

Sterile Technique

Good sterile technique is the first and most important step in insuring consistent results when employing recombinant DNA and protein expression techniques. Sterile technique refers to procedures by which cultures may be manipulated without infecting the worker or contaminating the cultures or the laboratory environment.

Because contaminating bacteria are ubiquitous and are found on fingertips, bench tops, etc., it is important to minimize contact with these contaminating surfaces. When students are working with the inoculation loops and agar plates, you should stress that the round circle at the end of the loop, the tip of the pipetter, and the surface of the agar plate should not be touched or placed onto contaminating surfaces.

The flaming of lips of tubes and flasks must ALWAYS be done whenever culture liquid is to be poured from a container (e.g., pouring plates). Flaming should be routinely done when caps are removed from tubes during transfer of cultures. The purpose of flaming is not to sterilize, but to warm the tube and create warm air convection currents up and away from the opening. This "umbrella" of warm, rising air will help to prevent the entrance of dust particles upon which contaminating bacteria reside.

Petri dish lids prevent dust from falling directly onto plates but allow diffusion of air around the edges. There are no direct air currents into the plate, and to enter, dust particles would have to rise vertically more than a centimeter. This does not often occur because of the density of the particles. Whenever the lid is removed, it should be held over the plate as a shield. Do not place the lid on the bench top. Do not leave plates uncovered. Do not walk around the room with an open plate.

When working with cultures in test tubes, work as rapidly as is consistent with careful technique. Keep the tubes open a minimum amount of time. While the tubes are open, hold them at a 45 degree angle so that dust cannot fall into the open tube. Hold the tubes away from your face while transferring.

Test tubes are handled in the following manner:

- The test tube is held in the left hand (for a right-handed person).
- The instrument (loop, pipette, or needle) is held in the right hand.
- The test tube cap is grasped by the little finger of the right hand, and removed.
- While continuing to hold the cap with the little finger, the tube is lightly flamed and the instrument is manipulated appropriately, and withdrawn.
- The cap is replaced on the test tube and the test tube is put back into the rack.

Label all cultures with the name or number of the organism, and your name.

Always clean all work areas (your bench, balance area, sink area, gel area, etc.) thoroughly before leaving the laboratory! The last step before leaving the lab is to wash your hands thoroughly.

These are guidelines. You may find a set of techniques that best suite your working style. This is fine as long as you adhere to the basic concepts of good sterile technique.