



Biological Risk Management and Containment

Biological Safety Cabinets

Class II - Equipment Placement and Use

Containment Laboratory Guidelines

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This document was originally Version 1 which was extensively reviewed and approved in February 2021.

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Date	Page number	Nature of amendment
31 July 2024	11	Decontamination steps added
31 July 2024	All	University Logo updated

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1. Who are these guidelines for?

These guidelines are intended for **principal investigators (PIs), designated persons in charge, designated laboratory person (DLPs)**, technical staff and students trained in the safe use of **risk biologicals** in appropriate containment facilities.

It is strongly recommended that all users view the video presentation prepared by WHO Collaborating Centre for Biosafety in Microbiology at Victorian Infectious Diseases Reference Laboratory (VIDRL) on location, use, decontamination of Biological Safety Cabinets.

This video is available as part of the Canvas Containment course.

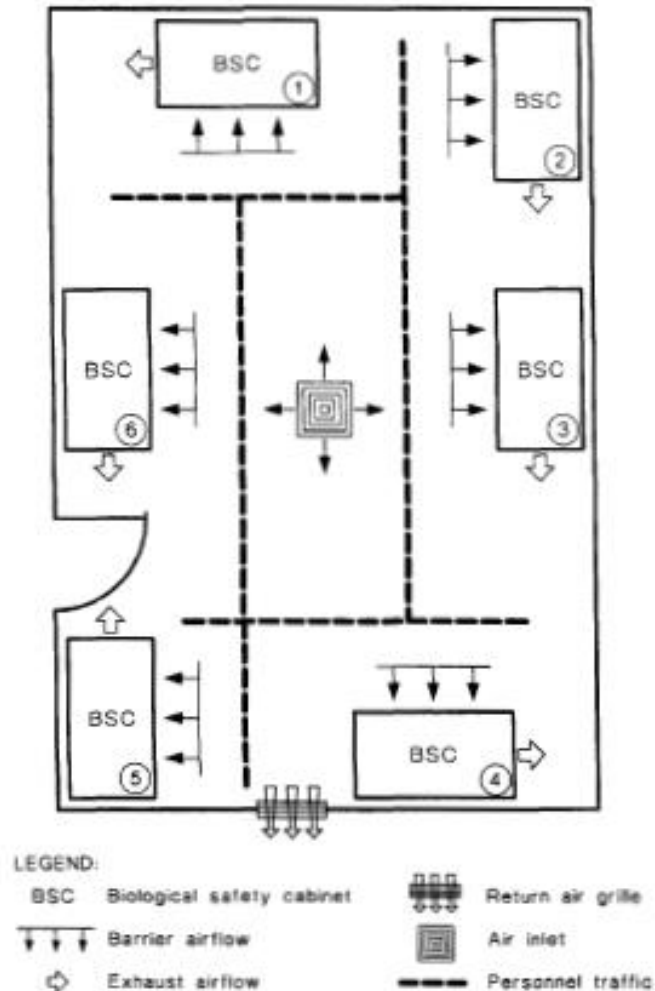
2. Biological Safety Cabinet Placement

Biological Safety Cabinets (BSC) must be situated so that there is adequate clearance behind and on each side of each cabinet to allow easy access for maintenance and to ensure that the cabinet air re-circulated to the laboratory is not hindered.

There must be 300-350mm clearance above the cabinet to provide for accurate air velocity measurement across the exhaust filter surface and for exhaust filter changes.

The air curtain created at the front of the cabinet is quite fragile, amounting to a nominal inward and downward velocity of 1.5 kph.

Open windows, air supply registers, portable fans or laboratory equipment that creates air movement (e.g., centrifuges, vacuum pumps) should not be located near the BSC. Similarly, chemical fume hoods must not be located close to BSCs. The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from foot traffic), to minimise disruption since people walking parallel to the face of a BSC can disrupt the air curtain.



NOTES:

- 1 The location of cabinet 1 is appropriate with respect to the avoidance of air movements which could influence cabinet airflows, and the installation provides the specified exhaust discharge clearance.
- 2 Although otherwise well-sited, cabinet 2 will direct exhaust airflow towards cabinet 3.
- 3 The airflow of cabinet 3 could be influenced by exhaust airflow from cabinet 2, and by the air inlet.
- 4 Cabinet 4 is well-sited, providing that the adjacent return air grille does not influence cabinet airflow.
- 5 Cabinet 5 is too close to the doorway.
- 6 Cabinet 6 is too close to the doorway, and could be influenced by the air inlet. The specified exhaust discharge clearance will not be maintained when the door is open.

Figure 1.

British Standard 5726:2005 requirements, while not mandatory in NZ, do provide useful guidance. The requirements of BS 5726 are depicted in the following 10 figures.

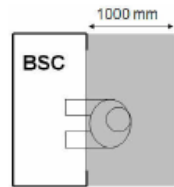


Figure 1. There should be an undisturbed area of 1 m in front of the cabinet.

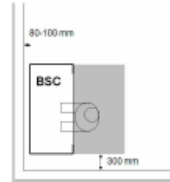


Figure 2. Cabinets should be kept clear of adjacent walls.

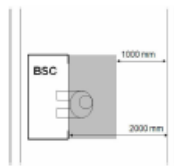


Figure 3. Cabinets should be kept clear of opposite walls.

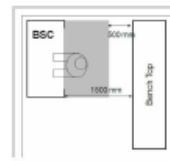


Figure 4. Cabinets should be kept clear of opposite benches.

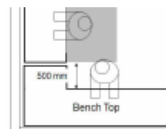


Figure 5. Adequate room for workers at nearby benches should be provided.

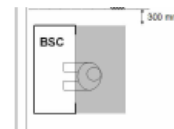


Figure 6. Cabinets should be kept clear of adjacent columns and structures.

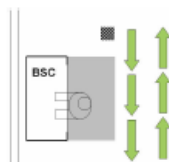


Figure 7. Through ways must be at least 1 m away from the cabinet. Columns and other structures can be helpful to define traffic routes.

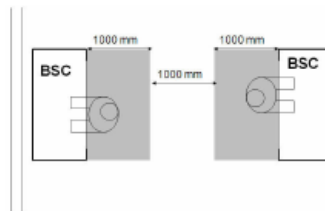


Figure 8. A distance of 3 m must be maintained between two opposite cabinets.

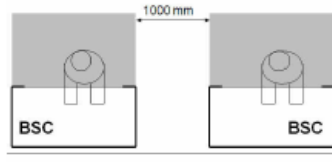


Figure 9. A distance of 1 m must be maintained between two cabinets along the same wall.

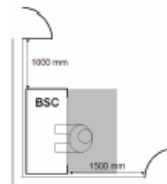


Figure 10. Cabinets must be kept clear of doors.

Figure 2

3. Preparing for Work in a Class II BSC

Check the cabinet is still within certification

Prepare a written checklist of materials necessary for a particular activity and place necessary materials in the BSC before beginning work. This preparation minimizes the number of movements across the air barrier and hence disruption to air barrier of the cabinet.

Materials or equipment placed inside the cabinet may cause disruption of the airflow, resulting in turbulence, possible cross-contamination and/or breach of containment. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

Before beginning work, the investigator should adjust the stool height so that his/her face is above the front opening.

Laboratory coats should be worn buttoned over street clothing; latex, vinyl, nitrile or other suitable gloves can be worn to provide hand protection. Increasing levels of PPE may be warranted as determined by an individual risk assessment.

If the cabinet has just been started, the fans should be operated at least four minutes before beginning work to allow the cabinet to “purge.” This purge will remove any suspended particulates in the cabinet. The work surface, the interior walls (except the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH)

Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize, to “air sweep” the hands and arms, and to allow time for turbulence reduction.

4. Work in a Class II BSC

All operations should be performed on the work surface at least 300mm in from the front grille.

All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet.

Similarly, aerosol-generating equipment (e.g., vortex mixers) should be placed toward the rear of the cabinet to take advantage of the greater airflows at the back of the cabinet.

Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection.

Horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within the cabinet.

Potentially contaminated materials should not be brought out of the cabinet until surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or another part of the laboratory.

Bulky items such as biohazard bags, discard pipette trays and vacuum collection flasks should be placed to one side of the interior of the cabinet. If placing those items in the cabinet requires opening the sash, make sure that the sash is returned to its original position before work is initiated. The correct sash position (usually 250-300 mm above the base of the opening) should be indicated on the front of the cabinet. On most BSCs, an audible alarm will sound if the sash is in the wrong position while the fan is operating.

5. Operations Within a Class II BSC

Class II cabinets are designed so that horizontally nebulized spores introduced into the cabinet will be captured by the downward flowing cabinet air within 350mm of travel. Therefore, as a general rule, keep clean materials at least 300mm away from aerosol-generating activities to minimize the potential for cross-contamination.

Rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and compromise the partial containment barrier provided by the BSC.

Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce this risk.

Other personnel activities in the room (e.g., rapid movements near the face of the cabinet, walking traffic, room fans, open/closing room doors) may also disrupt the cabinet air barrier.

When the user's arms rest flatly across the front grille, occluding the grille opening, room air laden with particles may flow directly into the work area, rather than being drawn down through the front grille. Raising the arms slightly will alleviate this problem.

The front grille must never be blocked with towelling, research notes, discarded plastic wrappers, or pipetting devices

6. Reducing Contamination Within a Class II BSC

Workflow should be from "clean to dirty" (Refer to Figure 1). Materials and supplies should be placed in the cabinet in such a way as to limit the movement of "dirty" items over "clean" ones.

Clean cultures (left) can be inoculated (centre) and then contaminated pipettes can be discarded in the shallow pan or contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed persons

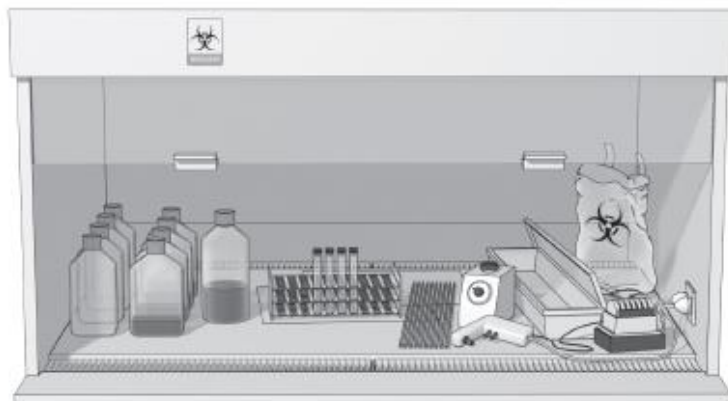


Figure 3. A typical layout for working "clean to dirty" within a Class II BSC.

Several measures can be taken to reduce the chance for cross-contamination of materials when working in a BSC.

Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impact of downward air. Bottle or tube caps should not be placed on the towelling. Items should be recapped or covered as soon as possible.

Open flames must never be used in a biological safety cabinet. They are not necessary in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current that prevents microorganisms from falling into the tube or flask.

An open flame in a BSC creates turbulence that disrupts the pattern of HEPA-filtered air being supplied to the work surface.

When deemed absolutely necessary, touch-plate micro burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat build-up will be minimized. The burner must be turned off when work is completed. Small electric “furnaces” are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable or recyclable sterile loops should be used whenever possible.

7. Aspiration Equipment



Figure 4

Aspirator bottles or suction flasks must be connected to an overflow collection flask containing approved disinfectant (refer to Quick Reference Guide), and to an in-line HEPA or equivalent filter (Figure 4).

This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation

of aspirated materials can be accomplished by placing sufficient approved decontamination solution into the flask to inactivate the microorganisms as they are collected.

Proprietary vacuum aspiration systems (e.g. 'Vacusafe') fitted with 0.22um hydrophobic filters as part of the assembly are commonly used in University laboratories and provide a superior solution.

Once inactivation occurs, liquid materials can be disposed of as non-infectious waste.

8. Cleaning and decontamination

The procedure below is for decontamination after use with no evident spills. In case of spills please use the relevant guide for '[Microbiological spills](#)' instead.

Decontamination steps:

1. After use enclose or remove items which have been in contact with the agent and cover any waste container (if any).
2. It is recommended that the cabinet is left to operate for 5 minutes with no activity before proceeding with the decontamination. This should purge airborne contaminants from the work area.
3. Clean and decontaminate all containers and equipment and then remove from the cabinet
4. Once the cabinet is empty and ON proceed to clean from top to bottom and from back to front. Use University approved decontaminating agents only and allow the effective contact time.

Tip: If you are using a chloride type disinfectant, wipe down interior surfaces with a 70% alcohol solution. This will protect stainless steel interiors from corrosion.

If the cabinet has a catch basin beneath the work surface and more extensive decontamination is planned then follow the steps in green below. Otherwise move on to number 5.

- Ensure the drain valve under the cabinet is closed.
- Using University approved decontaminating agents only apply to the work surface and through the front and rear grills into the drain pan. Allow relevant contact time.

- Lift the grill and proceed to decontaminate the under bench surface in the same way. Absorb spilled fluid-disinfectant from work surface with paper towels & discard in biohazard bag.
 - Visually check the pre-filter to make sure no objects would impede the flow. This does not need to be removed by the users.
 - Wash hands after gloves are removed.
 - Run BSC at least 10 minutes after cleanup, before resuming activity in the cabinet, or turn off if work is completed.
5. The cabinet can be turned OFF and the vertically sliding window, or equivalent, closed.
6. If desired and available, the UV light might be turned on.

Interesting fact: The Centre for Disease Control (CDC), National Institute of Health (NIH) and the National Science Foundation (NSF) all agree that UV lamps are neither recommended, nor required in Biological Safety Cabinets. To the point that NIH does not allow to purchase BSC with UV lights and banned the retro-installation of UV light on existing cabinets. On top of the decontamination inefficiency, there are maintenance costs and safety concerns related to UV light.

9. Definitions

Designated laboratory person (DLP) means the trained person in each research group who has been given the authority to receive purchase requests made in SQERM and to make a formal request for a purchase order via PeopleSoft. In containment and transitional facilities DLPs will have additional training to enable them to scrutinise documentation for restricted items and provide support to researchers.

Designated person in charge means a staff member in any of the following roles: sector manager, facility manager, floor manager, technical manager or an appointed delegate.

Principal Investigator (PI): In the context of hazard containment and transitional facilities, a principal investigator is the holder of an independent grant administered by the University and the lead researcher for the grant project, usually in the sciences, such as a laboratory study or a clinical trial. The phrase is also often used as a synonym for "head of the laboratory" or "research group leader." The PI is responsible for assuring compliance with applicable University standards and procedures, and for the oversight of the research study and the informed consent process. Although the PI may delegate tasks, they retain responsibility for the conduct of the study.