DB440 FACILITY

STANDARD OPERATING PROCEDURES

BIOSAFETY

Chemistry
UNIVERSITY OF TORONTO

1. DB440 CL2 Facility Access

2. Entry & Exit Procedures

3. Offboarding the Department

4. Administrative Controls

5. Equipment Decontamination

6. Biological Spills

7. Biohazard Waste Disposal

8. BVC Aspiration System

9. Broken Glass Disposal

10. Biosafety Cabinet (BSC) Use

11. BSC Booking

12. Centrifuge Use

13. Autoclave Use

14. Handling/Storage of Compressed Gas Cylinders

15. Liquid Nitrogen Use

16. CO2 Switch-Over Manifold

17. Safe Sharps Use

18. Aerosol Risk Reduction

19. Removing Items from CL2 Facility

20. Transfer of Biological Agents

21. Packaging Infectious Substances

22. Cleaning of DB440

23. Sanitation of Millipore Water System

24. Defrosting Freezers

25. Incubator Routine Maintenance

26. BSC Routine Maintenance

27. Working with Virus

DBB440 CL2 Facility Access

1. Purpose:

To provide a step-by-step guide to Principal Investigators (PI) and laboratory personnel on how to access the Containment Level 2 (CL2) biological facility located in DB440.

2. Scope:

Applies to all PIs and laboratory personnel who will work in the CL2 facility.

3. Prerequisites:

PIs: Current Biosafety permit. For more information, please see the Procedure section below

Lab Personnel: Fit criteria for authorized personnel and be included in PI's biosafety permit, mandatory training (both the LM onboarding safety training and DB440 onboarding safety training), and the hands-on DB440 SST.

4. Responsibilities:

PI: To follow and enforce this SOP and ensure that all laboratory personnel under your supervision have completed all mandatory training.

Lab Personnel: To complete all mandatory training in order to gain access to the CL2 facility, and to follow this SOP.

For both PIs and lab personnel: To review the **DB440 Governance document**, understand and agree (by signed agreement) with the terms and conditions for the use of the CL2 facility (DB440)

5. Procedure: describe step by step (as applicable for SOP)

Procedure for Principal Investigator

1. Request to become a member by emailing the Facility Permit Holder, Professor Haissi Cui (haissi.cui@utoronto.ca) with the following:
    i. Subject Line: Request: DB440 Membership

2. Obtain or update your CL2 Biosafety permit.
    i. **If you have a CL2 Biosafety Permit, please complete the following steps:**
        i. Send an amendment to include the room number (DB440)
        ii. List all lab personnel who will work in the facility in your permit.
    ii. **If you have a CL1 permit or no permit, please apply by:** visiting the EHS website. For further inquiries, please contact: ehs.biosafety@utoronto.ca
        i. For further assistance, please email the Department of Chemistry's Chief Administrative Officer, Grace Flock (grace.flock@utoronto.ca)
            - Subject line: Request: Help with CL2 Permit
    iii. **If you need any important documentation** (ie. biosafety cabinet certification): please contact Professor Cui.
        i. Subject Line: Request: DB440 Necessary Documentation
3. Ensure lab personnel take all relevant training (ie. EHS and DB440-Site Specific Training).
4. Email the names, and start and end dates of your lab personnel BEFORE their expected start date to chem.keys@utoronto.ca
    i. Subject line: CL2 Facility: Names and Dates of the Lab Personnel
5. Ensure authorized personnel read the DB440 Governance document, and sign and submit the lab personnel memorandum of understanding (MOU).
6. Visit the Rates page on the CL2 Cell Culture Facility website for more information about membership and fees.

NOTE: Lab personnel will not be issued a fob to access DB440 until they have completed the full requirements of training and agreement with the DB440-Governance terms.

Procedure for Laboratory Personnel requesting access to DB440

1) Email Professor Haissi Cui (haissi.cui@utoronto.ca) to briefly discuss the planned experiments.
    i) **Subject Line:** DB440 Laboratory Personnel Experimental Plans
    ii) **Note:** Please update Professor Cui if you plan experiments with biological agents that are not previously approved (ex. bacteria or viruses that are not yet used in DB440).
2) Email Logan Zettle (logan.zettle@utoronto.ca) to schedule the DB440-Site Specific Training.
    i) **Subject line:** Scheduling DB440-Site Specific Training
3) Complete training by registering for the following courses at My EHS Training:
    i) Mandatory:
        i) EHS601: Laboratory Biosafety Training or EHS602: Biosafety Refresher
        ii) EHS630: Safe Use of Biological Safety Cabinets
    ii) Optional (Based on Lab-Specific activities)
        i) EHS603: Blood Borne Pathogens
        ii) EHS620: SARS-C0V-2 Biosafety Training Course
4) Read the **DB440 Governance document** and sign the lab personnel MOU
5) Email the following to chem.keys@utoronto.ca:
    i) Proof of training

ii) Lab personnel MOU
    i) **Subject line:** DB440 Proof of Training + Signed Lab Personnel MOU
6) Follow the Key Pickup SOP.
7) Once you become a user, you will be added to Slack to coordinate communication and announcements.

Note: Please ensure that your supervisor is an approved member. Lab personnel can only access this facility if their supervisor requests it on their behalf. For more information, please see the Principal Investigator section.

Entry and Exit Procedures for the LM-CL2 Facility (DB440)

1. Purpose:

To provide step by step guidance on what to do before entering and exiting the LM-CL2 biosafety facility located in DB440.

2. Scope:

Applies to all authorized faculty, staff and students who have access to, and work in the LM-CL2 biosafety facility located in DB440.

3. Prerequisites:

You are an authorized user of DB440 and are either included in your PI's permit or you possess a CL2 permit for DB440.

4. Responsibilities:

It is the responsibility of all faculty, staff, and students to follow the procedures described in this SOP.

5. Personal Protection Equipment (PPE):

+ ANY OTHER PPE APPROPRIATE FOR THE PROCEDURE BEING PERFORMED/BIOLOGICAL MATERIAL BEING HANDLED

6. Procedure:

Entry Procedure

1) Ensure that you have brought all required materials, solutions and media at volumes required for your work.

i) NOTE: Keep stocks of your solutions and media in your lab. Only bring diluted solutions, concentrated chemicals are not allowed.

2) Ensure that you do not bring your personal belongings to the LM CL2 facility and that they are stored in your respective laboratories.
   i) Under no circumstances are you allowed to bring:
      i. Your phone or earbuds
      ii. Backpacks, Jackets, Scarves, Mittens, etc.
      iii. Food or drinks
      iv. Other items not related to the facility
   ii) You are allowed to bring:
      i. Any personal belongings required for work e.g. Writing equipment and notebooks
3) Upon entry, put on all mandatory PPE.
   i) Ensure that your lab coat is properly fastened.
   ii) Take off jewellery that can come into contact with biological agents, can become caught in equipment, or can puncture gloves.
   iii) Ensure the gloves have no tears or holes in them before using. Depending on the agents you're working with, consider using double gloves.
   iv) Ensure that you are wearing closed-toed shoes, which are not high-heeled and have non-slip soles.
   v) Ensure that your clothes cover your legs up to your ankles.
   vi) If you have long hair, ensure that it is tied back. If it is likely that it will be contaminated when working in the facility, restrain or cover it.
   vii) Cover open wounds, cuts, scratches, and grazes with waterproof dressing.

Exit Procedure

If leaving the facility momentarily:

1. Take off all PPE by following the procedures outlined on the CDC website or watching the procedures on the CDC website
   i) Ensure that your skin, hair, and clothing do not come into contact with the contaminated PPE as you are taking it off.
   ii) Always hang your lab coat on the hooks provided near the entrance and ensure that there is one lab coat per hook.
      i) If all the hooks are occupied, you can hang your lab coat on top of another lab coat BUT the outsides of the lab coats have to be touching each other.
2) Upon removing all your PPE, wash your hands with soap and water at the hand-washing sink.

NOTE: Under no circumstances should you wear your lab coat or any other PPE equipment outside of the facility or in public areas (e.g. washrooms).

If leaving the facility for the day:

1) Clean/disinfect work surfaces with either 70% ethanol, 1% sodium hypochlorite or 30% water peroxide, ensure that the work environment is clean and uncluttered (e.g. floors), ensure that your solutions and media are labelled and stored appropriately.
2) Ensure that you have followed the "Shutting down the BSC" procedures under the **"Biosafety Cabinet (BSC) Use" SOP**
3) Ensure that the refrigerated centrifuge and the microscope is turned off.
4) Follow Steps 1 and 2 under "If leaving the facility momentarily".

STANDARD OPERATING PROCEDURE: Offboarding the Department of Chemistry

1. Purpose:

To provide step by step guidance on what to do when someone is permanently leaving the Department of Chemistry.

2. Scope:

Applies to all faculty, staff, and students, who are either leaving themselves, or know of someone under their supervision leaving the Department of Chemistry.

3. Prerequisites:

N/A

4. Responsibilities:

It is the responsibility of all faculty, staff, and students to follow the procedures described in this SOP every time they have someone under their supervision, or when they themselves are permanently leaving the Department of Chemistry.

5. Personal Protection Equipment (PPE):

As required when handling hazardous materials.

6. Procedure:

Procedure for Principal Investigators when someone under their supervision is leaving:

1) Inform chem.safety@utoronto.ca of the name of the personnel, under their supervision, leaving the Department of Chemistry and their last day at the Department of Chemistry. ☐
2) Update all relevant records (ex. Biosafety permit, Radioactive work permit). ☐
3) Ensure that the person leaving follows the checklist below. ☐

Procedure for Faculty, Staff and Students leaving: Checklist

1) On the last day, before leaving the Department of Chemistry, the personnel must return the keys Key Return SOP. ☐
2) The personnel must: ☐
   i) Vacate their lab bench and all other belongings (including any chemical/biological samples, solutions, and media)
   ii) Dispose of any chemical waste, expired solutions, expired chemicals following the Biohazard Waste Disposal SOP. ☐
   iii) Transfer accurate inventories of biohazardous group 2 stocks, if applicable. ☐
   iv) Following your research group data management plan, transfer all relevant datasets to the appropriate person. ☐

Procedure for Faculty leaving the department:

1) Complete the lab decommissioning form. ☐
2) Contact chem.safety@utoronto.ca if you require assistance. ☐

Controls Applicable to DB440 (CL2 Facility) Operations

1. Purpose:

To provide instruction on how to properly and safely use DB440.

2. Scope:

Applies to everybody using DB440.

3. Prerequisites:

WHMIS, EHS601 Laboratory Biosafety, DB440 SST

4. Responsibilities:

Principal investigators are responsible to enforce this SOP and lab-personnel are responsible to comply.

5. Personal Protection Equipment (PPE):

Lab coat, nitrile gloves, safety glasses/goggles



6. Procedure:

DB440 does not have a safety shower, therefore experimental procedures can only take place in this room if the following conditions are adhered to:

1) Hazardous chemicals (stocks) cannot be stored in DB440
2) Only small volumes of already diluted solutions/reagents can be brought to/used DB440 (ex. Already prepared 70% ethanol, already prepared 1% sodium hypochlorite, and commercial sodium hypochlorite previously aliquoted in small volumes for daily use)
3) All dilutions of concentrated stocks and aliquoting of stocks (sodium hypochlorite) should be performed in each research group laboratory
4) All squeeze bottles containing 70% ethanol or 1% sodium hypochlorite should be labelled with worksite WHMIS labels

5) Reagents such as trypsin, PBS, cell culture media are allowed to be stored in DB440 in the quantities needed for experiments. Larger stocks should be kept in each research group lab.
6) Stocks of DMSO should be kept in each research group lab and properly labelled small aliquots can be brought into DB440
7) **Cardboard boxes are prohibited in DB440** (source of spores = cell culture contamination)
8) Larger stocks of plasticware should be stored in each research group lab. Small stocks can be brought and stored within plastic containers on the shelves available in DB440. Plastic containers must be labelled with the research group name.
9) Lab coats should be stored in DB440. They should have a nametag, and they should be stored one lab coat per hook.

Mandatory Lab Duties (Rotation Schedule):

There is a requirement that everyone will participate in a shared responsibility to maintain equipment and housekeeping of DB440.

1) Once access to DB440 has been granted to a new lab member, this person will be included in the weekly lab duties rotation schedule. Participating in the maintenance of shared equipment, resources, and the overall housekeeping to avoid cross-contamination
2) When it is your turn based on the schedule, fulfilling the DB440 Lab Duties Checklist is mandatory
3) At the end of your week, you must submit the signed DB440 Lab Duties Checklist to chem.safety@utoronto.ca

Best Practices to Prevent Cross-Contamination Among Research Groups

- Do not use the biosafety cabinet (BSC) as a storage area
- Designate one BSC for bacterial work and use ONLY the designated BSC for this purpose
- Use 70% ethanol to wipe down the BSC's work surfaces before and after each use, and between cell lines
- Do not open/use other people's solutions
- Do not open/use other people's plasticware
- Be very careful when labeling solutions, cultures, etc.
- Routinely wipe floors and work surfaces to keep down dust
- Incubators, especially those that maintain high humidity levels, require periodic cleaning and disinfecting
- If you spill media in the incubator when bringing your cell cultures in you must clean and disinfect the incubator shelves, remove the water tray, clean and disinfect the tray, and put it back in the incubator with a sterile water supply
- Water baths should be emptied and cleaned on a regular basis, well before odor or visible turbidity develops

Equipment Decontamination

1. Purpose:

To provide instruction on how to properly and safely decontaminate equipment

2. Scope:

Applies to everybody working in CL2 Lab

3. Prerequisites:

WHMIS and Laboratory Biosafety Training (EHS601)

4. Responsibilities:

It is the responsibility of the PI and lab personnel to ensure that equipment is properly decontaminated, and in the case of transport, properly prepared.

5. Personal Protection Equipment (PPE):

Lab coat, nitrile gloves, safety goggles/glasses



6. Procedure:

1) Before beginning decontamination:
    - Equipment should be unplugged
    - All materials should be removed and properly discarded or stored
    - Refrigerators should be allowed to reach room temperature prior to decontamination and freezers must be defrosted
    - All materials used to soak up thawed water should be collected and treated as biohazardous waste
    - Incubators and other equipment with water temperature jackets must be drained if they are to be moved and the water drained from the jacket should be treated as biohazardous waste
2) In most cases, the following decontamination and contact times are appropriate:
    - *Tissue specimens, viruses (non-retroviral viruses) and non-spore forming bacteria:* 1% sodium hypochlorite for 30 minutes, or 70% ethanol for 20 min (read manual to choose compatible disinfectant)

- *Spore forming bacteria:* 1% sodium hypochlorite (freshly prepared) for ≥30 min
- *Fungi:* 1% sodium hypochlorite (freshly prepared) for 20 min
- *Others:* Virox, Clidox, gaseous Formaldehyde or H2O2 - discuss with the Biosafety Officer

3) Prepare a sufficient amount of decontaminant and transfer to a squeeze bottle (do not use spray bottle)

4) Squeeze covering all inner surfaces of the item with the decontaminant including door, handles, etc., and leave for the appropriate contact time
5) Wipe with absorbent material, collect and treat as biohazardous waste
6) Repeat steps 4 and 5 if the surfaces are particularly covered with dirt or "grime"
7) Following cleaning with 1% sodium hypochlorite, rinse well with water to prevent corrosion
8) Once the equipment is decontaminated, label with a "Decontaminated" sign that includes the date and the name of the person who was responsible for the decontamination process.

Biological Spills

1. Purpose:

To provide step by step guidance on responding to spills or release of biological materials.

2. Scope:

Applies to all faculty, staff and students who work with biological materials.

3. Prerequisites:

Applies to all authorized Principal Investigators (PIs) and authorized laboratory personnel working in the LM-CL2 facility (DB440).

4. Responsibilities:

It is the responsibility of faculty, staff and students to follow the procedures described in this SOP.

5. Personal Protection Equipment (PPE):

Personal Protective Equipment (PPE)	Spill Kit Contents
	  **+ Biological Spill SOP + Spill use report form**

- Items in the spill kit: Goggles, disposable suit, shoe covers, dust pan and broom, caution tape, absorbent powder

6. Procedure:

S.T.O.P

S – Stop. Secure the area.
T – Think about the material that was spilled and its associated hazards. Consult the material's SDS or PSDS for information about associated hazards.
O – Observe what has spilled: biological material, sharps, potential sources of fire, etc.
P – If safe to do so, proceed with spill cleanup.

Personal Exposure:

In the case of a biohazard spill, always check first to see if there was personal exposure.

i) If this is the case, take off your PPE if necessary, wash the contaminated areas at the sink with soap and water. Rinse at the eyewash for 15 minutes if spill came into contact with eye(s). If needed, the nearest safety shower is located in LM438. Immediately notify the PI and chem.safety@utoronto.ca. Seek medical attention if necessary.

Spill outside the Biosafety Cabinet (BSC):

1) If personal exposure, follow the steps above.
2) If risks of biohazard aerosols are present, leave the lab immediately. Place the sign found in ***Appendix 1*** on the lab's door to prevent people from entering.
    i. Wait 20 minutes before re-entering to proceed with cleaning up the spill.
    ii. **DO NOT** leave the spill unattended.

Clean up procedure:

3) Use the spill kit available in DB440. Put on shoe covers, double gloves, goggles, and the disposable suit found in the spill kit.
4) Secure the area using the caution tape found in the spill kit.
5) Remove any visible sharps using the broom and dust pan found in the spill kit.
6) For small volumes of liquid spill (less than 50 mL), use paper towels as absorbent material. For larger volumes, use the absorbent powder found in the spill kit.
7) Dispose the contaminated absorbent materials (paper towels or powder) using the broom and dustpan as solid biohazard waste. **Do not** use your hands to do this due to the potential of sharps.
    i) NOTE: Throw away the contaminated broom and dustpan in the Bio waste pail.
8) Decontaminate the spilled surface using freshly prepared 1% sodium hypochlorite solution. Soak paper towel with 1% sodium hypochlorite leaving it in contact with the contaminated surface for 30 minutes. Repeat the process twice.
9) Afterwards, contact chem.safety@utoronto.ca to request caretaking services.
10) Dispose of all PPE worn during spill cleanup with the exception of the lab coat in the Bio waste pail. Wash your hands with soap and water.
11) Follow Steps 1 and 2 under "**Reporting Spill Incidents**"

Spill inside a Biosafety Cabinet (BSC):

1) If personal exposure, follow the steps under the **Personal Exposure** section.
2) While you get the supplies needed to clean the spill, secure the area by closing the sash of the BSC and placing the sign found in ***Appendix 2*** on the BSC.

Clean up procedure:

3) Before you start cleaning the spill, observe potential splashes on the BSC's inner walls or contamination under the grills. Ensure all surfaces are taken care of. **Do not leave the spill under the grill unattended.**
4) Put on double gloves.
5) If there are contaminated broken sharps, use forceps/tweezers to pick them up and dispose of them into the sharps container. **Do not** use your hands to do this.
6) For small volumes of liquid spill (less than 50 mL), use paper towels as absorbent material. For larger volumes, use the absorbent powder found in the spill kit.
7) Dispose the contaminated absorbent materials (paper towels or powder) as solid biohazard waste. **Do not** use your hands to do this due to the potential of sharps.
8) Decontaminate the spilled surface using freshly prepared 1% sodium hypochlorite solution. Soak paper towel with 1% sodium hypochlorite leaving it in contact with the contaminated surface for 30 minutes. Repeat the process twice.
9) Wipe the spill area with 70% ethanol and then with water. IMPORTANT, THIS STEP IS NECESSARY TO PREVENT CORROSION OF THE BSC.
10) Dispose of your gloves into the Bio waste pail. Wash your hands with soap and water.
12) Allow the BSC to run for at least 10 minutes. Then follow Steps 1 and 2 under "**Reporting Spill Incidents**".
13) See video for more information on small spills inside BSC:

Spill inside the Eppendorf™ 5810R Centrifuge:

Centrifugation is a procedure that produces aerosols. To prevent personal exposure to aerosolized biohazards, follow Step 1.

1) If during a run you suspect a spill has occurred (noise of broken tube) then stop the run immediately and **DO NOT** open the lid for 30 minutes.
   OR
   If you discover that a spill has occurred after a run, immediately put the lid back on and **DO NOT** open for 30 minutes.
2) While waiting, secure the centrifuge by placing on it the sign found in ***Appendix 2***.
3) Put on double gloves and obtain the following supplies to clean the spill:
   i) 1% sodium hypochlorite, 70% ethanol, paper towel, forceps, sharps container and Bio waste pail
4) After 30 minutes, open the lid, turn the centrifuge off and **disconnect it from the power plug**.

If biosafety caps used:

5) Take the buckets with the biosafety caps on to the nearest BSC.
6) Open the caps and take out the contents. Wipe the tubes exteriors with 70% ethanol.
7) If applicable, use forceps to pick up sharps fragments/debris in the buckets and discard them in the sharps container.
8) Use paper towels as absorbent material. Dispose the contaminated paper towels as solid biohazard waste.
9) Decontaminate the bucket and biosafety caps by soaking paper towel/lint-free cloth with 1% sodium hypochlorite leaving it in contact with the contaminated surface for 20 minutes.
   i) Clean thoroughly with soap and water. Rinse a minimum of 3 times with water.
   ii) Do a final disinfection rinse with 70% ethanol.
   iii) Dispose of the contaminated cleaning materials as solid biohazard waste.

If biosafety caps were not used:

10) Take the bucket and rotor out.
11) If applicable, use forceps to pick up sharps fragments/debris in the centrifuge and discard them in the sharps container.
12) Use paper towels as absorbent material. Dispose the contaminated paper towels as solid biohazard waste.
13) Decontaminate the body of the centrifuge and the exterior of the buckets by soaking paper towel/lint-free cloth with 1% sodium hypochlorite and leaving it in contact with the surface for 20 minutes.
   i) Clean thoroughly with soap and water. Then rinse a minimum of 3 times with water.
   ii) Do a final disinfection rinse with 70% ethanol.
   iii) Dispose the contaminated cleaning materials as solid biohazard waste.

Final Steps:

14) Clean the centrifuge by following the manufacturer's instructions, which are also posted on-site.
15) Dispose of your gloves in the Bio waste pail. Wash your hands with soap and water.
16) Follow Steps 1 and 2 under "**Reporting Spill Incidents**".

Reporting Spill Incidents:

1) Notify your supervisor and chem.safety@utoronto.ca. Your supervisor should fill out the Accident/Incident report form.
2) To ensure replenishing of spill kit supplies, complete the **"spill kit usage"** form using the dry erase marker found in the biological spill kit. Take a picture of it and send it to chem.safety@utoronto.ca

Appendix 1

SPILL INSIDE.
DO NOT ENTER.

Appendix 2

SPILL INSIDE.
DO NOT OPEN/USE.

Biohazard Waste Disposal

1. Purpose:

To provide step by step guidance on properly disposing liquid, solid, and sharps biohazardous waste. Biohazardous waste is any liquid, solid or sharp that has come into contact with:

Risk Group 1 or Group 2 agents	Viral vectors and Aerosolisable Bioagents
DNA Staining Reagents	Toxins and Human Tissues
Animal Tissues	Etc.

2. Scope:

Applies to all authorized Principal Investigators (PIs) and authorized laboratory personnel working in the LM-CL2 facility (DB440).

3. Prerequisites:

You are an authorized user of DB440 and are either included in your PI's permit, or you possess a CL2 permit for DB440.

4. Responsibilities:

It is the responsibility of all faculty, staff, and students to follow the procedures described in this SOP.

5. Personal Protection Equipment (PPE):

6. Procedure:

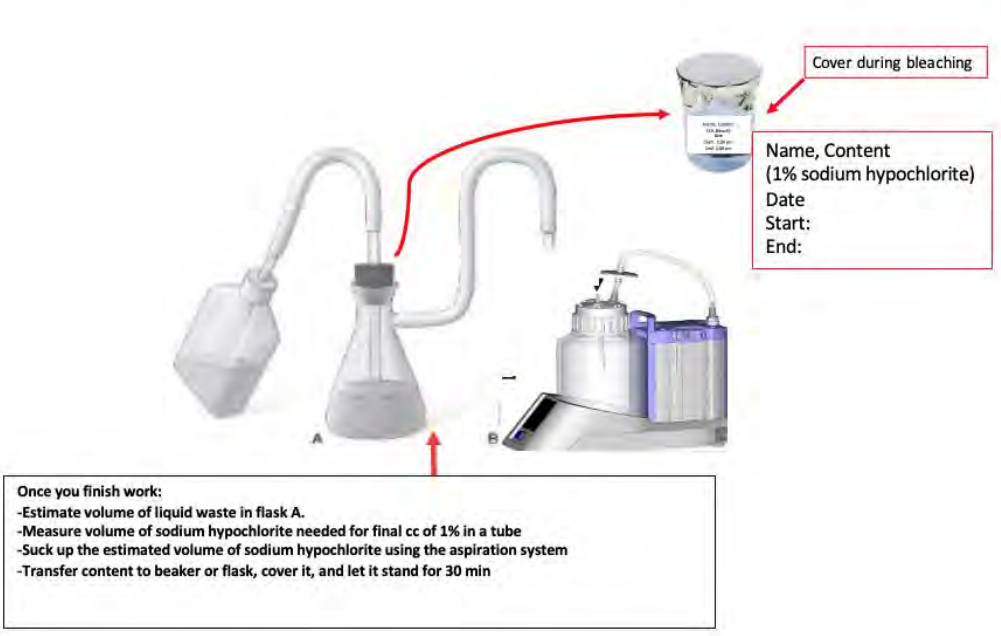
Liquid Biohazardous Waste Disposal

NOTE: Biohazardous liquid waste must be pre-treated with 1% sodium hypochlorite before disposal, as described below.

1) Wear PPE as described above. Label liquid waste container with the following:
   i) Name of user, Content, Date, Time sodium hypochlorite added
2) Determine the volume of sodium hypochlorite needed for your liquid waste to get a final concentration of 1% sodium hypochlorite.
   i) C1: Commercial sodium hypochlorite is usually 10%, but always check the concentration of your sodium hypochlorite stock and modify C1 if required.
   ii) C1V1 = C2V2: (0.10) × (Volume of sodium hypochlorite stock to add to liquid waste) = (0.01) × (Total volume of liquid waste)
3) Add sodium hypochlorite to the liquid waste. Contact time is 30 minutes.
4) After 30 minutes, pour the liquid waste down the sink and run tap water.
5) Wash the container with soap and water and leave it to dry.
6) Remove and throw away your gloves in the Bio waste pail. Wash your hands with soap and water.

BSC vacuum filtering system waste disposal:

- Label container for liquid biohazardous waste with the following:
  - Name of user, Content, 1% sodium hypochlorite, Date, Time sodium hypochlorite treatment started and ended



Solid Biohazardous Waste Disposal

- Examples include: Broken glassware, empty cell culture flasks, plates, tubes and petri dishes, agar plates, serological and Pasteur pipettes, micropipette tips, contaminated gloves, contaminated paper towels. Ensure that you've removed all left-over liquids before disposal.

- Use lined Bio Waste pails, shown below, to dispose solid biohazardous waste.

  - If it is not lined, line with a yellow biohazard bag.
- When the bags are full, tie them. Replace full pails for new ones.
  - **DO NOT** remove the bags from the pails.
  - Contact chem.safety@utoronto.ca to request new pails if not available or if stocks are low.
- When the pails at the BSC's are full, contact chem.safety@utoronto.ca to request pick up and keep the full pails near the entrance.

Sharps Biohazardous Waste Disposal

For needle and blade waste

- Use sharps containers, shown below, to dispose needle and blade waste.

Maximum capacity line

  - Do not fill the container beyond its maximum capacity (fill line on container).
  - Ensure that needles are empty of liquids before disposal. Follow the "Liquid Biohazardous Waste Disposal" procedures if necessary.
- Close the container when full and replace it for a new one.
  - Contact chem.safety@utoronto.ca to request new containers if not available or if stocks are low.
- When containers are full, contact chem.safety@utoronto.ca to request pick up and keep the full containers near the entrance.

For glassware and plasticware waste (even if broken):

- Follow the "Solid Biohazardous Waste Disposal" procedure above.

Using the BVC Pro Aspiration System

1. Purpose:

To provide step by step guidance on how to use the BVC Pro Aspiration System.

2. Scope:

All DB440 lab personnel.

3. Prerequisites:

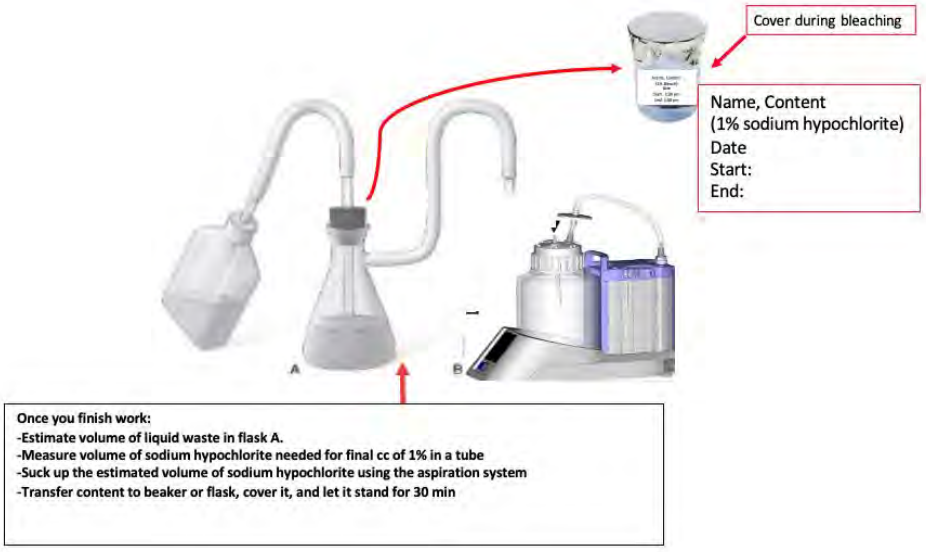
You are an authorized user of DB440 and are either included in your PI's or you possess a CL2 permit for DB440.

4. Responsibilities:

It is the responsibility of all lab personnel who use the aspiration system to follow this SOP.

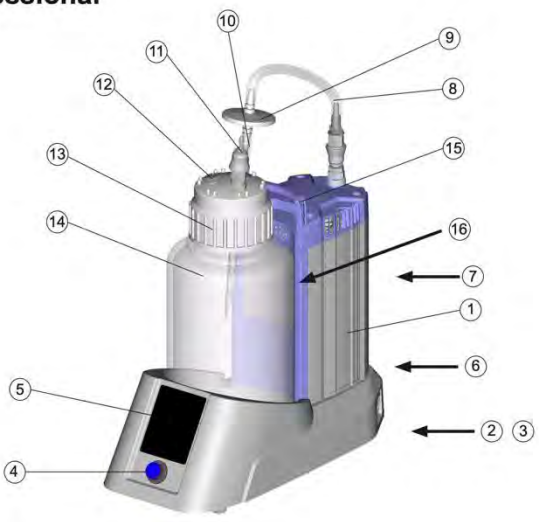
5. Personal Protection Equipment (PPE):

6. Procedure:



Using the BVC Pro Aspiration System:

BVC professional



Position	Designation
1	Pump ME 1C
2	Mains connection
3	Fuse holder
4	On / Off switch
5	Touch panel
6	Rating plate
7	Outlet
8	Connection tubing
9	Hydrophobic protection filter
10	Connection filter
11	Connection VacuuHandControl VHC^{pro}
12	Closing screw (optional: connection second VHC^{pro})
13	Screw cap / bottle cap with insert
14	Collection bottle
15	Handle
16	Level sensor

Safety during operation

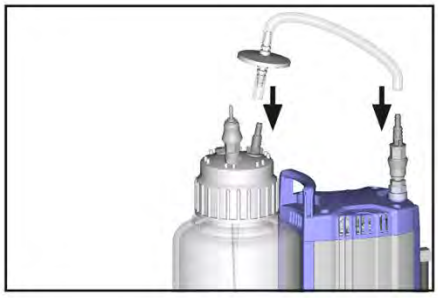
- Avoid interactions of media in the collection bottle. Comply with material safety data sheets and notes on safe use from the manufacturer. **Do not mix incompatible disinfectants and/or unknown substances.**
- For example, sodium hypochlorite (chlorine bleach) (see below):

Incompatible chemicals and agents	Possible results of mixing with sodium hypochlorite (chlorine bleach)
Acids or acidic compounds (e. g. hydrochloric acid, aluminium chloride)	Release of chlorine gas
Ammonia containing compounds (e. g. ammonium hydroxide, quarternary ammonium salts)	Formation of explosive compounds, release of chlorine gas and other hazardous gases
Organic chemicals (e. g. solvents, polymers, amines, oils)	Formation of chlorinated organics, release of chlorine gas and other hazardous gases
Metals (e. g. copper, iron) Hydrogen peroxide	Release of oxygen, overpressure, rupture of a closed system
Reducing agents (e. g. sodium thiosulfate)	Production of heat, boiling
Guanidine salts (e. g. guanidine hydrochloride, guanidine thiocyanate)	Release of toxic gases, e. g. chlorine, chloramine, hydrogen cyanide

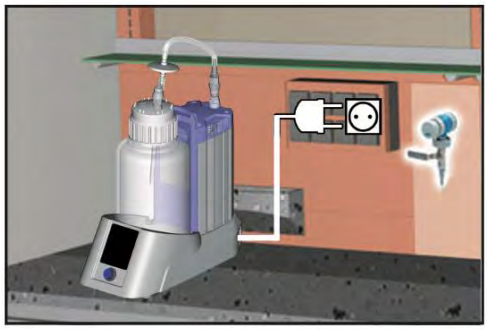
Avoid the formation of dangerous reactions/gases in the BVC, especially in the collection bottle. If this is not possible, dispose of dangerous gases at the outlet of the pump appropriately.

Procedure:

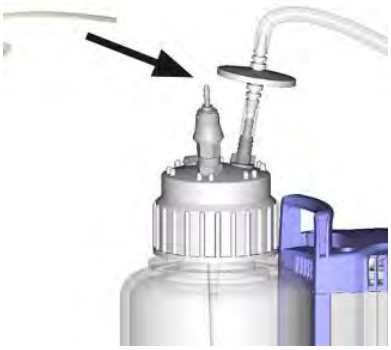
1. Assemble tubing with filter



2. Connect to power supply. Check line voltage and current prior to switching on.



3. Attach tubing to the hose nozzle of the bottle head



Operation

- The unit has a touch panel, so the keys should be touched gently. The “+” and “-“ keys have to be touched > 0.25 seconds to be activated. The other keys have to be touched > 0.5 seconds. Touch the LED keys **below** the LED. A successful action is confirmed by a blip and the flashing of LEDs.

Position	Designation
1	Key to select bottle size and level sensor
2	Key to reduce suction power
3	Key “bottle change”
4	Display suction power
5	Key to increase suction power

- Use the keys to set suction power of the system. The suction power can be set linearly in a range from 150 mbar (1 LED flash) to 850 mbar (8 LEDs flashes) underpressure (relative to atmospheric pressure).
- A flashing LED indicates that the actual available suction power differs from the pre-set suction power.

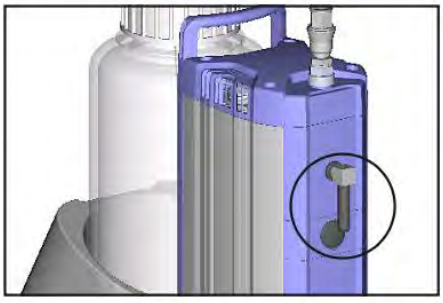
- NOTE: If the collection bottle is under vacuum and the vacuum demand is reduced, the existing underpressure inside the bottle remains until the vacuum is reduced (pressure rises) by further aspiration or venting with the VHCpro. See procedure for venting in "Disinfection routine".
- Use the keys to select the bottle size (4L PP bottle), and with it, the activation of the corresponding level sensor. To operate the key "4l PP" touch the key > 1 second. The LED of the selected bottle flashes blue. Use the key to turn off the level sensor alarm and to start or stop the pump during bottle change. To operate the "bottle change" key, touch the key below the LED for > 0.5 seconds.

Level sensor

- The sensor foil is located on the bottle support. The level sensor gives an alarm and switches off the pump to avoid overfilling the collection bottle if the liquid level in the collection bottle reaches the height of the level sensor- approximately 80% of the maximum bottle capacity (grey marked range with bottle symbols on the sensor foil, for both bottle types).
- Do not fix adhesive foil or anything near the bottle side next to the sensor foil.

During operation

- Use the system only with the integrated hydrophobic filter to protect the vacuum supply from aspirated liquids and aerosols, and to protect the environment/user from contamination risk.
- Silencer at the outlet: Dust-laden gases, deposits and condensed solvent vapor can restrict air flow out of the silencer. The resultant back pressure can lead to damage of the pump bearing, diaphragms, and valves. Under those conditions, a silencer must not be used. Check the silencer regularly and replace if necessary. In case of harmful gases or condensate at the outlet, remove silencer and replace with exhaust tube.



- Removing the connection tubing from the screw cap leads to immediate venting of the collection bottle. In systems without quick couplings, removal of the tubing at the pump inlet will also vent the system. Do not remove connections in case of liquid in the tube.

Emptying the collection bottle

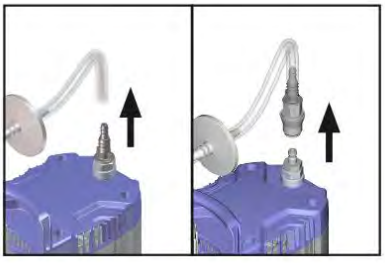
- Check the liquid level in the collection bottle regularly. Maximum admissible liquid level in collection bottle is approx. 80% depending on application, and if correct bottle size is selected and if correctly adjusted.
- Once at 80% capacity, the level sensor will switch off the pump. This is indicated by “blips” and by a red LED inside of the bottle symbol on the bottle change key. Proceed with disinfection routine prior to emptying collection bottle. See below.

Disinfection routine

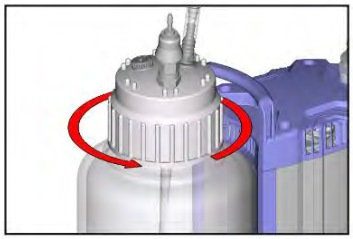
- Switch off the BVC to avoid running of the pump while no bottle is connected.

- Vent the collection bottle. Venting the BVC: Press the lever of the VHCpro or set the VHCpro to continuous aspiration.

- Remove the tubing at the inlet of the pump or disconnect coupling.

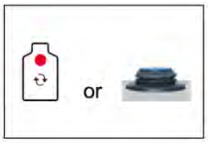


- Remove screw cap from the collection bottle after venting.



- Remove bottle from the support. Determine the volume of bleach needed for your liquid waste to get a final concentration of 1% sodium hypochlorite.

i) C1: Commercial sodium hypochlorite is usually 10%, but always check the concentration of your sodium hypochlorite stock and modify C1 if required.
ii) C1V1 = C2V2: (0.10) × (Volume of sodium hypochlorite stock to add to liquid waste) = (0.01) × (Total volume of liquid waste)
iii) Ex. If the volume is at 80% capacity, volume will be 3.2L, in which case 320 ml of sodium hypochlorite is needed.
iv) Add sodium hypochlorite to the liquid waste. Contact time is 30 minutes.
v) After 30 minutes, pour the liquid waste down the sink and run tap water.
vi) Wash the bottle with soap and water and leave it to dry.
vii) Remove and throw away your gloves in the Bio waste pail. Wash your hands with soap and water.

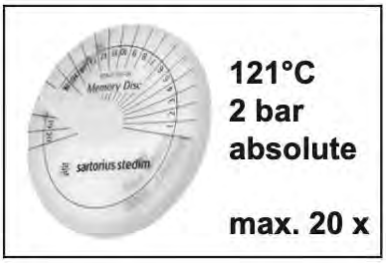


- NOTE: After changing the bottle, touch the key to restart the pump or switch on the pump.
- NOTE: The use of sodium hypochlorite (chlorine bleach) can corrode the materials of the 4l polypropylene bottle and other components (e.g. quick-coupling accessory sets between the pumping unit and VHCpro). Therefore, **after disinfection, rinse bottle thoroughly to avoid leaving residues of disinfectant in the bottle.** If there is residue on the bottle, make sure you scrub it and remove it properly to avoid build-up that could interfere with the level sensor of the system.

Autoclaving

- Autoclaving should be done each time the collection bottle reaches maximum capacity (80%) and disinfection is required.
- The collection bottle with bottle head and screw cap, the quick coupling and the filter are designated for steam sterilization at 121°C and 2 bar absolute (1 bar overpressure). Time of exposure according to DIN 58946 t_e = 20 minutes.
- Prior to autoclaving, **loosen or remove the bottle head from the bottle.**

- The number of autoclaving cycles can be marked on the plastic disc (memory disc) of the filter (max. 20 autoclaving cycles).

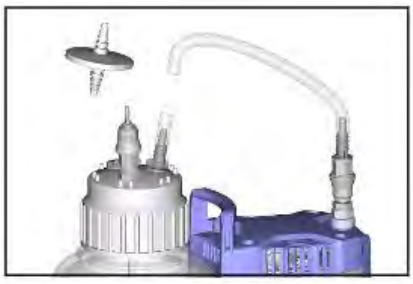


- Over time, discoloration and material changes (e.g. resiliency, elasticity, cracking) due to repeated steam sterilizations/autoclaving and/or chemical disinfection, may occur. Check all parts regularly and replace defective parts.

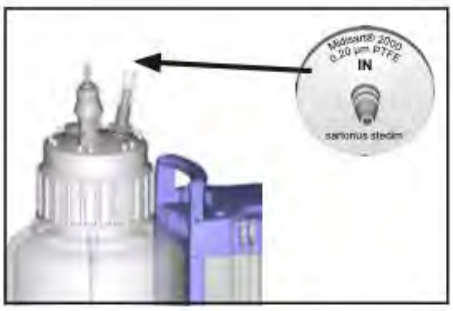
Replacing filter

- Vent the collection bottle. Ensure that there is no liquid in the tube to avoid risk of contamination.

- Remove connecting tube from the filter. Remove the filter from the piece of tube at the hose nozzle.



- Attach new filter. Observe flow direction. Position filter with the printed side “IN” towards the bottle. Attach the connecting tube.



Broken Glass Disposal

1. Purpose:

To provide step by step guidance on how to handle broken glass.

2. Prerequisites:

You are an authorized user of DB440 and are either included in your PI's permit, or you possess a CL2 permit for DB440.

3. Responsibilities:

It is the responsibility of all faculty, staff and students to follow the procedures described in this SOP.

4. Personal Protection Equipment (PPE):


5. Procedure:

Broken Glass

1) Wear PPE as described above. Use a brush and dust pan (in Spill kit) or tongs, etc. to pick up broken glass pieces.
   i) **DO NOT** pick up broken glass with your hands.
2) Dispose chemically contaminated broken glass in the chemical waste pail. Dispose biologically contaminated broken glass in the Bio waste pail as per the **"Biohazard Waste Disposal" SOP**.
3) Remove and discard your gloves. Wash your hands with soap and water.

STANDARD OPERATING PROCEDURE: Biological Safety Cabinet (BSC) Use

1. Purpose:

To provide step by step guidance on how to safely use a Biological Safety Cabinet (BSC).

2. Scope:

Applies to all authorized Principal Investigators (PIs) and authorized laboratory personnel who use a BSC in a Lash Miller CL1-CL2 laboratory.

3. Prerequisites:

You are an authorized user of DB440 and are either included in your PI's permit or you possess a CL2 permit for DB440.

4. Responsibilities:

It is the responsibility of all faculty, staff and students to follow the procedures described in this SOP.

5. Personal Protection Equipment (PPE):

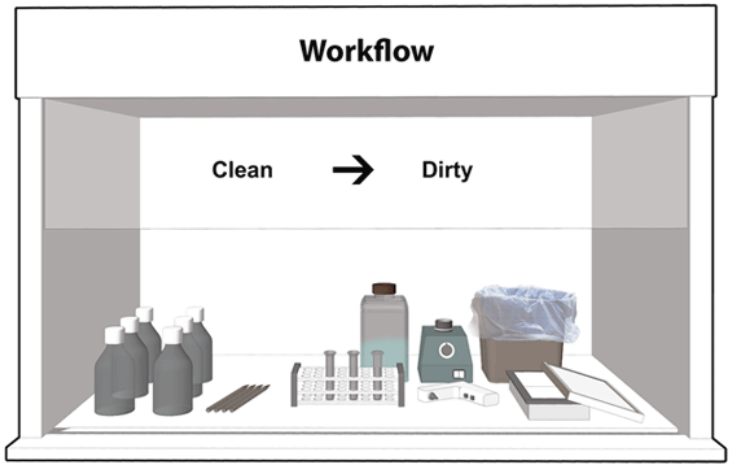
6. Procedure:

Watch this video on working in a BSC.

Before you begin work in the BSC:

- Plan ahead, schedule uninterrupted work times by using the BSC Booked Scheduler.
  People walking or doors opening disturb airflow in the cabinet
- Check that the BSC has been certified to NSF/ANSI 49 within the same year you are using it.

- Open the sash to the appropriate height (10 inches) and ensure that the stool height is set so that your underarms are at the same height to the bottom of the sash position.
- Turn on the BSC's power switch (located on the top of the right-hand side), the light, and then the blower switch.
    - NOTE: Ensure that the intake and exhaust grilles are not blocked before turning the blower switch on.
- While waiting for the BSC to purge the inside air, creating a laminar flow of HEPA filtered air (10 min), disinfect the work surface of the BSC with 70% ethanol.
- Hold a tissue at the middle of the edge of the sash and ensure that it is drawn in.
- Gather all materials you will be loading into the BSC and wipe them with 70% ethanol before bringing them inside the BSC.
    - NOTE: Do not load paper, writing utensils, and other contaminated objects in the BSC.
- Arrange materials to minimize movement within the cabinet. Segregate clean items from dirty items (see diagram below).
- Place aerosol-generating equipment (e.g. Vortex) closer to the back of the BSC (see diagram below).



- **Never block the grilles at the front or rear of the cabinet**
    - Doing so will disrupt the laminar flow and could potentially result in personal exposure and/or sample contamination.
- After loading all materials, wait an extra 5 minutes for the air to purge in the BSC, before starting work.

Working in the BSC:

- Wash hands thoroughly with soap and water before and after procedures.
- Avoid rapid movements during procedures.
- Minimize entering and exiting the BSC, therefore as feasible, try to keep all biohazardous waste within the BSC until work is completed.

1) Wear sterile gloves. Consider double gloving.
2) Move hands and arms straight (perpendicular) into and out of the BSC.

- Avoid resting your elbows/arms on the work surface and front grille.
- While performing procedures, separate non-contaminated and contaminated items (see diagram above) in the BSC. This will then dictate your workflow, which should be from the clean to the dirty area. Following this best practice will help prevent sample contamination.
- UV lamps are not recommended nor required in BSCs. Proper cleaning and disinfection using liquid disinfectant negates the need for use of UV lamps.
- **DO NOT** use open flames (e.g. Bunsen Burners), on-demand open flames, natural gas, and propane inside the BSC.
    - Use non-flame alternatives instead. For more information about these options, consult chem.safety@utoronto.ca
- **DO NOT** use equipment which create air movements (e.g. Centrifuge).
- **DO NOT** overflow the collection flask.

See "Using BVC Pro Aspiration System SOP" for instructions on how to use the aspiration system

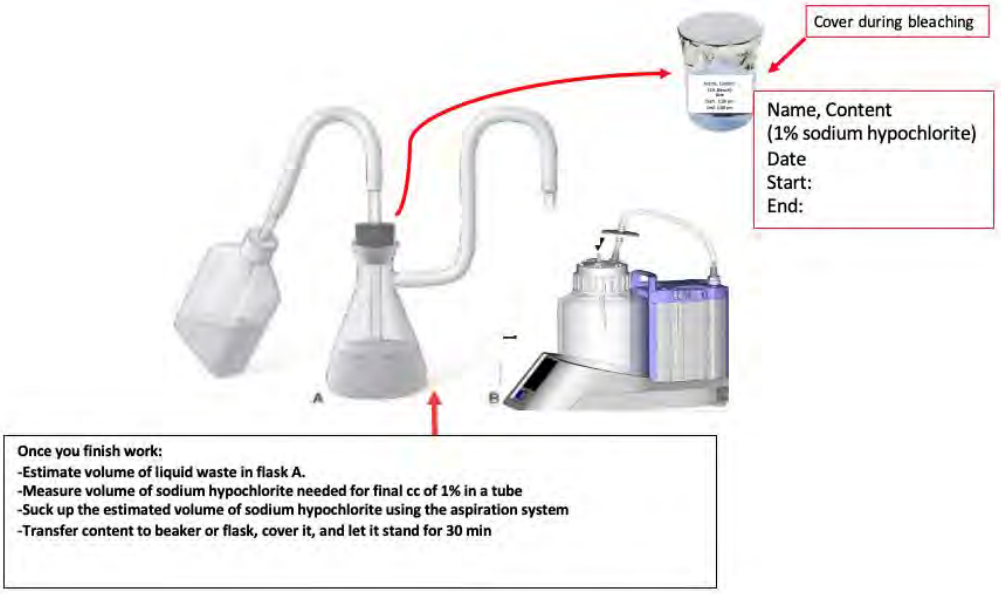
After completing work in the BSC:

- Wipe the exterior of all materials with 70% ethanol before unloading them from the BSC.
    - NOTE: Materials and equipment cannot be stored inside the BSC.
- Remove and dispose biohazardous waste (follow **"Biohazard Waste Disposal SOP"**).
- Remove liquid waste as per ***Appendix 1*** or by following the **"Biohazard Waste Disposal" SOP.**
- Remove and throw away your contaminated gloves into the Bio waste pail and wash your hands.
- Put on new gloves and disinfect the interior surfaces of the BSC with 70% ethanol.
- Turn off the lights, blower, and the power of the BSC. Once done, close the sash.
- Remove and dispose your gloves, wash your hands at the sink.
- Follow the **"Entry and Exit Procedures" SOP** to leave the room.

In Case of BSC Failure or Alarm:

- Remain calm. Immediately stop work.
- Close all primary containers if not working with biohazardous materials, surface decontaminate items, remove them from BSC, post "Do Not Use" signage found in Appendix 2, and report problem to supervisor.
- If working with biohazardous materials, continue to follow instruction below:
    - Remove gloves and discard in BSC waste
    - Turn off BSC and close sash
    - Wash hands
    - Post "Do Not Use" signage
    - After 30 minutes, don clean PPE, surface decontaminate, then remove items in BSC
    - Decontaminate work surface, close sash, leave "Do Not Use" signage on BSC
    - Report problem to supervisor

Appendix 1: Liquid waste removal using the vacuum line in the BSC (from "Biohazard Waste Disposal" SOP)



Appendix 2: "Do Not Use" BSC Sign

CAUTION

DO NOT USE THIS BSC UNTIL REPAIRS ARE MADE AND PROPER FUNCTION IS VERIFIED

DATE:……

- **CLOSE CHEMICALS & STORE THEM AWAY**
- **CLOSE SASH**
- **IF AN EXPERIMENT IS IN PROGRESS, KEEP THE SASH CLOSED**
- **CALL 8-3000 & ALWAYS CONTACT THE CAO: grace.flock@utoronto.ca**

- **If you do not see a note from Facilities and Services (proof that they have attended to the issue) within 24 hours, please contact the CAO**
- **If a resolution is not achieved within 72 hours, please contact the CAO**

Chemistry
UNIVERSITY OF TORONTO

Booking Biological Safety Cabinets (BSC)

1. Purpose:

To provide instruction on how to access and use the BSC booking scheduler.

2. Scope:

Applies to everybody who uses the BSCs.

3. Prerequisites:

A UTORid is needed to make reservations in the booking scheduler

4. Responsibilities:

It is the responsibility of everyone to follow this SOP. It is the responsibility of everyone using BSCs to use the booking scheduler, and everyone using the booking scheduler should cancel bookings if they are no longer needed.

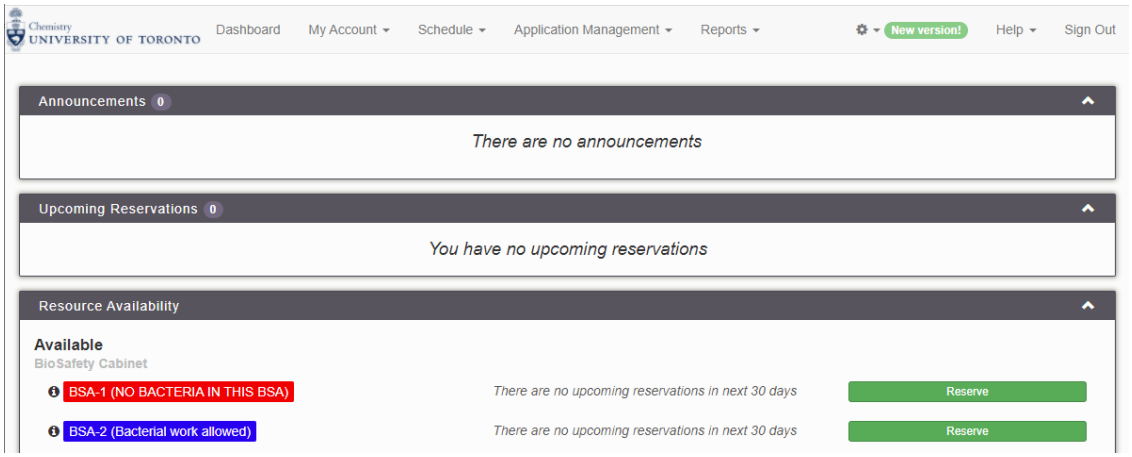
5. Procedure:

Accessing the Booking Scheduler

1) Go to https://trackit.chem.utoronto.ca/booked/Web/dashboard.php
2) You will be prompted to enter your UTORid and password. If you have UTORMFA enabled, you will be required to use the DUO Mobile app or an MFA Token Key.

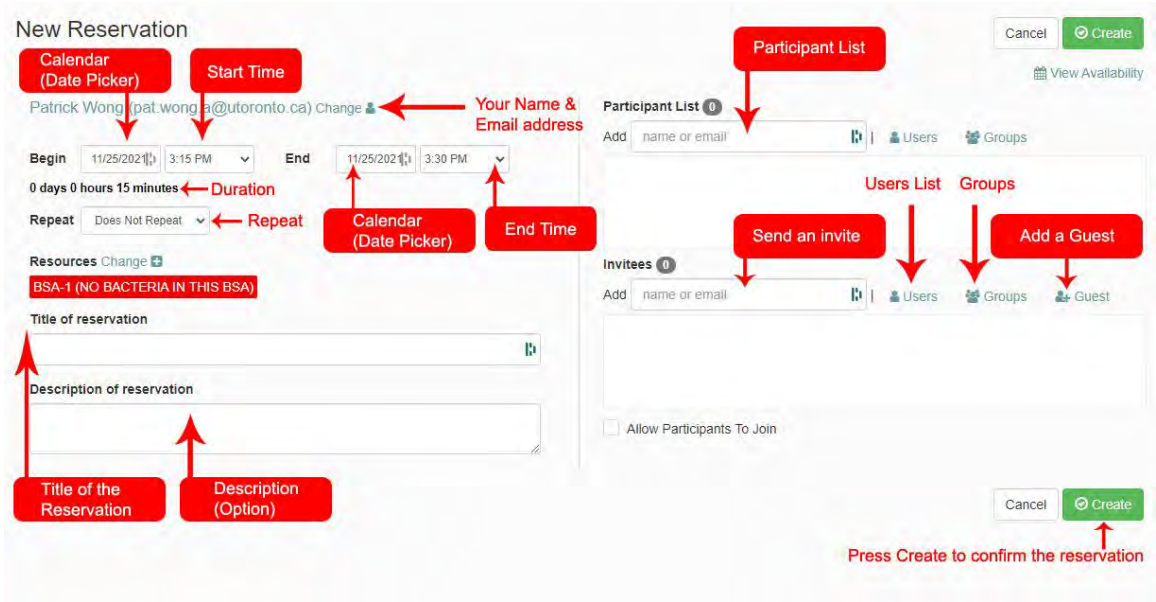
Booking

1) You will be taken to a Dashboard, which is your quick view into your day.
2) Announcements will include any recent announcements posted by the administrator, located on the top section.
3) Upcoming Reservations will show your reservations for the next 2 weeks.
4) Resource Availability will display the booking resources. Click on the Reserve button on the right side for the associated resource to book it. Note: The Biosafety cabinets are colour-coded. Hover your cursor to the resource name to view the reservation summary and booking information.



Make a Reservation

1) Click on the date and a calendar pop-up will appear. Choose your date. Click on the dropdown menu for the start time and end time. Note, all the time slots are 15-minute incremental intervals.
2) You can book multiple resources by clicking on the Change [+] icon next to the Resources.
3) You can repeat the reservation every day, week, month, or year if there's no conflict. Note: Reservations can only be made up to 14 days in advance (can be made the week of, or for the following week).
4) Enter the title of the reservation, the description is optional.
5) Add other people to participate by entering their name or email in the participant list.
6) For the invitees, inviting a user will send an invitation email and give the user an option to accept or decline the invitation. You can also invite unregistered users as guests by entering an email address.
7) When you click Create, it will verify if there are any reservation conflicts. If everything is good, you will see the booking is successful.



Eppendorf™ 5810R Centrifuge

1. Purpose:

To provide step by step guidance on using and maintaining the Eppendorf™ 5810R Centrifuge.

2. Scope:

Applies to all authorized Principal Investigators (PIs) and authorized laboratory personnel using the Eppendorf™ 5810R Centrifuge in the LM CL2 facility (DB440).

3. Prerequisites:

You are an authorized user of DB440 and are either included in your PI's permit, or you possess a CL2 permit for DB440.

4. Responsibilities:

It is the responsibility of all faculty, staff and students to follow the procedures described in this SOP.

5. Personal Protection Equipment (PPE):


6. Procedure:

Before a Centrifugation run

1) Go through the pre-centrifuge run inspection checklist below:
   i) Visually check that there are no cracks/damages on the bottles/tubes/plates/rotor.
   ii) Ensure that the tubes and rotors are dry and clean.
   iii) Visually check if the overall centrifuge is in good condition.

2) Turn on the centrifuge and open the centrifuge lid.
3) Put the rotor onto the motor shaft, place the rotor key into the rotor nut and turn the rotor key clockwise until the rotor nut is tightened.
    i) NOTE: The rotor has been inserted properly if you are able to see its maximum speed on the display (displays for 2 seconds).

Preparing samples for centrifugation

4) Fill plates/tubes working inside a BSC and wipe their exterior with 70% ethanol before taking them out of the BSC.
    i) Ensure that you do not fill them past 3/4 of their maximum capacity.
    ii) Ensure that all the filled tubes/plates are roughly the same mass.
5) If loading plates:
    i) Ensure that the plates perfectly fit into the bucket (not too big/small).

    If loading tubes:
    i) Ensure that you fasten lids on the tubes and label them before loading.

Loading your samples

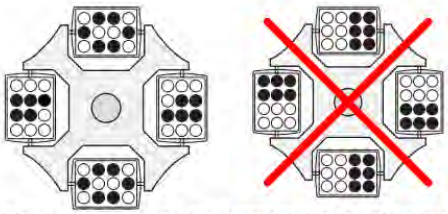


Fig. 4: Incomplete, but symmetric loading of the buckets. The pegs of each bucket must be loaded equally.

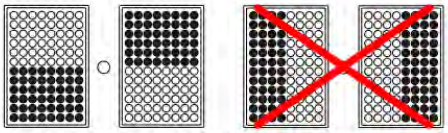


Fig. 5: Symmetrical loading of the plates.

The plate arrangement shown on the right-hand side is incorrect, as the buckets will not swing properly.

6) Ensure that your samples are balanced (see above).
7) Place aerosol-tight caps (biosafety caps), shown below, on the buckets by following the instructions on the Eppendorf Canada website.
    i) Ensure that they are properly secured before starting the run.

Starting the run

8) Push the centrifuge lid down and hold it until the lid latch engages and the lid is closed automatically.
   i) To ensure that the lid is closed, the "Open" button will turn blue and the display will show this: 
9) Select the speed/g-force (set the radius if setting the g-force), run time, temperature, and press the "start/stop" button to start the run.
   i) Ensure that the temperature is set between -9 °C and +40 °C.
   ii) **Remain on site until the centrifuge reaches the set speed to monitor performance and to ensure the run is balanced (running safely without noise and vibration).**
      i. If unusual noises/vibration or other unusual conditions occur, immediately stop the run by pressing the "start/stop" button.
   iii) **Log-in your use of the centrifuge using the log provided on site. Copies can be found in Appendix.**
      i. This helps determine when preventative maintenance should be done to the centrifuge.

After a Centrifugation run

1) When the centrifuge stops and the "open" button lights up, wait at least 10 minutes before opening the lid (if you have not used biosafety caps).
2) Check to see if there are spills in the centrifuge. If so, follow the **"Biological Spill" SOP**
3) Remove the buckets with the biosafety caps on and bring them to a BSC as a unit. Unload the tubes/plates.
   i) NOTE: Never remove the biosafety caps outside of a BSC or you will be exposed to aerosols generated during the run.
   ii) Follow the **"Biological Spill" SOP** if you notice any spills in the buckets due to broken tubes/plates.
4) Wipe the exterior of the buckets and the inside of the biosafety caps with 70% ethanol before taking them out of the BSC.
5) Ensure that the centrifuge lid is completely opened and that it can stay in that position without falling. Switch the centrifuge off.

Centrifuging infectious materials or human samples

- Place a biohazard label on the centrifuge.
- Always wear gloves when handling tubes or rotors.
- Avoid the use of celluloid tubes with biohazards. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to decontaminate them.
- **Always use sealed safety cups**, safety buckets, or sealed rotors with O-ring as secondary containment if available.
- Fill centrifuge tubes, load into rotors, remove from rotors, and **open tubes within a biological safety cabinet**.
- Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or bucket.
- Seal rotor or bucket, remove outer gloves, and transport to the centrifuge.

- **Always wait at least 10 minutes after the run to allow aerosols to settle before opening the centrifuge**. Check for possible spills or leaks. See emergency procedures, below.

Emergency Procedures:

1. Emergency Situations

The following events are considered an emergency:

- If there is a spill in the centrifuge
- If centrifuge malfunctions
- If there is rotor failure
- If there is tube breakage

2. Emergency Procedures

- Turn centrifuge off immediately, close the centrifuge lid.
- Notify others, evacuate the facility, close the door, post a biohazard spill sign at the facility door.
- Leave for 30 minutes to reduce the risk of aerosols. For spill clean-up, the operator should wear proper gloves, remove debris, clean, and disinfect centrifuge interior, rotors, safety cups or buckets following the manufacturer's instructions/instructions posted on-site.
    - Use 1% sodium hypochlorite to disinfect the centrifuge and its parts. Contact time of 20 minutes.
    - Then thoroughly clean with soap and water, and rinse a minimum of 3 times with water.
    - Do a final decontamination rinse with 70% ethanol.
- Place any contaminated protective clothing, gloves, and all clean-up materials in a biohazard bag. Wash hands and any exposed skin surfaces with soap and water.

Call 911 or seek immediate medical attention if overtly exposed to recombinant or synthetic nucleic acid molecules or Risk Group (RG) 2 infectious agents.

Report incidents to P.I.

Centrifuge Maintenance:

- Moisture, chemicals, strong cleaning agents, and other substances can promote corrosion of centrifuge parts and cause centrifuge failure. The following are general maintenance recommendations:
    - Follow manufacturer instructions for maintenance and cleaning.
    - Keep the centrifuge clean and dry. Only use cleaning agents that are compatible with the centrifuge. Avoid aggressive or corrosive cleaning and disinfecting agents.
    - Clean the centrifuge and its accessories by wiping with 70% ethanol. Use a damp cloth/paper towel. Complete cleaning procedure can be found in the Facility Duties Checklist and follow instructions posted on-site.
    - Cleanup all non-infectious spills immediately. For infectious spills see Emergency Procedures above.
    - Never clean rotors and associated parts with abrasive wire brushes.
    - Store the rotor upside down in a dry place, with lids or plugs removed, to prevent condensation.
    - Remove adapters after use and inspect for corrosion.
    - Inspect rotor regularly. Remove rotors from use that show any sign of defect and report it to a manufacturer's representative for inspection.

Maintaining a Logbook:

To avoid rotor failure, keep a logbook for high-speed rotors, recording the length of time and speed for each use. Track and discard rotors according to the manufacturer's recommended schedule. See logbook below in Appendix.

Appendix: Centrifuge use log

Name	PI	Date	Speed	Length of run time	Set Temperature

STANDARD OPERATING PROCEDURE: Autoclave Usage

1. Purpose:

To provide step by step instruction on how to safely use an autoclave.

2. Scope:

Applies to everybody using an autoclave.

3. Prerequisites:

WHMIS, Laboratory Biosafety course (EHS601), and autoclave training session

4. Responsibilities:

Principal investigators are responsible for enforcing this SOP and ensuring that those operating the autoclave are properly trained. Lab-personnel are responsible for complying with this SOP.

5. Personal Protection Equipment (PPE):

Eye/face protection (if necessary), nitrile gloves, insulated gloves, lab coat

Insulated gloves

6. Procedure:

Before Using Autoclave/Sterilizer

1) Prior to using the autoclave, verify that it has been functioning correctly by reviewing previous cycle log recordings (time, temperature, pressure), and the results of efficacy testing with biological indicators if available.
2) Follow the steps below before using autoclave:
    1. Open chamber door and check drain strainer is clean and in place.

2. Verify chamber interior is clean and close chamber door. Refer to Section 8 of autoclave manual if cleaning is necessary.
3. Open front cabinet panel on load end of autoclave. Verify steam and water supply valves to sterilizer are ON. Close cabinet panel.
4. For *Manual Flush option only*, if autoclave is equipped with an integral electric steam generator, flush and start up generator as outlined in Section 4.7 of manual. This is done by opening the front panel of autoclave. Only manual flush when autoclave is cool.
5. Open printer access door and verify amount of printer paper is sufficient. A colored warning stripe is visible when paper roll is near end. Refer to Section 8 of manual if paper roll needs replacement, or to Replacing Paper Roll section of this SOP.
6. Close printer access door. Printer records sterilizer type.
7. Enter Operator Mode (refer to Section 5.1 of manual). NOTE: Once operating mode is entered, steam enters sterilizer jacket and heats jacket to 115°C. Also, the isothermal mode does not turn jacket on.
8. Load chamber as outlined below and in Appendix 1.

Using Autoclave

1) Wear the appropriate PPE required to safely handle the material being loaded into autoclave
2) Prepare materials to be autoclaved following the guidelines shown in Appendix 1
3) Place material in autoclave ensuring it is evenly spaced out and not overloaded, or **fully sealed**. Containers holding liquids should not be more than 75% full.
    a. Do not mix solid and liquid materials (they use different autoclave cycles)
    b. Place packages on their edges, and empty flasks/tubes horizontally
    c. Ensure all containers allow steam penetration (**slightly open autoclave bags and bottles**) (see Appendix 1 for more information)
    d. Primary containers must be placed into secondary containers, which must be made of a material that can withstand repeated autoclaving
    e. When sterilizing liquids, ensure only Type 1 borosilicate glass bottles are used, and not ordinary glass bottles

Types of Cycles

1. Pre-Vacuum Cycle
    - Rack item, peel pouches (wrapped)
    - Can extract air from inside

2. Gravity Cycle
    - Unwrapped items eg. Glass items

3. Liquid Cycle
    - Vented containers, loose caps
    - Exhaust slowly to ensure liquid doesn't turn to gas

4. Waste Cycle

Loading Autoclave

a. Open chamber door.
b. Slide shelf half way out of sterilizer chamber.
c. Place load on shelf and slide shelf back into chamber. Ensure shelves are completely inside chamber before closing door.
d. Close chamber door. Sterilizer is now ready to run a processing cycle. Refer to appropriate Cycle Operation Instructions included in manual, for instruction on running the cycle. Ensure cycle is long enough to allow for it to reach appropriate temperature.

4) Close and latch autoclave door firmly
5) Choose the appropriate cycle for the material (to determine which cycle and time is best consider):
    a. Whether it is to be decontaminated or sterilized
    b. Composition of the load (solid or liquid)
    c. Density of material
    d. Volume and viscosity of liquids (larger volumes require more time)

6) Cycle selection includes: slow exhaust (for liquids), fast exhaust (for glassware), fast exhaust and dry (for wrapped items)
    a. Consult autoclave manual for assistance in choosing a cycle
    b. **When sterilizing liquids, always use Liquid cycle**
7) Do not open the door while the autoclave cycle is occurring. If a problem with the autoclave is perceived, abort the cycle and contact the person in charge immediately
8) When unloading autoclave, wear necessary PPE (ex. Heat-insulating gloves)
9) Ensure that cycle is complete and both the temperature and pressure have returned to a safe range. **Check chamber pressure gauge before opening door- it should be zero**
10) Carefully open door a little and avoid the steam. Allow steam to escape and the pressure within liquids and containers to stabilize.
11) Do not disturb containers of super-heated liquids or remove caps prior to unloading these materials. Gently transfer containers to trolley.
12) Check autoclave tape for colour change and cycle log recorder for time and temperature attained.
13) If disposing of biological liquid waste after autoclaving, first allow to cool, then pour down the drain.
14) Ensure door is closed after unloading autoclave.

Important Functions/Buttons

- Button on top left of screen puts unit on stand-by
- Inverted triangle at the bottom of the screen is the abort button
- Red button on right is emergency button, which needs key
- Emergency exhaust is the red handle, which should only be used if the autoclave needs to be opened in an emergency

Replace Printer Paper Roll

- Replace the roll whenever a colored stripe is visible on one or both edges of the printout paper.

1. Open thermal printer front cover. Note: cover is magnetically held closed.
2. Lift Inner cover.
3. Position paper roll as follows:
    a. Position paper roll in bottom of printer compartment, paper coming from bottom of roll.
    b. Ensure at least 8 inches of paper extends from printer compartment.
4. Close inner cover.
5. Remove take-up spool from printer and carefully pull apart.
6. Place paper between take-up spool; then push spool halves together.
7. Tightly wind paper around spool two to three times; then place spool back in original position (at top of printer compartment).
8. Close printer cover.

Clean Chamber Drain Strainer

Allow autoclave to cool before performing any maintenance.

1. Remove drain strainer from drain in chamber bottom.
2. Remove any obvious debris from strainer. If necessary, clear screen in strainer using a brush, wire, or similar tool.
3. Once strainer has been cleared of obvious debris, reverse/rinse strainer under running water.
4. Replace strainer in chamber drain.

Flush Chamber Drain

Flush chamber drain as follows whenever line becomes clogged:

1. Turn OFF steam supply valve. Wait until jacket pressure is zero. Wait until chamber has cooled to room temperature.
2. Remove chamber drain strainer. Clean strainer using procedures given above, if necessary.
3. Pour hot solution of 15mL of tri-sodium phosphate to 500mL of hot water.
4. Open door and return strainer to drain.

7. Procedure for Injuries/Spills:

1) All incidents, including spills must be reported to the supervisor and department (Please refer to the Spill Reporting Procedure webpage on the EHS website
2) If any injury occurs, seek first aid and/or medical assistance depending on injury

    a. Accident report must be filled out in the event of injury (Please refer to either the Accident Form for Students webpage or the Accident Form for Employees webpage on the EHS website)
    b. If clothing absorbs hot water/steam, remove clothing and apply cool water/ice to affected area. Use safety shower if necessary (use with clothes on).
3) Notice must be placed on autoclave to indicate unit is out of service until the cause of incident is identified, pro-active measures are taken to prevent future accidents, and autoclave is deemed safe for operation (see Appendix 2 for sign to place on autoclave)
4) No operation of the autoclave is allowed until the spill is cleaned up, investigation of the incident has been completed, and the autoclave has been found safe to use
5) Operator is responsible for the clean-up. Wait until the autoclave and materials have cooled down to room temperature before attempting to clean-up.
6) If spill of biological material occurred before autoclaving (during loading), follow biological spill SOP. If spill is found after autoclaving, then any biological material should no longer be hazardous.
7) Dispose of any cracked glassware properly

Appendix 1:

All containers must have a loose seal, which can be achieved by:

- Loosening screw caps or using self-venting caps
- Capping open containers for sterilization with aluminum foil (see Image 1 below)
- Opening plastic (autoclave) bags slightly prior to loading them into the autoclave
- Using envelope folds for wrapping Kraft paper or muslin

Indicators

- Physical Indicators: pressure and temperature recording devices. Thermocouples can be placed inside the load to determine the temperature achieved in the bag itself.
- Chemical Indicators: change colour after being exposed to specific temperatures e.g. heat sensitive tape.
- Biological Indicators: bacterial spores are autoclaved along with the load, and incubated for period stated by the manufacturer, and observed for any sign of growth that would indicate that autoclave is not sterilizing properly. *Bacillus stearothermophilus* spores are used, as they are most resistant to steam autoclaving.

Autoclave Tape

- Temperature sensitive tape should be affixed to each item to be autoclaved (see Image 2 below)
- Lines will appear when this tape is exposed to high temperatures (see Image 2 below)
- **It is not proof that the autoclave cycle was successful at decontaminating or sterilizing the contents** (just means the outside of the container got hot)
- A biological indicator or other means should be used to validate the efficacy of the sterilization procedure

Image 1

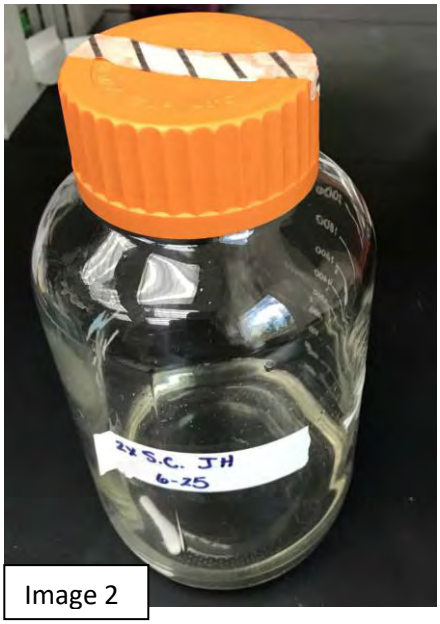


Image 2

Appendix 2:

AUTOCLAVE IS OUT OF SERVICE

Handling and Storing Compressed Gas Cylinders

1. Purpose:

To provide instruction on how to properly and safely handle and store compressed gas cylinders

2. Scope:

Applies to everybody handling and storing compressed gas cylinders

3. Prerequisites:

WHMIS and EHS113 Compressed Gases Safety Training

4. Responsibilities:

Principal investigators: are responsible for enforcing this SOP, ensuring that all gas cylinders are used, stored, and transported according to applicable legislation and guidelines, and providing training to cylinder users. They are also responsible for ensuring that the right regulators are provided and maintained/serviced when needed, that the cylinders are in good condition, and that the proper storage is provided.

Lab-personnel: are responsible for being aware of hazards associated with compressed gasses, following this SOP, and receiving appropriate training prior to handling compressed gasses. They are also responsible for checking cylinder and regulator conditions, and for handling and storing cylinders per this SOP.

5. Personal Protection Equipment (PPE):



6. Procedure:

Ensure you review the hazards associated with cylinders in Appendix 1 and 2.

See video for more information on handling compressed gas cylinders:
https://www.youtube.com/watch?v=uOvwDbNDdWA

Moving Cylinders:

1) Remove the regulator and make sure the valve protection cap is in place or that the smart-top valve is closed before moving a cylinder. Never move a cylinder with the regulator attached. See Figure 1 for valve protection cap image (below).

Figure 1

2) Do not lift a cylinder by the valve caps. Never sling with ropes or chains or lift with electromagnets.
3) Elevators must be used in all cases to transport cylinders.
4) Move cylinders with appropriate trolleys/carts (see Figure 2 below). Use proper lifting cradles. Cylinders can be rolled for short distances **on their base** in order to mount them on the cart.
5) Move cylinders individually onto the cart (in the upright position). Avoid striking other objects. Place the cylinder on the cart in an upright position. The cylinder must now be tightly secured with straps or chains (Figure 2 below). Do not ever transport cylinders that have not been strapped.
6) Lecture size or small compressed gas cylinders less than 3 inches in diameters and less than 20lbs can be hand carried.
7) While holding the cylinder, tilt the cylinder so that the rear wheels of the cart are touching the floor. It is now secure to transport the cylinder (Figure 3 below).
8) Cylinder MUST BE SECURED WITH STRAPPING once in position at the storage or point of use area (Figure 4 below).

Figure 2

Figure 3

Figure 4

Storing Cylinders:

1) Cylinders must be stored in dry, ventilated areas
2) Must be stored upright and capped when not in use (to protect valve from damage). Keep valves closed. Use the valve to shut off gas flow, not the regulator.
3) Cylinders must be labelled
4) Cylinders should usually be strapped securely to an immovable object.
5) Must be kept away from fire, sparks and electricity, including intense sources of heat (ex. radiators, welding, and Bunsen flames). Ambient temperature should not exceed 52°C.
6) Compressed gasses that may react with one another either need to be stored in separate fire compartments or they need to be separated by specific distances.
7) Do not lay acetylene cylinders on their sides. If an acetylene tank has accidentally been left on its side, set it upright for at least 1 hour before it is used.
8) Do not try to refill a cylinder or mix gases in a cylinder
9) Call the supplier to remove leaky cylinders immediately. **Disposal** of gas cylinders should be arranged with the gas supplier for empty or otherwise unneeded cylinders. In the event that this is not possible, follow the UofT Hazardous Waste Disposal Procedures found at http://www.ehs.utoronto.ca

Cylinder Use:

Before commencing connection to the regulator:

1) Ensure the gas cylinder contains the gas of interest and it is securely fastened
2) Remove the valve protective cap. It may require some force to remove.
3) Ensure the appropriate gas regulator is available
4) The appropriate tubing (chemically resistance, proper sizing, pressure sustainable) must be available to connect to the outlet valve/port

Connecting the regulator to the cylinder:

1) Please note that some gas nut tightens clockwise (helium, nitrogen, argon) and some have gash which denotes they tighten counterclockwise (hydrogen, methane, air).
2) Teflon tape can be placed along the threads of the nut (Figure 5).
3) Take the regulator in your hand and place the nut onto the nipple of the regulator, to ensure a tight seal. HAND TIGHTEN the nut as far as possible. The appropriate gas regulator is available.
4) Once the regulator is secured tightly by hand, use the palm of the hand to firmly tap the wrench to secure an even tighter seal. When a dull metal thud sound is heard, similar to a muffled bell, then the regulator is attached properly (Figure 6).

Figure 5 and 6

Connection from the regulator:

1) Insert an equipment specific outlet valve to one (or more) of the outlet ports. Securely tighten the connection and check for leaks.
2) The outlet port should have an outlet valve to control the gas flow to your system
3) Connections to the outlet valve can be quick-connect, hose bard and Swagelok (1/8", 1/4").
4) Set up the appropriate tubing to the valve that will be able to sustain the pressure and chemical interaction with the gas
5) Consult the gas supplier or the equipment manufacture
6) Connect the tubing from the equipment to the outlet valve

Operating the regulator:

1) Do not face the regulator when you turn the gas valve and regulator on
2) Two major types of regulators exist. Single stage and Dual Stage
    - Single stage is less accurate when reducing the source pressure down to the desired delivery pressure in one step
    - Dual stage is more accurate in its ability to deliver a constant pressure, even with a decrease in inlet pressure
3) Turn on the flow of gas by turning the valve on the top of the gas cylinder counterclockwise in the direction of the arrows labeled "Open".
4) When using SmartTops ™, the flow of gas is 'opened' when raising the red valve at the top of the cylinder. See Figure 8 on page 5.
5) The cylinder regulator has two pressure gauges. The gauge to the right will read the pressure remaining in the cylinder. This gauge will IMMEDIATELY become active. NOTE: In most cases the gas supplier only guarantees the purity of the gas in the cylinder so long cylinder pressure is above 500psi. See Figure 7 (below) for diagram.

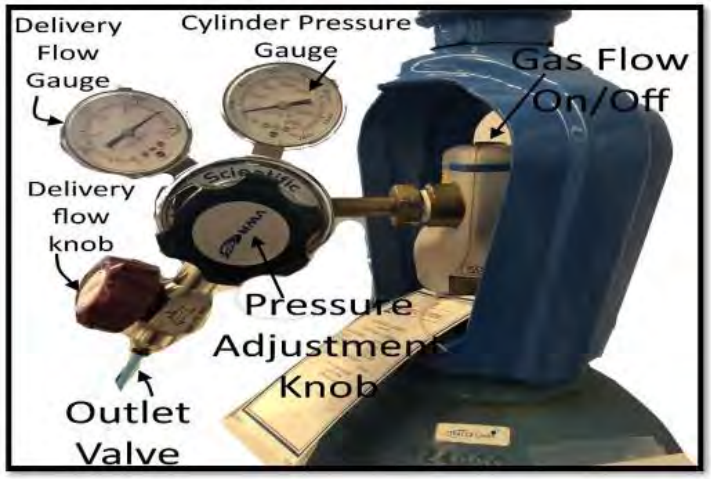


Figure 7

6) The gauge to the left is the delivery pressure. This gauge should be set to the required delivery pressure by turning the pressure adjustment knob.
7) Turn the delivery flow knob to "Open" and at this point the flow of gas should commence into the desired system.

8) In many cases the instrument/equipment must be operational to correctly 'fine tune' the delivery pressure.
9) If the gas is not needed it should be turned off by closing the valve on the top of the gas cylinder clockwise in the direction of the arrows labeled "Close".
10) When using SmartTops ™, the flow of gas is 'closed' when lowering/dropping the red valve at the top of the cylinder. See Figure 8 below.

Figure 8

Removing the regulator:

1) Cylinder should be replaced once the pressure inside the cylinder has dropped below 500psi
2) Ensure that the valve on the top of the gas cylinder is turned clockwise in the direction of the arrows labeled "Close"
3) At this point, allow the gas to purge into the system until it reads zero in BOTH gauges
4) Alternatively, close the delivery flow knob
5) Disconnect the tubing from the outlet valve
6) Slowly release the pressure by opening the delivery flow knob and release the pressure until it reads zero in BOTH gauges. CAREFUL. THIS SHOULD ONLY BE DONE WITH INERT GASES.
7) Disconnect the tubing from the outlet valve
8) Place the wrench onto the nut of the regulator, where it meets the cylinder. Firmly work the wrench in the opposite direction that it was secured.
9) Once it is loosened with the wrench, it is preferable to loosen the regulator by hand
10) Remove the regulator and store for future reuse.

Regulators should be connection leak tested on a regular basis, and creep (internal leak) tested at least annually. The frequency of testing depends on factors such as the toxicity of the gas, the corrosivity of the gas, and the use of the gas. More toxic, more corrosive and use of the gas in a critical system increase the frequency of tests. For low hazard situations, it is recommended by manufacturers that external leak testing be conducted monthly, and creep testing annually. Environmental conditions can dramatically affect the life of a regulator; of particular note is use outside or in salt spray (ocean) conditions.

Appendix 1: Title

Compressed Gas Hazards

Figure 9 (Compressed Gas WHMIS Symbol 2015)

All compressed gases are under pressure and a great deal of potential energy is contained within the walls of each cylinder. This danger stores a potential rocket or bomb if the pressure is released through rupture of the valve or container failure.

Fire and Explosion Hazards

Flammable Gas

Fire and explosion may occur when gas concentration is within the flammable or explosive range in the presence of an ignition source or when the auto-ignition temperature is reached. MUST BE KEPT AWAY FROM Oxidizing Gases.
Examples: Acetylene, Hydrogen, Methane

Oxidizing Gas

Gas can react rapidly and violently with combustible materials and result in fire and explosion when mixed with oxygen at or above atmospheric concentrations. MUST BE KEPT AWAY FROM Flammable Gases.
Examples: Oxygen, Nitrous Oxide, Xenon

Reactive Gas

When exposed to slight temperature or pressure increases, or mechanical shock, these can readily undergo certain types of chemical reactions such as polymerization or decomposition leading to fire or explosion.
Examples: Vinyl Chloride, 1,3 Butadiene, Acetylene

Health Hazards

Inert Gas

The largest group of gases. They will displace air thus reducing oxygen levels and can cause loss of consciousness or even death. Examples: Nitrogen, Helium, Argon

Toxic Gas

These gases can cause various health problems (including death) upon inhalation, eye, or skin contact. Examples: Hydrogen Sulphide, Suphur Dioxide, Silane

Corrosive Gas

Typically, the gas attacks and corrodes metals and in turn can destroy and burn body tissues on contact. Examples: Hydrogen Chloride, Ammonia

Poisonous (e.g. chlorine, fluorine), corrosive or dangerously reactive compressed gas The Principal investigator or manager of a lab that has the above compressed gasses must contact EHS to conduct a further assessment to ensure all procedures outlined in Section 5.6 Compressed Gas Cylinders of the Ontario Fire Code 213/07 and industry best practices are followed.

Compressed Gas Groupings

- *Non-liquefied gases* are also known as compressed, pressurized, or permanent gases. These gases do not become liquid when they are compressed at normal temperatures, even at very high pressures. Common examples of these are oxygen, nitrogen, helium, and argon.
- *Liquefied gases* are gases which can become liquids at normal temperatures when they are inside cylinders under pressure. They exist inside the cylinder in a liquid-vapour balance or equilibrium. Initially the cylinder is almost full of liquid, and gas fills the space above the liquid. As gas is removed from the cylinder, enough liquid evaporates to replace it, keeping the pressure in the cylinder constant. Anhydrous ammonia, chlorine, propane, nitrous oxide and carbon dioxide are examples of liquefied gases.
- *Dissolved gases* as gases dissolved in an inert material. Acetylene is the only common dissolved gas. Acetylene is chemically very unstable. Even at atmospheric pressure, acetylene gas can explode. Nevertheless, acetylene is routinely stored and used safely in cylinders at high pressures (up to 250 psig at 21°C). This is possible because acetylene cylinders are fully packed with an inert agamassan. The filler is saturated with acetone or other suitable solvent. When acetylene gas is added to the cylinder, the gas dissolves in the acetone. Acetylene in solution is stable.

Appendix 2

Cylinder Safety and the Importance of It

Hazard – Cylinder Contents

- Example: 2010 - Missouri
  - Lab using hydrogen gas; gas leak led to explosion
  - 4 injured, lab destroyed

Hazard – High Pressure

- Example: 2008 – United Kingdom
  - 80 cylinders stored in hallway; no caps; not properly secured; contained argonite - argon/nitrogen mix
  - One fell over, hit another, setting off chain reaction of 66 cylinders rocketing through hall.
  - Estimated speeds of up to 170 mph.
  - One person killed.

Reference Documents

- Ontario Fire Code O. Reg. 213/07, s.5.6
- How to Work Safely with Fact Sheets | Canadian Centre for Occupational Health and Safety
- Regulator Maintenance | Air Liquide
- Transporting Chemicals Within Buildings | The University of Toronto's The Office of Environmental Health and Safety
- Compressed Gas Use Program | The University of Michigan's Environmental Health and Safety Department
- CGA P-1, Safe Handling of Compressed Gases in Containers
- Compressed Gas Cylinder Use and Safety SOP | The University of Toronto's TRACES Centre
- Gas Cylinder Safety | Massachusetts Institute of Technology's Department of Materials Science and Engineering

Liquid Nitrogen (LN2) Use

WARNING: LN2 expands 700 times its volume, displacing O2, and may cause asphyxiation. IT DOES NOT SUPPORT LIFE.

1. Purpose:

To provide instruction on the safe handling of liquid nitrogen.

2. Scope:

Applies to all users.

3. Prerequisites:

WHMIS and site-specific training

4. Responsibilities:

Principal investigators are responsible to enforce this SOP and lab-personnel are responsible to comply. It is everybody's responsibility to report any equipment misuse and or deficiency to the lab manager.

5. Personal Protection Equipment (PPE):




6. Procedure:

Refer to Appendix to review Liquid Nitrogen hazards.

Liquid Nitrogen Storage:

1) Large amounts (more than 2L) of LN2 should always be stored in a well-ventilated area. Request a risk assessment from EHS to determine the need for an oxygen sensor.

Handling/Dispensing LN2:

<u>Transferring from primary container (Dewar) to large secondary containers (ex. 30L)</u>.

1) Put on PPE (as shown in above PPE section)
2) Always use the specially designed containers when transporting and handling LN2 (see below for examples)

3) Open valves of primary Dewar slowly to minimize thermal effects and control gas escape
4) **Do not** fill secondary containers to more than 80% of capacity; expansion of gases may cause pressure buildup
5) If the container tips over, let it go and evacuate (yourself and all people nearby).
6) Following, contact your PI

<u>Bench top containers: utilized for small scale use in the labs/pods</u>

1) **Never** dispense liquid into an unapproved container, such as a Thermos® bottle. It will shatter.
2) Transfer of LN2 can cause splashing, wear long pants, face shield and appropriate gloves
3) Utilize specialized withdrawal devices instead of pouring (LN2 Pump)
4) Transfer liquid slowly to prevent thermal shock, pressure buildup, and splashing. Always wear appropriate PPE.

Snap Freezing: https://m.youtube.com/watch?v=Qb6h4k2kLwM

Handling/Transporting/Thawing Cryotubes:

Cryotubes used to contain samples stored under liquid nitrogen may explode without warning when handling and thawing.

1) When thawing cryotubes, wear a face shield and safety goggles
2) Wear appropriate insulated gloves
3) Wear a buttoned lab coat, pants, and closed-toe shoes
4) Place the cryotube in a secondary tube (example, falcon tube), as a shield, while transporting and/or thawing

Emergency Response Procedure:

1) If there is a large spill or rupture of a Liquid N2 container, evacuate. Spill may induce oxygen deficiency.
2) Notify Campus Safety Special Constables (82323)

Personal injuries:

Minor injuries:

1) Cold burns should be immediately flushed with tepid water or placed in a warm water bath.
2) Notify your supervisor and fill out Incident Report
3) DO NOT RUB SKIN – may damage tissue
4) Contact first aid (emergency contacts posted at entrance of every lab)
5) Seek medical attention immediately for assessment and follow-up: **Ex: emergency department at nearby hospital (Mount Sinai or Toronto General Hospital) or walk-in clinic (depending on severity).**

Major injuries: Call 911 for major injuries.

Appendix

Liquid Nitrogen Hazards

Extreme Cold: The vapor of liquid nitrogen can rapidly freeze skin tissue and eye fluid, resulting in cold burns, frostbite, and permanent eye damage even by brief exposure.

Asphyxiation: Liquid nitrogen expands 700 times in volume when it vaporizes and has no warning properties such as odor or color. Hence, if sufficient liquid nitrogen is vaporized so as to reduce the oxygen percentage to below 19.5%, there is a risk of oxygen deficiency which may cause unconsciousness. Death may result if oxygen deficiency is extreme. To prevent asphyxiation hazards, handlers have to make sure that the room is well ventilated when using cryogens indoors.

Oxygen Enrichment: When transferring liquid nitrogen, oxygen in the air surrounding a cryogen containment system can dissolve and create an oxygen-enriched environment as the system returns to ambient temperatures. Since the boiling point of nitrogen is lower than oxygen's, liquid oxygen evaporates slower than nitrogen and may build up to levels which can increase the flammability of materials such as clothing near the system. Equipment containing cryogenic fluids must be kept clear of combustible materials in order to minimize the fire hazard potential. Condensed oxygen in a cold trap may combine with organic material in the trap to create an explosive mixture.

Pressure Buildup and Explosions: Without adequate venting or pressure-relief devices on the containers, enormous pressures can build upon cryogen evaporation. Users must make sure that cryogenic liquids are never contained in a closed system. Use a pressure relief vessel or a venting lid to protect against pressure build-up.

References and Additional Information

- Liquid Nitrogen Handling | The University of Iowa's The Environmental Health & Safety Office
- Standard Operating Procedure: Stores Operation|University of Toronto's Department of Chemistry
- Standard Operating Procedure: Transporting Cryogenic Liquids |University of Toronto's Department of Chemistry

CO2 Switch-Over Manifold for TC Incubators

1. Purpose:

To provide step by step instructions for the proper use and maintenance of CO2 switch over manifold that supplies gas to incubators.

2. Scope:

Applies to all users.

3. Prerequisites:

WHIMIS, EHS113 Compressed Gases Safety Training

4. Responsibilities:

Principal investigators are responsible to enforce this SOP and lab-personnel are responsible to comply

5. Personal Protection Equipment (PPE):



6. Procedure:

1) The protocol station should always be hooked up to two CO2 tanks: the left hose to the "Mother" tank, and the right hose to the "Secondary" tank.

2) The protocol station has three pressure indicator dials:
    a. Lower Left ("Mother Tank Pressure"): indicates tank pressure of the "Mother" tank.
    b. Lower Right ("Secondary Tank Pressure"): indicates tank pressure of "Secondary" tank.
    c. Top Middle ("Flow Pressure"): Indicates flow pressure feeding the incubators. Should be between 12 and 15 pSi. 3) There are two knobs on the protocol station:
        - Lower Left ("In Service Knob"): Indicates the tank that is service: should be turned to point left if the "Mother" tank is feeding the incubators. When the "Mother" tank empties, as indicated by the "Mother Tank Pressure" indicator dial reading 0, turn this dial to point to the right, towards the "Secondary" tank, to indicate the flow is coming from the "Secondary" tank. If this occurs, switch the "Mother" tank as outlined below (Point 4).
        - Upper Middle ("Flow Regulator Knob"): Regulates flow pressure to incubators. Leave at current setting. If the flow pressure drops noticeable, inform the facility manager.
3) If the "Mother" tank has gone empty and the protocol station is drawing from the "Secondary" tank, change "Mother" tank immediately.

To change the empty "Mother" tank to the full backup tank:

a. Using an adjustable wrench, loosen and remove the hose from the empty "Mother" tank.
b. Remove the "In Use" tab from the tag on the empty "Mother" tank. This tag should now only say "Empty".
c. Screw lid back onto the empty tank, ensuring the tag is tucked into the lid. Using chalk, write "Empty" on the tank.
d. Unscrew the lid from the "Backup Mother" tank that is present on location in the tank holder. Remove the "Full" tab from the tag.
e. Screw the Manifold's flexible hose removed from the empty "Mother" tank onto the "Backup Mother" tank. Using the adjustable wrench, tighten the hose well.
f. Turn the Lower Left Knob to point to the left, away from the "Secondary" tank. The protocol station is now drawing from the "Backup Mother" tank, which has now become the "Mother" tank. Turning the Lower Left Knob to the left now indicates this.
g. Print off a new "Mother" tank tracking log sheet and replace the "Mother" tank tracking log sheet from the old/empty "Mother" tank. Write down the date the tank was changed and the starting pressure of the new "Mother" tank.
h. Using the cylinder cart located in DB440, remove the empty "Mother" tank and bring it to the loading dock. Note: Ensure the chains on the cart are snug to hold the tank on.
i. Load a full tank onto the cart and bring it back to DB440 and place it in empty cradle spot where "Mother" tank was removed. Secure with chains. This new tank is now the "Backup Mother" tank.
j. Inform chem.safety@utoronto.ca that there is an empty CO2 tank from DB440, so the loading dock stock can be replaced.
k. Submit a copy or screenshot of the old "Mother" tank tracking log to chem.safety@utoronto.ca

Safe Sharps Use

1. Purpose:

To provide step by step guidance on safely using sharps.

2. Scope:

Applies to all authorized Principal Investigators (PIs) and authorized laboratory personnel working in the LM-CL2 facility (DB440).

3. Prerequisites:

You are an authorized user of DB440 and are either included in your PI's permit, or you possess a CL2 permit for DB440.

4. Responsibilities:

It is the responsibility of all faculty, staff and students to follow the procedures described in this SOP.

5. Personal Protection Equipment (PPE):

6. Procedure:

NOTE: In the event of an injury, refer to the **First Aid SOP**.

Sharps General Safety Procedures

- DO NOT shear, bend, recap or break needles during and after use.
- Place uncapped or exposed needles in a shallow tray during use.
    - NOTE: Never leave them unattended or out of your sight.
- Keep the needle away from your body during use.

- Do not pick up sharps with your hands. Instead use other methods such as a brush and dust pan or using either tongs or forceps.
- Do not clip used needles.
- When discharging liquids into a container using a needle, discharge the liquid against the side of the container to prevent exposure to aerosols.
- DO NOT recap disposable needles and syringes into their sheath or guard. They should be disposed of in a sharps container.

Sharps Disposal

- Immediately dispose of used needles and other sharps into the sharps container.
- Follow the "Sharps Biohazardous Waste Disposal" procedure in the **Biohazard Waste Disposal SOP**.

Standard Operating Procedure: Aerosol Risk Reduction Risk Group 2 (RG2) Biological Agents

1. Purpose:

To provide guidance on risk reduction techniques and safety precautions when working with aerosolizable biological agents. Aerosolizable Risk Group 2 (RG2) biological agents may include bacteria, fungal spores, toxins, viruses and viral vectors.

2. Scope:

Applies to those working with RG2 biological agents.

3. Prerequisites:

WHMIS, EHS601: Laboratory Biosafety Training, DB440-SST

4. Responsibilities:

It is the responsibility of all PIs to enforce this SOP, and it is the responsibility of all personnel working in the facility (DB440) to follow this SOP, and review all appropriate literature, product guides and Pathogen Safety Data Sheets (PSDS) before working with biologicals.

5. Personal Protection Equipment (PPE):



6. Procedure:

Aerosolizable biological agents are defined as the following: biological particles or liquids that have the potential to become suspended in a gaseous medium (e.g. air), that can be created by any activity that imparts energy into a liquid/semi-liquid material (CBS). Energy can be imparted through sudden movements including shaking, transferring, or dropping RG2 biological matter or liquid.

- Safe operational practices and the use of primary containment devices (i.e. Biological Safety cabinets (BSC), Gloveboxes etc.) can limit the creation, and prevent exposure to potentially infectious aerosols or aerosolized toxins.

Risks and Hazards

- The main mode of dispersal for aerosolizable RG2 biological agents is in a gaseous medium such as the air. Aerosols may remain suspended in the air for long durations of time.
- Exposure can occur by inhalation of the suspended biological agents or by indirect contact with contaminated surfaces on which the droplets produced by aerosol-generating procedures have settled and subsequent transfer to mucosal surfaces of personnel.
- If the primary mode of transmission and route of exposure/infection for a pathogenic RG2 biological is inhalation of infectious aerosols, then steps must be taken to prevent aerosol formation or release.
- Local Risk Assessments should also be done before working with biologicals.
- Refer to **Best Practices to Avoid Contamination Guidelines** for good general laboratory practices

DOs and DON'Ts

- Use plastic labware rather than glass (less likely to break which generates aerosols)
- Designate an incubator or at a minimum, assign dedicated shelves within the incubator for the work. The use of secondary containers/trays to hold the plates is also recommended.
- Where feasible assign other dedicated equipment such as microscopes, plate readers etc. for the work. Care should be taken when using equipment.
- Streak plates where the surface of the medium is smooth (i.e. avoid bubbles)
- Avoid using tubes with push-in closures (when opened, the film of liquid trapped between tube and closure breaks and releases aerosols)
- Use a vortex mixer instead of inverting tubes. Allow a settling time of 30 seconds after vortexing before opening the tube.
- Avoid pouring off supernatant – use pipettes instead (see below for safe practices using pipettes)
- Pour infectious liquid waste through a funnel where the end is below the surface of the disinfectant in the discard container; pour disinfectant through the funnel after use
- Avoid hastily opening ampoules of lyophilized cultures by snapping the neck, which can lead to a sudden inrush of air and dispersal of contents:

- Instead make a file mark near the middle of the cotton plug and apply a red-hot glass rod to crack the glass, allow time for air to seep into the ampoule and gently remove the top and plug
- Add liquid for re-suspension slowly to avoid frothing

Working in a BSC

The following provides a brief overview of proper technique and safety practices while working inside a BSC. Ensure all handling/loading/unloading of aerosolizable RG2 biologicals is done inside a BSC, or other primary containment device (e.g. Glovebox).

- Aerosol-generating equipment should be placed towards the rear of the work area inside the BSC. Do not block the rear BSC grille. Keep clean materials at least 30 cm from any potential aerosol-generating equipment to avoid cross contamination.
- Avoid resting arms and elbows on the grille or work surface
- Avoid frequent movements in and out of the BSC
- Avoid sweeping movements of the arms and hands while working inside the BSC. Hands should enter and exit carefully, straight in and out.
- During work ensure that some disinfectant is kept inside the BSC for easy access
- Segregate non-contaminated (clean) from contaminated (dirty) items. Set up workflow from “clean” to “dirty” areas in the BSC.
- Ensure all waste is discarded in waste containers containing the appropriate disinfectant inside the BSC. Waste containers should be placed in the rear of the workspace but away from equipment. Do not discard contaminated material into containers outside the BSC.
- All waste, both liquid and solid, must be decontaminated inside the BSC prior to removal
- In the event of a spill, decontaminate all surfaces including all objects in the BSC and the inside of the BSC window, while the BSC remains in operation.
- Inside the BSC, natural gas and propane should not be used and sustained open flames are prohibited, see section below on Bunsen burners.
- Only one user should operate inside the BSC at any time (BSCs are designed and certified for single person use). The user should be seated at the middle of the BSC.
- Equipment with the potential to create air movement that could disrupt air flow in the BSC should not be used.
- Close all windows when the BSC is in use
- Upon completion of work, allow time for any potential aerosols to be purged by BSC before removing hands and other materials. Close/cover all containers and surface decontaminate items before removal from BSC.
- Remove gloves inside the BSC before withdrawing hands. If 2 pairs of gloves are worn discard outermost layer in the BSC.

Working with Centrifuge

- Aerosol-tight centrifuge capability is required when working with aerosolizable RG2 biologicals. Check with the manufacturer of the centrifuge, if the centrifuge has aerosol-tight capability. The manufacturer’s instructions on how to ensure this capability is maintained through servicing and maintenance must be followed.

- Only centrifuge tubes equipped with O-rings are aerosol tight (Eppendorf tubes and screw cap tubes are not). Even with the use of tubes equipped with O-rings, a centrifuge that has aerosol-tight capability is required as the tubes may crack or break, leading to the release of aerosols.

Benchtop Centrifuge

- Centrifugation of any aerosolizable biological agents must be conducted in an aerosol-tight centrifuge (centrifuge rotor or bucket is o-ringed) which is loaded and unloaded within a BSC (and prior to removal from the BSC, is appropriately disinfected).
- Aerosols may be produced in bench top centrifuges if using poorly sealed test tubes or Eppendorf tubes

Microcentrifuges:

- If spinning aerosolizable materials in a microcentrifuge without aerosol-tight capability, then the microcentrifuge must be used in a BSC. However, equipment which creates air movement may affect the integrity of the airflow and should not be used within the BSC.
- While not recommended, if it is necessary to proceed with the placement of the microcentrifuge in the BSC, ensure that the microcentrifuge causes no or very minimal air flow disturbance, is placed towards the back of the BSC (without blocking the rear grille), and have your BSC certifier recertify the BSC while the centrifuge is running to ensure that the integrity of the airflow is not compromised.

Working with Blenders, Sonicators, Homogenizers, Shaking Incubators, Lyophilizers and Mixers

- Use laboratory equipment and associated accessories that are specially designed to contain infectious aerosols, e.g. cup horn sonicator
- If specially designed equipment is not available, then equipment should be used in a BSC (only if their use will not disrupt air flow patterns) or other primary containment device
- If blender, grinder or sonicator cannot be used in a primary containment device, then move the equipment into a fume hood and where possible, place a towel moistened with disinfectant over them
- Use a laboratory blender with a tight-fitting gasketed lid and leak-proof bearings (domestic kitchen blenders leak and release aerosols)
- If kitchen type blenders must be used, avoid glass blenders and check for leakage regularly, wait at least 10 minutes before opening lid
- Filter lyophilizer vacuum pump exhaust through HEPA filter or vent into BSC
- Open equipment in BSC or wait a sufficient time for aerosols to settle (at least 10 minutes)
- Autoclave or disinfect equipment after every use

Working with Bunsen Burners and Inoculation Loops

- Single-use, disposable inoculation loops, and inoculating needles are recommended when working with aerosolizable RG2 bioagents
- If not using disposable loops:

    - Use a cooled loop for insertion into a culture
    - Ensure loop is completely closed
    - Use short loops, the shank should be no more than 6 cm long to avoid vibrations
    - Use a shielded microincinerator rather than a Bunsen burner to sterilize

- Sustained open flames (i.e. operating Bunsen burners) are prohibited in a BSC since they disrupt airflow patterns decreasing user protection and may damage the BSC's filters.
- Avoid using Bunsen burners to sterilize inoculation loops as this can generate aerosols
- Disinfect the centrifuge, rotors and buckets with an appropriate disinfectant; allow at
- least 20 to 30 min of contact time. Wipe down all parts including the lid and bowl
- Microincinerators are a recommended alternative to Bunsen Burners as they have shields which can decrease aerosol dispersal. They may be used in a BSC if placed toward the rear.
- If absolutely necessary, touch plate micro burners (flame on-demand) may be used in a BSC if placed toward the rear. Use of on-demand open flames in a BSC must be strictly limited and avoided if suitable alternatives are available (i.e. disposable loops or microincinerator). If used in BSC the unit must be able to be easily disinfected.

Pipetting

- Work inside a BSC when pipetting aerosolizable RG2 biologicals
- Mouth pipetting is prohibited; mechanical pipetting devices must be used
- Use "to deliver" pipettes to avoid blowing out the last drop
- Use plastic serological pipettes instead of glass to reduce ability to break if dropped
- Use filtered serological pipettes with pipette aids and filtered pipette tips with micropipettes to reduce contamination of the pipetting device
- Some micropipettes contain internal filters, replace filter as appropriate and document change outs
- Electronic serological pipettes, if used for this work, must be dedicated to this work only, and the filter replaced as appropriate and change outs documented.
- Ensure pore size of the in-line filter in serological pipettors match the required size for biological handled (0.2 um or less depending on aerosolizable agent)
- Work over an absorbent, plastic-backed pad to avoid aerosol dispersion from drops falling on hard surfaces
- Hold micropipettes in a vertical position during use and store the micropipette in an upright position so that liquids do not run down the body of the instrument
- Drain pipettes gently with the tip against the inner wall of the receiving vessel
- Do not mix materials by alternate suction and expulsion through a pipette (use vortex mixer)
- Do not aspirate or expel liquid forcefully from pipette
- Used pipette tips should be discarded into a container containing disinfectant in the BSC before disposal
- Place used serological pipettes horizontally in a pan or tray containing enough disinfectant to cover them in the BSC before disposal. Some disinfectant may need to be

sucked up into the pipettes to ensure interior is disinfected and to stop pipettes from floating in the disinfectant.

Needles and Syringes

- Sprays or aerosols may be produced when removing a needle from a serum vial that has been pressurized by injecting more air than the volume of liquid withdrawn. Before withdrawing the needle from the vial, wrap the needle and top of the rubber diaphragm lid with a disinfectant-soaked absorbent pad.
- Needle-locking syringes or syringe-needle units are recommended to reduce the possibility of aerosol production (Luer lock connectors)
- Dispose of needles directly into sharps waste container without further manipulation
- Depending on aerosolizable agent used, some may require (based on LRA) disinfectant to be sucked up into the syringe prior to disposal
- Do not clip used needles as this may produce aerosols
- Aerosols can be produced if the needle separates from the syringe or if the plunger separates from the syringe barrel
- Aerosols may be produced if liquids are forcibly discharged into containers with a syringe.
- Gently direct liquids against the side of containers.
- Work over an absorbent, plastic-backed pad to avoid aerosol dispersion from drops falling on hard surfaces

Vacuum Pumps and Systems

- Vacuum systems should not be used with aerosolizable RG2 bioagents, but if you need to, a documented maintenance schedule including vacuum trap maintenance and filter change out schedule is required
- For instructions on how to set up your vacuum line system please refer to the Vacuum Line Hazards webpage on the EHS website
- Aspiration may cause the aerosolization of biological materials which can contaminate both the vacuum line and pump
- Vacuum systems must be equipped with a mechanism (in-line filter) that prevents internal contamination
- Properly sized in-line filters must be used based on the biological agents handled. For example, some viruses require 0.1um filters while for others 0.2um filters may be used
- Vacuum line traps must be in place and properly maintained
- Ensure regular inspection and keep maintenance documentation

Cell Sorters

- Droplet based cell sorters which use jet-in-air technology have a capacity to aerosolize biological matter at rapid rates and in large volumes
- An LRA must be done to determine the physical containment and operational procedures to safely work with infectious bioagents or toxins

- A cell sorter may need to be housed in a custom-built ventilated enclosure if it cannot be housed in a BSC
- Any custom-built ventilated enclosure must be certified

Fermenters

- Use double mechanical seals or a top-mounted agitator on motor shafts
- HEPA filters or equivalent method of preventing pathogen
- release should be equipped to exhaust vents
- Sampling ports should be fitted with a sterilizable closed sampling system
- Validation of the relief system should be done regularly
- Anti-foam products are recommended to prevent blockage of the exhaust air vent

Other Aerosol-Producing Lab Activities

These are some examples of other potential aerosol-producing activities:

- Carelessly removing gloves
- Flaming slides or lips of flasks
- Dropping/breaking culture containers

Resources

- University of Toronto EHS Safe Work Practices: Aerosol Risk Reduction RG2 Biological Agents

Removing Items from Containment Level 2 (CL2) Facility

1. Purpose:

To provide step by step guidance for the removal of items from the facility (DB440) for use in other facilities, the office space, or for final disposal.

2. Scope:

Applies to everyone working in CL2 facility (DB440).

3. Prerequisites:

PI: possession of Biosafety Permit. Lab-personnel: Biosafety Training & WHMIS

4. Responsibilities:

It is everyone's responsibility to follow this SOP every time you need to remove items from a CL2 lab (DB440).

5. Personal Protection Equipment (PPE):

6. Procedure:

1) Disinfect item/items that you will be removing from CL2 facility (wipe down all surfaces of the item with 70 % ethanol)
2) Place item inside a clean plastic bag or sealed plastic secondary container (ex. Tupperware)
3) Always place group 2 cell cultures inside sealed secondary container(s) before removing from CL2 facility

Disposing or transferring equipment:

1) Equipment: must be emptied and fully decontaminated. Depending on the type of equipment, the interior will need decontamination with 10 sodium hypochlorite (example: fridges, freezers, incubators).
2) Wipe down the exterior surface with 70 % ethanol.
3) Need to place safe to remove tag (Link to tag can be found here: Safe to remove tag)
4) Inform chem.safety@utoronto.ca if you require assistance for the disposal of the equipment

Transfer of Biological Samples Between Institutions in the GTA

1. Purpose:

To provide step by step guidance for the transfer of biological agents to and from other institutions.

2. Scope:

Applies to everybody working in the CL2 Facility (DB440), all PIs and lab-personnel requesting, transporting and storing biohazard materials from other institutions.

3. Prerequisites:

PI: possession of biosafety permit. Lab personnel: Biohazard training. For risk group 2: TDG Class 6 training

4. Responsibilities:

Principal investigators are responsible for enforcing this SOP, and for updating their biosafety permit with the Biosafety office. They are also responsible for updating the group 2 biohazard inventory to reflect any new changes in biological agent acquisitions or removals. Lab personnel are responsible for transporting biohazard materials as per this SOP, and for handling and storing as per CL2 practices.

5. Personal Protection Equipment (PPE):

When preparing biohazards for shipment, receiving, unpacking, and handling biohazard materials:

6. Procedure:

Principal Investigators:

1) Update biosafety permit with Biosafety office

2) Update the group 2 biohazard inventory to reflect any changes in biological agent acquisitions or removals
3) Obtain Biohazard Agent Transfer Notification form

Lab-personnel:

Risk Group 1: Biohazards can be transported from other Institutions without further training and legal requirements. If they are genetically modified, TDG training is required.

Risk Group 2: the person transporting biological risk group 2 agents must have received full TDG Class 6 training (opening, transporting, packaging and receiving) training. For TDG Class 6 training go to: Biosafety Training Courses

Transferring Cell Cultures:

1) Growing cell culture in flasks with non-filtered lids
2) Before transferring, fill the flasks to 3/4th its total capacity with fresh media and close the flasks with the non-filtered lids
    a. Wrap parafilm around the mouth of the flask to secure the lid
3) Place biological sample in a primary container (tube, flask) which is leak proof, and can be capped
    a. Have to wipe the outside of the containment device with 70% ethanol
4) Wrap the primary container with paper towels before placing it into the zip-lock/sealable bag or a sealable plastic container (becomes the secondary container)
5) Better to have more layers of protection so that the spill/leak is contained better and there isn't a greater area of contamination
6) If the sample needs to be kept frozen then place ice cubes/dry ice in a Styrofoam box and then place the containment device with the biological sample in it
7) Label the box with: Biological agent name, risk group classification, receiver's information
8) Wipe exterior of box with 70% ethanol before leaving the lab with the box

Receiving Biological Agents:

1) Review documentation that came with the box to determine its risk group
2) Open the box inside a BSC
    a. Inspect for spills and take care of the spills by following the Biological Spills SOP
3) Take out contents inside the box and throw out the things not required such as absorbent material
    a. Throw away the box as solid biohazardous waste
4) Wipe materials with 70% ethanol before taking it out of the BSC
5) Handle/store the samples/items as per the CL2 guidelines (applicable to group 2 biohazard)

Transportation of Dangerous Goods (TDG) – Packaging Infectious Substances

1. Purpose:

To provide instruction on how to properly and safely package infectious substances (dangerous goods).

2. Scope:

Applies to everybody working in the CL2 facility who will be packaging infectious substances for transport.

3. Prerequisites:

WHMIS, Laboratory Biosafety Training, TDG training, Biosafety Permit, and relevant import/export documents which can be found here: Biosafety Website

4. Responsibilities:

Principal investigators are responsible to enforce this SOP and lab-personnel are responsible to comply. It is the responsibility of those packaging the goods, that they use the appropriate type of packaging.

5. Personal Protection Equipment (PPE):



6. Procedure:

1) Determine whether Risk Group 2 organism belongs to **Category A or B**, by consulting Appendix 3 SOR/2008-34 of the TDG Regulations. Infectious substances in Category A are **high risk**.

a. If **Category A**, determine proper shipping name. Note: Category A infectious substances that are infectious to both humans and animals are classified as UN2814.
    - UN2814, Infectious substance, affecting humans, or
    - UN2900, Infectious Substance, affecting animals only
b. If **Category B**, the proper shipping name is:
    - UN3373, Biological Substance, Category B

2) Choose appropriate packaging based on Category and shipping name:
    a. **Type P620** (used for UN2814, 2900, 3291 and 3373);
        - UN2814- Category A
        - UN2900- Category A
        - UN3291- Waste
        - UN3373- Category B
    b. **Type P650** (used for UN3291 and 3373); OR
        - UN3291- Waste
        - UN3373- Category B (including Category A infectious substances that can be shipped as Category B)
    c. Standardized and non-standardized packaging permitted in Part III of the CAN/CGSB-43.125 standard for the transport of infectious substances intended for disposal (UN2841 or 2900) or clinical, (bio) medical or regulated waste (UN3291)

3) Packaging must protect the material from damage during shipping and conform to UN requirements (must have the UN safety mark on the outside) and must meet the shipping criteria of the International Civil Aviation Organization (ICAO). In most circumstances, combination packaging is used (a leak-proof container which cushions and stabilizes the contents from shifting or movement inside a box. The dangerous good(s) are within a sealed container, which is then placed in an outer package that protects it from damage). See Appendix Figure 1 for example.

4) A **Type P620** Packaging is a triple packaging system consisting of:
    a. Inner packagings:
        - leakproof primary receptacle(s);
        - leakproof secondary packaging(s);
    b. a rigid UN Standardized outer packaging
    c. a UN packaging symbol, packaging code, the text "CLASS 6.2", the last two digits of the year of manufacture, the country authorizing the allocation of the marking, and the name or symbol of the manufacturer and other identification of the container as specified by the country authorizing the allocation of the mark (e.g., design registration number).

Note: The assembled packaging must be capable of successfully passing the performance tests set out in section 7 of the CAN/CGSB-43.125 standard. Refer to Appendix Figure 2 for Type P620 Packaging example.

5) A **Type P650** packaging is a triple packaging system consisting of:
   a. Inner packaging comprising:
      - Primary receptacle(s) (leakproof or siftproof);
      - Secondary receptacle(s) (leakproof or siftproof) with a list of contents on the outside of the secondary receptacle is required;
   b. An outer packaging with at least one surface having a minimum dimension of 100 mm x 100 mm designed to protect contents from outside influences, such as physical damage, while in transit.

Note: Either the secondary packaging(s) or the outer packaging shall be rigid. Refer to Appendix Figure 3 for Type P650 Packaging example.

6) The primary receptacle is restricted to contain less than 1 L, and absorbent material must be placed between the primary and secondary packaging in sufficient quantity to absorb the entire contents of the primary receptacle. The outer packaging must not contain more than 4 L for liquids or 4 KG for solids.

Labelling requirements

7) For labelling, there is a set of requirements for what must appear on the outside of a package of dangerous goods which include:

- Shipping Name
- UN Identification Number
- Hazard Class Label(s)
- Packaging Certification Mark
- Ship to address

Note: The manufacturer of the carton will typically print on the orientation mark and packaging certification – the shipper usually applies the shipping name, UN number, the hazard class label stickers and the shipping address. See Appendix Figure 4 for example.

8) **Shipments of Category A**, Infectious Substances must have a Category A label, which reads: INFECTIOUS- IN CASE OF DAMAGE OR LEAKAGE IMMEDIATELY NOTIFY LOCAL AUTHORITIES AND CANUTEC 613-996-6666. See Appendix Figure 5 for example.

9) **Shipments of Category B**, Infectious Substances must have diamond hazard label identifying the UN number on the outer container. The shipping name, Biological Substance, Category B must be written in font at least 6mm high, instead of displaying the Class 6.2, Infectious Substances label. In the case of Category B materials, a 24h emergency response number must also be provided. This would be the telephone number of a responsible person, knowledgeable about the shipment. See Appendix Figure 6 for example.

References

- AAC (Advanced Analysis Centre) Transportation of Dangerous Goods SOP
- Transportation of Dangerous Goods Regulations | Government of Canada

Appendix

Figure 1 Combination Packaging Examples

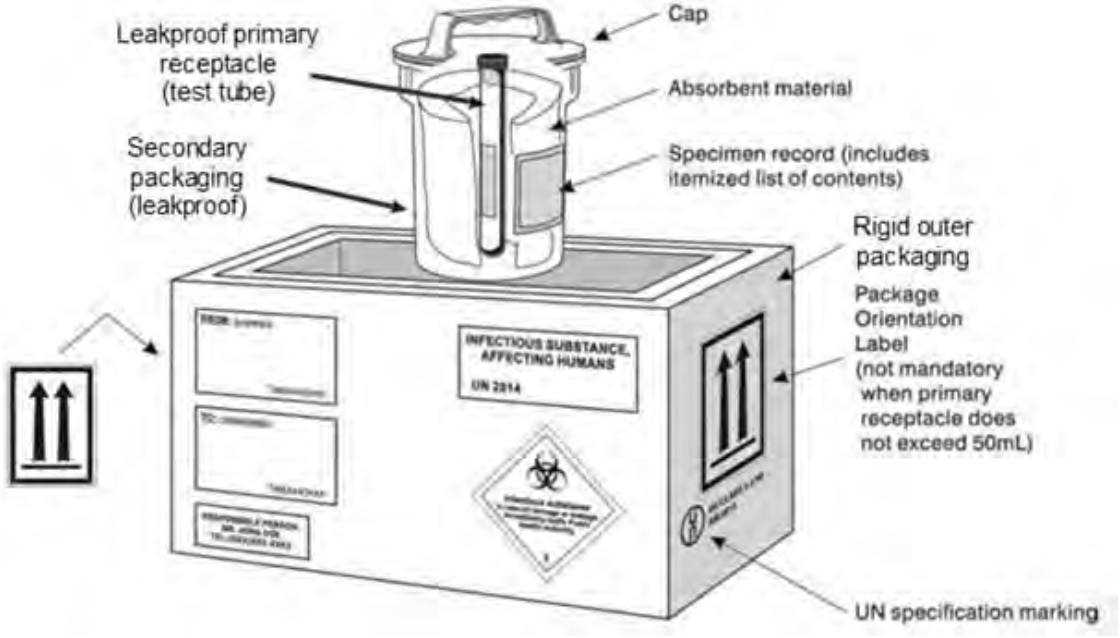


Figure 2 Type P620 Packaging

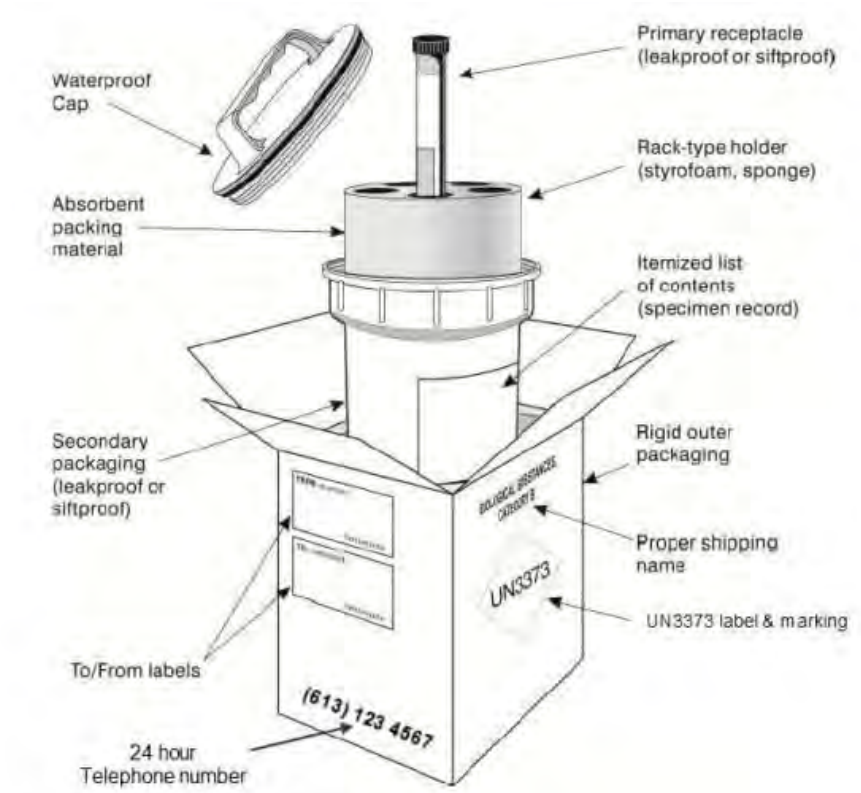


Figure 3 Example of Type P-650 Packaging

Figure 4 Labelling

Figure 5 Category A Label

Figure 6 Category B Label

Cleaning of DB440 (CL2 Facility)

1. Purpose:

To provide instruction on how to properly clean and disinfect DB440.

2. Scope:

Applies to all custodial staff

3. Prerequisites:

WHMIS

4. Responsibilities:

It is the responsibility of custodial staff to follow this SOP.

5. Procedure:

1) Always wear the appropriate protective equipment as provided by your supervisor to clean.
2) Custodians should only enter the laboratories if they have been properly trained.
3) Floor should be mop cleaned every two months using 5% Lysol (provided by the department and available in DB440). Floor should not be swept or waxed.
4) To prepare the Lysol working solution required for disinfection, take half a bottle of the concentrated Lysol provided and add water to fill half of the bucket.

Half

Half

5) The lab should have a mop and bucket set and reagents allocated to them, which should only be used for cleaning of the lab and should not be removed from the lab.

6) Fresh cleaning reagents should be prepared before each cleaning and should not be stored in diluted form because their activity will diminish with time.
7) Communicate with chem.safety@utoronto.ca to request more supplies when required.
8) Do not touch anything while in the laboratory unless required to do so to carry out your work and you have been told it is safe to do so by your supervisor or lab members. In particular, do not touch anything on the benches and only move things on the floor if you have been told it is safe for you to do so. Do not touch, empty, or move things in the laboratory sinks unless you have been told exactly what you can or cannot do.
9) Use the Cleaning Log provided to check off completed tasks (see Page 3)
10) Mopping of DB440 will be scheduled through the property manager and the lab will be prepared for such cleaning ensuring no items are on the floor and facilitating the custodian's work.

Cleaning Log

- Clean every first Friday of every two months
    - Prepare sufficient fresh cleaning solutions and mop the floor using supplies available in DB440
    - Do not remove supplies from DB440

Date	Name	Notes

Sanitation of RO (Type 3) Water Reservoir

1. Purpose:

To provide instruction on how to properly sanitize MilliQ-RO (Type 1 & 3) Water System.

2. Scope:

Applies to everybody using the MilliQ Water System.

3. Prerequisites:

WHMIS, EH601 Laboratory Biosafety Training, DB440 SST

4. Responsibilities:

Principal investigators are responsible to enforce this SOP and lab-personnel are responsible to comply. It is the responsibility of the designated person to follow this SOP during PM duties (yearly, prior to changing filters).

5. Personal Protection Equipment (PPE):


6. Procedure:

Treatment: Hydrogen peroxide to a final concentration of 0.5% (from 30%)
Materials needed: 1 bottle of 500 ml each of 30% Hydrogen peroxide per 30 L reservoir

Procedure

1) Fill-up 50 % of the reservoir total volume (15 L)
2) Add 500 ml of 30% Hydrogen Peroxide (check expiry date)
3) Complete filling reservoir to maximum volume (30 L)
4) Flush about 200 ml of water using tap (cleaning lines)
5) Let stand reservoir (water + hydrogen peroxide) for 4-6 hours
6) Empty tank
7) Refill completely and empty completely
8) Refill: Tank is now ready to use with RO water (Type 3)

Defrosting Freezers

1. Purpose:

To provide step by step guidance on defrosting freezers in the LM-CL2 facility (DB440).

2. Scope:

Applies to all authorized Principal Investigators (PIs) and authorized laboratory personnel working in the LM-CL2 facility.

3. Prerequisites:

You are an authorized user of DB440 and are either included in your PI's permit, or you possess a CL2 permit for DB440.

4. Responsibilities:

It is the responsibility of all faculty, staff and students to follow the procedures described in this SOP.

5. Personal Protection Equipment (PPE):

6. Procedure:

NOTE: If it is your first time defrosting the freezer, ask your PI or a senior student/PDF to help guide you through the procedure.
NOTE: Have all the materials available before defrosting the freezer. Some of these materials include: absorbent materials for water spills (e.g. paper towels, absorbent pads) and trays that fit inside the freezer to collect melting ice.

1) Unplug the freezer as early as possible **in the morning**. **DO NOT defrost overnight.**
2) Wear PPE as described above. Remove all the items from the freezer and place them in another freezer (you can request a loaner by contacting chem.safety@utoronto.ca).
   i) NOTE**:** Ensure that the secondary freezer is at the desired temperature (plan ahead).
3) Scrape loose ice pieces with a plastic scraper into a tray and dispose them into the sink.
   i) Ensure that you do not damage the interior of the freezer during this process.
   ii) Ensure that you do not use sharp tools to scrape.
4) Place trays on the bottom shelf of the freezer in order to collect any melting ice from the top shelves and walls.
5) Place absorbent material on the floor around the freezer. **DO NOT use supplies from the spill kit (each group is responsible for obtaining their own absorbent materials).**
   i) If you're using absorbent pads, make sure the absorbent side is facing upwards.
6) If necessary, to prevent water spillage on the floor due to melting ice, place a tray on the floor outside the freezer and use absorbent materials to guide the water to the tray.
   i) Refer to the photo below on how this can be set up.

7) When all the ice has become soft enough, scrape the ice pieces with a plastic scraper into a tray and dispose them into the sink.
   i) Ensure that you do not use sharp tools to scrape.
8) When all the ice has melted and there is no more water leaking, dry the interior surfaces of the freezer with paper towels and plug it back on.

Routine Maintenance of TC Incubator

1. Purpose:

To provide instruction on how to properly clean TC incubator

2. Scope:

Applies to everybody working in DB440

3. Prerequisites:

WHMIS, EHS601 Laboratory Biosafety, DB440 SST

4. Responsibilities:

Principal investigators are responsible for enforcing this SOP and lab-personnel are responsible for complying

5. Personal Protection Equipment (PPE):

6. Procedure:

Weekly Maintenance

1) Check incubator for any signs of contamination.
2) Empty water tray. Wash with soap and water. Wipe with 70% ethanol.
3) Change the incubator water (not just refill it, but empty and add fresh, sterile, distilled water). Do not use tap or ultra-pure water.

4) Ensure nothing is stored on top of the incubator and clean the top of the unit every two weeks to remove dust. Wipe down the doors and handles with 70% ethanol or 2% quaternary ammonium.

Every 6 Months

1) Clean the incubator every 6 months. It is not necessary to autoclave everything; spray or wipe down the incubator with 70% ethanol or 2% quaternary ammonium, especially the water tray (do not spray ethanol on sensors). Allow to air dry. Water tray may be autoclaved.
2) Clean the incubator exterior with a damp sponge or soft, well-wrung cloth and mild detergent dissolved in water. Dry with a soft cloth.

Disinfecting the Incubator Interior (Thorough Cleaning) (Annual Maintenance, or for Recurrent Contamination)

1) Turn the incubator off and disconnect the plug from the power source.
2) Remove the shelves
3) Wipe the shelves with disinfectant (2% quaternary ammonium) and rinse with water. Wash with soapy solution, and rinse with water. Spray with 70% ethanol or 2% quaternary ammonium and leave to air dry. Option: Autoclave shelves (recommended).
4) Wash the cabinet interior with disinfectant starting at the top and working down. Wash the inner door both inside and out. The cabinet and door must be rinsed with sterile water until the disinfectant has been removed. After the cabinet has been rinsed, spray with 70% ethanol or 2% quaternary ammonium.
5) Install the shelves and spray with 70% ethanol or 2% quaternary ammonium.
6) Plug the incubator in and turn the power switch on. Allow the unit to run empty for 24 hours before returning to service.

A service contract for annual replacement of the HEPA filter and calibration is recommended (if done in-house, refer to the operation manual).

References

- Best Practices for CO2 Incubator Maintenance | American Laboratory
- Thermo Electron Corporation: Model 3110 Series*Forma Series II Water Jacketed CO2 Incubator Operating and Maintenance Manual

STANDARD OPERATING PROCEDURE: Routine Maintenance of Biosafety Cabinet (BSC)

1. Purpose:

To provide instruction on how to properly clean BSC.

2. Scope:

Applies to everybody working in DB440.

3. Prerequisites:

WHMIS, EHS601 Laboratory Biosafety, DB440 SST

4. Responsibilities:

Principal investigators are responsible to enforce this SOP and lab-personnel are responsible to comply.

5. Personal Protection Equipment (PPE):


6. Procedure:

For Labconco BSC

The following should be done every 6 months:

1) Make sure BSC is empty of any items, equipment, or waste containers.
2) Allow the cabinet to operate for 5 minutes with no activity, which should purge airborne contaminants from the work area.

3) Wet wipe the top surface of the outside edge of the BSC and front grille a total of 3 times with the pre-soaked paper towels with 1% sodium hypochlorite. Place used paper towels into waste bag.
4) Decontaminate work surface before removing it from the cabinet by spraying/wiping with 70% ethanol.
5) Remove work surface:

   a. Lift the front edge of the work surface straight up by grasping the knob handles at either front corner.
   b. Pull the work surface straight out, letting its rear edge rest on the center support underneath.

Prop-Up Work Surface

6) This procedure must be performed with a partner. This procedure must be listed in your 'working alone policy/procedure' for your laboratory or area.
7) With a partner, lift up the work surface and prop it up securely. Refer to image below.

8) The prop should be something that is strong enough to hold up the metal surface and wide enough that it will not slide and let the surface fall.

Cleaning Front Grille

9) At one end of the grille, grip the front of the grille with one hand, and the back with the other hand. Pivot that end of the grille upward and inward, paralleling the angle of the sash.
10) Pull the other end of the grille up and away from the bottom edge of the cabinet.
11) Wet-wipe the underside of the grille for a total of 3 times with pre-soaked paper towels. Place used paper towels into waste bag.
12) Scrub any gross contamination remaining on the grille with appropriate scrubbing tool. Remove all loosened debris by wet-wiping another three times.
13) Spray/rinse with water to remove sodium hypochlorite residue.
14) Spray top of grille and underside of grille thoroughly with 70% ethanol. Let sit for 5 minutes.

Cleaning Work Surface

15) Thoroughly spray the underside of the work surface with 70% ethanol. Let sit for 5 minutes.
16) Assess the underside of the work surface and clean any gross contamination present by wet-wiping 3x the entire surface with paper towels pre-soaked with decontamination solution. Rinse by wet-wiping with paper towels pre-soaked with tap water if necessary.
    - Tongs or another long reaching tool is recommended to clean hard to reach surfaces.
17) Collect all paper towels into a bag.

Cleaning BSC Catchbasin

1) Using a flashlight and an extendible mirror, examine the extent of cleaning required. Note any sharps or broken glass.
2) Saturate the entire surface of the catch basin by misting with decontamination solution to prevent fly away debris. You want to capture as much solid material and not let it get sucked up into the HEPA filters.
3) Let sit for 5 minutes, keep spraying, do not let it dry out
4) Using an appropriate tool (such as: tongs, plastic scraper, mini dustpan, toilet brush or long handled scrub brush) remove as much loose solid material, sharps or broken glass. Place sharps or broken glass into a sharps container.
5) Collect debris into a plastic bag inside the BSC if you are positive no sharps are present. If the debris contains sharps that you cannot pick out, place the wet, sharps-containing muck into a wide mouth sharps container.
6) For dried on gross contamination, scrub catchbasin with a plastic scrub brush. A toilet brush is ideal because of the long handle.
7) Place paper towels soaked in decontamination solution in the catchbasin. Make sure to cover entire surface.

8) Let sit for 15 minutes or the recommended contact time for the decontamination solution. Remove paper towels and dispose of in regular waste bins.
9) Repeat steps 6 to 8 to ensure all debris is removed.
10) Rinse catchbasin with tap water three times. **Failure to thoroughly rinse corrosive chemicals will result in damage to the entire catchbasin.**
11) Spray catchbasin with 70% ethanol and let it air dry.
12) Replace front grille by **reversing** the following sequence, ensuring that the grille properly engages the bottom edge of the cabinet: At one end of the grille, grip the front of the grille with one hand, and the back with the other hand. Pivot that end of the grille upward and inward, paralleling the angle of the sash. Pull the other end of the grille up and away from the bottom edge of the cabinet.
13) Replace work surface by resting the bottom on the center rail while pushing it back into the cabinet. Be sure to engage the tabs on the back corners of the work surface with the slots on the rear wall of the work area.
14) Take care when handling paper towel when the BSC is on. They can get sucked up into the HEPA filter and you will have to have it removed.

Cleaning the Rest of the BSC

1) Surface disinfect using 70% ethanol to decontaminate all interior work surfaces, making sure to clean the back and side of the cabinet (sidewalls, back wall), inside of sash (interior of the glass), and work surface.
2) If using a chloride type disinfectant, after contact time, wipe down interior surfaces with a 70% alcohol solution to protect stainless steel interiors from corrosion.
3) The drain pan should be emptied into a collection vessel containing disinfectant; and the drain valve can be disinfected using a flexible tube.
4) Any spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag.
5) Hands should be washed whenever gloves are changed or removed
6) If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary.

For Nuaire BSC

The following should be done every 6 months:

1) Make sure BSC is empty of any items, equipment, or waste containers.
2) Allow the cabinet to operate for 5 minutes with no activity, which should purge airborne contaminants from the work area.

3) Cleaning front grille:

Figure 1

- Wet wipe the top surface of the outside edge of the BSC and front grille a total of 3 times with the pre-soaked paper towels with 1% sodium hypochlorite. Place used paper towels into waste bag.
- Remove the grille, turn upside down and place onto the work surface.
- Wet-wipe the underside of the grille for a total of 3 times with pre-soaked paper towels. Place used paper towels into waste bag.
- Scrub any gross contamination remaining on the grille with appropriate scrubbing tool. Remove all loosened debris by wet-wiping another three times.
- Spray/rinse with water to remove sodium hypochlorite residue.
- Spray top of grille and underside of grille thoroughly with 70% ethanol. Let sit for 5 minutes.

Prop-Up Work Surface

- This procedure must be performed with a partner. This procedure must be listed in your 'working alone policy/procedure' for your laboratory or area.
- With a partner, lift up the work surface and prop it up securely. Refer to images below.

Figure 2

Figure 3

Figure 4

- The prop should be something that is strong enough to hold up the metal surface and wide enough that it will not slide and let the surface fall.
- Thoroughly spray the underside of the work surface with 70% ethanol. Let sit for 5 minutes.
- Assess the underside of the work surface and clean any gross contamination present by wet-wiping 3x the entire surface with paper towels pre-soaked with decontamination solution. Rinse by wet-wiping with paper towels pre-soaked with tap water if necessary.
    - Tongs or another long reaching tool is recommended to clean hard to reach surfaces.
- Collect all paper towels into a bag.
- Make note of Figures 2-4 above for following instructions.

Cleaning BSC Catchbasin

Figure 5- work surfaced propped up and front grille removed

1) Using a flashlight and an extendible mirror, examine the extent of cleaning required. Note any sharps or broken glass.
2) Saturate the entire surface of the catch basin by misting with decontamination solution to prevent fly away debris. You want to capture as much solid material and not let it get sucked up into the HEPA filters.
3) Let sit for 5 minutes, keep spraying, do not let it dry out
4) Using an appropriate tool (such as: tongs, plastic scraper, mini dustpan, toilet brush or long handled scrub brush) remove as much loose solid material, sharps or broken glass. Place sharps or broken glass into a sharps container.
5) Collect debris into a plastic bag inside the BSC if you are positive no sharps are present. If the debris contains sharps that you cannot pick out, place the wet, sharps-containing muck into a wide mouth sharps container.
6) For dried on gross contamination, scrub catchbasin with a plastic scrub brush. A toilet brush is ideal because of the long handle.
7) Place paper towels soaked in decontamination solution in the catchbasin. Make sure to cover entire surface.
8) Let sit for 15 minutes or the recommended contact time for the decontamination solution. Remove paper towels and dispose of in regular waste bins.
9) Repeat steps 6 to 8 to ensure all debris is removed.
10) Rinse catchbasin with tap water three times. **Failure to thoroughly rinse corrosive chemicals will result in damage to the entire catchbasin.**
11) Spray catchbasin with 70% ethanol and let it air dry.
12) Replace work surface
13) Replace front grille
14) Take care when handling paper towel when the BSC is on. They can get sucked up into the HEPA filter and you will have to have it removed.

Cleaning the Rest of the BSC

1) Surface disinfect using 70% ethanol to decontaminate all interior work surfaces, making sure to clean the back and side of the cabinet (sidewalls, back wall), inside of sash (interior of the glass), and work surface.
2) If using a chloride type disinfectant, after contact time, wipe down interior surfaces with a 70% alcohol solution to protect stainless steel interiors from corrosion.
3) The drain pan should be emptied into a collection vessel containing disinfectant; and the drain valve can be disinfected using a flexible tube.
4) Any spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag.
5) Hands should be washed whenever gloves are changed or removed
6) If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary.

7. References:

- 10 Step Guide for Biological Safety Cabinet Decontamination | LabRepCo
- Checklist for Safe Use of Biological Safety Cabinets | CDC Division of Laboratory Systems
- BSC Labconco Users Manual

Working with Viruses

1. Purpose:

To provide step by step guidance on how to safely use lenti- and adenoviral particles.

2. Scope:

Applies to Principal Investigators (PIs) and authorized laboratory personnel that have been pre-approved by the permit holder and the Department (the CAO) to work with virus while following strict operational protocols.

3. Prerequisites:

You are an authorized user of DB440 and either possess a CL2 permit for DB440 which explicitly lists viral work, or are included in your PI's. You have previously discussed viral work with the permit holder and with the Department (the CAO) and have reviewed additional safety precautions. Risk considerations must have been reviewed with the permit holder and the CAO. Only replication-defective lentiviral particles (2nd or 3rd generation lentiviral vectors) can be used. Nevertheless, as lentiviral particles are derived from HIV and stably integrate into the genome of the infected organism, risk consideration and increased safety measures must be taken seriously. Adenovirus must be E1 and E3 deleted to render viral particles replication defective and to reduce risk upon exposure. If you aim to work with viruses other than lenti- and adenovirus, the SOP must be updated accordingly.

4. Responsibilities:

It is the responsibility of all faculty, staff and students to follow the procedures described in this SOP. Working with viral vectors can be a real risk to the user and bystanders.

5. Personal Protection Equipment (PPE):




6. Procedure:

Before work:

Biosafety cabinets, tissue culture incubators, and low temperature storage units must be clearly labeled when used for working with viruses. Hang clearly readable signs on the BSC and the incubator. If possible, usage of the same compartment should be avoided by other users to minimize the risk of accidental spillage and handling.

Processing of adenovirus purification through cesium chloride (CsCl) density gradient combined with ultracentrifugation will take place in LM439. Please inform all other users 2 days prior and hang signs on the outside door to discourage other users from entering during this time due to the high risk of splashing.

Additional safety precautions:

All safety precautions as listed under 24 for working in Biosafety Cabinets apply. Additionally, the following precautions must be taken:

1. PPE: A lab coat and a double layer of nitrile gloves will be worn while working with viral vectors. If there is a splash risk, eye protection (safety glasses/goggles or a face shield) will also be worn. If there is high risk of exposure to aerosols, a N95 mask should be worn. All procedures will be performed in a biosafety cabinet. Upon contamination, the outer layer of the nitrile gloves only will be removed within the biosafety cabinet.

2. Surface Decontamination: Equipment and surfaces will be decontaminated with 70% ethanol prior to avoid contamination. The inside of the biosafety cabinet will be cleaned with clydox (freshly prepared following manufacturer instructions), or similar viricidal, with 10 min contact after working with viral vectors Clydox must be wiped out with sterile water, followed 70% ethanol (failure to do so, will corrode the surface of the BSC).

3. Liquid Waste: Generally, volumes of viral vector will be kept at < 10 ml to avoid splash risks and severe exposure. Viral particles will be stored in 1 ml aliquots. All waste, both liquid and solid, will be decontaminated inside the BSC prior to removal. A designated electronic serological pipettor with a 0.2 µm filter will be used to handle liquids with a volume of > 1 ml. A designated set of pipettes will be used with filter tips for volumes < 1ml. Liquid waste will be decontaminated by the following procedure: Liquid waste containers will be emptied before use of viral particles, filled with undiluted bleach to 10%, and medium will be directly with serological pipettes and ejected into bleach to inactivate particles. Liquid waste from mammalian tissue culture will be brought to a final concentration of ~1% sodium hypochlorite (1:10 dilution from 12.5 % sodium hypochlorite stock to ~1%, VWR) within the biosafety cabinet, left for 30 minutes and then disposed of down the drain. Vacuum aspiration may cause the aerosolization of biological materials which can contaminate both the vacuum line and pump and thus will not be used for viral work. Electronic serological pipettes will be dedicated to viral procedures work only, and the 0.2 um filter replaced as appropriate and change outs documented.

4. Solid Waste: Waste that was in direct contact with viral particles will be covered in sufficient 1% sodium hypochlorite (1:10 dilution from 12.5 % sodium hypochlorite stock to ~1%, VWR) to decontaminate the virus and then sealed in leakproof containers (50 ml tubes or screw top plastic bottles for tips, autoclavable plastic bags for dishes) inside the BSC. Pipettes will be flushed with 1% bleach to ensure deactivation of particles within the pipette and proper immersion in 1% bleach. All solid waste will be left in bleach for 30 minutes, then the bleach will be disposed of as described for liquid waste prior, and then disposed in yellow bags/lined buckets for disposal. Waste will only be brought outside after the surface of the container has been sprayed with ethanol and when sealed in an airtight, leakproof container. RG2 solid waste will be collected in yellow bags/lined buckets with the biohazard symbol (double bagged) and disposed as biohazard waste by the facility.

7. Sharp Waste: The use of glass pipettes will be avoided to prevent aerosols from infiltrating the pumps and to avoid handling broken glass.

8. Spills Outside the BSC: The SOP guides personnel to immediately leave the room and place a yellow tape preventing re-entry. Personnel will wait 20 min before coming back in to deal with the spill allowing aerosols to settle. A spill kit is located right outside DB440 and includes PPE. *In addition to the safety procedures outline at the beginning, a CAN-95 mask (available in the spill kit) should be worn to avoid infection through aerosol. CAN-95 masks must be worn by all personnel entering a room with viral particles spilled outside the BSC.*

9. Spills inside the centrifuge: Viral centrifugation is only allowed in buckets closed with aerosol-tight lids that are only opened within the BSC. Following spills within these buckets, buckets and lids must be opened inside the BSC and placed inside a container to soak for 30 minutes in 1% sodium hypochlorite (1:10 dilution from 12.5 % sodium hypochlorite stock to ~1%, VWR).