

STERILE TECHNIQUE: DELIVERY OF LIQUIDS BY PIPET page 40

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http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Yeast_Plate_Count/Sterile_Tech_pipet.htm

Pasteur observed that once a medium is sterilized, it remains sterile unless and until it is contaminated from an external source. He used this observation to reject spontaneous generation but it also lays the foundation for sterile (or aseptic) technique, a technique crucial to microbiology and medicine. Several practices are important to the success of this technique:

- 1) **Sterilize the instruments and keep them that way:** keep instruments off possibly contaminated surfaces, and flame them to incinerate loose material which might fall off (very briefly for plastic pipet tips). Never lie pipets down on the surface of a workbench.
- 2) **Sterilize the field** where work is to be performed with 70% EtOH.
- 3) **Exclude airborne contamination:** keep containers covered as much as possible
- 4) **Minimize drafts:** no breathing, coughing, *etc.*, over sterile materials.

Mastering sterile technique is one of the most important prerequisites to working in a microbiological lab. While the steps may seem overly detailed in the following narrative, early attention to learning proper technique establishes good technique for the rest of your life. Compare these detailed steps with the demonstration given by the professor. Patience pays off. Go slowly at the beginning, and verbally (*not physically*) assist your fellow students as they work through the steps.

PREPARATION:

1. **Write out the procedure** which you plan to perform in your notebook. Indicate how the sample was prepared and volumes to be transferred. **Construct a table** of plates to be spread, with plate number, specimen, plate type, dilution factor, aliquot, and sufficient blank columns for processed data (colonies counted and final calculated numbers, etc). Label tubes or plates appropriately.
2. **Loosen all caps, mentally review the steps** you will be performing and arrange materials to ensure they are available and easily accessible.
3. Select the appropriate pipet for volume to be delivered, and set the volume to be transferred by rotating the plunger. Pick up a sterile tip, reclose the lid on the box of tips immediately.
4. Hold displacement pipetter so that the thumb operates the plunger and you *keep your little finger on the pipetting hand free for grasping the cap of the vessel.*
5. Pick up specimen vessel with other hand, grip cap of vessel with little finger of pipet hand and twist the vessel and pull it down away from the cap. **Do not lay the cap down.**
6. Flame lip of vessel.

USING THE PIPET:

7. Pass sterile pipet through flame. (Very briefly so that the plastic tip is not melted...)
8. **DRAW UP SPECIMEN:** Immerse tip of pipet 1/2 cm below surface of liquid, brace in place by gently pressing pipet against the lip of the tube. Allow the thumb plunger to slowly draw the fluid up. (If you draw the fluid too quickly, you will get bubbles in the tip.)
9. **TRANSFER ALIQUOT:** Touch off excess fluid from tip of pipet, tilt pipet horizontally to prevent dribbling during transfer. (The fluid will draw up slightly into the tip pipet.)
10. Reflame the lip of specimen vessel, screw it back into the cap, set vessel down.
11. **DELIVER ALIQUOT:** Pick up receiving vessel. Remove its cap and flame its lip (as in steps 5 and 6). Insert pipet just below the surface of the liquid, return to vertical, deliver desired volume, press to blow out the fluid, *hold in that position*, withdraw the pipet, flame vessel lip, replace cap, discard tip into used pipet container for sterilization.