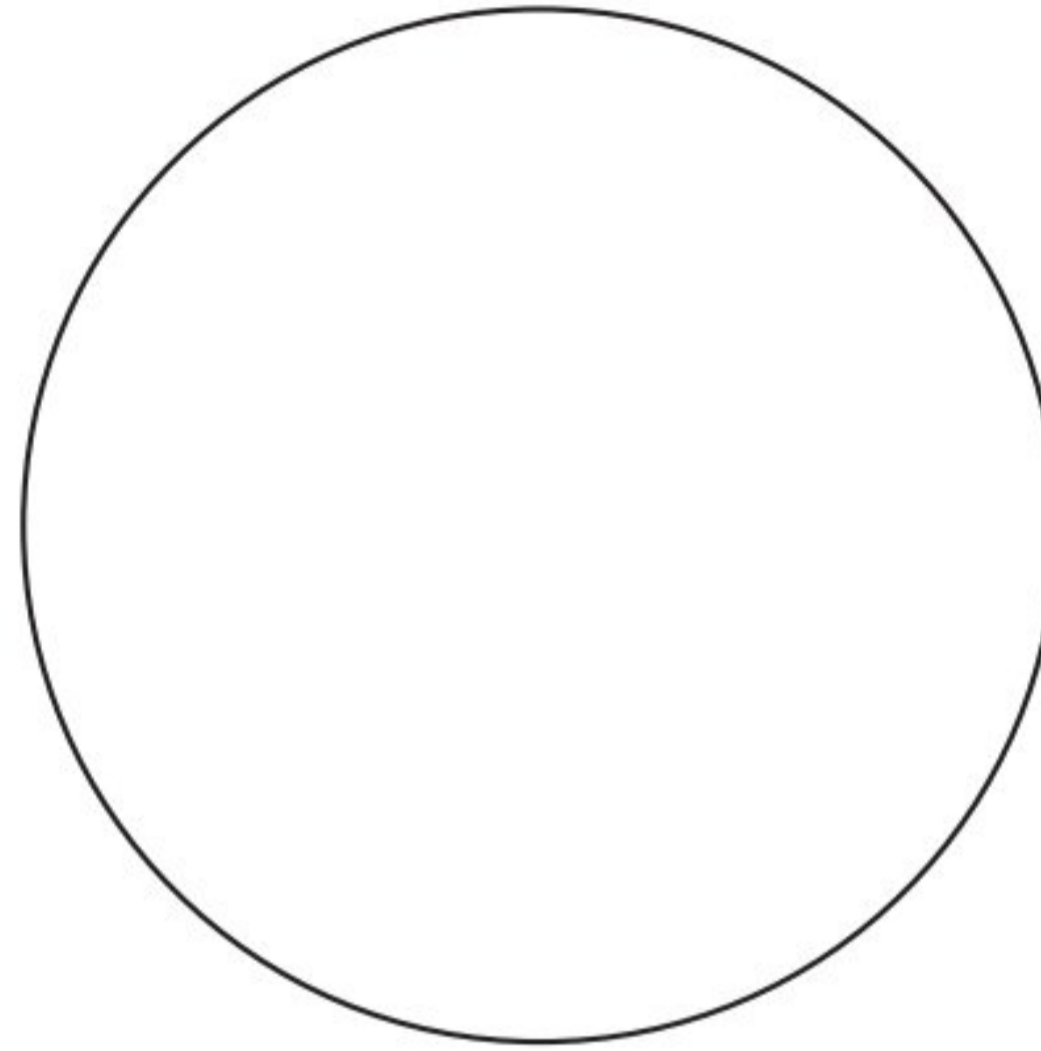
7. Where are the fusiform bacteria usually found in humans?

8. If you were working with an unlabeled simple stained smear, would you be able to identify the bacterial species by observing the slide under the microscope? Why or why not?

12 Negative Staining

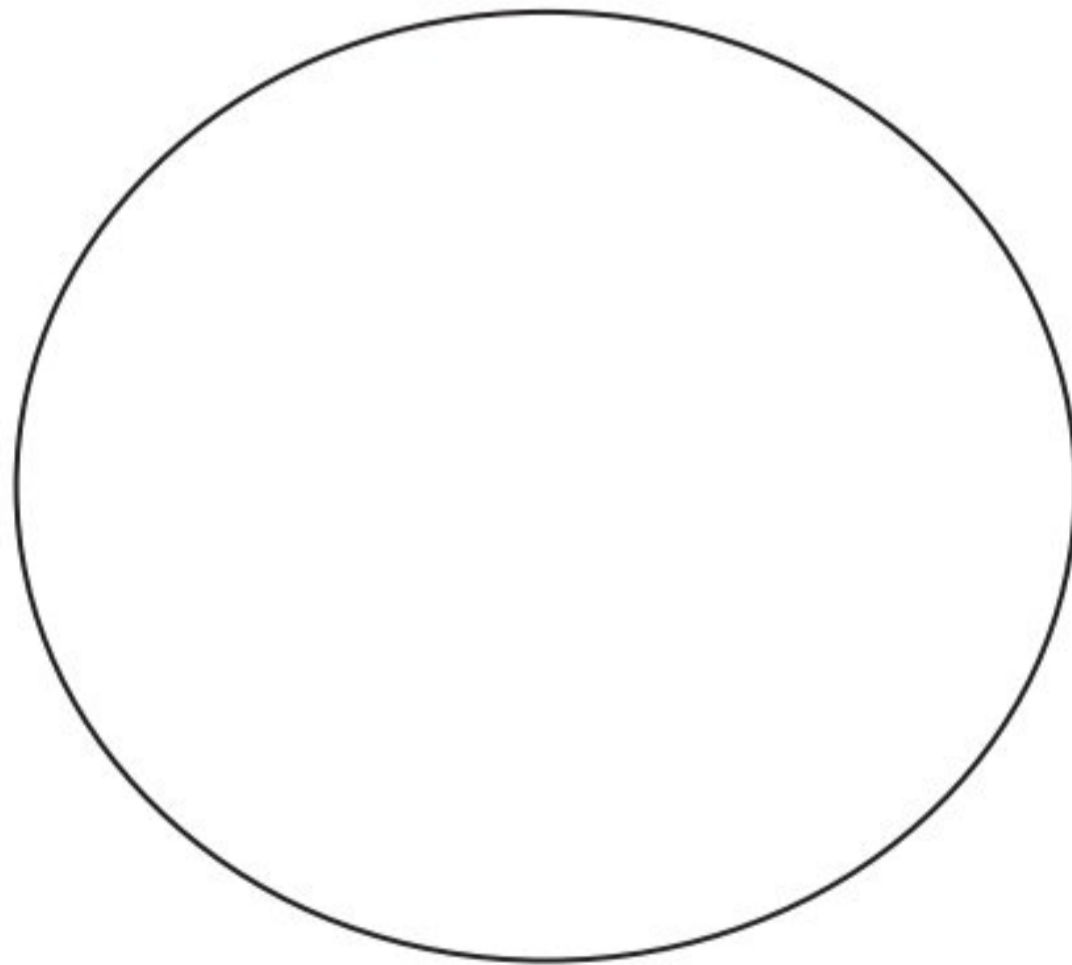
A. Results

1. Draw the different types of microorganisms that were found in the negative stain of the oral sample. How would you differentiate between oral streptococci, yeasts, and spirochetes in your sample?



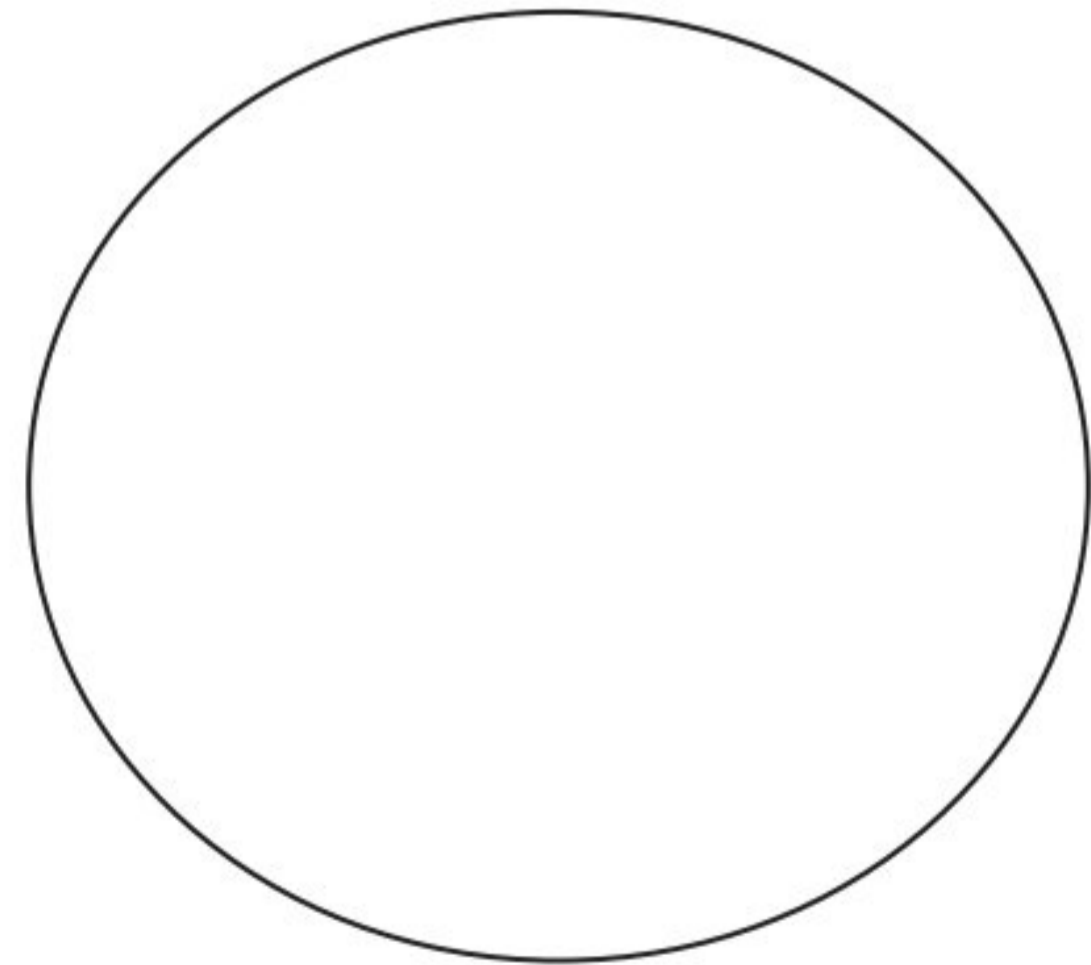
Oral organisms
(nigrosin stain)

2. Draw your slides made from bacterial cultures. Label the species under your drawing, and indicate the cell shape and/or arrangement.



Species: _____

Morphology: _____



Species: _____

Morphology: _____

Negative Staining (continued)

B. Short-Answer Questions

1. What type of chromophore is associated with a negative stain?

2. What is an example of a negative stain?

3. What step normally associated with staining bacterial cells is omitted when the dimensions of cells are determined? Why?

4. What external bacterial cell structures can be demonstrated by a negative stain?
