

# Standard Operating Procedures for Cell Culture Rooms

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The policies and recommendations in this guide pertain to routine use, maintenance and cleaning of the cell culture facilities. Some of these recommendations were taken from the Standard Operating Procedures of the University of North Carolina Tissue Culture Facility – see <http://www.unc.edu/depts/tcf/info.html>

## General Considerations

- 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 the fewer amounts of items and apparatus as possible.
- Keep the access door for the culture rooms closed when the incubator door is open.
- Clean gloves should be worn to open and manipulate culture plates inside the incubator.
- For the Class II, Type A hood in Rm 238 499, the hood should be left on 24 hours a day. The room UV light is turned on from 2-5 am each day
- For hoods in Rm 123 (IVF Culture Lab), hoods should be turned on 15 minutes before use. The UV light in the laminar flow hood in the centrifuge area should also be turned on 15 minutes before use.
- No items should be stored inside the hoods (Exception: pipettor and Bunsen Burner in IVF Lab).
- All glassware, pipette tips and containers used in the hoods during media preparation or cell culture manipulation must be sterile.
- All other items such as pipetting devices, bottles of pre-warmed media, aliquots, etc. must be (Rm 238 499) or should be (IVF Lab) wiped down with 70% ethanol when practical before being placed inside the hoods.

## Daily Cleaning

1. Hood work surfaces must be cleaned and disinfected before and after every use.
2. All bench and work areas must be cleaned and disinfected before and after each use. The same applies for the microscope working areas.
3. For Rm 238, biohazard bags must be sealed and removed from the hood at the end of the workday and new empty bag placed in the receptacle. If not full, the bags may be folded, closed and opened and used the following day.
4. For the IVF Culture Room, tips are thrown in the red bags and media, oil, and plates are thrown in a zip-lock bag and the bag sealed and thrown in the biohazard box located in the outer culture area. The large biohazard box in the outer culture area will be thrown away each week.
5. The temperature of the incubators should be confirmed and recorded. Any anomalies in the readings should be reported immediately

## Weekly Cleaning and Maintenance - Rm 238

1. Every other Monday, disinfectant solutions such as 70% ethanol and 1% benzalconium chloride are prepared fresh.
2. Every Monday, water baths must be completely emptied and cleaned. The water used for replacement should be either fresh low hardness city treated tap water or distilled deionized water. Few drops of a commercial water bath microbicide or benzalconium chloride (diluting the 1% working solution 1:50 to 1:100) can be added and a sterile piece of cooper pipe can be placed inside of the water bath container.
3. Every Monday, incubator humidification pans must be emptied and cleaned. The same rules for the water baths apply for the incubator humidification pans. FOR NOW, we are excluding the benzalconium chloride from incubators used for IVF.

4. Every Monday, the incubator CO<sub>2</sub> readings should be confirmed with Fyrite Test and recorded. Any anomalies in the readings should be reported immediately.
5. Every Friday, regardless of the amount of waste in the biohazard bags, they must be sealed and taken out of the cell culture rooms. Under no circumstances any waste in the biohazard bags should be left in the cell culture room areas over the weekends.
6. Every Friday, the incubator door rubbers and the refrigerator rubber must be cleaned with 70% ethanol.
7. Every Friday, at the end of the work day, floors should be swept and mopped with diluted bleach (10%) using a clean/disposable head. A sticky mat can be placed in the floor, right before the entrance to the cell culture rooms and the top layer may be removed as need it.
8. All centrifuge balance tubes should be discarded and replaced. Centrifuge rotors and carriers must be cleaned and disinfected using 70% ethanol as well as the outside area of the centrifuge.

### **Weekly Cleaning and Maintenance – IVF Lab**

Mop floor with 10% bleach  
 Throw out large biohazard bag in outer culture room and replace  
 Change water bath  
 Wipe the surface of the hood with ethanol  
 Wipe the inside of the centrifuge with ethanol  
 Wipe the benches, refrigerator and incubator tops and other horizontal surfaces with ethanol  
 Wipe the refrigerator door and seal on the refrigerator door with ethanol  
 Clean the incubator doors with 70% ethanol  
 First Week of Month: Change water in incubator pan – add piece of copper to each pan  
 Make sure nothing is stored in hoods and little is stored on benches  
 Fill out checklist in the IVF Media and Cleaning Log  
 Check CO<sub>2</sub> and O<sub>2</sub> levels with fyrite  
 Check whether temperature in each incubator is accurate

### **Monthly Cleaning (Rm 238) or Quarterly to Semi-Annual Cleaning (IVF Lab) - by lab technician or dishwashers under lab technician's supervision**

1. Clean hoods monthly 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 and 1% benzalconium chloride before and after reassemble. Let the fumes to dissipate overnight before any work.
2. Clean incubators monthly 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 and 1% benzalconium chloride. Let the fumes to dissipate. 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.
3. The vent cover for the A/C duct should be cleaned, specially when there are season changes (switch from the A/C to the heater system)

