

Standard Operating Procedures (SOP) for B360C BSL2 Cell Culture Room

Vj k'r tqegf wtg'ku'c'b of k'ef 'h'ito 'qh'

1. Standard Operating Procedures (SOP) for Cell Culture Rooms James I. Moss, Maria B. Padua and Peter J. Hansen May 2, 2010
2. R. Ian Freshney (2005) **Culture of Animal Cells** a manual of basic technique. *Willey-Liss*. 642, 79-80.

GENERAL

No lab coats or any equipment contaminated with blood or any other animal tissue 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 room closed when the incubator door is open.

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

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

Wipe down the hood's working surface with disinfecting wipes before and after each use.

DAILY

Check daily the level of liquid nitrogen in the LN₂ cell storage vessel. If lower than the marked level immediately contact

Dr. Lyubovitsky (julial@ucr.edu, cell: 949-302-1866)

Check daily the high-pressure CO₂ gas cylinder. If the incubator incoming pressure reads below 4 Psi immediately contact

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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.

WEEKLY

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 autoclaved deionized water.

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 mat on the floor should be removed and replaced with a fresh mat.

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

MONTHLY

Incubator humidification pans must be emptied, cleaned and refilled with autoclaved 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.

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. 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 CO₂ 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.

STANDARD OPERATING PROCEDURES FOR 360C CULTURE ROOM WHEN BIOSAFETY LEVEL 2 (BSL2) PROCEDURES ARE CARRIED OUT

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) below before using B360C space.

The Biosafety Level Two (BSL2) is displayed on the door at all times, therefore, ALL personnel must be registered prior to entry into the lab.

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 leak proof red container next to the biological safety cabinet, which must be closed for transport.

Report spills and accidents that may result in exposures to infectious materials to Dr. Lyubovitsky. 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 Dr. Lyubovitsky.

OTHER USEFULL CELL CULTURE TIPS

- Wash hands with the soap before to wear the gloves.
- If you have long hair, tie it back.
- When working aseptically on an open bench, do not talk. Talking is permissible when you are working in a vertical laminar-flow hood, with a barrier between you and the culture, but should still be kept to a minimum.
- If you have a cold, wear a face-mask, or better still, do not do any tissue culture during the height of the infection.