**Negative and Simple Staining**

**Background on Negative Staining:** As any microbiology student will tell you, finding bacteria on a slide is not an easy task. It demands a great deal of practice, a fair amount of patience and a little bit of luck. Fortunately there is a simple method that we can use to detect microorganisms on a slide and it is called **negative Staining**. The process involves mixing microbes with a dark stain and spreading the mixture over a clean slide. Since living cells are transparent and do not take up the stain, they are visible as “bright dots” against a darkly stained background as shown in Figure 1.

Cell

Figure 1. Negative Stain Negative staining is primarily used for:

1. revealing the presence of a capsule
2. providing accurate cell size
3. specimens that are difficult to stain

# Negative Staining Protocol

1. Clean microscope slide **thoroughly**.
2. Place a small drop of **nigrosine** near one edge of the slide.
3. Mix a loopful of bacterial culture into the drop of nigrosine as shown in Figure 2. Use aseptic technique.

Nigrosine

Figure 2

1. Place a second slide lengthwise in front of the drop of stain as shown in Figure 3. Starting in the middle of the slide which contains the stain, move the other slide back at a 45o angle until it touches the stain, then push forward to spread the stain.

Figure 3

1. Remember that the slide used to spread the stain is now contaminated with microbes and should be handled appropriately.
2. Air dry (5 - 10 min.) **DO NOT heat fix!**
3. Examine under oil immersion and record your observations in the space provided below.

Culture #1 Culture #2 Culture #3

## Negative Staining Results

**Background on Simple Staining:** Now that you are convinced (I hope) that microorganisms do in fact exist, it is time to learn how to stain them. We will begin by staining them with a single stain, a process called **simple staining**.

At some time in your distant past you have probably been subjected to a high-school physical science course.

During this course you learned a valuable lesson, which will help you to stain microorganisms. Yes, that age-old physical axiom “opposites attract”, the one piece of information that you gleaned from this course, is actually useful! Bacteria, unbeknownst to them, carry a slightly negative charge. Many stains, such as methylene blue, have a positively-charged portion (chromophore) which is attracted to and sticks to the negatively charged bacterial surface thereby “staining” the cell.

Now that you understand how simple staining works you are probably anxious to get started, but you are not yet ready. A first step in most staining techniques (with the notable exception of negative staining) is the preparation of a bacterial smear. This technique basically sticks the bacteria to the slide so they won’t come off while you are staining them

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# Procedure for Making a Smear

1. Clean and dry a glass slide.
2. Using aseptic technique, transfer a loopful of a bacterial broth culture to the center of the slide. You need to smear the material out into an area no larger than a dime. If you suspect there are not many organisms in the culture you may need to add several loopfuls of organisms to the slide being sure to place them in the same spot.
3. Allow to air dry (5 - 10 min.)
4. Holding the edge of the glass slide with a clothespin or forceps, pass the slide (smear side up) through a Bunsen burner 3 or 4 times. This fixes the cells to the slide so that subsequent staining and washing will not remove them.
5. You are now ready to stain!

## NOTES:

**Simple Staining Protocol**

**Q: Describe the shapes of the bacterial cells in the culture.**

**LIST the major bacterial shapes – See text for more information**

**1.**

**2.**

**3.**

1. Cover the smear with a few drops of **methylene blue**. Let sit for one minute.
2. Rinse with water and blot dry with bibulous paper.
3. Examine under oil-immersion and record your observations below.

## NOTES:

Culture #1

Culture #2

Culture #3

# Simple Stain of Mouth Organisms

The little critters we have been looking at so far are tame laboratory pets I keep around for your culturing and viewing pleasure. What I would like for you to do now is to do a little exploring and see what you can find lurking around the base of your teeth.

1. Using a sterile swab, gently scrape around the base of your teeth and gums for about 30 seconds.
2. Smear the material you collected on the swab onto a clean microscope slide and allow to air dry for a few minutes and then heat fix.
3. Stain with Methylene blue as described above and observe.
4. Record your results below.

**What did you see in the stain of the material from your mouth?**