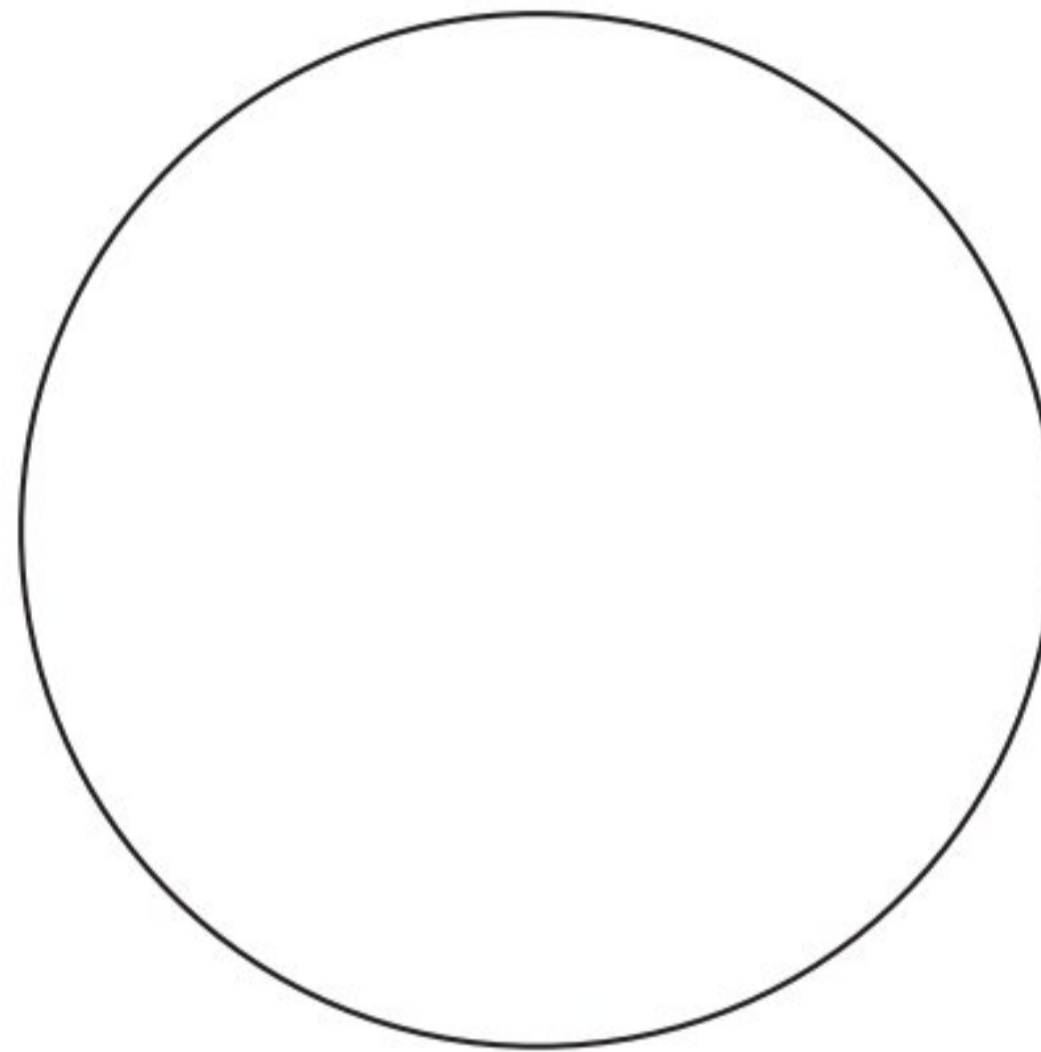


13 Capsular Staining

A. Results

1. Draw cells that display a capsule from your stained slide of *Klebsiella pneumoniae*. Explain how the capsule is visualized without the use of dyes that adhere to a capsule.



Klebsiella pneumoniae
(capsular stain)

B. Short-Answer Questions

1. What are two functions of the capsule or slime layer in bacterial cells?

2. What biological molecules can make up the bacterial capsule or slime layer?

3. What function does the capsule have for *Streptococcus mutans*?

Capsular Staining (continued)

4. Look up the term *virulence factor* if you do not already know its definition. Explain why the bacterial capsule is considered a virulence factor.

5. A student heat-fixes his smear intended for capsule staining. What result might the student expect?

Laboratory Report

14

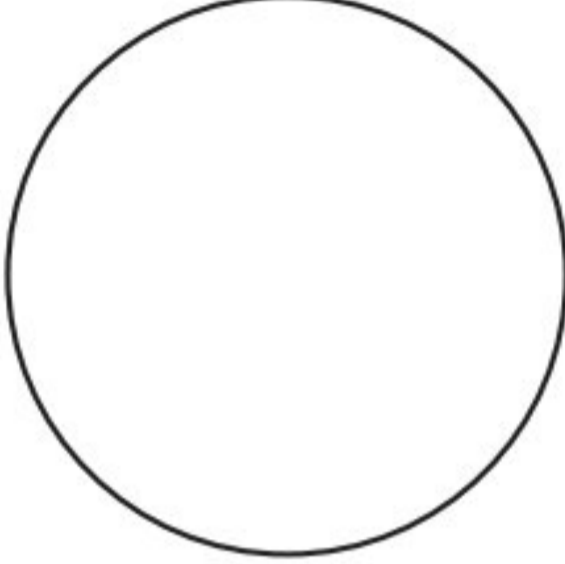
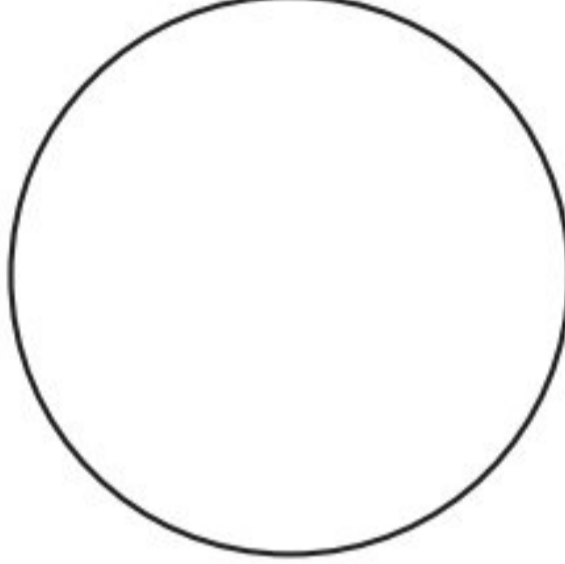
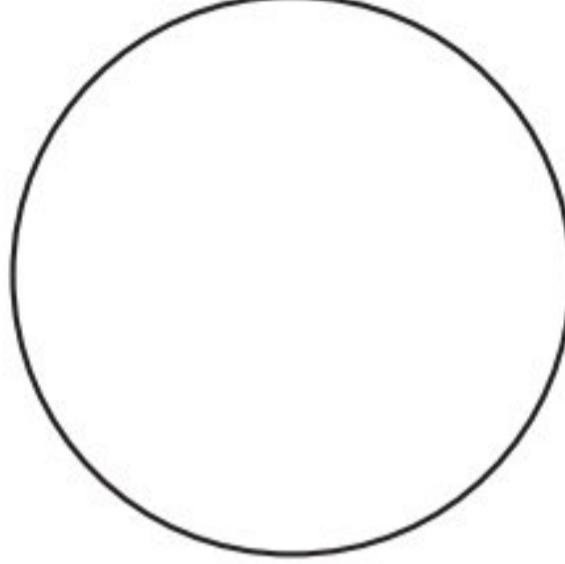
Student: _____

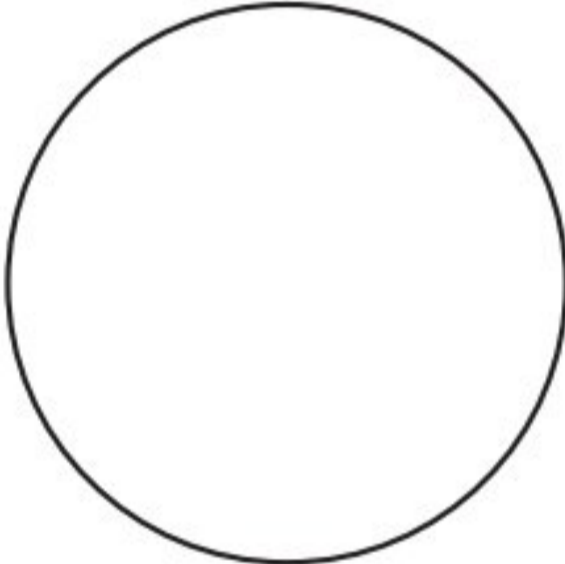
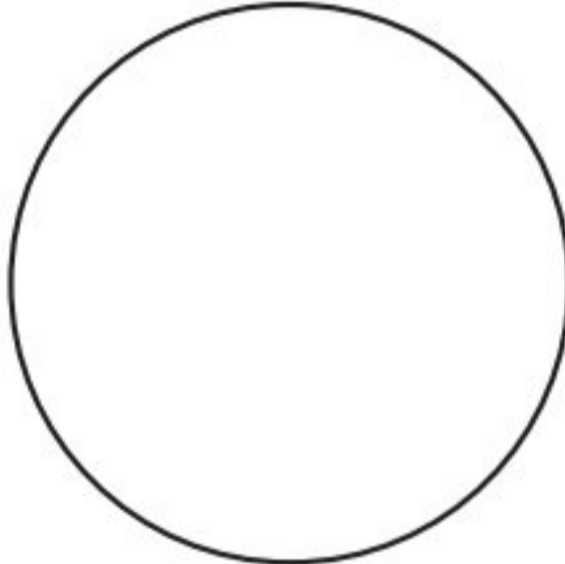
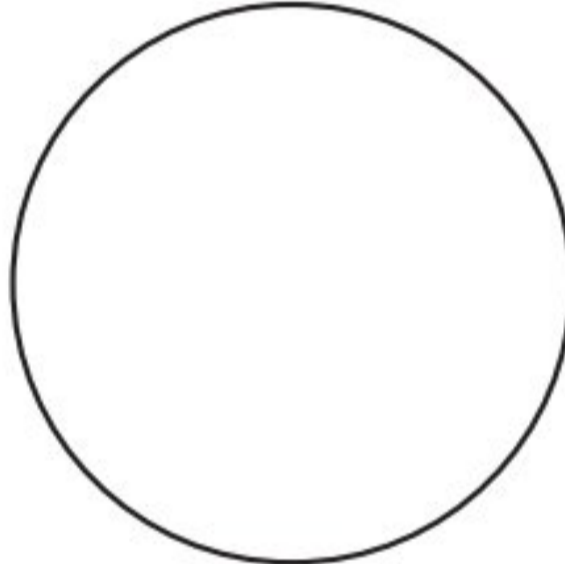
Date: _____ Section: _____

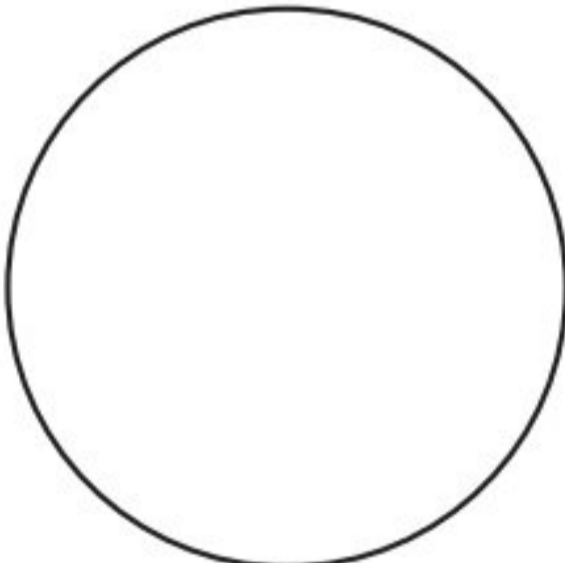
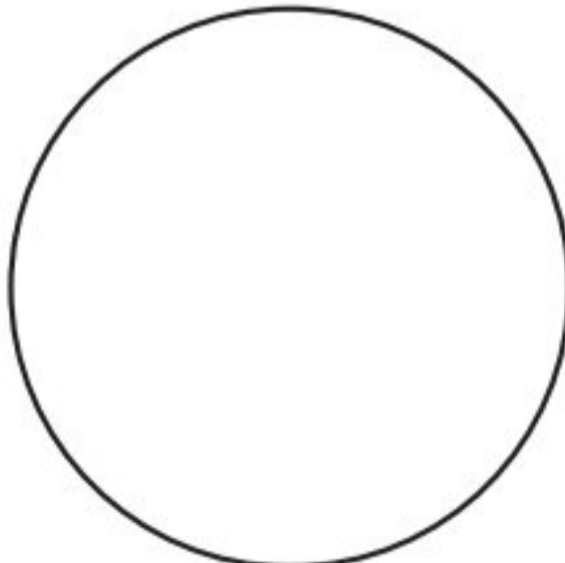
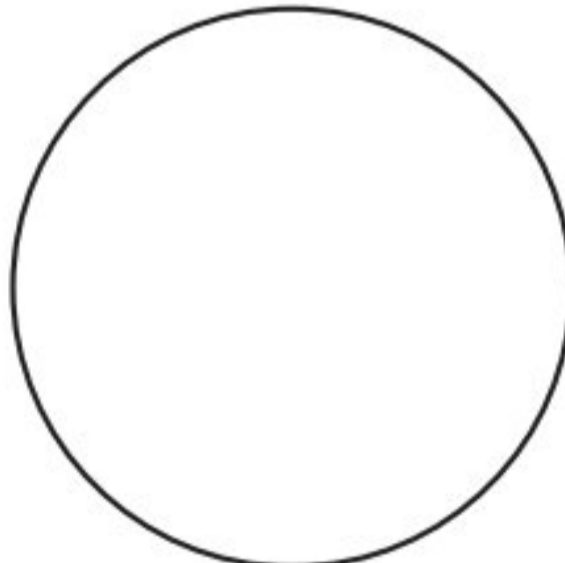
14 Gram Staining

A. Results

Draw cells from the Gram-stained slides in the figures below. Based on your results, note the Gram reaction, cell shape, and cell arrangement of each bacterium in the given table. Verify these results using figure 14.5 to determine if you are correct. If your control organisms do not display the correct color or shape, draw what you do see on the slide and make a note that the slide was not stained correctly.

		
<i>S. epidermidis</i>	<i>E. coli</i>	<i>P. fluorescens</i>

		
<i>S. epidermidis</i>	<i>M. catarrhalis</i>	<i>P. fluorescens</i>

		
<i>S. epidermidis</i>	<i>B. megaterium</i>	<i>P. fluorescens</i>

BACTERIAL SPECIES	GRAM REACTION	CELL SHAPE	CELL ARRANGEMENT
<i>Staphylococcus epidermidis</i>			
<i>Pseudomonas fluorescens</i>			
<i>Escherichia coli</i>			
<i>Moraxella catarrhalis</i>			
<i>Bacillus megaterium</i>			

B. Short-Answer Questions

1. Why is the Gram stain considered a differential stain?

2. How do gram-positive and gram-negative bacteria differ in cellular structure, and how does this contribute to their differential staining properties?

3. How does the age of a culture affect the Gram stain reaction? What is an optimum culture age for a valid Gram reaction?

4. Which step in the Gram stain procedure is most prone to error? If done incorrectly, how might that step affect the end result?

5. What is the function of a mordant, and which reagent serves this purpose in the Gram stain procedure?

6. List the reagents of the Gram stain technique in order and their general role in the staining process.

7. In what type of cell, gram-positive or gram-negative, would you find lipopolysaccharide in its cell wall? Where is this found, and why is it clinically relevant?

8. Conducting additional research, why are beta-lactams more effective on gram-positive infections?

9. What additional compounds are found associated with the cell walls of gram-positive bacteria but are absent in gram-negative bacteria?

C. Matching Questions

Match the expected result (purple, red, or colorless) to the following descriptions of Gram-stained cells. Consult your chart at the beginning of this lab report if you need help remembering the correct Gram reaction for each species.

Choices may be used more than once.

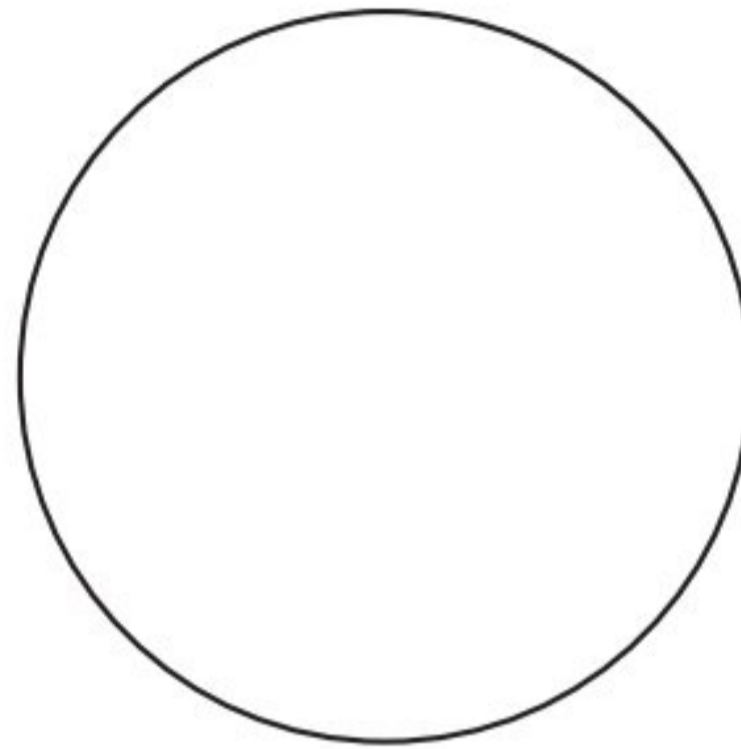
1. _____ *Staphylococcus epidermidis* before the primary stain
2. _____ *Pseudomonas fluorescens* after the primary stain
3. _____ *Bacillus megaterium* after the addition of the mordant
4. _____ *Staphylococcus epidermidis* after decolorization
5. _____ *Moraxella catarrhalis* after decolorization
6. _____ *Pseudomonas fluorescens* after decolorization
7. _____ *Bacillus megaterium* after adding the counterstain
8. _____ *Escherichia coli* under the microscope if you forgot to apply safranin
9. _____ *Escherichia coli* under the microscope if you forgot to apply decolorizer
10. _____ *Bacillus megaterium* under the microscope if you forgot to apply iodine

- | |
|--------------|
| A. Purple |
| B. Red |
| C. Colorless |

15 Endospore Staining

A. Results

Draw cells from the endospore slide. Label your drawings to differentiate endospores from vegetative cells.



B. cereus

(_____ method)

B. Short-Answer Questions

1. What are the functions of endospores in bacteria?

2. What external structure on the endospore acts as a protective barrier? What is its composition?

3. Compared to a vegetative cell, how much less water is present in an endospore?

4. What is the mordant in the endospore stain?

5. What is the stimulus for endospore production in bacteria?

Endospore Staining (continued)

6. What conditions are necessary to destroy endospores? In what device are these conditions achieved?

7. What is the color of endospores after Gram staining? After Schaeffer-Fulton endospore staining?

8. What is the secondary stain in the Schaeffer-Fulton endospore stain?

9. What is the color of the vegetative cell after the Schaeffer-Fulton endospore stain?

10. Of these three genera of bacteria, which does not produce endospores: *Clostridium*, *Mycobacterium*, or *Bacillus*?

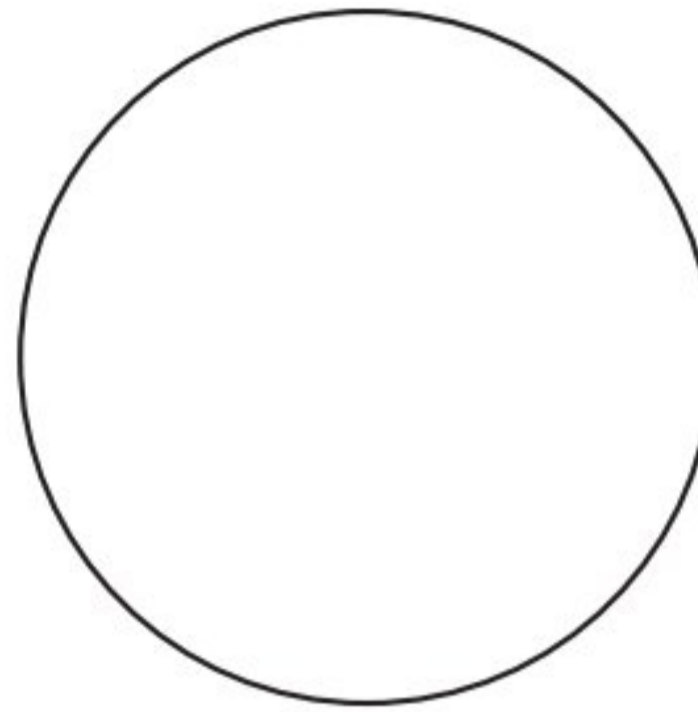
11. Based on the sporulation cycle, are bacterial endospores reproductive structures? Explain why or why not.

12. List and briefly describe three examples of diseases caused by endospore-forming bacteria and the names of the specific bacterial agents involved.

16 Acid-Fast Staining: Kinyoun Method

A Results

Draw cells from the acid-fast stained slide. Label your drawing to differentiate acid-fast from non-acid-fast cells.



M. smegmatis and *S. epidermidis*
(Kinyoun method)

B. Short-Answer Questions

1. What makes *Mycobacterium* resistant to staining?

2. Name another acid-fast bacterial genus.

3. What is the primary stain in the Kinyoun acid-fast staining method? How does this differ from the primary stain in the Ziehl-Neelsen method?

4. What is the secondary stain in both acid-fast stain methods?

5. In the Ziehl-Neelsen acid-fast stain, what is the mordant?

Acid-Fast Staining: Kinyoun Method (continued)

6. What is the advantage of the Kinyoun staining procedure over the Ziehl-Neelsen method?

7. If you conducted an acid-fast stain on a sputum sample from a person with tuberculosis, what would you expect to see on the slide?

17 Motility Determination

A. Results

1. Which bacterial species exhibited true motility on the slides?

2. Draw the appearance of the inoculated motility tubes. Did the semisolid medium inoculations concur with the slide results?



Staphylococcus epidermidis



Pseudomonas fluorescens

B. Short-Answer Questions

1. Describe the structure of a flagellum. How do flagella generate cell motility?

2. If you compared two motile bacterial species and determined one was considerably more motile than the other, which arrangement of flagella would you expect to be associated with the highly motile species? How would you confirm this?

Motility Determination (continued)

3. Differentiate between the following types of movement observed on a wet mount or hanging drop slide:

a. directional motility

b. Brownian movement

c. water current movement

4. What concentration of agar is used in a semisolid medium for motility determination? How does that compare to a typical solid medium (see Exercise 18)? Explain.

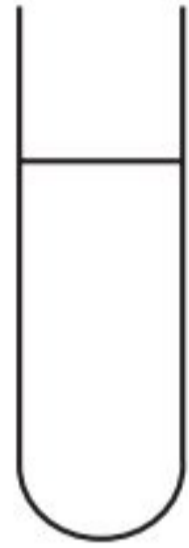
5. Why are semisolid media sometimes preferred over slide techniques for evaluating bacterial motility?

24 Effects of Oxygen on Growth

A. Results

1. FTM Tube Inoculations

After carefully observing the growth of the FTM cultures, sketch the appearance of the growth in the tubes below.



E. coli



E. faecalis



S. epidermidis



B. subtilis



C. sporogenes

2. Plate Inoculations

After comparing the growths on the two agar plates with the growths in the five tubes above, classify each organism based on its oxygen requirements (obligate aerobe, facultative anaerobe, etc.).

Escherichia coli: _____

Bacillus subtilis: _____

Enterococcus faecalis: _____

Clostridium sporogenes: _____

Staphylococcus aureus: _____

B. Short-Answer Questions

1. What is the role of oxygen in aerobic respiration?

2. What type(s) of metabolism can occur in the absence of oxygen?

3. Name two enzymes that are present in obligate aerobes but lacking in obligate anaerobes. What is the function of each enzyme?

4. Differentiate between a microaerophile and an aerotolerant organism.

Effects of Oxygen on Growth (continued)

5. Why is resazurin a useful media additive for the study of anaerobes?

6. Why is a GasPak anaerobic jar necessary for the culture of anaerobes on nutrient agar plates but not in tubes of FTM?

7. Where in the human body would you expect to find an obligate aerobe? Where would you expect to find an obligate anaerobe? Look up some examples of each, and indicate where they may be found in the body.

23 Phage Typing

A. Results

1. To which phage types was this strain of *S. aureus* susceptible?

2. To what lytic group does this strain of staphylococcus belong?

B. Short-Answer Questions

1. *S. aureus* is gram positive. What differences in strains might account for differences in phage susceptibilities?

2. Why is phage typing an important clinical tool?
