Standard Operating Procedure

Task/Activity/Equipment: Use of biological safety cabinets	
Purpose: To outline the situations where use of a biosafety cabinet is recommended or required, and to outline procedures for safe use and routine decontamination.	
Location: PC1, PC2, BC1 and BC2 facilities at Flinders University	**Reference Number:** IBC-SOP-26 **Version:** 1.0
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
Chemical disinfectants	Flammable substances (e.g., ethanol)
Electric shock from biosafety cabinet	

2. TERMS & ACRONYMS

BSC	Biological safety cabinet
UV	Ultraviolet

3. RELEVANT LEGISLATION, GUIDELINES & STANDARDS

- *Australian/New Zealand Standard 2243.3 Microbiological Safety and Containment*
- *Australian Standard 2252.2 Controlled Environments Part 2: Biological Safety Cabinets Class II Design*
- *Australian Standard 2252.4 Controlled Environments Part 4: Biological Safety Cabinets Classes I and II – Installation and Use*
- *Australian Standard 2252.5 Controlled Environments Part 5: Cytotoxic Drug Safety Cabinets (CDSC) – Design, Construction, Installation, Testing and Use*

4. SWP SCOPE AND COVERAGE

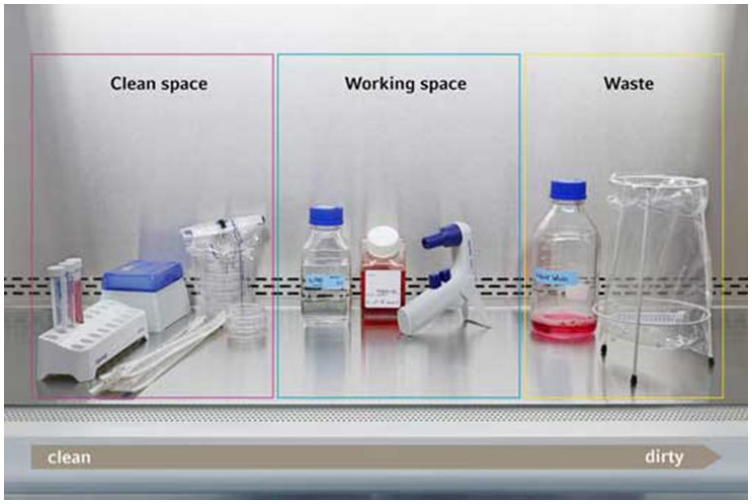
Refer to Annex 8 of the Flinders University Biosafety Manual for information about the purpose and function of biological safety cabinets.

As each biosafety cabinet model has its own operating conditions, these procedures outline general principles of use and not step-by-step details of how to turn on and operate each cabinet. If you would like a Word document copy of this document to modify for your own lab, please contact the Biosafety Officer (ibcadmin@flinders.edu.au).

5. PRE-ACTIVITY CHECKS

Do not use defective or faulty equipment.
Ensure that you have read and understood risk assessments and Safety Data Sheets (SDS) for all substances, processes and plant equipment being used.
Ensure that you are aware of the locations of the following: • Eye wash • Emergency exits • Required PPE
Ensure that the biosafety cabinet certification is current and that the cabinet is operating correctly.
Do not use a cabinet that has not been NATA-certified or that has any signage indicating that the equipment is out-of-order. Cabinet certification status can be checked by reviewing the certificate on the front of the cabinet.
Ensure that you have been trained in the safe and correct operation of the biosafety cabinet.
Check clearance zone behind BSC; this area should not be used as a thoroughfare while BSC is in use, to avoid disruptions to airflow and containment.

6. PREPARING BSC FOR USE

1	Wear eye protection, a lab coat, enclosed shoes, and disposable gloves when working in a BSC.
2	Turn off UV lamp, if one is used (NOTE: It is recommended that use of UV lamps be avoided, as UV is generally ineffective for adequate disinfection).
3	Open front sash of the biosafety cabinet to operating position.
4	Turn on the BSC fan 10 minutes before beginning work to allow adequate air filtration.
5	Turn on fluorescent light, inspect air intake grilles for obstructions and foreign materials, and remove any obstructions.
6	Decontaminate the cabinet work area, and surface-decontaminate all materials to be placed inside the BSC using an appropriate disinfectant (80% v/v Ethanol, or 1% Virkon). See Section 8 ‘Cleaning and Disinfection’ section for further instructions.
7	Place all items required towards the rear of the BSC (waste receptacles, pipettes and tips, reagents, and other necessary items). Items should be organised in a manner to reduce movement within the BSC. Bulky items such as waste containers should be placed to one side of the interior of the cabinet.  **Figure 1: Example of work zone layout incorporating clean to dirty workflow within the cabinet.**
8	Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC impedes proper airflow and affects personnel protection.
9	If vacuum-connected liquid waste traps are used, ensure that an in-line HEPA filter is installed to protect the vacuum line. Where working with infectious microorganisms or GMOs, the waste trap must be placed within the BSC for use.

7. WORKING WITHIN THE BSC

1	Work as far back as possible, but within comfortable reach. At minimum, all work should be performed at least 10 cm back from the inside edge of the front grille.

Figure 2: Demonstration of working within the work zone of the cabinet, showing a 10 cm distance from the front grille (ruler provided to show 10 cm distance).

2	After placing hands inside the cabinet, allow air to stabilise before beginning work.
3	Segregate clean and dirty items within the cabinet workspace. Active work should flow from clean to contaminated areas across the work surface.
4	Minimise movements inside the cabinet. Arms should be moved slowly, perpendicular to the front opening to minimise disruption of the air curtain. Avoid moving your hands in and out of the BSC.
5	When exiting the cabinet, do so in a motion that is slow and directed away from the cabinet. Upon re-entering, wait a few minutes for the air to re-stabilise.
6	When finished working, seal all biologicals and waste, surface-decontaminate all items and leave within the running cabinet for 3 minutes prior to removal from the cabinet. Remove items slowly and away from the cabinet.
7	Disinfect all surfaces following completion of work. See 'Cleaning and Disinfection' below for further instruction.
8	Turn off fluorescent light an allow blower to continue running for at least 10 minutes after work has ceased.
9	Turn off the blower.

If an airflow alarm sounds whilst working in the cabinet, work should cease immediately. Leave cabinet running, seal all samples and check that the front air grille is not blocked or obstructed. If no obvious obstructions are present, cease all work in the cabinet then decontaminate items in the cabinet and remove them before decontaminating the cabinet following instructions in Section 8. Close the front sash of the cabinet and notify the Laboratory Manager to contact a service technician.

8. CLEANING AND DISINFECTION AFTER USE

- Use of UV light as a sole means of decontamination is **NOT** sufficient or acceptable.
- **Do not spray ethanol within biosafety cabinets** – this may damage mechanics and presents a flammable risk. Always use a drench bottle to apply ethanol disinfectant to surfaces within the cabinet.

1	Use of UV light as a sole means of decontamination is NOT sufficient or acceptable.
2	With the cabinet blower on, wet all materials, reagents, and waste containers in the cabinet with disinfectant and remove from the cabinet. Store or dispose of appropriately.
3	Interior surfaces should be wetted with disinfectant.
4	Liberally apply disinfectant on the work surface, side walls, back wall, and inside of sash. Allow disinfectant to remain on surfaces for at least 10 minutes.
5	After sufficient contact time has elapsed, wipe down all surfaces with paper towel. Wipe towards

	yourself, in straight horizontal lines. Each time, overlap the previous wipe by 10-25%. Also wipe from the top of the cabinet to the bottom on all sides, and on the interior of the sash.
6	If 1% Virkon or sodium hypochlorite has been used for cleaning and disinfection of the cabinet, step 3 should be repeated with a wipe-down with sterile water or 80% v/v Ethanol to remove Virkon residue
7	Allow the cabinet to run for at least 10 minutes after disinfection, then turn blower off
For instructions on cleaning and disinfection following a biological spill, Refer to IBC-SOP-29.	

9. CLEANING & DISINFECTION PRIOR TO SERVICING, RELOCATION, REPAIR OR DISPOSAL

- Use of UV light as a sole means of decontamination is **NOT** sufficient or acceptable.
- **Do not spray ethanol within biosafety cabinets** – this may damage mechanics and presents a flammable risk. Always use a drench bottle to apply ethanol disinfectant to surfaces within the cabinet.

1	With the cabinet blower on, wet all materials, reagents, and waste containers in the cabinet with disinfectant and remove from the cabinet. Store or dispose of appropriately.
2	Apply disinfectant to the front grill of the cabinet. Liberally apply disinfectant to the work surface, side walls, back wall, and inside of sash. Allow disinfectant to remain on surfaces for at least 10 minutes.
3	Raise the workspace grill and work surface and liberally apply the disinfectant to the underside, drain pan and all exposed surfaces. You may need something or someone to hold the work surface up while you apply the disinfectant. Allow disinfectant to sit for at least 10 minutes.
4	If any sharps are present, use forceps to collect and dispose into a sharps container.
5	Using paper towel, wipe down all surfaces (including underneath the work surface and grill).
6	If the drain pan was soiled/dirty, repeat steps 3 and 5.
7	After decontaminated surfaces have dried, replace the work surface and grill, close the sash, and turn off the blower.
8	For relocation, repair, or decommissioning, also wipe down all external surfaces of the biological safety cabinet with an appropriate disinfectant, allowing 10 minutes contact time.
9	Within PC2 and BC2 facilities, the cabinet must also undergo gaseous decontamination prior to servicing relocation, repair, or decommissioning. This must be arranged with the NATA certified cabinet testing who will undertake the gaseous decontamination. Do not attempt to undertake this procedure yourself.
10	**Following decontamination, DO NOT** use the BSC until completion of annual servicing, relocation, or repair. Place a sign on the BSC indicating date of decontamination (see Appendix A for a sample sign).
11	**Important:** If being decommissioned/discarded, be sure to remove any biohazard stickers after decontamination is completed
12	When the BSC is moved to a new facility or a new location, it **MUST NOT** be used until recertified by an authorised BSC technician.

10. FORBIDDEN ACTIVITIES

1	Heat sources such as Bunsen burners are strictly prohibited in the BSC unless a risk assessment has been undertaken, as they significantly disrupt the airflow and reduce personnel protection.
2	Do not work in the BSC if a warning light or alarm is signalling, or if the equipment is 'out-of-order'.
3	Do not work in a BSC that has not passed annual NATA certification, as shown on the certificate on the front of each cabinet. Do not use a BSC if the date of annual certification has expired.
4	Do not work in a BSC that has been relocated unless the BSC has undergone and passed NATA certification in its new location.
5	Ultraviolet (UV) lamps are not permitted to be used as a sole-disinfection method in the BSC. Cleaning and disinfection procedures outlined above must be followed at the completion of each work session.
6	Large pieces of equipment (e.g., bench-top centrifuges, cell sorters or microscopes) must not be placed or operated within the biological safety cabinet, as they significantly affect airflow and reduce personnel protection. • Where such equipment must be placed within a cabinet, either a customised cabinet is required, or the cabinet must undergo testing with the equipment in situ. In some instances, this may require

	an alternate test method (e.g., KI Discus test) to confirm that the cabinet is operating correctly. Discuss with the NATA certifier in these instances.

11. APPLICABILITY

These procedures are applicable to all persons working with biological safety cabinets.

12. CONTACTS

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13. DEFINITIONS

Biological safety cabinet	A piece of equipment designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating substances containing infectious agents, such as viruses, bacteria, and primary tissue cultures.
High Efficiency Particulate Air (HEPA) filter	Filter that traps 99.97% of particles of 0.3 µm in diameter, and 99.99% of particles of greater or smaller size, thus capturing all infectious agents and ensuring only microbe-free air is exhausted from the cabinet or directed to the work surface.