Chapter 19 **Bacteria and Viruses** **Name _____**

Lab Identifying Bacteria

Background Information

Bacteria are divided into different groups based on a number of characteristics. One way to distinguish bacteria is by shape. Bacteria that resemble spheres are called *cocci. Bacilli* are bacteria that look like rods. Bacteria that resemble spirals are called *spirilla.*

Another way to distinguish bacteria is by their ability to accept a specific type of stain called a Gram stain. The Gram-staining method uses two stains - a dark purple stain and a light pink stain. Bacteria that are Gram-positive retain the dark purple stain. Bacteria that loose the dark purple stain and show only the light pink stain are called Gram-negative. The difference in staining characteristics is due to differences in the bacterial cell wall.

In this investigation, you will identify bacteria by their shape, mobility, and ability to accept a stain.

Problem

How are different types of bacteria identified?

Materials (per group)

microscope
inoculating loop
test tube rack
simple stain
bibulous paper
prepared slides of bacteria
glass-marking pencil
culture of *Bacillus subtilis*
culture of *Escherichia coli*
culture of *Micrococcus luteus*
bunsen burner
microscope slides
flint striker or matches
slide holder

Safety

Put on a laboratory apron if one is available. Put on safety goggles. Handle all glassware carefully. Use extreme care when working with heated equipment or materials to avoid burns. Always use special caution when working with laboratory chemicals, as they may irritate the skin or cause staining of the skin or clothing. Never touch or taste any chemical unless instructed to do so. Use special caution when working with bacterial cultures. Always handle the microscope with extreme care. You are responsible for its proper care and use. Use caution when handling glass slides as they can break easily and cut you.

Procedure

Part A Identifying Bacteria by Shape

1. Obtain prepared slides of different-shaped bacteria from your teacher. Bacteria have three characteristic shapes: round, or *coccus;* rod-shaped, or *bacillus;* and spiral-shaped, or *spirillum*. These three basic shapes can be arranged singly, in pairs (*diplo-*), or in chains (*strepto-*), and in clusters (*staphylo-*).

2. Observe a prepared bacteria slide under high power. Make a drawing of what you observe in the appropriate place in Observations. Write the name of the bacteria. Observe how the bacteria on this slide are arranged and record this information in Data Table 1.

3. Repeat step 2 with the other prepared slides obtained from your teacher.

Part B Staining Bacteria

1. Obtain bacterial cultures A, B, and/or C. **CAUTION:** *Use extreme care when working with bacterial cultures. Avoid spills. If a spill does occur, immediately call your teacher for assistance. Do not try to clean it up by yourself.* With a glass-marking pencil, label one glass slide A and the other glass slide either B or C.

2. Put on safety goggles. Light the bunsen burner. **CAUTION:** *Use extreme care when working with or near an open flame. Tie back loose hair and clothing.*

3. From culture A make a sterile transfer from the Petri dish to a clean glass slide in the following manner. Hold the inoculating loop in the flame until it is red hot. Pick up a loopful of bacterial culture from culture A. Touch the loop with the bacterial culture to the center of slide A. Spread the bacteria into a circle about the size of a dime, as shown in Figure 1A. The area of bacteria on the slide is called a smear. Set slide A aside to air-dry. Flame the inoculating loop until it is red hot. **CAUTION:** *Do not touch the bacterial smear to determine if it is dry. Always avoid direct contact with bacterial cultures.*

4. Repeat step 3 making a transfer from culture B or C to another slide.

5. Fasten a slide holder onto the end of slide A. Pass the slide, *smear side up,* three times through the flame of the bunsen burner, as shown in Figure 1B. **CAUTION:** *Do not touch the hot glass slide. Handle it only with a slide holder.* Set the slide aside to cool. Repeat this procedure with the other slide. Passing the slide through the flame kills the bacteria and attaches, or fixes, the bacteria to the slide.

6. Place both slides in the center of a staining tray. Find the dropper bottle marked "simple stain." Flood the smears on both slides with the simple stain, as shown in Figure 1C. **CAUTION:** *Handle the simple stain with care. It is a stain and is difficult to remove from hands and clothing.* Leave the stain on the smears for **60 seconds**.

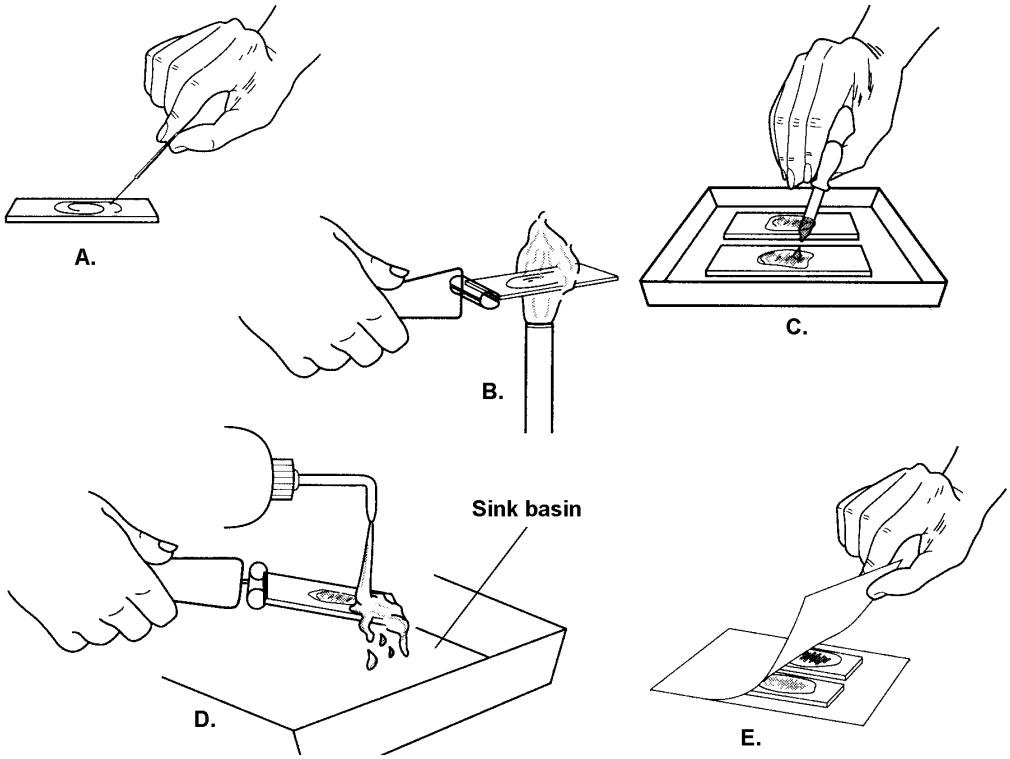


Figure 1

7. As shown in Figure 1D, use a wash bottle filled with water to rinse all excess stain from the slides.

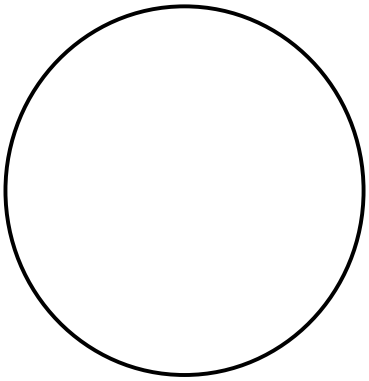
8. Place the slides between two sheets of bibulous paper, as shown in Figure 1E. Close the sheets and carefully press on the slides to blot them dry. **CAUTION:** *To avoid breaking the slides, use only gentle pressure.*

9. Observe both slides under the oil immersion lens. Your teacher will show you how to do this procedure. Record your observations in Data Table 2.

10. Return the slides and bacterial cultures to your instructor for proper disposal. Wipe the surface of your work area with disinfectant and allow it to air dry. Thoroughly wash your hands with soap and water.

Observations

Prepared Slide 1 or Area 1



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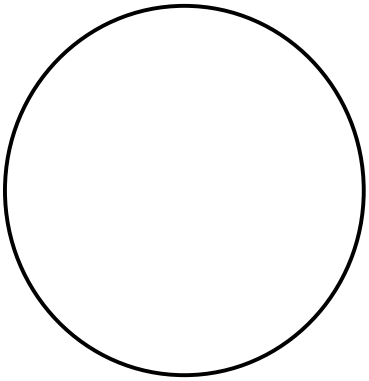
Prepared Slide 2 or Area 2



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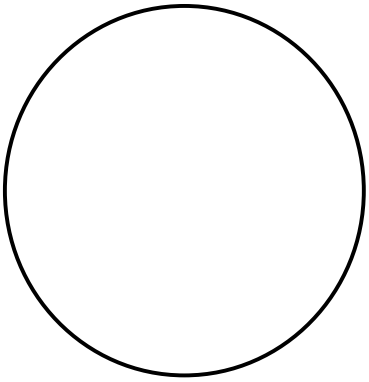
Prepared Slide 3 or Area 3



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Data Table 1

		Arrangement (✓)				
Slide or Area	**Basic Shape**	**Single**	**Pair (diplo-)**	**Chain (strepto-)**	**Cluster (staphylo-)**	**Other (draw)**
1.						
2.						
3.						

Data Table 2

Bacterium	Basic Shape	Color	Name of Stain Used
A (*Bacillus subtilis*)			
B (*Escherichia coli*)			
C (*Micrococcus luteus*)			

Analysis and Conclusions

1. Why do you think you flame the slide with the smear facing away from the flame?

2. Why do you think you do not run water directly onto the smear?

3. What are the two reasons for staining bacteria?

4. Does a bacterium's stain reaction seem to be directly related to its shape? How could you prove your hypothesis?

Critical Thinking and Application

1. Penicillin works by interfering with a bacterium's ability to build a cell wall. Using this information, explain why Gram-positive bacteria are more susceptible to destruction by penicillin than Gram-negative bacteria are. *(Hint:* Review the structural differences between Gram-positive and Gram-negative bacteria in your text.)

2. You are given an unknown bacterial culture by your teacher. After completing the Gram-staining technique, you observe both pink and purple bacteria in the same smear. What could be a possible explanation for this?

3. Occasionally after a flood or the breaking of a large water pipe, people living in the area are advised to boil their drinking water. Why is this done?

4. It has been only in recent years that scientists have discovered fossilized bacteria. Why might this discovery have taken so long to occur?