

STERILE TECHNIQUES

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Hood Preparation

1. Wear gloves to protect yourself but also to prevent dry skin and micro-organisms from contaminating your samples.
2. Swab down the work surface liberally with 70% ethanol. Start from the back and proceed forward. Swab during work if necessary.
3. Swab any instruments that will be used in the hood with 70% ethanol, particularly the pipettes, which will often be used above biological samples.
4. Keep sterile pipette tips in “Hood Only” boxes that are opened only in a sterile environment. Swab the exterior of the box with 70% ethanol.
5. Bottles should always be tightly capped when outside the hood (*i.e.*, they should have been tightly capped the last time they were *in* the hood).
6. Dry bottles thoroughly if they have been taken out of the water incubator. Swab them with 70% ethanol, especially at the neck and the bottom, and place them directly into the hood. Avoid shaking them vigorously during handling.
7. Bring only the items you need for a particular procedure into the hood to prevent cluttering your working space. Having a clear working space will significantly reduce the chance of contamination! Ensure easy access to items in the hood and maintain plenty of clear space in the center of the hood to work in.

Sterile Handling

1. Spray gloves with 70% ethanol as often as necessary.
2. The indicator stripes on the autoclave tape should turn black if an object has been properly autoclaved.
3. Never block the negative pressure zone (also the frontal non-sterile area) of the vertical laminar flow hood with objects (*i.e.*, notebooks, pipette handle).

4. Avoid working too closely to the front, or the non-sterile area, of the hood. Keep working area at the center or towards the back. Keep the objects needed for the current procedure within reach; keep the others in the back.
5. Avoid working above an open bottle or dish in vertical laminar flow. Always work around them unless they are capped or covered.
6. Avoid leaving bottles, dishes, and flasks open when they are not in use. If the cap must be laid down, place it face-up/face-down towards the back of the hood where there is less traffic and less chance of being touched or crossed over. Correct cap placement has been debated. Having a cap facing up can potentially introduce airborne particles and drive non-sterile lid liquid onto the interior face of the cap, where contaminations can fall into the bottle upon recapping. If face-down placement is preferred, then make sure to swab the area specifically and thoroughly before the cap is placed down there. Conversely, if hood surface sterility cannot be *absolutely* guaranteed due to high traffic or cluttering, then face-up is a better option. The best placement, however, is to place the cap on its side and towards the back of the hood. This way the interior is not in contact with the air flow or with the work surface. However, this is not possible with dishes. Therefore, exercise good judgment in light of individual operating style and the hood setup.
7. Never pour from one sterile container to another. Pouring will generate a liquid path to introduce infection from the outside to the inside. Always pipette or use filters when transferring from one bottle to another.
8. Mop up any spillage immediately and swab with 70% ethanol to avoid the chance of growth of micro-organisms.
9. Withdraw a pipette from its wrappers at the center of the working area, tilt it such that the tip (bottom end) is pointing away from the frontal non-sterile area and away from other objects in the hood.
10. Withdraw the pipette such that it slides through the sterile interior of the wrapper without touching the outside of the wrapper.

11. Avoid contact between the tip of the pipette and the mouth of the bottle. The mouth and neck of the bottle (both inside and out) present a potential source of contamination.
12. When working with Pasteur pipettes, do not remove pipettes directly from the box with fingers. Shake the tube gently to cause the pipettes to slide out slightly, and then withdraw a pipette with fingers without touching the other pipettes and the tube interior.
13. To keep the hood from being cluttered, do not leave any trash in the hood. Immediately discard uncontaminated wrappers in the regular trash. Put all pipette tips and biologically contaminated sharps in the sharps biohazard waste container. Put all biologically contaminated tissue culture plates, flasks, and other non-sharps in the non-sharps biohazard waste container. However, an effort to minimize entry/exit from the hood should be made to minimize disturbances in the laminar flow at the entrance, which may create the potential to waft in contaminants.
14. Handle the pipette with a steady hand. Avoid large motions and do not let the tip touch anything non-sterile. Keep the tip away from the front and far above the objects in the hood.
15. Do not fill a dish/flask so full or swirl it such that the medium spills over the edge. This will introduce a path of infection via liquid and may cause cross-contamination.

Cleaning up

1. Cap bottles tightly before removing them from the hood.
2. Swap down the work surface liberally with 70% ethanol.
3. Turn off the vacuum, if used.