Aseptic Technique and Cell Culturing Protocol (2017)

This protocol describes basic procedures for aseptic technique for cell culture in biotechnology. This protocol should be used along with pages 65-66 in your textbook. One basic concern for successful aseptic technique is personal hygiene. The human skin harbors a naturally occurring and vigorous population of bacterial and fungal inhabitants that shed microscopically and ubiquitously. Most unfortunately for cell culture work, cell culture media and incubation conditions provide ideal growth environments for these potential microbial contaminants. This procedure outlines steps to prevent introduction of human skin flora during aseptic culture manipulations. Every item that comes into contact with a culture must be sterile. This includes direct contact (e.g., a pipet used to transfer cells) as well as indirect contact (e.g., flasks or containers used to temporarily hold a sterile reagent prior to aliquoting the solution into sterile media). In the biotechnology pathway we will mostly use equipment that will need to be sterilized using the techniques of this protocol.

Materials: Antibacterial soap**,** 70% isopropanol, 91% isopropanol**,** clean laboratory coats or gowns**,** non-latex surgical gloves**,** clean and quiet work area**,** Bunsen burners

Take personal precautions:

1. Just prior to aseptic manipulations, tie long hair back behind head. Vigorously scrub hands and arms at least 2 min with an antibacterial soap. Superficial lathering is more prone to loosening than removing flaking skin and microbial contaminants. Loosely adhering skin flora easily dislodges and can fall into sterile containers.
2. Gown appropriately. For nonhazardous sterile-fill applications, wear clean laboratory coats and latex gloves. Safety glasses should be worn when manipulating biological agents.
3. Frequently disinfect gloved hands with 70% isopropanol while doing aseptic work. Although the gloves may initially have been sterile when first worn, they will no doubt have contacted many non-sterile items while in use. Wash hands after removing gloves.

Prepare and maintain the work area:

4. Perform all aseptic work in a clean work space, free from contaminating air currents and drafts. For optimal environmental control use a Bunsen burner to maintain a constant upward air current (figure 1). Never leave the burner unattended when using this technique. One student must always be present, if not, turn the burner off.
5. Clear the work space of all items extraneous to the aseptic operation being performed.
6. Wipe down the work surface before and after use with 70% isopropanol or other appropriate disinfectant.
7. Wherever feasible, wipe down items with disinfectant as they are introduced into the clean work space. Arrange necessary items in the work space in a logical pattern from clean to dirty to avoid passing contaminated material (e.g., a pipet used to transfer cultures) over clean items (e.g., flasks of sterile media).
8. When the aseptic task has been completed, promptly remove any larger contaminated items or other material meant for disposal (e.g., old culture material, spent media, waste containers) from the work space and place in designated bags or pans for autoclaving. Disinfect the work space as in step 6.

Flame sterilize the opening of a vessel:

9. For a right-handed person, hold the vessel in the left hand at ~45° angle (or as much as possible without spilling contents; figure 2) and gently remove its closure. Do not permit any part of the closure that directly comes in contact with the contents of the vessel to touch any contaminating object (e.g., hands or work bench). Ideally, and with practice, one should be able to hold the closure in the crook of the little finger of the right hand while still being able to manipulate an inoculating loop or pipettor with the other fingers of the hand. Holding the vessel off vertical position while opening will prevent any airborne particulates from entering the container.
10. Slowly pass the opening of the vessel over the top of (rather than through) a Bunsen burner flame to burn off any contaminating matter. Be careful when flaming containers of infectious material. Any liquid lodged in the threads of a screw cap container will spatter as it is heated. Aerosols thus formed may actually disseminate entrapped biological agents before the heat of the flame is hot enough to inactivate them.

11. While still holding the vessel at a slant, use a sterile pipet and pipettor to slowly add or remove aliquots to avoid aerosol formation.
12. Flame-sterilize again as in step 10, allow the container to cool slightly, and carefully recap the vessel.

Flame sterilize small hand instruments:
13. Dip critical areas of the instrument (i.e., those that come into contact with the material) in 91% isopropanol. Make certain that the alcohol is in a container heavy enough to support the instrument without tipping over. CAUTION: 91% isopropanol is flammable; keep the container at a safe distance from any open flame.
14. Remove the instrument from the alcohol, being careful not to touch the disinfected parts of the instrument. Allow excess ethanol to drain off into the container.
15. Pass the alcohol-treated part of the instrument through the flame of a Bunsen burner and allow residual alcohol to burn off.
16. Do not let the sterilized portion of the instrument contact any nonsterile material before use. Let the heated part of the instrument cool for ~10 sec before use.

Flame sterilize inoculating loops and needles:
17. Hold the inoculating wire by its handle and begin in the center of the wire to slowly heat the wire with the flame of a Bunsen burner. Proceed back and forth across the wire's full length until it glows orange.
18. While still holding the handle, allow the inoculating wire to cool back to room temperature (~10 sec) before attempting any transfer of material. If tranfers are made while the inoculating wire is hot, cells will be killed by the hot wire, and aerosols created from spattering material can disperse contaminants throughout the workspace.
19. After the transfer is made, reheat the inoculating wire as in step 17 to destroy any remaining biological material. Let cool to room temperature before putting aside for next use.

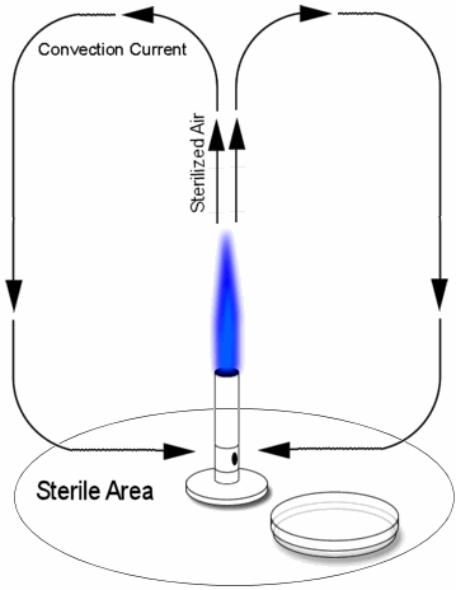

Figure 1

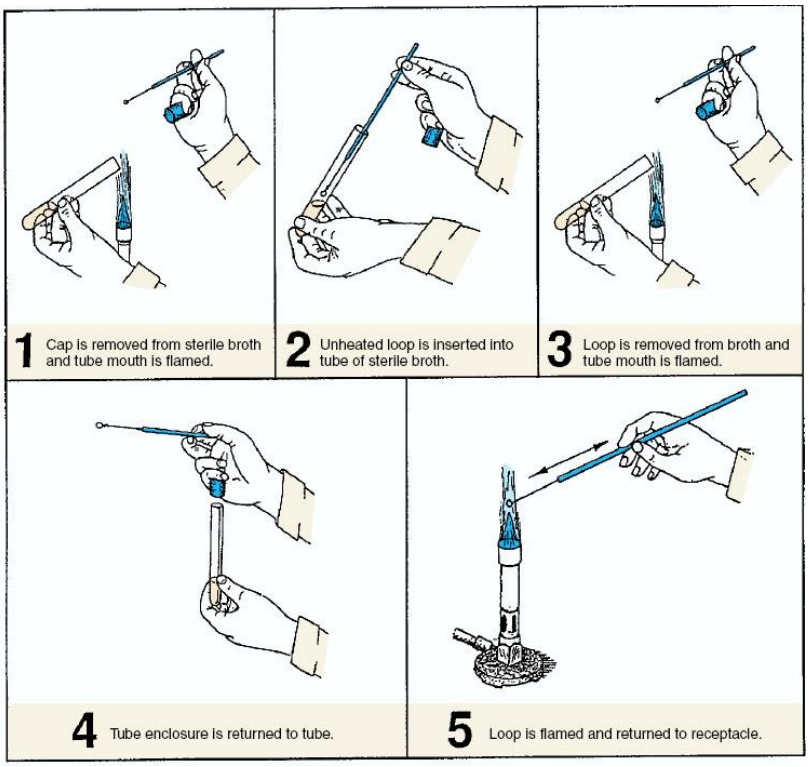

Figure 2

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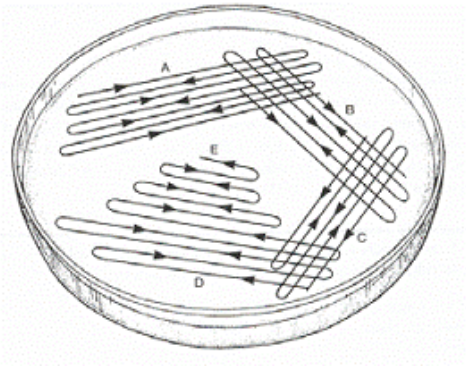

Figure 1: Streaking an agar plate is done in 3-4 sections. The goal is that sections "D" and "E" will yield isolated, individual colonies. **Flame loop between each section.**

Figure 3: Always label the bottom of the petri before pouring media.

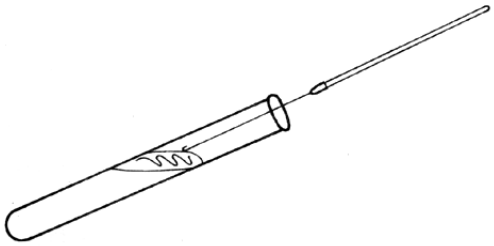

Figure 2: Streaking and inoculating a slant.

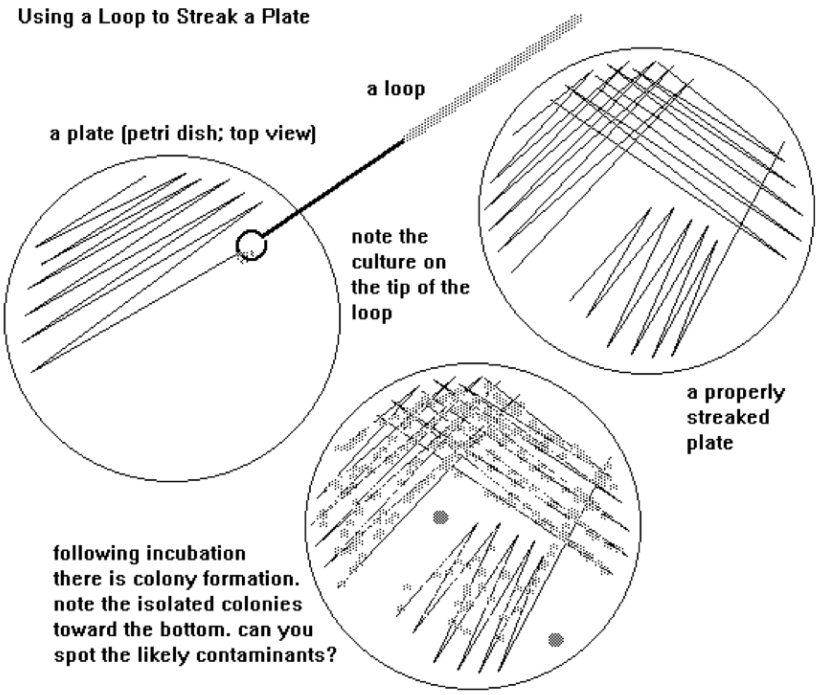

Figure 4: Technique for streaking a plate in three sections using an inoculating loop.