

**Standard Operating Procedures (SOP) for Cell Culture Rooms**  
**James I. Moss, Maria B. Padua and Peter J. Hansen**  
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The policies outlined here are to be used for common routine maintenance and cleaning of the cell culture facilities. Some of these recommendations were taken from the University of North Carolina Tissue Culture Facility.

**General**

No farm cloths, boots, lab coats or any equipment contaminated with blood or any other animal tissue should be permitted in the cell culture room areas.

All bench and work areas should be kept with as few items and pieces of equipment as possible.

Keep the access door for the culture rooms closed when the incubator door is open.

For the Class II, Type A hoods (the air blower hoods that cannot be completely closed), hoods should be left on 24 hours a day.

No items should be stored inside the hoods unless the supplies are off the surface of the hood (Exception: sterile pipettes in the IVF Lab).

It is encouraged to wipe down with 70% ethanol all other items placed in the hood such as pipetting devices, bottles of pre-warmed media, aliquots, etc.

**To be done daily**

Hood and bench work surfaces (including microscope working areas) must be cleaned and wiped down with water and then ethanol before and after every use.

If full, biohazard bags and other trash receptacles must be sealed and removed from the lab at the end of the work day and new empty bag placed in the receptacle. If not full, the biohazard bags may be folded, closed and opened and used the following day.

**To be done weekly**

Once a week, water baths must be completely emptied and cleaned. The water used for replacement should be distilled or deionized water. A piece of copper pipe or a copper penny should be placed inside of the water bath. A few drops of a commercial water bath microbicide or benzalconium chloride (a 1:50 or 1:100 dilution of 1% solution) can be added if needed.

Once a week, incubator humidification pans must be checked and new distilled or deionized water added if needed. The water used for replacement should be distilled or deionized water.

Once a week, the incubator CO<sub>2</sub> readings should be confirmed with Fyrite test and recorded. Any anomalies in the readings should be reported immediately.

Every Friday, regardless of the amount of waste in the biohazard bags, bags must be sealed and taken out of the cell culture rooms. Under no circumstance should any waste in the biohazard bags be left in the cell culture room areas over the weekends.

Once a week, the incubator door rubbers and the refrigerator rubber must be cleaned with 70% ethanol.

Every Friday, at the end of the work day, floors should be swept and mopped with diluted bleach (10%).

Every Friday, the sticky mat on the floor should be removed and replaced with a fresh mat.

Once a week, centrifuge rotors and carriers must be cleaned and disinfected using 70% ethanol as well as the outside area of the centrifuge.

Once a week, the temperature of the incubators should be confirmed and recorded. Any anomalies in the readings should be reported immediately

### **To be done monthly**

Incubator humidification pans must be emptied, cleaned and refilled with distilled or deionized water. A piece of copper pipe or a copper penny should be placed inside of the water bath unless the incubator is copper lined.

### **To be done every 6 months (June and December)**

Clean incubators and ovens every 6 months or after any evidence of contamination in the cell cultures. Shelves, side supports and the humidification pans should be removed, cleaned with soap and water and autoclaved. The rest of the chamber should be cleaned and disinfected with 70% ethanol. After that, shelves and the humidification pan can be reinstalled. The chamber should be left overnight to equilibrate and readings of temperature and CO<sub>2</sub> should be checked before placing any cell culture dishes inside.

Clean hoods every 6 months or after any evidence of contamination in the cell cultures. The work surface and the air intake grills should be removed and cleaned with soap and water. The area under the work surface should be also cleaned (the bottom area of the hood) as well as the interior walls and the glass window (front and rear side). Disinfect with 70% ethanol before and after reassemble. Let the fumes dissipate overnight before any work.

Defrost freezer in outer culture room and freezer compartment in culture room refrigerator.

### **Standard Operating Procedures (SOP) for cell culture rooms when Biosafety Level Two (BSL2) procedures are performed**

All personnel are required to ensure that the Biosafety Level Two (BSL2) placard is displayed before commencing BSL2 procedures. All personnel are required to read and follow instructions on practices and procedures (SOPs) before using this space when the Biosafety Level Two (BSL2) placard is displayed.

When the Biosafety Level Two (BSL2) is displayed on the door, you may not enter this area without permission of Dr. P. J. Hansen, or Jim Moss (lab manager).

If you are immunocompromised, immunosuppressed, pregnant, or might be pregnant, do not enter this room.

Wash your hands after handling viable materials, after removing gloves, and before leaving the laboratory.

Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. No food is allowed in this work area.

Mouth pipeting is prohibited.

Wear laboratory coats while in the laboratory. Remove coats and leave in the laboratory before leaving for non-laboratory areas. If necessary, decontaminate protective clothing before laundering.

Wear gloves. Dispose of gloves when overtly contaminated, when working with infectious materials is completed or when the integrity of the glove is compromised. Do not wear disposable gloves for touching "clean" surfaces (keyboards, telephones, etc.), and do not wear them outside the lab. Wash hands following removal of gloves. Remove protective clothing and leave it in the laboratory before leaving for non-laboratory areas. Decontaminate protective clothing before laundering.

Maintain a separate biohazard bag labeled as BSL2 and remove at the end of each day. Decontaminate before disposal.

Use biological safety cabinets (BSC) when manipulating infectious materials. If centrifuging, use sealed rotor heads or centrifuge safety cups, and open only in a biological safety cabinet. Use face protection (goggles, mask, face shield or other splatter guard) when the microorganisms must be manipulated outside the BSC.

Decontaminate work surfaces on completion of work or at the end of the day and after any spill or splash of viable material with 10% bleach. Autoclave all cultures, stocks, and other regulated wastes before disposal. Put potentially infectious wastes in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping. Materials to be decontaminated outside of this laboratory shall be placed in a durable, leak proof container which will be closed for transport.

Report spills and accidents that may result in exposures to infectious materials to the laboratory director and contain and clean up the spill, and decontaminate with 10% bleach. Decontaminate contaminated equipment before it is removed from the facility with 10% bleach, or autoclave.

Report incidents that result in exposure to infectious agents or materials to the lab manager.