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 aerosolgenerating 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 aerosoltight 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 aerosoltight 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 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