Smear Preparation

Not only are most bacteria very small, they are also very clear and difficult to view under a microscope without first staining. You must firmly attach your bacteria to a glass slide before you can stain them. There are two important things to consider when preparing a slide for staining:

1. The bacteria must be evenly and lightly dispersed. If there are too many bacteria on the slide they will form a big glob and you will not be able to see the morphology of the individual cells. Large blobs of cells also do not stain properly and could yield erroneous results from the improper staining.
2. The bacteria need to be firmly attached to the slide so they are not washed off during the staining procedures. All procedures that attach the bacteria to the slide result in some morphological changes. The cells typically shrink in size and will exhibit some changes in shape and extra-cellular matrixes.

You will be preparing slides for staining from both broth and agar surfaces. While the goals are the same for both, evenly and lightly dispersed cells firmly adhered to the slide surface, the techniques are slightly different. Staining is as much art as science. It will undoubtedly take you several tries before you are successful.

Materials

- Clean glass slides
- Inoculating loops or needles
- Sterile water
- Marking pen
- Assorted broth and plate cultures

Safety Considerations

Be careful of aerosols when transferring bacteria from your loop to the slide. The loop is very flexible and it is easy to zing off a loop-full of organisms. Do not assume your organism is dead. Heat or methanol fixation is not guaranteed to kill the organism. Dispose of your completed slides in the disinfectant bucket at your bench.

General Considerations

You are striving for a light suspension of cells that will leave a faint cloudy deposit on your slide. You have lots of room on your slide; use it! It helps to initially draw a circle on the bottom of the slide so you know where to look for your smear. It is very easy to get confused which side of the slide your smear is on. Be sure to label the far edge of the slide. Do this consistently on the same end of the slide to help orient your slide.

Be patient and take the time to let your slide air dry before proceeding with adhering it to the slide. If your slide is wet and you heat fix it, the bacteria will boil and the cellular morphology will be lost. If your slide is wet and fix it in methanol, it will most likely wash off the slide. Smears that are too thick will most likely wash off the slide regardless of the fixation method.

Smear from Broth

Broth cultures are usually easier to work with because the cells are already diluted in the broth. Be sure to carefully mix the culture tube to suspend the bacteria in the broth.

1. Label your slide. Aseptically transfer a loop-full of organism onto the center of your slide.
2. Use the flat part of the loop to smear the broth drop around the slide. Use a spiraling, circular motion to spread out the drop. Because the broth is full of protein, the smear will usually stay spread out and not bead up on the surface of the slide.
3. Set the slide aside to air dry. This will take several minutes at least. Do not rush this step.

Smear from Plate

You can scoop a lot of organisms off with your loop. You may want to use an inoculating needle to transfer your organism to the slide. Be sure to use sterile water to dilute your samples. Regular tap water or the de-ionized water in your rinse bottles are often contaminated with bacteria.

1. Label your slide. Aseptically transfer a loop-full of sterile water to the center of the slide.
    - This serves to both dilute your bacteria and give you something to spread around.
2. Pick a well-isolated colony.
3. Prick it with your sterile needle, or slightly scoop the edge of the colony with your sterile loop.
4. Place your needle/loop in the center of the drop and with a spiraling circular motion spread the bacteria on the slide.
5. Set the slide aside to air dry. This will take several minutes at least. Do not rush this step.

Fixation

The fixation procedure is the same regardless of smear source, plate or broth. There are two methods of adhering your bacteria to the slide, heat fixation or methanol fixation. Heat fixing is only used with BSL 1 organisms. The organisms we will be working with are BSL 2, so you will need to use the methanol fixation technique. Heat fixing the slide can create aerosols and with BSL2 organisms, we need to prevent this as much as possible. Methanol fixation causes fewer changes in cellular morphology and creates no aerosols. Please be careful when working with the methanol, if you forget you have fixed it with methanol and your slide isn't totally dry, the remaining methanol will catch on fire.

Methanol Fixing (BSL 2)

1. Be sure your slide is totally dry. Set it on the staining rack over the sink.
2. Carefully flood the slide with 95% methanol. Let it sit for two minutes.
3. Tilt the slide and pour off the methanol.
    - Touch the edge of the slide to a paper towel to wick off the excess methanol.
4. Set the slide aside to air dry before staining.