Microbiological Safety Cabinets

This guidance is provided to help you carry out biological agent (BA) risk assessments and control the risks of the work to protect humans, animals, plants and any other aspects of the environment. Microbiological safety cabinets are intended to offer protection to the user and the environment (which will include other people in the laboratory) from the **aerosol** hazards arising from the handling of infected and other hazardous biological material. Some types of cabinet may also protect the materials being handled in them from environmental contamination and cross contamination within the cabinet. Air discharged from the exhaust of the cabinet, which is either ducted to outside or recirculated into the laboratory, is filtered to remove microbial contamination.

Microbiological safety cabinets are intended to reduce the risk to the user when handling hazardous biological materials but they do not necessarily protect the user from all hazards involved. There may, for example, also be radioactive, toxic or corrosive substances present. Similarly the exhaust HEPA filters will not remove these types of contaminants from the exhaust air and particular care must be taken to ensure these are not discharged into the laboratory environment from cabinets that are not ducted to outside.

The following describes some of the main factors that should be taken into account in selecting the correct safety cabinet for its intended use, where it should be positioned within the laboratory and venting arrangements. Since microbiological safety cabinets are pieces of local exhaust ventilation (LEV) equipment for controlling exposure to hazardous substances, there is a statutory requirement under the COSHH Regulations for regular maintenance examination and testing to be carried out at least every 14 months. Further information on this is provided below.

The relevant British Standards covering microbiological safety cabinets are

- BS EN 12469 Biotechnology – Performance criteria for microbiological safety cabinets 2000 (this standard supercedes BS 5726 Microbiological Safety Cabinets 1992, Parts 1 & 3), and
- BS 5726 Microbiological safety cabinets – Information to be supplied by the purchaser to the vendor and to the installer, and siting and use of cabinets – Recommendations and guidance 2005 (this standard supercedes BS 5726 Microbiological Safety Cabinets 1992, Parts 2 & 4),

When users are purchasing microbiological safety cabinets or arranging maintenance work they should always check the cabinets and the associated installation and servicing complies with the British Standard specifications.

In relation to safety cabinets, the terms user, worker and operator are synonymous. Materials handled within a safety cabinet are commonly described as the work or, less obviously, as the product.

1. Types of Cabinet

There are three types or "Class" of microbiological safety cabinet, which differ significantly in design and mode of operation. These are referred to as Class I, Class II and Class III cabinets. All provide protection to the user (operator protection), with Class II and Class III cabinets also providing a clean working environment to protect the work from contamination (termed product protection).

The British Standard defines the three types of cabinet as follows. A diagrammatic representation of the airflow patterns in the different Classes of cabinet is provided in Appendix 1.

Class I: Safety cabinet with a front aperture through which the operator can carry out manipulations inside the cabinet and which is constructed so that the worker is protected and the escape of airborne particulate contamination generated within the cabinet is controlled by means of an inward airflow through the working front aperture and filtration of the exhaust air.

Class II: Safety cabinet with a front aperture through which the operator can carry out manipulations inside the cabinet and which is constructed so that the worker is protected, the risk of product and cross contamination is low and the escape of airborne particulate contamination generated within the cabinet is controlled by means of an appropriate filtered internal airflow and filtration of the exhaust air.

Note: A typical way of achieving this is by means of a uni-directional downward (laminar) airflow inside the cabinet and an air-curtain at the front aperture.

Class III: Safety cabinet in which the working area is totally enclosed and the operator is separated from the work by a physical barrier (i.e. gloves mechanically attached to the cabinet). Filtered air is continuously supplied to the cabinet and the exhaust air is treated to prevent release of micro-organisms.

In both Class I and II microbiological safety cabinets the inward airflow protects the user by minimising the escape of any airborne particulate contamination generated within the cabinet. In Class II microbiological safety cabinets the downflow of filtered air affords protection to the work minimising contamination during manipulations. In Class III cabinets the physical barrier protects the user from the work and the air going in to the cabinet is filtered to protect the work.

Some manufactures also produce a hybrid Class I/III cabinet but this is not described within the British Standard. The hybrid, as the name suggests, can be used as either a Class I or a Class III cabinet by the use of a removable port that attaches to the front aperture. However the construction and testing of these cabinets is such that

when used in Class III mode it is not equivalent to the specification of a standard Class III cabinet.

2. Cabinet selection for particular applications

A risk assessment should be undertaken to determine the Class of cabinet appropriate for a particular work activity. This should take into account the nature of the potential hazards in terms of not only the micro-organisms involved and their route of infection but also the techniques to be carried out and whether protection of the work (product protection) is needed.

The Class of cabinet required is not linked to the Containment Level assigned to the work. It is a commonly made mistake to think these are connected and it can lead to inappropriate selection of cabinet. The following sets out some general guidance on selecting a cabinet.

Modern Class II microbiological safety cabinets designed to meet the current British Standard give a high degree of protection to the user. This type of cabinet is suitable for most pathogens apart from those in Hazard Group 4.

Class II cabinets will probably be the cabinets of choice for most applications in the University as these provide both operator (user) and product protection (protection of the work) and so allow for flexibility in future use when the nature of the research work may change. However where an older Class II cabinet is already in situ, care should be taken to ensure its performance is adequate for purpose.

Class I cabinets should be used if procedures within the cabinet are likely to generate a significant aerosol and/or disrupt the air flow pattern within a Class II cabinet and so compromise operator (user) protection. An example would be use of a homogeniser to break up tissues. A Class I cabinet would be preferentially selected over a Class II for work with certain pathogens that infect via the airborne route (for example Neisseria meningitidis) if there is no need for protection of the work (product protection).

Since a Class III cabinet is totally enclosed this offers the highest level of protection to both the user and the work. However, in practice this level of protection tends only to be required for the most hazardous work i.e. for certain Hazard Group 3 and Hazard Group 4 pathogens in Containment Level 3 or 4 facilities.

Some general comments on selections likely at the different containment levels:

i) Containment Level 1 – a cabinet is unlikely to be required for operator (user) protection as any micro-organisms involved are unlikely to cause harm (otherwise the work would be assigned to a higher containment level). Class II cabinets can be used to provide protection of the work (product protection), for example for tissue culture work; a Class I cabinet will not provide product protection.

ii) Containment Level 2 – usually a Class II cabinet would be used to provide both operator (user) and product protection (protection of the work) unless the procedures are likely to generate a significant aerosol or compromise air flow pattern in which cases a Class I cabinet should be used. If a respiratory pathogen is being used then consideration should be given to using a Class I cabinet.

iii) Containment Level 3 – select cabinet according to nature of work, see additional guidance in Appendix 2.

Where operator (user) protection is required for work with hazardous micro-organisms the cabinet should meet the requirements of the current British Standard relating to microbiological safety cabinets (BS EN 12469). There are other types of cabinets available but these do not provide operator protection. Examples of such types of cabinet include laminar flow or clean cabinets which are used to reduce non-hazardous materials. Laminar flow / clean cabinets may use either horizontal or vertical laminar flow. These types of cabinet must not be used for protection against microorganisms or other biological hazards. Since horizontal laminar flow cabinets blow air from the back of the cabinet across the work and into the face of the user it is entirely inappropriate to use this type for work handling anything other than clean, non-hazardous materials. Fume cupboards should not be used to provide protection against biological hazards.

3. Venting Arrangements

Microbiological safety cabinets may vent in the lab or to the outside. Within the University the following approaches should be taken:

i) If it is not possible to vent to the outside, a recirculating cabinet fitted with double HEPA filters on the exhaust may be considered in Containment Level 2 facilities providing there are no other hazardous contaminants in the discharged air. Consideration must be given to a safe method of fumigating the cabinet.

ii) In Containment Level 3 facilities cabinets must exhaust via a HEPA filter to the outside. The output duct must be taken to roof level exhausts.

4. Siting, Installation and Commissioning

The siting of a microbiological safety cabinet is extremely important. Air currents and movement of people in the laboratory can adversely affect the performance (operator protection) of a cabinet. Factors to be considered include the proximity of cabinets to doors, windows, ventilation ducts and to movement routes – see Appendix 3. Positioning of cabinets within laboratories should meet the guidelines set out in the current British Standard (BS5726), some of which are reproduced here in Appendix 3. For new cabinets the supplier should always visit the site, undertake a site survey and advise on installation and meeting BS5726 prior to contracts being placed. If the proposed sitting does not meet the recommendations set out in

BS5726 and there is no suitable alternative then the University Biological Safety Adviser should be contacted for advice.

Cabinets must be properly installed and commissioned. Prior to use the cabinet must pass the performance tests specified in the British Standard. The test requirements are quite detailed, need specialist equipment and competent persons to undertake the work properly. This therefore forms part of the service offered by the supplier. Schools should note however that similar requirements apply when cabinets are moved or relocated and so a specialist contractor will need to be appointed to undertake such works.

If a School chooses to install a cabinet itself then the requirements of the British Standard must be met. A specialist contractor must be appointed to undertake the operator protection (KI Discus) test prior to use. It is strongly recommended that Schools ask the contractor to comment on the installation at that time, specifically as to whether it meets the BS requirements. Particular points to note are in relation to siting, incorporation of anti blow back valves and the need for additional fans if ducting is longer than two metres or bent in any way.

5. Routine Maintenance, Examination and Test

In order to meet the British Standard specification, cabinets undergo various testing when manufactured. Within the British Standard there are also requirements for tests on installation and regularly thereafter to demonstrate performance under conditions of use.

Most importantly, microbiological safety cabinets constitute local exhaust ventilation (LEV) systems in that they offer protection to the worker (user) from airborne hazards. As such there is a requirement for regular maintenance, examination and test under the COSHH Regulations. Therefore, all microbiological safety cabinets should be serviced on an annual basis and undergo examination and test at that time. MSC in CL3 laboratories (full or derogated) must be serviced every six months. It is a requirement of the COSHH Regulations that a record be kept for 5 years of the examinations and tests and of repairs. Health and Safety Executive Inspectors are likely to request sight of, or copies of, records during visits to the University.

The certificate should show tests results for:

i) Volumetric airflow measurements and airflow patterns

These include various measurements of face velocity (inward airflow) at the front aperture and, in Class II cabinets, the velocity of the laminar downflow.

For Class I cabinets the measured face velocity should be between 0.7 m/s and 1.0 m/s at all points. For Class II cabinets this should be not less than 0.4 m/s.

The downflow in a Class II cabinets (not applicable in a Class I) should be between 0.25 m/s and 0.5 m/s.

ii) Exhaust HEPA filter test

The HEPA filters on the exhaust are there to ensure that any contamination in the airstream is filtered prior to discharge. It is therefore important to check the integrity of the filters to ensure there are no holes and the filter is properly located so there are no leaks around the edges. The test is undertaken by introducing an aerosol challenge to the airstream upstream of the filter and testing to see if there is any penetration downstream.

Filters should have an efficiency of at least 99.995% (or penetration of <0.005%).

iii) Operator Protection Factor (KI) Test

As part of the inspection, a containment test for operator (user) protection should be undertaken. This is usually by the KI Discus method where an aerosol of potassium iodide is generated within the operating cabinet and sampling devices are placed in front of the cabinet to capture any aerosol escaping from the working area. The operator protection factor (OPF) is defined as the ratio of exposure to airborne contamination generated on the open bench to the exposure resulting from the same disposal of airborne contamination generated within the cabinet.

When tested in accordance with the British Standard all cabinets in use should have an operator protection factor of at least 1.0×10^5.

Within the University the following approaches should be taken:

i) All cabinets must have an operator protection (KI Test) test included as part of the commissioning process for new or relocated cabinets.
ii) All cabinets must be tested for operator protection (KI Test) on an annual basis, or every six months if in Containment Level 3 facilities.
iii) Operator protection tests are to be carried out in such a way as to ensure the cabinet and the laboratory are as representative as possible of normal working conditions, that is
    a. with any air conditioning units or other ventilation systems in the laboratory switched on;
    b. with other safety cabinets and fume cupboards within the laboratory switched on;
    c. with the cabinet loaded with a typical arrangement of equipment and samples;

d. with a person moving around the laboratory, particularly if any pedestrian traffic is near the cabinet; and
e. with doors (laboratory, nearby incubators and fridges etc) being opened and closed.

Copies of KI test certificates must be kept for at least 5 years (a requirement under the COSHH Regulations).

6. Training and correct use of cabinets

The effectiveness of the microbiological safety cabinet depends on

- good design;
- suitable installation;
- ongoing maintenance; and
- correct use.

Comments on the first three items in this list have been covered in earlier sections. It is important users of microbiological safety cabinet are trained in correct use not only in order to understand how the cabinet works but also because poor technique can compromise the operator protection afforded by the cabinet.

The University Health and Safety Department provide general training. However, this must be supplemented with practical training provided by local personnel dealing with the specifics of the particular equipment, location, work, etc.

Training should be provided to cover

- principles of how the different classes of cabinets work including airflow patterns;
- suitability of different cabinets for particular types of work;
- principles of airflow, operator protection factor and filter penetration tests;
- limitations of cabinet performance;
- how to work at cabinets safely;
- operation and function of all controls and indicators;
- how to decontaminate the cabinet after use (routine cleaning); and
- requirements for fumigation and, where appropriate, how to do this.

The incorrect use of microbiological safety cabinets can compromise their performance and adversely affect the level of operator protection afforded by the cabinet. Some of the most common factors that users should pay attention to are

- the user should avoid sudden and sweeping movement of their arms to minimise disturbance of the air flow patterns;
- large and bulky equipment should not be placed in the cabinet, nor should equipment be placed on air grilles as both these will disturb air flow patterns;

- centrifuges, including microfuges, should not be placed in a safety cabinet unless an operator protection factor (KI) test has been carried out with it running in situ and it shown not to compromise operator protection;
- bunsen burners should not be used in safety cabinets, particularly Class IIs, because of the concern about the effect of the heat rising from the flame on the laminar downflow of air in the cabinet. However, if they are used, they should be placed towards the back of the cabinet and a low profile type used. If the bunsen is used in conjunction with alcohol etc for flaming, then the alcohol pot should always be placed to the far side of the bunsen in order that any drips from the item being flamed do not drop in the pot and ignite it; and
- cabinets should always be installed in appropriate locations to ensure any traffic movement within the laboratory does not cause draughts to disturb the airflow patterns at the front of the cabinet (see Appendix 3) and affect performance. Users should be aware of this requirement and should ensure the 1 metre clear behind rule is observed when they are using the cabinet.

A checklist of Dos and Don'ts for users when working at a safety cabinet is provided in Appendix 4.

7. Fumigation of cabinets

Fumigation must be carried out only by a trained responsible person with adequate knowledge of the procedure and the precautions to be followed.

Fumigation with hydrogen peroxide or formaldehyde vapour are commonly used method for this type of fumigation procedures. Today the most common methods use vapourised hydrogen peroxide (VHP). Both are very effective but VHP is generally recommended to avoid the hazards related to potential exposure to formaldehyde which is a sensitizer and carcinogen.

Microbiological safety cabinets, if they have been used for hazardous micro-organisms, must be fumigated in the following circumstances:

i) before any maintenance or testing work on the cabinet where access to potentially contaminated parts is necessary (including filter and pre-filter changes);
ii) after a major spillage or a spillage where inaccessible surfaces have been contaminated;
iii) when there are any changes in the nature of the work that result in significantly different risks.

Where the cabinet has been used for hazardous micro-organisms, HEPA filters should be handled only with appropriate protective clothing (laboratory coat and heavy duty gloves) even after fumigation. After fumigation, such filters must be securely wrapped in yellow bags for disposal as hazardous waste.

The cabinet must be sealed before fumigation to prevent leakage of formaldehyde into the laboratory. It should be checked to ensure the closure panel/night door has been properly and securely located and a good seal has been achieved. Where necessary, sealing tape should be used to ensure there is no leakage. With Class III or hybrid (Class I/Class III) cabinets a blanking plate should be fitted over the inlet filter.

Manufacturers of microbiological safety cabinets should provide detailed instructions for fumigation of their particular cabinets and these should be followed. This is particularly important when the cabinet has an automatic fumigation cycle. An outline of the main principles of fumigation has been provided above and manufacturers' instructions should be consistent with these (if there are serious discrepancies please contact the University Health and Safety Department for advice).

Schools must ensure they develop a safe system of work for any fumigations with written standard operating procedures in place for cabinet fumigations. The written procedure must identify those individuals competent and authorised to carry out the fumigation process.

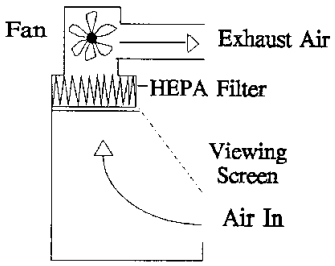
Appendix 1

MICROBIOLOGICAL SAFETY CABINETS - Summary of Types

Operations where there is a risk of airborne infection must be performed in microbiological safety cabinets or under equivalent containment. Three types of cabinet are specified by the current British Standard:

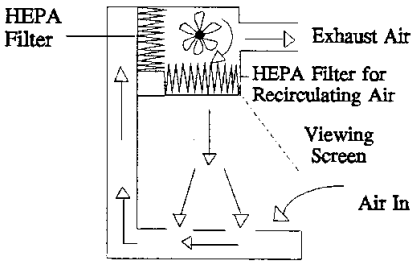
CLASS I

An open fronted cabinet through which air is drawn at a sufficient rate to minimise aerosol escape. The air is filtered by a high efficiency particulate absorbtion (HEPA) filter and is discharged to the exterior. **The worker but not the work is protected.** Suitable for work with Hazard Group 2 pathogens and most Hazard Group 3 pathogens.



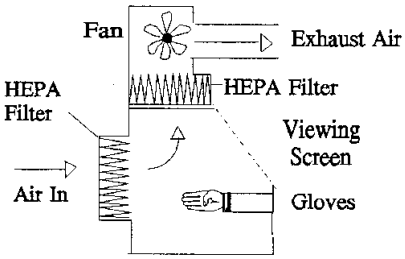
CLASS II

An open fronted cabinet where the working space is flushed with a downflow of sterile air which is HEPA filtered and recirculated. Some air is drawn in through the front of the cabinet and a corresponding amount discharged to the outside through a HEPA filter. **Both work and worker are protected.** Suitable for Hazard Group 2 pathogens and, in some circumstances, for Hazard Group 3 pathogens.



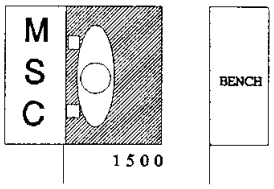
CLASS III

A totally enclosed cabinet where the operator is separated from the work by gloves attached to ports and the incoming and outgoing air is HEPA filtered. **Gives a high degree of protection to work and worker.** Suitable for Hazard Group 3 and Hazard Group 4 pathogens.

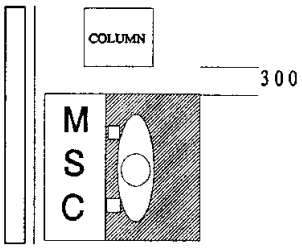


Appendix 2

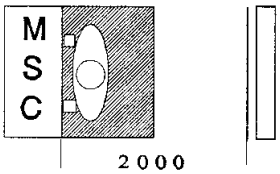
The distance between the front of the microbiological safety cabinet and the bench opposite should be at least 1500mm.



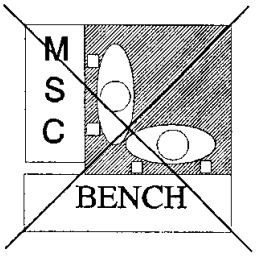
Large obstructions, for instance architectural columns should not be within 300mm of the side of the microbiological safety cabinet as with walls.



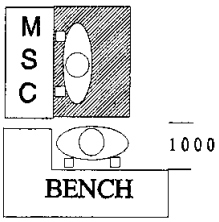
There should be no opposing wall within 2000mm of the front of the microbiological safety cabinet.



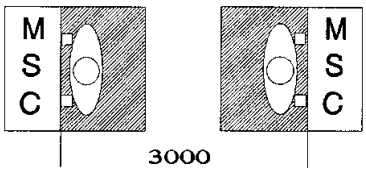
A bench at right angles to a microbiological safety cabinet may keep traffic away from the front of the cabinet, but any other person working at that bench will cause disturbances in airflow.



A projecting bench will help to keep traffic clear of the microbiological safety cabinet and work at the bench will have little effect on airflow if sited at a minimum of 1000mm form the side of the cabinet.



The distance from the front of an opposing microbiological safety cabinet or fume cupboard should be at least 3000mm.



Source: the above layouts are based on the recommendations for avoiding disturbances given in BS5726: 2005.

Appendix 3

The Dos and Don'ts When Working at a Microbiological Safety Cabinet

Dos

- Make sure the particular cabinet is suitable for your work (risk assessment)
- Organise and plan your work
- Keep the inside of the cabinet free of clutter
- Always wear a lab coat
- Check the indicators/ dials show its safe to use
- Sit comfortably at the cabinet centre
- Use good aseptic technique
- Allow to purge before switching off
- Always clean up after use

Don'ts

- Do not obstruct the air intake grilles in Class II cabinets
- Avoid use bunsen burners or centrifuges in Class II cabinets (and if used ensure included during service tests)
- Do not use until the cabinet has warmed up
- Do not work with the UV light on
- Do not let others in the lab intrude in your space – keep 1 metre clear behind
- Do not put any paperwork in the cabinet
- Do not use if in any doubt about cabinet performance
- Do not rely on the cabinet to cover up poor technique

Remember

- A cabinet only offers protection against infectious aerosol hazards
- The level of protection offered by a cabinet relies heavily on good working practices

Document version

Version number	Summary of change	Date and by whom
V1.0	**New template**	**June 2023 HE**

Alt format

If you require this document in an alternative format please contact The Health and Safety Department on health.safety@ed.ac.uk or call (0131) 651 4255