Safe Work Practices
Aerosol Risk Reduction RG2 Biological Agents

Scope and Purpose

This Safe Work Practices document discusses some of the risk reduction techniques and safety precautions that should be used in laboratories working with potentially aerosolizable bioagents. Aerosolizable Risk Group 2 (RG2) biological agents may include, bacteria, fungal spores, toxins, viruses and viral vectors.

Biologicals can vary in ability to aerosolize, duration of suspension and aerosol spread. These characteristics can be influenced by a variety of environmental factors including temperature and humidity. As such, please reference all appropriate literature, product guides and Pathogen Safety Data Sheets (PSDS) (https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html) when working with biologicals. These references will contain instructions and safety measures specific to the biological agent.

The purpose of this document is to assist supervisors in the development of their lab-specific Standard Operating Procedures (SOPs) for laboratory operations and equipment where aerosol production is a risk. This document does not take the place nor does it fulfill the role of a detailed work-specific safety-focused SOP. Before personnel work with any biologicals, they must have received in-house training and have reviewed their lab's specific work SOPs.

Introduction

All RG2 biologicals must be handled in accordance with the guidelines outlined in the Canadian Biosafety Standard (CBS) and the Canadian Biosafety Handbook (CBH). 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.

Canadian Biosafety Standard:

https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html

Canadian Biosafety Handbook:

https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition.html

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.

In work with viral vectors, the nature of the insert is a factor that must be considered in risk assessments. Known oncogenes or genes with high oncogenic or toxic potential may require additional or modified biosafety practices. Vector modification in the form of pseudotyping may lead to vectors becoming capable of infecting a broader range of human and mammalian cells. Some aerosolizable biological components of a system (i.e. vesicular stomatitis virus GP (VSV-G)) in which pseudotyping has expanded the host range may also increase inhalation risk. Following exposure to viral vectors, there may be a potential for oncogenesis due to insertional mutagenesis or transactivation of adjacent gene sequences from the integration of viral genetic sequences into the host genome. The possibility of generating replication competent viruses during vector production or the possibility of recombination with wild type virus should also be considered in your risk assessment. See CBH 4.3.8.3 for risks associated with viral vectors.

Local Risk Assessment (LRA)

Permit holders/Principal Investigators are responsible for their site-specific Local Risk Assessments (LRAs) and for informing their personnel of any risks or hazards they may encounter in the lab, including potential effects of exposure (i.e. the potential viral vector risk of insertional mutagenesis). The LRA should identify the hazards based on the biological agent(s) in use and the activities to be performed in their laboratories. LRAs should identify not only the potential biological hazards but any other hazards such as chemical, radiological, or physical risks and outline measures to be used to reduce the risk of injury, and in the case of potentially aerosolizable agents or material, methods of reducing/preventing

the production of aerosols. This risk assessment can then be used to create their work-specific, safety-focused SOPs.

For more details on LRAs see CBH 4.4.1 and Canadian Biosafety Guideline – Local Risk Assessment: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/canadian-biosafety-guidelines.html

Permit holders that plan to work with viral vectors are recommended to review the Canadian Biosafety Guideline – Lentiviral Vectors: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/lentiviral-vectors/document.html

Standard Operating Procedure (SOP)

A Standard Operating Procedure (SOP) is a detailed step-by-step procedural document on how to safely perform the activities and procedures done in your lab. It should contain precise, practical instructions on how to use instruments, handle bioagents, perform experiments and all the safety measures that should be followed including PPE requirements. It should also include maintenance and documentation requirements, waste and decontamination instructions, emergency procedures and accident reporting instructions.

Principal Investigators are responsible for reviewing and approving all SOPs relevant to their laboratory operations. SOPs should be introduced to personnel during training and must be reviewed, understood and followed by all laboratory users. The SOPs should be included in your lab-specific biosafety manual and be available for review by all users. SOPs should be reviewed and amended whenever there are changes to equipment or procedures. Personnel need to be advised of any changed or updated SOP.

For more information on SOPs see CBH 5.3.5

SOPs should contain the following (if applicable) and any other instructions needed to perform lab activities safely, based on your LRA:

- safety considerations/potential hazards/risks based on risk assessment, any special handling procedures
- PPE requirements
- entry/exit procedures
- use of primary containment devices
- instructions on where the work is to be carried out, e.g. BSC, Glovebox
- animal work considerations
- decontamination, cleaning procedures and waste (disposal) procedures for both liquid and solid waste
- safe and secure movement/transportation of infectious materials or toxins and storage requirements

- any procedure/task involving infectious material, toxins, and/or infected animals, as determined by an LRA
- spills, accident procedures and exposure response (must be included in your emergency response plan)

Personnel Training and Clearance

Personnel must know and understand the potential hazards of their work, and follow all operational practices and procedures. The fundamentals of safe RG2 biological work are discussed in the Laboratory Biosafety course (EHS601). Supervisors must also ensure that all personnel have successfully completed an in-house training session on their lab's SOPs including equipment use, waste procedures and emergency response. Personnel must show competence in those procedures prior to working with aerosolizable RG2 agents. This requirement is applicable to both new and experienced personnel. All in-house training must be documented, dated and signed by both the trainee and trainer, and available to view upon request by EHS personnel or external regulators. Documentation of all in-house training should be kept by the supervisor for a minimum of 5 years after the personnel has left the lab.

Review aerosol reduction techniques used for your lab procedures and equipment as part of your annual emergency response review (CBS 4.3.10).

General Safety

Personal Protective Equipment (PPE)

The PPE to be worn when working in any lab should be in accordance with the highest risk or possible hazard for the biological agents, material or chemical used in the procedure. The supervisor must advise personnel on what PPE is required based on a LRA to identify potential risks in any lab procedure.

PPE must be worn at all times when working with RG2 biological agents and stored within the containment zone (lab) (CBS 4.4.1). Check the Safety Data Sheet(s) (SDSs) for the chemicals that will be used in the procedure, to ascertain if any additional PPE is required.

- Lab coats must be long sleeved and knee length
- Long pants or skirt (the entirety of the legs must be covered)
- Shoes are to be closed toe and heel, low heeled (or no heeled) and have non slip soles (CBS 4.6.3)
- Gloves (CBS 4.4.4). Specific glove types may have to be specified for different procedures (based on your LRA). Some examples include nitrile/latex laboratory gloves for handling specimens,

and insulated utility gloves for handling freezing materials. Ensure gloves are compatible with possible hazards

- Safety goggles or face shield if there is a possibility of ocular splash, or flying debris (CBS 4.4.2)
- Respirators may be required due to the potential exposure to aerosols outside of a primary containment device based on your LRA. Those personnel that must wear respirators must be fit tested every 2 years, see: https://ehs.utoronto.ca/training/respiratory-protection-training-fit-testing/
- Personnel to remove PPE carefully to minimize possible contamination of their skin, hair or clothing when leaving the containment zone (lab) (CBS 4.5.14)
- Potentially contaminated clothing articles and PPE should be decontaminated prior to washing (CBS 4.8.5, 4.8.6):): https://ehs.utoronto.ca/wp-content/uploads/2015/10/Lab-Coat-Washing-Guidelines.pdf

Additional information and resources on PPE are provided below:

General Laboratory PPE Assessment tool: https://ehs.utoronto.ca/wp-content/uploads/2016/06/Laboratory-PPE-Assessment-Tool.pd-Updated.pdf

General information on PPE: https://ehs.utoronto.ca/resources/personal-protective-equipment-ppe/

Protective Glove Standard: https://ehs.utoronto.ca/wp-content/uploads/2015/10/Hand-Protection-Gloves.pdf

Protective Eye and Face wear Standard: https://ehs.utoronto.ca/wp-content/uploads/2015/10/Eye-Protection-Standard.pdf

Respiratory Protection Program: https://ehs.utoronto.ca/wp-content/uploads/2015/10/Respiratory-Protection-Program.pdf

General Good Microbiological Laboratory Practices

Good microbiological laboratory practices are an effective method for the prevention and reduction of contamination by aerosolizable RG2 biological agents. They help safeguard the user from biological agents and help protect the purity and integrity of the product. The following provides an overview of some of the good microbiological laboratory practices for handling RG2 bioagents, see Chapter 4 of the CBS for a complete list.

- Waterproof dressings (i.e. adhesive bandages) must be applied to all skin injuries (open wounds, cuts, abrasions) prior to work (CBS 4.6.6)
- Contact with the face or mucous membranes (mouth, nose, ears, and eyes) is prohibited. This includes activities such as eating, drinking, chewing gum, applying makeup, inserting earbuds or inserting/removing contact lenses (CBS 4.6.1)
- Oral pipetting of any substance is prohibited (CBS 4.6.5)

- Long hair must be tied back (CBS 4.6.2), and jewelry removed if it may become caught in equipment, come into contact with biologicals/chemicals or may puncture gloves (CBS 4.6.4)
- Benchtops and other work surfaces must be decontaminated with appropriate disinfectants when work is completed (CBS 4.6.11)
- All personal belongings (including electronic devices) and clothing must be stored away from PPE and separate from areas where biologicals are handled or stored (CBS 4.5.10; 4.5.11)
- Waste, both solid and liquid, that comes into contact with biologicals must be fully decontaminated before disposal – see Waste section below for detailed instructions on requirements for aerosolizable RG2 agents (CBS 4.8.7, 4.8.8)
- Hands must be washed after handling infectious material and before exiting work area (CBS 4.5.16, 4.6.27)

Additional Good Laboratory Practices for RG2 Aerosolizable Bioagents

A certified BSC (or other primary containment device) must be used for all procedures involving open containers (i.e. pipetting, pouring off of liquids, loading syringes etc.) of infectious material or toxins that may produce infectious aerosols or aerosolized toxins, when aerosol generation cannot be contained through other methods (CBS 4.6.24).

With any work with aerosolizable agents, SOPs that outline how your lab conducts this work are required. Keep in mind that should your protocols/work change then the SOPs must change accordingly and documentation may be required for some aspects (e.g. centrifuge O-ring maintenance). Personnel are to be notified of any changes in SOPs and retraining may be required. Some considerations to keep in mind when writing your SOPs:

- Sharps prohibition or clear, concise directions on usage and procedures for safe handling/disposal (see below for safe practices using needles and syringes)
- 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 and SOPs on the decontamination procedures following use are needed
- 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

Containment Level required

All handling of risk group 2 biologicals must take place in a commissioned Containment Level 2 (CL2) laboratory attached to your biosafety permit.

The determination for the requirement of Inward directional airflow (IDA) for a CL2 designated room is based upon a LRA which includes the specific biological agents in use and the risk of contracting an infection by aerosolized biological agents. For animal or plant pathogens not covered under the Human Pathogen and Toxins Act (HPTA), the Canadian Food Inspection Agency (CFIA) requires labs to have IDA, regardless of the risk to plants and animals outside the laboratory, including established cell lines commercially available.

Work with potentially aerosolizable pathogens should be carried out in CL2 labs where IDA can be maintained (CBS 3.5.1).

Inward Directional Airflow (IDA)

Work with aerosolizable bioagents requires enhanced CL2 operational practices that are aimed at mitigating the risk of exposure to these aerosolizable agents and relevant liabilities. This work should be carried out in a CL2 lab that is not heavily used by other users and that has reliable IDA (also called negative air flow) (CBS 3.5.1; CBH 10.1). IDA should be confirmed prior to starting any work with aerosolizable agents.

IDA means that air is flowing **into** the lab rather than out of the lab into hallways or other public areas. There are monitoring devices (e.g. floating ball, differential pressure gauges, alarms) which may be installed in some labs. If your lab does not have a monitoring device, then IDA may be tested manually.

Testing IDA Manually

Using Smoke Pencil:

With all lab doors shut hold a smoke pencil (these can be purchased at hardware stores) near the bottom of the lab entrance door and observe direction of the smoke. If the smoke travels into the lab, then IDA is confirmed. If your lab door is equipped with door sweeps, then air flow may be impeded at the bottom of the door. In this case, move the smoke pencil slowly along the sides of the door to discover the air flow direction.

Using tissue paper:

With all the lab doors shut, take a small piece of 1-ply tissue (i.e. Kimwipe) and place at the bottom of the entrance door. If the tissue moves into the lab, then IDA is confirmed. If the door has door sweeps, then the air flow may be impeded at the bottom of the door. In this case, try to find an area of the door along the sides that allows air flow and hold a long thin piece of tissue there and observe any direction of movement of the end of the tissue that may indicate the air flow direction.

Note – If work with aerosolizable agents is being done in labs without reliable IDA then extra precautions, schedules and SOPs may be required based on your Risk Mitigation Plan cleared through the biosafety office. These added SOPs may include the use of plastic labware with aerosol barriers including filter barrier pipette tips and filter vented tissue culture flasks. Contact your Health & Safety Officer (HSO) for further details on submitting a Risk Mitigation Plan to the Biosafety office.

Aerosol Reduction Techniques for Specific Equipment/Procedures

The following outlines some aerosol reduction techniques for common laboratory procedures and equipment.

Use of Primary Containment Devices: Biological Safety Cabinet

Class II BSCs provide personnel, product and environmental protection. The most common type of BSCs are Class II, Type A which allow for safe handling and containment of RG2 biological material (CBH 11.1.2). BSCs or other primary containment devices (e.g. Glovebox) are required to safely handle aerosolizable RG2 biologicals when not in a sealed container. (CBS 4.6.24)

Working Safely in a Biological Safety Cabinet (CBH 11.4)

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. For more information please visit www.ehs.utoronto.ca/our-services/biosafety/biosafety-manual/biosafety-cabinets/

- 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 work flow 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 work space 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. For further information on spills in a BSC see: https://ehs.utoronto.ca/our-services/biosafety/biological-spills/ and CBH 17.3.2
- Inside the BSC, natural gas and propane should not be used and sustained open flames are prohibited (CBS 4.6.30), 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 inside the laboratory 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 (CBS 4.6.26)

Centrifuge (CBH 12.1)

Aerosol-tight centrifuge capability is required when working with aerosolizable RG2 biologicals (CBS 4.6.28). Permit holders/Principal Investigators must determine, with the manufacturer of their centrifuge, if their 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.

Ultracentrifuges:

- Read and follow all manufacturer instructions to prevent damage or malfunction of device. Follow manufacturer's advice on required maintenance for centrifuge and rotors (i.e. O-ring cleaning and lubrication, rotor retirement)
- Do not overfill centrifuge tubes. Wipe the outside of tubes with disinfectant after they are filled and sealed

- The centrifuge must be equipped with sealed cups (equipment with O-rings/gaskets) which are loaded and unloaded in the BSC (CBS 4.6.28)
- Check the integrity of the cups, O-rings and gaskets before use. If any of these components are defective, they must be replaced
- A maintenance log must be kept to document O-ring change-outs and periodic inspections of the devices. (CBS 4.6.14) A maintenance schedule for the centrifuge outlining all this and other maintenance must be documented in your permit-specific biosafety manual
- Decontaminate the outside surface of cups and rotors after each use
- It is recommended that the centrifuge be located within the cell culture room where the bioagents are being handled and that the room has IDA. If the centrifuge is located in another lab, then methods for the safe transportation of the bioagents must be included in your SOP (see below for instructions regarding safe transport)

Bench Top Centrifuges:

- 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). See ultracentrifuge instructions above for further details
- 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 effect the integrity of the airflow and should not be used within the BSC (CBH, chapter 11)
    - 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 (CBH 11.4.1)), and have your BSC certifier recertify the BSC while the centrifuge is running to ensure that the integrity of the airflow is not compromised

Spill in a centrifuge:

All personnel must be familiar with the location and use of your biological spill kit and safety features such as emergency eyewashes and showers. For further information on spill procedures see: https://ehs.utoronto.ca/our-services/biosafety/biological-spills/ and CBH 17.3.

In the event of a spill or tube breakage (actual or suspected):

- If the centrifuge is in operation, switch it off immediately

  - Leave lid closed for at least 30 minutes to allow aerosols to settle, if breakage is discovered after the centrifuge is opened, replace lid immediately and leave closed for the required time
  - Affix signage not to open the centrifuge
  - Move to BSC if possible (see CBH 17.3.3 for detailed instructions)
  - 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
  - Rinse with water if bleach was used
  - Report incident to supervisor

Blenders, Sonicators, Homogenizers, Shaking Incubators, Lyophilizers and Mixers (CBH 12.3)

- 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 can not 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

Bunsen Burners and Inoculation Loops (CBH 12.4; 12.5; 12.6)

- 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 (CBS 4.6.30)
- Avoid using Bunsen burners to sterilize inoculation loops as this can generate aerosols

- 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) (CBS 4.6.30). If used in BSC the unit must be able to be easily disinfected

Pipetting (CBH 12.7)

- Work inside a BSC when pipetting aerosolizable RG2 biologicals (CBS 4.6.24)
- Mouth pipetting is prohibited (CBS 4.6.5); 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 SOPs 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 (CBH 12.8)

- Vacuum systems should not be used with aerosolizable RG2 bioagents, but if you need to, then detailed SOPs and 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 see: https://ehs.utoronto.ca/our-services/biosafety/vacuum-line-hazards/
- 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 (CBS 3.7.17)
- 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 (CBH 12.11)

- 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 can not be housed in a BSC
- Any custom built ventilated enclosure must be certified

Fermenters (CBH 14.3)

- Use double mechanical seals or a top-mounted agitator on motor shafts
- High Efficiency Particulate Air (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. Where possible, aerosol reduction procedures/techniques should be explored and included in your SOPs.

- Carelessly removing gloves
- Flaming slides or lips of flasks
- Dropping/breaking culture containers
- Intranasal inoculation of animals
- Cage cleaning and bedding changes (use BSCs or other primary containment devices)
- Harvest of infected materials from animals, eggs or other virology procedures
- Necropsies of infected animals

Transporting Materials (CBH 20.1)

The proper transportation of biologicals is a key step in preventing contamination and spread of any potential pathogens.

For movement within the containment zone (lab), ensure that all precautions are taken to avoid spills and the release of aerosolized biologicals. The precautions taken to prevent mishaps should correspond to the inherent risk associated with the bioagent being moved. In other words, greater care should be taken with bioagents with higher inherent risk (CBH 2.1.1). Bioagents should be moved in closed containers (primary containment) which are leak-proof and impact-resistant. Screw top containers should be used rather than snap-cap tubes.

For movement outside of the containment zone (lab) but still within the same building, ensure that all biologicals are secured in labelled, closed and leak-proof secondary containers. The surfaces of all transportation containers must be decontaminated prior to use (CBS 4.8.8). Movement of biologicals out of the containment zone must only be done when transporting to a decontamination area, another appropriate containment zone or storage area (CBS 4.6.19,4.8.8). Use a cart with raised edges and guard rails when moving heavy containers or a large number of samples (CBH 2.1.2). Avoid using passenger elevators, where possible use freight elevators.

Waste Management

When working with aerosolizable RG2 bio agents all biological contaminated waste (both liquid and solid) must be fully decontaminated within the BSC prior to removal.

- Follow all SOP instructions regarding required steps for decontamination
- Place solid waste i.e. tips, pipettes, tubes etc. in containers that contain enough of the appropriate disinfectant to cover them
- Pipettes may require some disinfectant to be sucked up so that the interior is disinfected and to stop pipettes from floating in the disinfectant
- Disinfectant may need to be sucked up in syringes prior to disposal to fully disinfect
- Wait the required contact time then pour off the disinfectant and place the decontaminated solid waste in biohazardous waste containers
- All sharps must be disposed of in approved sharps waste containers
- All liquid waste must be placed in containers with the appropriate amount of disinfectant to meet the required final dilution of disinfectant (e. g. 1% sodium hypochlorite) Wait at least 30 minutes before pouring the liquid down the drain

For an overview of waste procedures in Bio labs see: https://ehs.utoronto.ca/wp-content/uploads/2019/04/Waste-Information-and-Procedures-for-Bio-Labs_v3.1-09-14-2020.pdf

Ensure that waste is disposed into the correct containers. This is a link to the university's various waste disposal streams: https://ehs.utoronto.ca/wp-content/uploads/2015/10/Bucket-List-Poster.pdf

Information regarding Biological Waste packaging and collection can be found at https://ehs.utoronto.ca/laboratory-hazardous-waste-management-and-disposal-manual/biological-waste-disposal/

St. George campus:

To set up a pickup service and if you have any questions on hazardous material disposal/waste contact Rob Provost, Manager, Environmental Protection Services (EPS) at 416-978-7000 or rob.provost@utoronto.ca . For chemical/biological waste buckets or a waste collection call the EPS directly at 416-946-3473,or hazwaste.ehs@utoronto.ca . EPS website: https://ehs.utoronto.ca/our-services/environmental-protection-services/

UTM and UTSC campuses:

Waste is received at established storage areas at either the Central Stores or Receiving Areas at both UTM and UTSC. If unaware of the proper contact at UTM and/or UTSC contact Rob Provost, Manager, Environmental Protection Services (EPS) at 416-978-7000 or rob.provost@utoronto.ca .

Resources

George Washington University, Lab Safety, Biosafety Program, Preventing Aerosol Production: https://labsafety.gwu.edu/preventing-aerosol-production-0

Public Health Agency of Canada, Canadian Biosafety Guideline – Lentiviral Vectors: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/lentiviral-vectors/document.html

Public Health Agency of Canada, Canadian Biosafety Guideline – Local Risk Assessment: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/canadian-biosafety-guidelines.html

Public Health Agency of Canada, Canadian Biosafety Handbook: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition.html

Public Health Agency of Canada, Canadian Biosafety Standard: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html

Public Health Agency of Canada, Pathogen Safety Data Sheets (PSDS): https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

University of California, Davis, Safety Services, Safety Net #21, Minimizing Aerosol Exposure: https://safetyservices.ucdavis.edu/safetynet/minimizing-aerosol-exposure

University of Tennessee, Environmental Health & Safety, Biosafety Program, Biosafety Basics Fact Sheet, Aerosol Production and Exposure Control: http://biosafety.utk.edu/wp-content/uploads/sites/30/2012/12/AerosolGuidance.pdf

University of Toronto, Environmental Protection Service, Laboratory Hazardous Waste Management and Disposal Manual: https://ehs.utoronto.ca/laboratory-hazardous-waste-management-and-disposal-manual/

University of Toronto, Environmental Health & Safety, Biosafety: https://ehs.utoronto.ca/our-services/biosafety/