

# Sterile Technique

**Purpose:** Sterile, or aseptic, technique is a central concept in microbiology and good sterile technique is a very important part of working in a microbiology lab. The goal of sterile technique is two-fold. The first part is to prevent the contamination of a bacterial culture with bacteria from the environment. Clinical and research microbiology labs both depend on a bacterial culture containing only the type or types of bacteria of interest, and not other types of bacteria which entered the culture from the environment. Equally important is the prevention of possible contamination of the lab worker, or others, by the potentially pathogenic bacteria he/she is working with.

**General Principles:** Achieving an effective aseptic technique may be one of the most difficult skills to learn in a microbiology lab, but it is also one of the most important. The difficulty lies in remembering to apply the fundamental principles of sterile technique. With practice, sterile technique will become second nature; until then, it requires constant attention.

1. Disinfect the work area before starting to reduce potential contaminants on the benchtop, and after work is finished to protect others from possible contamination.
2. Flame the inoculating loop before and after making a transfer of bacteria from one container to another. Never lay an inoculating loop on the benchtop if you are not sure it has been flamed first. When in doubt, flame the loop again.
3. Flame the opening of glass containers before removing bacteria from them and again after bacteria have been removed. Likewise, flame the opening before transferring bacteria to a container and again after the transfer is completed.
4. Do not lay the cap to containers of bacteria on the benchtop while bacteria are removed from or transferred to the container. The cap should remain under your control throughout the transfer.
5. Work quickly and efficiently to minimize the time the culture is exposed to the environment.

**Example:** As an example of a typical transfer, these are the steps required for good sterile technique in transferring bacteria from one capped test tube to another. Note that once the transfer is started, nothing is laid on the benchtop and everything is flamed before and after.

1. Flame the inoculating loop
2. Flame the mouth of the tube while holding the cap
3. Remove some bacteria with the loop
4. Flame the mouth of the tube and replace the cap
5. Flame the mouth of the tube to which the bacteria are being transferred
6. Inoculate the tube with bacteria from the loop
7. Flame the mouth of the tube and replace the cap
8. Flame the inoculating loop