

DETERMINING MOTILITY

One characteristic that is useful in helping to identify an unknown organism is whether or not the organism is motile. Described below are three methods which can be used to determine motility.

Organisms to be studied: *Escherichia coli*, *Staphylococcus aureus*, or whatever your instructor assigns.

Note: Be especially careful when handling slides with live culture as this poses a risk of contamination.

The Wet Mount

Materials: Clean slides, cover slips, nutrient broth cultures, inoculating loop.

Procedure:

- Use the vortex mixer to mix the broth culture.
- Place several loopfuls of a pure bacterial culture on a clean slide and cover with a cover slip.
- Observe under the microscope. Reduce lighting to a minimum for better contrast.
- Begin focusing with the lowest power objective, as always, and work up to the oil immersion objective.

If the organism is motile, you should see some of the bacteria darting about. In some cases, only a few bacteria will be moving, while the others are still. The organism must still be considered motile.

Caution: A common mistake is to confuse Brownian motion (or movement) with motility. Brownian movement is a continuous vibrating motion caused by invisible molecules striking the bacteria. If the bacteria are truly motile, their movement will be over greater distances and will be multi-directional, not just back and forth.

Advantages: This method is the simplest and quickest way to determine motility. It is also useful for determining cellular shape and arrangement which is sometimes destroyed during the staining process.

Disadvantages: This method is far too risky to use with highly pathogenic organisms.

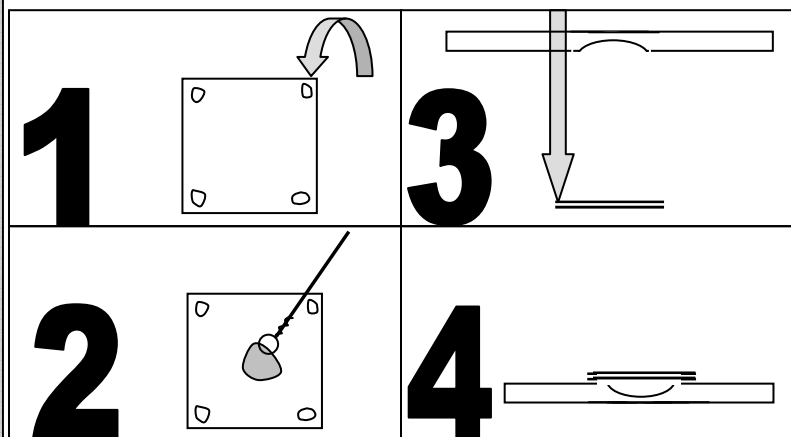
Disposal: Slide and coverslip should be placed in the broken glass biohazard box on the biohazard table.

The Hanging Drop Method

Materials: depression slide, coverslip, Vaseline, toothpicks, nutrient broth cultures.

Procedure:

- With the coverslip on a flat surface, place a small amount of Vaseline near each corner.
- Transfer several loopfuls of broth culture to the center of the coverslip.
- Take a depression slide and, with the concave portion over the drop, press the slide onto the coverslip.
- Invert the slide quickly to keep from disrupting the drop.
- Begin focusing with the lowest power objective and work up to the oil immersion objective. Be careful to not break or displace the coverslip.



The Hanging Drop Method, continued...

Advantages: Like the wet mount, the hanging drop method preserves cell shape and arrangement. The Vaseline-sealed depression also slows down the drying-out process, so the organisms can be observed for longer periods.

Disadvantages: The hanging drop method is also far too risky to use with highly pathogenic organisms.

Disposal: Place depression slide in the plastic beaker labeled “Depression Slides”; these will be autoclaved and reused. Place the coverslips in the broken glass biohazard box on the biohazard table.

Culturing Method Using a Semisolid Medium

Materials: Motility media, nutrient broth or nutrient agar slant cultures, inoculating needle.

Procedure:

- Use vortex mixer to mix the broth culture.
- Working in pairs, label three tubes of motility media—one for each organism to be cultured.
- Using an inoculating needle and the stab technique, aseptically transfer each organism to a tube of motility medium.
- Incubate 24-48 hours.

VERY IMPORTANT: Inoculation is with a straight wire that is stabbed two-thirds of the way into the media.

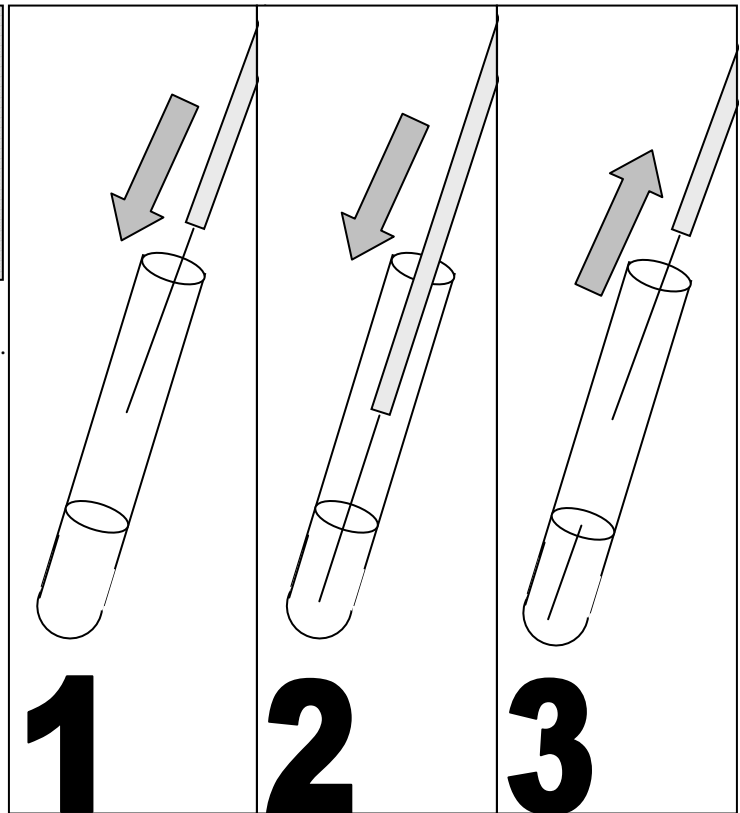
Reading the Motility Media:

Visible stab line, with cloudiness/pink color: motile
No distinct stab line, but with cloudiness/pink color elsewhere in the tube: motile
Visible stab line, clear media: non-motile

Advantages: This technique is useful for determining the motility of highly pathogenic bacteria. It is also easier to read than the other techniques.

Disadvantages: This technique takes longer to provide results than the wet mount or hanging drop.

Disposal: Place the tubes in the white baskets on the biohazard table, just like any other tubed media.



Making a Bacterial Smear

If you don't have a broth culture for the wet mount or hanging drop techniques, you can always make a smear from a slant culture. Directions for doing this are on page 150 of your lab manual.

When performing the hanging drop technique, it is recommended that you mix the bacteria and water thoroughly on a regular slide, as described on page 150. Be sure all the clumps are broken up. Don't try to use this slide for your hanging drop. Use your loop to move a drop or two of your mixture to the coverslip you will use for the hanging drop. Dispose of the slide you used for mixing your smear in the broken glass biohazard box on the biohazard table.