SW229A-Flow Facility Biosafety Rules and Standard Operating Procedures

Room SW229A is a biosafety level 2 containment area. Please abide by these rules when working in this room. You may be asked to leave the lab by the CNS personnel if you do not follow these guidelines.

GENERAL RULES WHEN WORKING IN THE FLOW CYTOMETER ROOM (SW229A):

1. When working in the Flow Facility, proper attire is mandatory (no sandals, shorts, skirts etc.). You may be asked to leave the lab if your clothing attire does not meet the guidelines. Also, when working in the lab, lab coats must be worn at all times.
2. Gloves are to be worn when working with your sample. Do NOT use gloves when using the computer, keyboard, mouse and logbook.
3. Do not use your cellphones when working in the lab. Do not put down your phone on the bench tops.
4. Remove bio-hazardous waste regularly (check the schedule). Keep space clean and tidy. Label all solutions in squeeze bottles.
5. Empty out the Flow Cytometer Waste and Fill the Sheath Fluid tank as needed at the end of your session.
6. *Decontamination:* Work surfaces must be cleaned and decontaminated with 70% Ethanol at the end of your session and after any spill of potentially hazardous material.
7. Do not leave the Fortessa unattended while running (must be on standby).
8. You must report any accident-incident immediately to CNS personnel.
9. For proper risk assessment, it is critical that relevant biohazard information about the samples be transmitted to CNS personnel before your flow cytometry experiments.

****Report any infractions to CNS Personnel Please****

List of Equipment in SW229A:

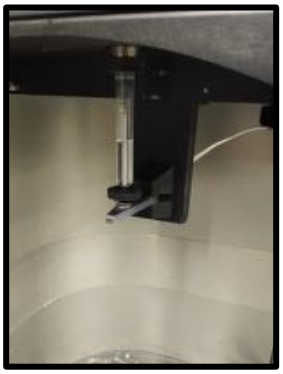
1. Bio Klone 2 BSC for Tissue Culture Work (see below for SOP)
2. Fisher Scientific hot water bath
3. Beckman Coulter Microfuge 22R Centrifuge
4. Fisher Scientific Dry Bath Incubator
5. Vortex Genie 2
6. Thermo Scientific Incubator
7. VWR Fridge
8. BD LSRFortessa and associated equipment
9. BD FACSAria III and associated equipment

Updated: August 2019

SW229A-Flow Facility Biosafety Rules and Standard Operating Procedures

1

5



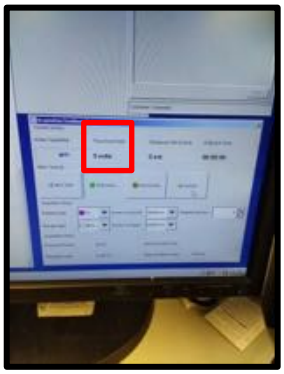
WORKING IN THE FLOW CYTOMETER

START UP

1. Turn on computer and cytometer
2. Launch FACSDiva
3. Open an experiment
4. Ensure the waste tank is empty and the sheath fluid is full
5. Ensure H2O is installed on the SIP
6. Open an experiment, select a sample and click acquire
7. Select run on the Fortessa
8. Record the starting event-rate in the log book
9. Acquire your samples

7

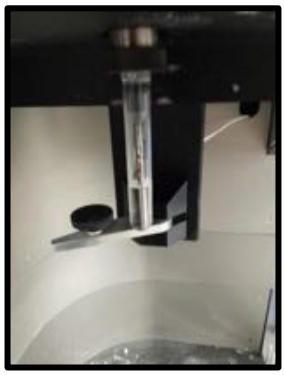
8



SHUT DOWN

1. Install a tube of FACS Clean on the SIP
2. Select any sample within your experiment and hit acquire to monitor the flow rate
3. Run on high for at least 5 minutes
4. Install a tube of FACS Rinse on the SIP
5. Run on high for at least 5 minutes
6. Install a tube of H2O on the SIP
7. Run on high for at least 5 minutes
8. Ensure that the event-rate has dropped to < or = 20 events/sec
9. **If the event-rate remains high after cleaning: install FACS Clean on the SIP and run on high with the SIP arm to the side for 30 seconds (this causes fluid to rush quickly through the flow cell and aids in cleaning)
    a. Return SIP arm and Run on high for 5 minutes or until event-rate drops
    b. Install a tube of H2O on the SIP
    c. Run on high for at least 5 minutes
10. **If the event-rate <u>still</u> remains high after cleaning: install FACS Clean on the SIP and Prime the machine, once air begins to be expelled from the SIP reselect Run to force FACS Clean into the flow cell
    a. Run on high for 5 minutes or until event-rate drops
    b. Install a tube of H2O on the SIP
    c. Run on high for at least 5 minutes
11. Record ending event-rate in the log book
12. Place the Fortessa in standby and turn off

9



10

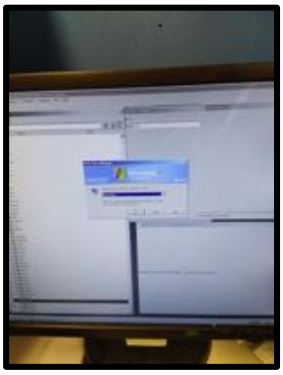
Updated: August 2019

SW229A-Flow Facility Biosafety Rules and Standard Operating Procedures

13. Empty the waste tank and re-fill the sheath fluid
14. Turn off the computer

13&14





SW229A-Flow Facility Biosafety Rules and Standard Operating Procedures

Tissue Culture SOPs

<u>ALL USERS</u> must read and abide by these guidelines to minimize cell contamination to the group. Failure to abide by these rules will result in losing privileges to this facility.

DO NOT USE THESE FACILITIES IF YOU HAVE NOT BEEN TRAINED

- The appropriate permits must be obtained to work with pathogens in this room and SOPs must be followed
- Primary cell culture and animal tissue work is to be completed in the BSC and incubator located in SW229A.

1. General Housekeeping of Tissue Culture Rooms

- NO food, water, or gum chewing in Biosafety Level II Rooms
- NO personal belongings in the tissue culture (TC) rooms, laptops, cellphones, earbuds or headphones in Biosafety Level II Rooms
- Please keep floor clean at all times
- Please do not leave bottles, dishes, or pipettes in the sink
- Please keep tissue culture bench clean and free of clutter, e.g. bleach and EtOH containers, DMEM bottles, falcon tubes, Kleenex boxes. Regularly disinfect the benches and door handles.
- Please ask for permission before using other lab's aspirator, disposal beaker, pipettes, dishes, lab coats, EtOH, bleach etc.

2. Booking

- A TC hood booking sheet is provided (above culture hood)
- Please do not over-book more than actually needed
- Please update booking sheet in advance for change of experimental plan
- Please ALWAYS write your name on the booking sheet when you use the hood, even for 5 minutes
- Greatly appreciated if you can:

a) Inform the next user ASAP in case of cancellation;
b) Inform the current user if you run late for your booking so they can use the hood longer if needed

3. Standard Operating Procedures for the Biosafety Cabinets (BSCs)

A. Start-Up:

1. Always wear a lab coat and gloves
2. Turn on the BSC and wait for 5 minutes for the airflow to stabilize
3. UV the hood for 5 minutes before using (if needed, see below for more details on the usage of UV irradiation)
4. Spray interior surfaces of TC hood with 70% EtOH and let it evaporate
5. Never leave hood cover completely closed when vacuum is on (This can cause damage to the vacuum motor)
6. Make sure to wipe/spray down everything with 70% EtOH before putting it in the hood. Avoid overcrowding or blocking the front or rear grilles to prevent the appropriate airflow patterns from being compromised. After loading material in the BSC, allow sufficient time for the airflow to stabilize before initiating work (3-5 minutes).
7. Check that the sash is at the appropriate height. Adjust stool height so that the user's underarms are level with the bottom of the sash.
8. Place aerosol generating equipment (e.g., vortex mixer, sonicator) towards the back of the BSC, without blocking the rear grille.

B. Working in the BSC

- Perform operations as far to the back of the work area as reasonable. Ensure that elbows and arms do not rest on the grille or work surface
- Avoid excessive movement of hands and arms through the front opening. Such movements disrupt the air curtain at the front of the BSC, which can allow contaminants to enter or escape the BSC. Arms should enter and exit the BSC slowly and perpendicular to the front opening.
- Minimize the stuff you put in the BSC while working in them
- Segregate non-contaminated ("clean") items from contaminated ("dirty") items. Work should always flow from "clean" to "dirty" areas.
- Discard material in a waste container located towards the rear of the cabinet workspace. Dispose the whole container and content in the level II garbage bins once you finish your work. Do not discard or store contaminated materials in containers outside of the cabinet (see below for more details on waste disposal)

- Aspirate cell culture media and biological wastes using the aspirators. The aspirators should be cleaned regularly and thoroughly. Never aspirate bleach as it will corrode the metal components of the aspirator lid.
- In case of spillage, if it is a biological agent, wipe clean with 70% EtOH. For sterile media or other liquids, first absorb with a Kleenex and then disinfect with 70% EtOH

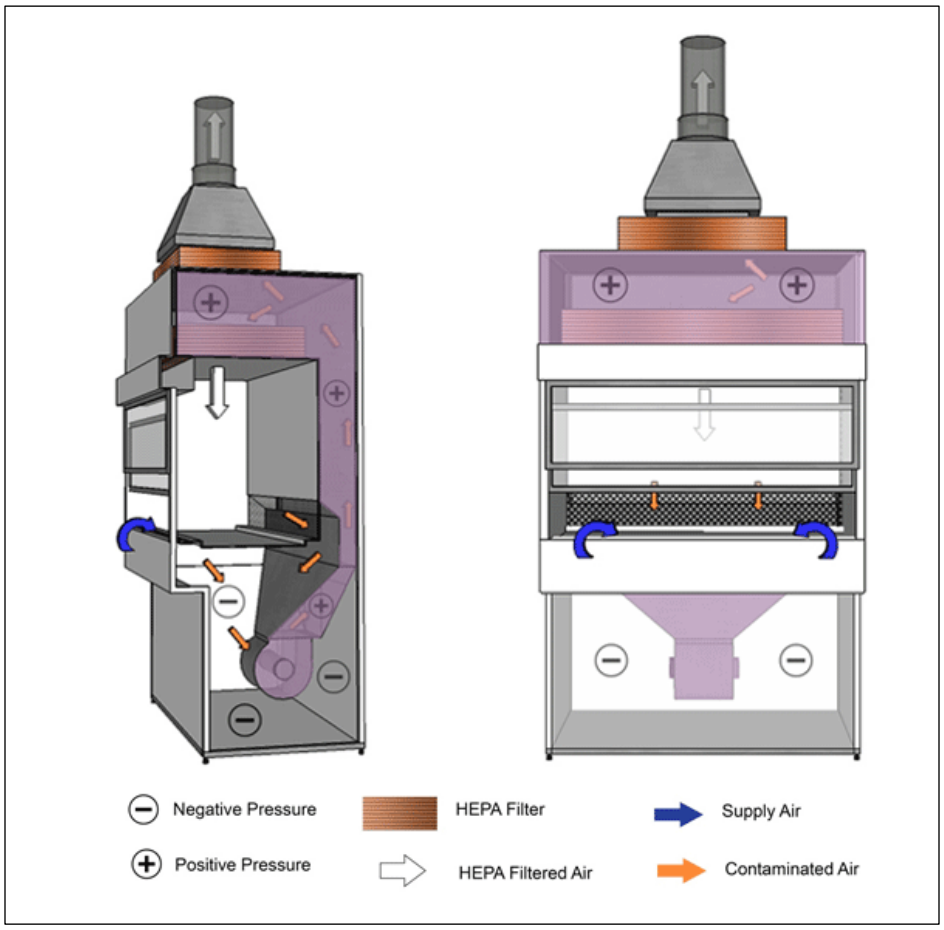


Figure 1. Diagram of the airflow in a Class II type A2 BSC retrieved from Public Health Agency of Canada, 2018.

C. Finishing Up:

- Used tips should be disposed into paper cups containing little amounts of bleach (just enough to cover the bottom surface). Used serological pipettes should be disposed into paper cups provided. When full, top with a second cup and dispose in yellow biosafety waste bin. Do not store any serological pipettes or scrapers beside the hood. Keep one biohazardous bin for just pipettes (see Figure 2). Place empty cups into the bin and dispose of used pipettes directly into the biohazardous bin once your work in the BSC is completed. Once cups are full, cap it with another cup and take the garbage down to SW111K at the end of the day.
- Used glass Pasteur pipettes are to be disposed of in a red sharps container or a cup (put lid on once full) and taken down to SW111K.
- Please remove all your material from TC hood and store away in appropriate cupboards.
- Spray interior surfaces of hood with 70% EtOH and let EtOH evaporate.
- Close the hood and turn off the vacuum.
- Gloves are to be disposed of in the yellow bins
- IF YOU ARE THE LAST PERSON:
    A. Please discard glass Pasteur pipettes into the red sharps container or a cup (cap it with a lid once full)
    B. Dispose biohazardous garbage (yellow bin) in SW111K on a daily basis. Garbage should not accumulate.
    C. Replace with new bags or new yellow biosafety bin if needed
    D. Ensure that the microscope, water bath and centrifuges are turned off. If you do not turn off the water bath you must check and maintain the water bath levels at around 60% and ensure that the water is clean.

Figure 2. As you work in the BSC, have a cup/milk jar inside the BSC as a temporary waste container for the serological pipettes and tips. Once your work is completed, directly transfer the serological pipettes and tips into the designated yellow bin. Once the cups are full, cap the top with another cup and take down to SW111K as soon as possible.

NOTE on the usage of UV irradiation as a method of disinfection:

- UV irradiation of the work area should only be used as a secondary method of disinfection in the cabinet. Never rely on UV irradiation alone to disinfect a contaminated work area.
- UV irradiation is ineffective if a microorganism is protected by dust, dirt, or organic matter. A liquid chemical disinfectant (e.g. 70% EtOH) should be the primary method of cleaning and disinfecting the interior of a BSC.
- UV irradiation does not penetrate into cracks or through the grilles of a BSC.
- UV irradiation can cause deterioration of various materials, including certain plastics and tubing

4. Incubators

- DO NOT keep the door open for too long
- If RH pan light is flashing add autoclaved ddH_2O immediately to the tray inside the incubator
- Avoid accidental spillage (Pay extra caution when transferring dishes/flasks)

5. 37°C water bath

a) Please help keep water bath clean
   - Water should be changed at least once a month
   - Add a drop of SigmaClean® to prevent growth of bacteria/fungi

b) Do not tamper with adjustment knob
   - Temperature may read below 37°C when you initially put your bottles in, but it will go back to 37°C shortly

6. CO_2 Tanks

a) Please help check CO_2 tank pressure regularly
b) Inform Bruno when the tank is running low to replace it

SW229A-Flow Facility Biosafety Rules and Standard Operating Procedures

7. Garbage disposal (Containment Level 2)

Biological waste includes:

- liquids such as used cell culturing media, supernatant, blood or blood fractions (serum), etc., which contain viable biological agents
- non-sharp, solid laboratory waste (empty plastic cell culture flasks and petri dishes, empty plastic tubes, gloves, wrappers, absorbent tissues, etc.) which may be, or is known to be, contaminated with viable biological agents
- all sharp and pointed items used during cell culturing

Biological waste must be disposed of as outlined below.

a) Please discard waste to the correct bin according to their nature:
   - Tissue Culture Garbage: anything that comes into contact with cells, media, or reagents should be discarded in yellow biosafety bins
     - IMPRORTANT: dispose of liquids by transferring to aspirator flask. Clean out aspirators with 6% bleach on a daily basis.
     - DO NOT leave large amounts (more than 25 mL) of liquids in T75 flasks or conicals when you dispose of them in the yellow biosafety bins!
   - General Garbage: all other wastes

b) Gloves MUST be disposed of in yellow biosafety bins!

c) Sharps should be disposed of in red sharps container or in a cup with a lid

d) Anything that is not considered a sharp (tips, pipettes, etc.) but may poke through bags should be placed inside secondary containers (paper cups) before being disposed in yellow biosafety bins (or regular garbage bins for Level 1 waste).

e) ALL biohazardous waste must be disposed WITHIN 24 HOURS in SW111K

f) All aspirator flasks must be emptied WITHIN 24 HOURS and DISINFECTED

Thank you for your cooperation!

Report any incidents, accidents, frequent contamination issues to CNS personnel and your supervisor. Accidents must be formally reported to the Environmental Health and Safety office on campus.