

Aseptic Technique

When performing cell culture work within a Biological Safety Cabinet Class II it is important to minimise the potential for contamination of the working environment and cross-contamination between cultures. This can be greatly assisted by the following:

- Wash or disinfect hands before and after handling cells.
- Wear gloves to protect yourself but also to prevent dry skin and micro-organisms from contaminating your samples. The gloves must be replaced immediately if torn or punctured or during extended work sessions.
- Remove or cover up all personal accessories (for example, rings, watches), which might compromise cell and tissue culture activities.
- Wear dedicated laboratory coat and remove it when you leave the Tissue Culture Room.
- Use of mobile phones in the TC area is forbidden.
- When handling cell and tissue cultures, avoid transferring contamination on the hands from the culture work to unprotected body parts (for example, eyes or mouth), clothing or items in the open laboratory environment. Do not talk, sneeze or cough towards the open hood.

Hood Preparation

1. Use 70% ethanol for sterilization of non-sterile equipment and surfaces
 - Swab down the work surface liberally with 70% ethanol. Start from the back and proceed forward. Swab during work if necessary.
 - Swab any instruments that will be used in the hood with 70% ethanol, particularly the pipettes, which will often be used above biological samples.
 - Dry bottles thoroughly if they have been taken out of the water bath. 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.
2. Open sterile equipment or culture dishes only inside the hood
 - 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.
 - 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).

3. 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, and/or change gloves frequently.
2. The indicator stripes on the autoclave tape should turn black if an object has been properly autoclaved.
3. Mop up any spills immediately and swab with 70% ethanol to prevent the growth of microorganisms.
4. 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.
5. Never have more than one cell line at a time in the cabinet.
6. Working with pipets
 - Withdraw a pipette from its wrapper at the center of the work area, tilt it so the tip (bottom end) is pointing away from the frontal non-sterile area and away from other objects in the hood.
 - Withdraw the pipette so that it slides through the sterile interior of the wrapper without touching the outside of the wrapper.
 - 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.
 - 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.
 - 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.
 - Manipulate fluids slowly and gently with the assistance of a pipetting aid to avoid the creation of aerosols.
7. Minimize disruption of work area and around samples
 - Never block the negative pressure zone (also the frontal non-sterile area) with objects (i.e., notebooks, pipets etc.).
 - Do not make rapid movements within the cabinet, as this may disrupt the airflow.
 - Avoid working too close to the front 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.

- Avoid working above an open bottle or dish in vertical laminar flow. Always work around them unless they are capped or covered.
- To keep the hood from being cluttered, do not leave any trash in the hood.
- Avoid leaving bottles, dishes, and flasks open when they are not in use.
- Organise the work area such that sterile reagents and cultures do not come in contact with each other.

Cleaning Up

1. Cap bottles tightly before removing them from the hood.
2. Remove all waste for sterilization and disposal.
3. Swab down the work surface liberally with 70% ethanol.
4. Remove gloves, wash hands.
5. If there is another booking in less than 1 hour => leave the hood working, otherwise:
6. Close the hood.
 - Turn off light.
 - Allow hood to run a further 5 minutes to effectively purge air.
 - Turn off mains.
 - Close window fully, or replace front guard.
 - Turn the UV light timer on.
7. Dispose of waste from the cell culture appropriately.
 - Chemically inactivate all liquid waste (by using 0.5-1% Virkon solution or sodium hypochlorite), soak overnight, and dispose of by the general sink the next day. **DO NOT LEAVE THE LIQUID WASTE SOAKING FOR DAYS.**
 - Collect all plastic waste in an autoclavable plastic bag (**DO NOT OVERFILL**), labelled it with your name and TC Room number and take to the wash-up room 2.

Maintenance of Tissue Culture Equipment

1. Every 2 weeks remove pans from incubators, empty them and clean thoroughly. Replace water pan and fill with 1L RO H₂O with F10 detergent (diluted 1:1000).
2. Every 2 weeks empty water bath and clean thoroughly. Fill the water bath with approximately 5L RO H₂O with F10 detergent (diluted 1:1000).
3. Every 3 months clean incubators thoroughly.
 - Turn the CO₂ off.
 - Remove shelves and water pan, empty and clean thoroughly.

- Wipe down all surfaces thoroughly with F10 detergent (diluted 1:1000) followed by 70% ethanol.
 - Re-assemble shelves. Replace water pan and fill with 1L RO H₂O with F10 detergent (diluted 1:1000).
 - Turn the CO₂ on.
 - Allow incubator to stabilise at the correct temperature and CO₂ levels.
4. Every 3 months clean cabinets thoroughly.
- With cabinet ON, remove work surfaces and any other removable components.
 - Wipe down all surfaces thoroughly with F10 detergent (diluted 1:1000) followed by 70% ethanol.
 - Dry any residual moisture from work surfaces and any other removable components and re-assemble the cabinet.
 - Turn cabinet off, close window or replace front guard and expose to UV for 15 minutes. Place a notice on the cabinet informing users of appropriate times for recommencing work in the hood.