Standard Operating Procedure

Task/Activity/Equipment: Procedures for minimisation of biological aerosols, splashes, and droplets.	
Purpose: To outline procedures that can be implemented for standard laboratory activities to minimise the risk of generation of and/or exposure to biological aerosols, splashes, and droplets.	
Location: PC1, PC2, BC1 and BC2 facilities at Flinders University	**Reference Number:** IBC-SOP-23 **Version:** 1
Written by: Dr Jess Hall, Biosafety Specialist	**Reviewed by:** Institutional Biosafety Committee
Approved by: Belinda Cox, Biosafety Officer	
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Replaces the version: Not applicable (1st version)	
Changes to the last approved version: Not applicable (1st version)	

1. POTENTIAL HAZARDS

Infectious substances	Genetically modified organisms
Risk group 1 or 2 microorganisms	Diagnostic specimens

2. TERMS & ACRONYMS

3. RELEVANT LEGISLATION, GUIDELINES & STANDARDS

- *Australian/New Zealand Standard 2243.3 Microbiological Safety and Containment*
- OGTR Guidelines for Certification of a Physical Containment Facility (PC1, PC2)
- Approved Arrangement Biosecurity Conditions (BC2)

4. SWP SCOPE AND COVERAGE

Aerosols are suspensions of finely dispersed solid particles or liquid droplets in air. This may include airborne biological particles, droplets, and microorganisms, which can remain airborne for long periods, spread across distances, or settle on surfaces causing contamination.

Inhalation of biological aerosols, exposure to splashes and droplets, or touching surfaces contaminated with biological aerosols has been associated with many confirmed laboratory-acquired infections worldwide. To minimise risk to workers in the lab, and to the community and the environment, it is important that we minimise the generation of biological aerosols, splashes and droplets in the lab and take steps to avoid exposure to these.

The purpose of this SOP is to provide information about common aerosol-producing activities in the lab, and to outline safe work practices to minimise the creation of and exposure to biological aerosols, droplets, and splashes.

5. BIOLOGICAL AEROSOL-PRODUCING ACTIVITIES IN LABS

A wide range of activities in the laboratory can generate biological aerosols, droplets, and splashes. The following list presents common examples but should not be considered comprehensive. An individual risk assessment is required for each project to identify risk points associated with specific methods.

Common aerosol-generating activities:

- Pipette mixing
- Serial dilution
- Vortex mixing
- Shaking tubes by hand
- Plating with drops, loops, or spreaders
- Flaming inoculating loops, spreaders, needles, and slides
- Inserting hot loops into a culture
- Pulling needles out of a septum, filling a syringe
- Removing (flicking or popping off) tube lids and stoppers
- Opening lyophilised/freeze-dried cultures, including snap top tubes
- Using freeze dryers
- Centrifuging – all steps involved in centrifugation are commonly associated with aerosols
- Tissue grinding, blending, and homogenising
- Use of cell sorters
- Use of vacuum and aspiration equipment
- Pouring liquids
- Intranasal inoculation of animals
- Cage cleaning
- Necropsies of infected animals
- Accidentally dropping a sample, resulting in spillage or splashes
- Carelessly removing contaminated gloves

6. SAFE WORK PRACTICES TO MINIMISE CREATION OF BIOLOGICAL AEROSOLS, DROPLETS & SPLASHES

Where possible, avoiding the use of biological aerosol generating procedures when working with infectious or potentially infectious materials is the best form of protection. Where this is not possible, the following suggestions for minimising creating of aerosols can be applied.
An individual risk assessment is required for each project to identify risk controls suitable for specific methods.

Pipetting

- Do not 'force expulsion' of the last drop of liquid in a pipette tip.
    - When using volumetric pipettes and burettes, use those marked 'TD'. This stands for 'To Deliver' and indicates that the volumetric markings account for the small amount of liquid that remains behind so that you do not need to force the last drop out to ensure that the full volume is delivered.
- Gently expel the contents of the pipette with the tip held against the side of the receiving vessel.
- Carefully eject disposable pipette tips. Where ejecting into an open container (e.g., a discard beaker or bucket), a wetted towel in the bottom of the container will help to prevent the pipette tip from bouncing around and creating splashes and aerosols.
    - Place the discard container for pipette tips inside the biosafety cabinet, not outside of it.
- Do not pipette mix samples outside of a biosafety cabinet.
- Decontaminate the work area after completion of pipetting to decontaminate aerosols or droplets that may have dispersed onto the work surface.

Mixing/shaking

- Ensure that the sample is in a well-sealed tube or container prior to undertaking mixing or shaking.
    - Knowingly infectious samples must be in a screw-capped tube or vessel for mixing or shaking procedures, flip-top lids shall not be used.
- Either allow the sample to settle for a minimum of 5 minutes (< 10 mL) or 30 minutes (> 10 mL) after mixing, vortexing or shaking, OR open the sample exclusively within a biological safety cabinet.
- Disinfect all surfaces of mixing devices and surrounding work areas thoroughly after use.

Plating and use of loops, spreaders, etc.

- Use disposable pre-sterilised inoculating tools or substitute an enclosed or shielded flameless steriliser (e.g., Bacti-Cinerator or hot bead steriliser) for a Bunsen burner.
- To avoid spitting and splashing, use cooled loops and spreaders for insertion into cultures or onto plates.

Use of needles and syringes

- Avoid use of needles with infectious materials wherever possible.
- Infectious material must only be used in syringes with a Luer lock connector.
- Discharge air from the syringe before inserting into a stopper, and fill syringe slowly and in a controlled manner to avoid introducing air bubbles.
- Do not use syringes to mix infectious liquids.
- Dispose of needles directly into sharps containers without further manipulation – needle cutting or removing devices release aerosols.

Opening tubes and sample containers

- Do not use containers with 'flip' or 'pop' open lids for infectious or potentially infectious materials. When these lids are opened, the film of liquid trapped between the tube and lid breaks and releases aerosols.
- Do not overfill tubes – leave an airgap at the top to prevent spillage or leakage when they are sealed or frozen.
- Open all tubes of infectious or potentially infectious materials within a biological safety cabinet.

Tissue grinding, blending, homogenising, sonicating, freeze drying

- Do not use kitchen blenders in a laboratory setting. Use a laboratory blender with a tight-fitting, gasketed lid and leakproof bearings. Avoid glass blenders.
- Place paper towel moistened with an appropriate disinfectant over the top of the blender, grinder or sonicator to trap and decontaminate any released aerosols. Dispose as biohazard waste after completion.
- Wait as long as possible, and for at least 5 minutes, before opening the lid after mixing. Where the contents are known to be infectious, open within a biological safety cabinet.
- Filter freeze dryer vacuum pump exhaust through an in-line HEPA filter.
- Autoclave or disinfect all equipment straight after use.

Centrifuging

- Do not overfill tubes or plates to be centrifuged. Wipe the outside of the vessel with disinfectant after they are filled and sealed.
- Use plastic tubes with screw-capped lids.
- When centrifuging infectious or potentially infectious samples, ensure that the centrifuge is fitted with internal aerosol containment devices (e.g., sealed rotors, safety cups or buckets with covers, etc.). These must be bioaerosol containment rated and must be used when centrifuging any infectious or potentially infectious sample.

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Examples of aerosol-containment devices for centrifuges. Image sources:
1: Beckman: https://www.beckman.com/resources/technologies/centrifugation/biosafety
2: Eppendorf: https://www.eppendorf.com/CA-en/applications/sars-cov-2-research-solutions/centrifuge-solutions/
3: Eppendorf: https://online-shop.eppendorf.com.au/AU-en/Centrifugation-44533/Microcentrifuges-1007183/Centrifuge-5430-5430R-PF-241000.html

- Do not open the lid of the centrifuge during or immediately after operation. Allow the centrifuge to come to a complete stop and wait at least 5 minutes before opening to allow aerosols to settle if a breakage occurred during operation.
- Aerosol-containment devices (where removable) and tubes/plates should be opened in a biological safety cabinet after centrifuging.
- Ensure that the centrifuge is cleaned and decontaminated if a sample has leaked or spilt during operation.
- Routinely inspect the centrifuge cups, buckets, and rotor seals for deterioration and have the equipment serviced, repaired, or replaced as needed.

Cell sorters

Only cell sorters contained within a purpose-designed biological safety cabinet may be used in association with infectious materials and GMOs.

Vacuum and aspiration equipment

- Use equipment designed for use under vacuum, and do not use breakable vessels or glassware.
- Ensure that the vacuum equipment is fitted with an in-line HEPA filter.
- Ensure that a liquid trap/interceptor vessel/overflow flask is installed with aspirating equipment, and that it is partially filled with disinfectant prior to operation.
- When working with infectious materials, ensure that the liquid trap/overflow flask sits within the biological safety cabinet.

Pouring infectious liquids

- Pour infectious or potentially infectious liquids within a biosafety cabinet.
- Do not pour off supernatant – use a pipette instead.
- When collecting liquid waste, pour through a funnel where the end of the funnel sits below the surface of the disinfectant in the discard container. Pour additional disinfectant through the funnel after use.
- Decontaminate the work area after completion to decontaminate aerosols or droplets that may have dispersed onto the work surface.

Cage cleaning

- Ensure that cages used with infectious studies are autoclaved prior to emptying.
- Use cage change stations and biological safety cabinets provided when undertaking cage changes.
- Empty cages at the bedding disposal station – the equipment provided draws aerosols and dust away from the operator and through a HEPA filter to minimise risk of exposure.

Spills Management

Refer to IBC-SOP-29 for details of spills management procedures.

Removing gloves

Refer to IBC-SOP-20 for details of safe removal of gloves to avoid generation of aerosols and cross-contamination.

7. EQUIPMENT TO MINIMISE EXPOSURE TO BIOLOGICAL AEROSOLS, DROPLETS & SPLASHES

The following laboratory equipment may be employed in applicable cases to minimise risks of exposure to biological aerosols, droplets, and splashes, as determined by risk assessment:

- A NATA certified biological safety cabinet is the primary device used to provide protection from biological aerosols and droplets. These must be used for any activity involving infectious samples and GMO microorganisms that may generate aerosols, and for all work with microorganisms infectious via the respiratory pathway.
- Centrifuges must be fitted with aerosol containment devices such as sealed rotors, safety cups or buckets with covers when used with infectious material.
- Liquid traps / interceptor vessels / overflow flasks and in-line HEPA filters must be installed for all vacuum systems.
- Fit-tested N95 or P2 masks may be used as an additional level of control where indicated by risk assessment. They should not be used as the sole source of protection.

8. APPLICABILITY

These guidelines are applicable to all persons working with biological samples in a laboratory setting.

9. CONTACTS

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10. DEFINITIONS

Aerosol	A suspension of finely dispersed solid particles or liquid droplets in air. For the purposes of biosafety, aerosol generally refers to airborne biological particles, droplets, and microorganisms, and includes but is not limited to airborne viruses, bacteria, spores, pollen, endotoxins, animal dander, or plant debris. Note: Standard laboratory procedures result in the generation of aerosols. Procedures including but not limited to shaking, mixing, centrifuging, pipetting, and ultrasonic disruption are common examples.
Biological safety cabinet	Also called a biosafety cabinet. An enclosed, ventilated cabinet providing a workspace in which to work safely with materials contaminated with (or potentially contaminated with) pathogenic microorganisms. The cabinet provides protection to the laboratory worker and surrounding environment from biological aerosols using HEPA filtration.
N95 or P2 mask	A respirator face mask designed to achieve a very close facial fit and used to protect the wearer from particles or from liquid contaminating the face, and to provide high efficiency filtration of airborne particles.