Guidelines for Working in the Eukaryotic Cell Culture Facility

The facility is a biosafety level 2 (BSL2) laboratory designated by JHU Environmental Health and Safety, according to the CDC/NIH Joint Guidelines on Biosafety in the Biomedical Laboratory. The facility has been designed specifically to facilitate eukaryotic cell culture projects by multiple users, who maintain and employ numerous mammalian and insect cell lines for research purposes. The mission to facilitate the large scale of expression of recombinant protein in eukaryotic cells, and to help client investigators achieve their research goals with the lowest possible cost in the shortest time.

Every user must take training on the biosafety procedures and equipment introduction before working in the facility (see requests). All cell culture must be undertaken in microbiological safety cabinet using aseptic technique to ensure sterility.

Basic safety procedures for cell culture

1. Wash your hands before and after.
2. Use of proper personal protective equipment (PPE). For example, gloves, labcoats are required. Protective glasses are advisable during particular work phases. Labcoats should be cleaned through a service such as Lord Baltimore offers.
3. Close toe shoes are required; if you have long hair, tie it in the back
4. Be aware of the potential for biohazard.
5. Decontaminate work surface before and after use.
6. Use sterile reagents and media and work to keep them that way (do not reuse tips, clean the outside of reagent bottles with 70% ethanol, autoclave or sterile filter as appropriate.
7. Keep bottles, flasks and tubes covered as much as possible.
8. HOODS are only intended for sterile work. Keep the hood as clear as possible and clean it (with ethanol 70%). Switch blower off after each use and turn on UV lamp.
9. Properly dispose of biological and chemical waste.
10. If any contamination suspected, please inform facility manager instantly.

Hood Preparation

1. Use 70% ethanol for sterilization of non-sterile equipment and surfaces
   1) Swab down the work surface liberally with 70% ethanol. Start from the back and proceed forward. Swab during work if necessary.
   2) Swab any instruments that will be used in the hood with 70% ethanol, particularly the pipettes, which will often be used above biological samples.
   3) 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.

2. Open sterile equipment or culture dishes only inside the hood
    1) 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.
    2) 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.
4. Leave the laminar flow hood properly set up.

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. Working with pipets
    1) 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.
    2) Withdraw the pipette so that it slides through the sterile interior of the wrapper without touching the outside of the wrapper.
    3) 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.
    4) 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.
    5) 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.
    6) When working with Pasteur pipettes, do not reach into the box to remove it. Instead, shake the box gently to cause the pipettes to slide out slightly, and then withdraw a pipette without touching the other pipettes or the tube interior.
6. Minimize disruption of work area and around samples
    1) Never block the negative pressure zone (also the frontal non-sterile area) of the vertical laminar flow hood with objects (i.e., notebooks, pipetteman handle).

2) 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.
3) Avoid working above an open bottle or dish in vertical laminar flow. Always work around them unless they are capped or covered.
4) 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. 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.\

Use of Cell counter

It is store in XXX Direct cell counts on samples obtained from dense populations can be accomplished using a hemacytometer. Each hemacytometer has two separate counting area etched into the metal strips. Each counting area has nine large squares used for counting large eukaryotic cells, and each is made up of smaller squares. The small squares are used for counting small cells like bacteria. Each of the large squares in the four corners has sixteen small squares, while the large square in the center has twenty-five small squares. A special coverslip is supported above the grid such that the volume above each large square is equal to 10^{-4} ml (0.1µl). The trick in using a hemacytometer is in getting an even distribution of cells over the squares. To do this, first the coverslip is placed on the hemacytometer. A drop of evenly suspended cells is then introduced to the edge of the grid. Fluid is drawn under the coverslip by capillary action. If the volume is too great and fluid overflows into the gutters, the preparation cannot be used. If it takes more than one application to cover the grid, it cannot be used. If the drop is held too long before applying it to the hemacytometer, it cannot be used. To check on accuracy, the numbers from several squares are obtained. If the numbers are, they can be averaged; if they are far apart, the hemacytometer should be cleaned and reloaded.

Cleaning Up and Before you leave

1. Cap bottles tightly before removing them from the hood.
2. Swab down the work surface liberally with 70% ethanol.
3. Turn off the vacuum, if used, and hang associated tubing inside the hood.
4. Switch hood blower off after each use and turn on UV lamp.
5. Turn off the centrifuge and microscope.
6. Make sure the CO2 incubator door is closed all the way.

7. Wash your hands

In case of contamination

Though quality of cell culture work correlates with experience, chances of contamination will never be eliminated especially in multi-user facility. If any contamination is suspected, please inform facility manager instantly.

1. Bottle caps should be tightened. Please, go to the facility and remove flask ASAP. After contamination events the cell culture work will be done at pre-arranged times under the supervision of the facility manager.
2. Users will arrange with the lab manager to do cell work under supervision to improve the technique to work under sterile conditions
3. Once every two months all users handling mammalian cell cultures would submit samples for mycoplasma contamination testing at their cost.

Other Rules of JHMI ETCF

- Users should be trained by the Eukaryotic Tissue CultureFacility Manager before using the facility.
- Each user would be charged per month a facility user fee besides the charges per use of equipment.
- Users should book the equipment (hood, stirrers, orbital shakers and centramate) using the icalendar.
- Users should request reagents (cells, cellfectin, media, etc) , services (autoclave, amplification of baculovirus by facility personnel etc) on ilab.

References

https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/introduction-to-cell-culture.html

http://www.vanderbilt.edu/viibre/CellCultureBasicsEU.pdf