

Safe Use of Microbiological Safety Cabinets Handbook

Introduction

Aerosols generated when handling micro-organisms and other hazardous biological material pose an invisible and potentially lethal threat to the scientist and other people in the vicinity. To protect these people from this threat a microbiological safety cabinet is used. Some types of microbiological safety cabinet also protect the materials being handled in them from environmental contamination. When using a microbiological safety cabinet there are three components that contribute to the safety of the scientist and other people:

- correct choice of cabinet
- satisfactory performance of cabinet within laboratory
- proper use of the cabinet through good operational technique.

These components are discussed in this Guidance Note.

Correct Choice of Microbiological Safety Cabinet

What is a microbiological safety cabinet?

A microbiological safety cabinet is a ventilated enclosure designed to offer protection to the user and the environment from aerosols generated when handling hazardous biological material. Air discharged from the safety cabinet to atmosphere passes through a high efficiency particle absorption (HEPA) filter to remove contaminants.

Microbiological safety cabinets must not be confused with laminar flow cabinets or tissue culture cabinets. These cabinets do not provide operator protection; they are designed to protect the work only. Laminar flow cabinets can be used only for animal derived material or tissue that is known to be non-pathogenic and non-allergenic. They cannot be used with human material or tissue.

Fume cupboards must not be used to protect against airborne biological hazards.

Types of microbiological safety cabinet

There are three types of safety cabinet that differ in mode of operation, and hence level of protection provided.

CLASS I

Class I cabinets have a front aperture through which the operator can carry out manipulations. The operator is protected by an inward flow of air through the front aperture but some airborne material can escape. This type of cabinet does not protect the work from external contamination.

CLASS II

Class II cabinets have a front aperture through which the operator can carry out manipulations but which allows some airborne material to escape. It is designed to protect the operator from the work being undertaken, and also the work from external contamination. This is achieved by a complex airflow pattern into and through the cabinet. Air drawn through the front aperture is directed downwards into a plenum below the work surface. Some of this air is filtered before being redirected into the working area as a laminar down flow of clean air to protect the work from external contamination. The balance of this laminar down flow with the incoming air provides an air curtain at the front aperture which provides the operator protection. The split between exhausted and recycled air on each cycle is typically in the range 20/80 or 30/70. With correct installation and use modern Class II cabinets give a similar value of operator protection as Class I cabinets.

CLASS III

Class III cabinets have a totally enclosed working area that prevents release of airborne material. This design provides maximum protection for the operator and the work. Access to the cabinet is through arm length gloves attached to ports in the front panel. Air drawn into the cabinet is HEPA filtered, and the working chamber is under negative pressure.

Making the correct choice

All cabinets purchased must comply with BS EN 12469:2000 that describes the specification for the design, construction, performance, maintenance and testing of microbiological safety cabinets. In addition, an operator protection factor test (OPFT) must be carried out at installation. Satisfactory performance of the cabinet in post installation tests must be a condition of your purchase contract. No payment should be made until satisfactory performance has been demonstrated.

The choice of cabinet class depends upon a risk assessment that considers:

- properties of micro-organisms including their route of transmission, infectivity, survival in environment, and susceptibility to disinfectants;
- nature of work eg are significant aerosols generated;
- is protection of the work required.

In most cases the following guidelines will be appropriate:

Containment Level 1 and 2 laboratories

A Class I cabinet should be used unless protection of the work is required when a Class II cabinet can be used.

Containment Level 3 laboratories

A Class I or Class III cabinet must be used:

1. if the micro-organism or genetically modified micro-organism has as an airborne route of transmission;
2. if procedure generates significant aerosol (eg ultrasonic disruption).

A Class III cabinet must be used with a micro-organism or genetically modified micro-organism considered to be high risk: for example multiply drug resistant strains.

A Class II cabinet may be used for work with:

- hazard group 3 pathogen with derogation such that use of a safety cabinet is not essential eg some blood borne parasites
- non-hazard group 3 pathogens that are assigned to containment level 3 under Scottish Executive Rural Affairs Department restrictions eg some animal pathogens
- materials known or suspected of containing hazard group 3 agents when there is no cultivation or concentration of those agents
- blood borne viruses in hazard group 3 that are unlikely to be transmitted via the airborne route (eg HIV, hepatitis)

Providing:

- the cabinet is tested for operator protection (KI discus) every 6 months
- all KI discus testing must be performed in accordance with the British Standard (4 head test) and no individual value of the protection factor shall be less than 1×10^5

- for work with blood borne viruses the operator protection test must be shown to be satisfactory under the conditions of use. An 'in-use' operator protection factor test must be carried out that mimics working condition, eg a second operator must move around the laboratory, open and close entry door, and stimulate normal working practices. All ventilation and other cabinets in the laboratory must be switched on for the duration of the test

In all cases the use of a Class II cabinet must be justified since the operator protection given by this type of cabinet is highly dependent on correct siting and installation, and on the proper use of the cabinet and the laboratory. For example opening doors and walking past a Class II may lead to a loss of operator protection. Class I and III cabinets are not so susceptible to these factors.

Performance of Microbiological Safety Cabinets in Laboratory

Siting, installation and commissioning of safety cabinets

The correct siting of a safety cabinet is essential for satisfactory performance. Air currents caused by movement of people in the laboratory, poorly located ventilation systems, and opening of doors and windows can compromise seriously the protection afforded by the cabinet. Operator protection given by Class II cabinets is especially susceptible to such disturbances. Therefore, cabinets should be sited to minimise disturbance of the airflow at the front of the cabinet. Key factors to consider when siting a cabinet are:

- distance from walls, corners, and doorways
- large objects that could interfere with air flow eg incubators, freezers
- amount of working space for operators
- position of ventilation supply and extract grilles

To ensure cabinets perform satisfactorily they must be installed and commissioned by a specialist contractor, and must pass performance tests as specified in BSEN 12469:2000 including an operator protection factor test. After commissioning the cabinet must not be moved to a new position either within the laboratory or to a different laboratory. If the cabinet is moved or if the laboratory is changed significantly (eg positioning of ventilation supply and extract grilles, altering number of air changes/hour, addition of a large piece of equipment or installation of air conditioning) then full commissioning tests must be carried out.

Venting arrangements

Exhaust air from safety cabinets should be vented to the building roof where possible for the following reasons:

- allows safe discharge of fumigant gas after decontamination
- allows safe discharge of small amounts of volatile, toxic substance used to treat cultures
- provides an additional safety margin in event of filter failure
- provides an inexpensive and simple method of maintaining negative pressure within a containment laboratory

For cabinets with integral fans the ductwork should be less than 2m and airtight. If the ductwork is longer than 2m then an additional exhaust fan should be located as near to the discharge point as possible.

At Containment Level 2 recirculating microbiological safety cabinets can be used provided that the exhaust air is filtered through two HEPA filters in series according to BS 5726 specification. Recirculating cabinets can only be used at Containment Level 3 with some derogated hazard group 3 pathogens eg *P.falciparum*, *L.donovani*, *T.brucei rhodesiense*. A safe method of fumigation must be available if this option is chosen.

Routine examination and testing

Regular examination and testing according to BSEN 12469:2000 is required to ensure safety cabinets continue to provide operator protection. Minimum testing frequency by operators and service engineers and the required performance for Class I and II cabinets are given below:

1. Operator tests

Test	Microbiological Safety Cabinet	
	Class I	Class II
Alarms/indicators	Before use	Before use
Face velocity/inflow	Monthly Velocity at all points should be between 0.7 and 1.0m/s	Monthly Not less than 0.4m/s

2. Engineer tests

At Containment Level 3 all microbiological safety cabinets must be examined and tested every 6 months: all other cabinets must be examined and tested at least annually.

Test	Microbiological Safety Cabinet	
	Class I	Class II
Alarms/indicators	Function as specified	Function as specified
Face velocity/inflow	Velocity at all points should be between 0.7 and 1.0m/s	Not less than 0.4m/s
downflow	N/a	0.25 to 0.5 m/s
Operator protection factor test	Greater than or equal to 1×10^5	
'in use' operator protection factor test (if required)	Greater than or equal to 1×10^5	
Filter integrity	Overall efficiency greater than 99.995%	
Mechanical integrity including visible ductwork	intact	
Mechanical and electrical function	Function as specified	

Proper Use of Microbiological Safety Cabinet

Local Rules

Improper use of safety cabinets can compromise significantly the level of operator protection. Operator protection afforded by Class II cabinets is especially susceptible to disruptive airflows created by improper use of the cabinet or the laboratory. A typical set of local rules for the safe use of safety cabinets is given below:

Before starting work:

1. Ensure laboratory door is closed.
2. Put on protective clothing.
3. Remove cabinet front.
4. Ensure the inside of cabinet is clean and free of clutter.
5. Switch on cabinet 5-10 minutes before starting work to allow air-flow to stabilise.
6. Do not use unless air-flow indicator is registering in the 'safe zone'.
7. Prepare for work eg obtain flasks, plates, pipettes and media/solutions.
8. Ensure appropriate disinfectants are readily available.
9. Ensure equipment required for work is available.
10. Place work in cabinet, keeping clean and dirty materials separate.

When working:

1. Keep materials within the cabinet to a minimum.
2. If alarm sounds, make work secure (eg put lids on flasks) and put front on cabinet.
3. Work as near to centre of cabinet as possible, and at least 15cm from front.
4. For Class 2 cabinets never obstruct air inlet grilles eg with notebooks or paper.
5. Do not make rapid arm movements.
6. Do not mix sterile with infected materials, avoid passing potentially infected material over clean material.
7. Dispose of waste inside cabinet.
8. Do not rely on cabinet to cover up poor technique.

On completion of work:

1. Remove samples for incubation, after wiping with appropriate disinfectant.
2. Remove all waste from cabinet in secure containers.
3. Wipe all surfaces with disinfectant.
4. Leave cabinet on for 5 to 10 minutes before finally switching off.
5. Replace cabinet front.

Training and competence

No one should be allowed to use a safety cabinet unless they have demonstrated competence. Training must be given when competence is lacking. Training should include instruction in the following:

- classification of cabinets;
- principals of airflow and operator protection factor tests;
- limitations of performance;
- appropriate and inappropriate use of cabinets;
- mode of operation and function of all controls and indicators;
- how to work at cabinets safely;
- how to decontaminate after use.

Decontamination

The interior of a safety cabinet should be washed with the appropriate disinfectant after use. If removal of the working surface and cleaning of exposed surfaces is necessary the following precautions should be adopted:

- heavy duty PVC or rubber gloves that can be disinfected after use must be worn over disposable gloves
- spay all exposed surfaces with appropriate disinfectant at correct dilution and allow sufficient time before proceeding
- lift working surfaces with handles if provided; or if not provided use a suitable implement to lever up
- spay all exposed surfaces with appropriate disinfectant at correct dilution and allow sufficient time before proceeding
- clean with a thick wad of absorbent material
- dispose of waste as Clinical Waste
- disinfect outer gloves before removal
- fumigate the cabinet if necessary

Microbiological safety cabinets should be fumigated with formaldehyde vapour:

- following a large spillage of infectious material resulting in contamination of inaccessible surfaces;
- before carrying out maintenance work requiring access to potentially contaminated parts (including filter and pre-filter changes;

- before carrying out filter penetration tests;
- when nature of work changes significantly eg use of different pathogen.

The cabinet must be sealed before fumigation to ensure formaldehyde cannot escape into the laboratory. Fumigation should be carried out overnight or at least for six hours. A safe method of fumigant dispersal must be adopted.

Equipment affecting performance of safety cabinets

Disturbance to the inward airflow of an open fronted Class I or II cabinet can reduce significantly the level of operator protection. For this reason the use of the following equipment within cabinets should be prohibited at Containment Levels 2 and 3:

- bunsen burners. If their use is essential at Containment Level 1 they should be placed towards the back of the cabinet, and the gas flow set at lowest possible level. Alcohol must not be used as a disinfectant if a bunsen burner is utilised;
- centrifuges.