Safety Services

Guidance on the Selection, Installation, Maintenance and Operation of Microbiological Safety Cabinets for use in containment level 2 (CL2) laboratories

Introduction

1. Microbiological safety cabinets (MSCs) are designed to protect the environment and the user from airborne hazards of biological agents or material that may contain such agents. They are not designed for the containment of radioactive, toxic and corrosive substances, unless specifically modified by the manufacturer.

2. Fume cupboards are not MSCs and must not be used as such. Horizontal and vertical laminar flow cabinets are designed for the preparation of material aseptically by forcing sterile air towards the operator. They must not be confused with Class II as they provide no operator protection.

3. The effectiveness of an MSC depends on:

   - Good design
   - Suitable installation
   - Ongoing maintenance
   - Correct use

4. The University aims to maintain the levels of containment so far as is reasonably practicable for work with biological agents to protect the health and safety of all staff, any other persons that might be affected by these work activities, and the environment. This should be achieved by suitable and sufficient risk assessment, provision of appropriate facilities and training to work with biological agents.

Responsibilities

5. It is the responsibility of all persons involved in selection, purchase, installation, use and maintenance to follow this guidance and to ensure that local Standard Operating Procedures (SOPs) are produced

Guidance

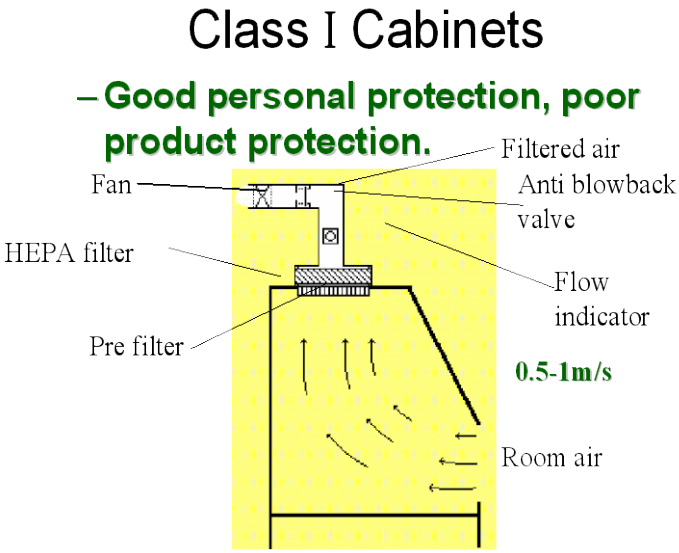
6. The University Biosafety Advisor must be notified prior to the purchase of all MSCs. All cabinets purchased must conform to British Standard BS EN 12469:2000 which covers three types of MSCs. Siting and use must comply with BS 5726:2005.

Types of MSCs

Class I

A cabinet with a front aperture through which the operator can carry out manipulations inside the cabinet.

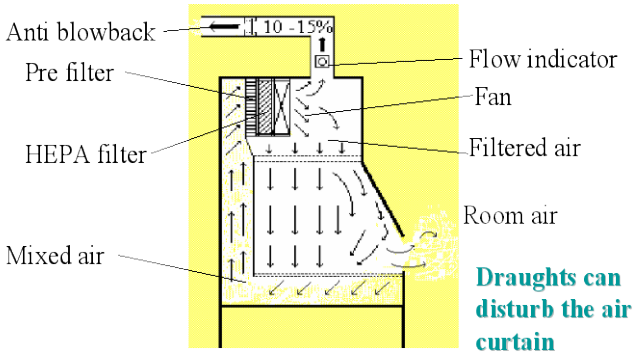
7. This type of cabinet is suitable for carrying out work on all **except** hazard group (HG) 4 pathogens, the use of which is **prohibited** at this University as there are no appropriate facilities. Most potentially airborne particles will be contained within the cabinet. The cabinet must exhaust through a high efficiency particulate absorption (HEPA) filter to the outside air or to the laboratory air extract system if used for handling samples that may contain HG 2 or 3 pathogens. This type of cabinet does not provide any protection of the work.



Class II

8. These are deigned to control airborne contamination of the work while at the same time controlling exposure of the operator. An inward airflow passed through a HEPA filter before circulation controls the escape of aerosols and the filtered down flow air passes over the work. Part of this airflow is exhausted to atmosphere through a HEPA filter and make-up air is drawn in through the front aperture. Modern Class II cabinets can provide operator protection of the same order as Class I cabinets but are more susceptible to disturbances of airflows. Class II cabinets should only be used where protection of the work is essential.

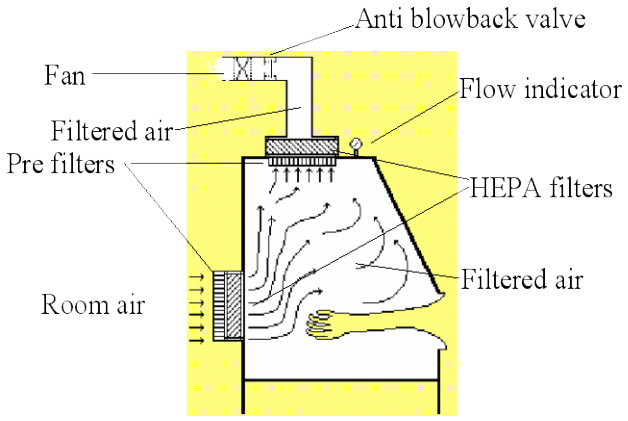
Class II cabinets



Class III

9. These are totally enclosed cabinets. The escape of airborne particles is prevented by a HEPA-filtered exhaust. A HEPA inlet filter supplies sterile air to the interior. Because of the airflow pattern there is a greater risk of contamination of the work following a spill than in a Class I cabinet. Class III cabinets are primarily designed for total containment of HG 4 pathogens although their use is advised for work with high titre cultures of some HG 3 pathogens.

Class III Cabinets



Class I / III Hybrids

10. These are Class I cabinets that are said to be modifiable to Class III. Prospective purchasers must be aware that these must be retested to demonstrate satisfactory operator protection in the Class III mode.

Installation of UV lights for biocidal purposes.

11. This is not recommended as they introduce a further hazard and risk of UV burns. They are ineffective unless kept meticulously clean and the effective lifespan of the lamps is short The radiation output should be at least 40microwatts/ cm2 at 254 nm to be effective .This is difficult to measure and the output can fall below this level yet still be capable of causing UV burns whilst being a totally ineffective biocide. A far more effective method to reduce or eliminate contamination is to use well-practiced microbiological procedures, good aseptic techniques, operational procedures as outlined in this document, and effective decontamination procedures before and after the use of MSCs.

Siting, Installation and Routine Maintenance

12. Installation and commissioning of cabinets is normally carried out by the supplier or an experienced agent. However, the siting of the MSC should be discussed with the customer/end-user(s) to ensure the position is consistent with maintaining safe operation. Space for access should also be allowed for connection of temporary ducting which may be required for recirculating, cabinets for discharge and clearance of fumigant. Factors that may affect the operation and should be considered include proximity to:

    - Doors
    - Windows
    - Walls
    - Ventilation systems
    - Other MSCs
    - Fume cupboards
    - Fridges/freezers
    - Centrifuges
    - Access routes and general movement of personnel within the laboratory

13. Once installed, commissioning tests should be conducted to verify the performance of the cabinet *in situ.* The importance of installation testing cannot be overemphasised. It demonstrates the cabinet's performance and level of protection achieved in practice. Testing should be conducted under conditions consistent with the normal operating procedures of the laboratory. It is also important to perform additional inspection and testing if modifications are made to the design or layout of the laboratory.

14. Installation and further routine maintenance testing of Class I and II MSCs should include the operator protection factor (OPF) for which the minimum standard is 1x 10^5 as defined in BS EN 12469:2000. This figure expresses the ratio of the number of airborne particles that would be generated in a procedure conducted on the open bench to the number resulting from the same procedure within a cabinet. This means that for every 100,000 particles used in a test as a challenge to the inward flow of air at the working surface, not more than one should escape. BS EN 12469:2000 differs from the previous British Standard in that the need to carry out an OPF is now optional. However, The Control of Substances Hazardous to Health Regulations 2002 (COSHH) in referring to "local exhaust ventilation"

requires a thorough examination and testing of equipment including safety cabinets on installation and as part of routine ongoing maintenance, at intervals not exceeding 14 months.

15. It is necessary to assess the containment under actual conditions of use if working with HG3 organisms. This will involve setting up a typical arrangement of equipment inside and around the cabinet, which should be switched on if they interfere with airflow currents. Other equipment such as adjacent MSCs, fume cupboards and fans which would normally function while the cabinet is in use should also be working during the test. Traffic around the laboratory should also be reproduced such as people entering and leaving the room and passing the cabinet.

Recirculation of Exhaust from MSCs

16. It is good occupational hygiene practice to discharge exhaust air from MSCs to atmosphere. However, because of building design constraints this may not be reasonably practicable. This must be discussed with the University Biological Safety Advisor (UBSA) and a member of the Estates Department prior to purchase and installation. If discharge to atmosphere is not reasonably practicable, recirculation of discharged air into the laboratory may be considered. If using this for HG3 biological agents it will be necessary to discharge the air through two HEPA filters in a way that the two filters can be tested independently.

Fumigation of Cabinets and Filters

17. If recirculation is considered issues regarding cabinet fumigation and clearance of fumigant must be considered as part of the risk assessment of the work. Before any service procedures are carried out other than minor work on external controls, fumigation must be carried out. This will be necessary when filters are changed and access to internal ducting and fittings is required or when the nature of the work or spillage requires it. The procedure adopted should ensure that both sides of the filter are effectively fumigated.

18. Although there are alternative methods such as vapour phase hydrogen peroxide, the most common method used for fumigation is the generation of formaldehyde vapour.

19. Formaldehyde vapour may be generated by different methods:

- from a mixture of formalin and water in a thermostatically controlled heating unit, which may be stand-alone or an accessory specific to the cabinet.
- depolymerisation of paraformaldehyde

Procedure

- Fumigation should be done by a responsible person with adequate knowledge of the procedure and precautions to be observed. A warning notice that the cabinet is being fumigated should be displayed.
- Decontamination of the whole cabinet, including filters, fan unit, and work surfaces is most frequently achieved by fumigation with formaldehyde vapour.

However, this may not be appropriate in certain cases, where local rules should be observed.

Warning: formaldehyde vapour is explosive at 7.75% (v/v) in dry air. Its ignition point is 430°C.

- For formaldehyde to act at maximum effect it must be able to penetrate. If pre-cleaning can be carried out without jeopardising safety this will aid fumigation. Water generated in the process of dispersing formaldehyde produces the, essential optimum level of relative humidity, greater than 35% and less than 80%.
- The volume of formaldehyde and water used should be determined from the manufacturer's recommendations. Too much formaldehyde will result in the deposition of sticky deposits of paraformaldehyde. As a guide, the volume of the cabinet should be calculated and sufficient formaldehyde should be used to provide an air concentration of at least 50 $mg^{-1}m^{-3}$. This can be achieved by evaporating 150 cm^3 of formalin (40% formaldehyde) per m^3 of cabinet.
- The fumigant must be generated with the night door closed and if necessary, depending on manufacturer's instructions additionally sealed with tape and if present the non-return valve left closed
- To ensure adequate fumigation after generating the formaldehyde vapour, the following procedure should be adopted unless the manufacturer gives specific recommendations:
    - After half the formalin has evaporated, turn the cabinet fans on for 10-15 secs to allow formaldehyde to reach all areas of the cabinet.
    - After evaporation is complete, switch the fans on again for 10-15 secs.
- Even after this procedure, the HEPA filters should only be considered "safe to handle using appropriate protective clothing" rather than sterile, and should be autoclaved or incinerated after removal.

20. Following a period of at least six hours and preferably overnight, the seals on the cabinet should be removed and the fans run for a period sufficient to remove the formaldehyde vapour before using the cabinet or doing maintenance. The fumigant must be exhausted to atmosphere from a ducted cabinet. A carbon filter extract system should be used for recirculating cabinets or temporary ducting attached and the fumigant exhausted to atmosphere via a fume cupboard.

21. Alternatively, formaldehyde vapour can be neutralised with either liquid ammonia in an open vessel, or with carefully controlled ammonia vapour generation following the fumigation step.

- **Formaldehyde has a workplace exposure limit (WEL), (long term 8 hour time average (TWA) and short term of 2ppm. A formaldehyde meter should be used to ensure the residual discharge concentration does not exceed this.**
- Respiratory protective equipment suitable for formaldehyde should be available in case of emergency. A face-fit test must be carried out on the people who may use this.

Routine Maintenance

22. This will normally be carried out by a qualified engineer but the user(s) must be aware of the frequency and type of test required as displayed in Table 1. Face velocities should be carried out by the user(s) and must be recorded.

Table 1. Frequency and type of testing

TEST	Microbiological Safety Cabinet Type		
	I	II	III
Alarms/indicators (Inspection)	Daily		
Face velocity inflow	Monthly		N/A
Inflow/down flow	N/A	Annually for CL2 6 monthly for CL3	6-monthly for CL3 and CL4
OPFT	12 monthly		N/A
In-use OPFT	As required by assessment(1)		N/A

(1)To assess the containment under actual conditions of use, it may be necessary to carry out “in-use” operator protection factor test. This may be required for example, when working with HG3 biological agents, particularly when there may be other sources of ventilation and movement of staff around the laboratory. This can result in alteration of air movements in the room which may reduce the containment ability of the MSC. I – use tests may also be required if laboratory set up changes significantly from the initial OPF, for example. if the layout of the laboratory has been changed or if new equipment has been installed

Use of MSCs

All users of MSCs must:

- Check that the cabinet work surface is easy to clean and disinfect
- Check the fan is switched on and the airflow indicator is in the safe position before starting work
- Check the airflow indicator regularly during use
- Keep any opening viewing panel closed during use
- Keep to a minimum apparatus and material in the cabinet during use
- Position apparatus and material so as not to disrupt airflows. In Class I cabinets, larger items should be placed towards the rear and in Class II cabinets, all materials should remain on the work surface and not obscure air vents.
- Not place large centrifuges in Class I or II cabinets
- Conduct work well within the inside of the cabinet away form the opening and view the work through the screen
- Run the cabinet for at least five minutes after completion of the work in the cabinet
- Before and after each work session, wipe the work surfaces with and appropriate disinfectant. Phenolics, quaternary ammonium compounds, and aldehydes can be used for disinfecting these surfaces. Users of chlorine

compounds should be aware of their corrosive nature. The use of alcohols should be undertaken with caution because of the risk of fire

23. Test the airflow with an anemometer at least once a month for HG2 and 3 organisms

24. Bunsen burners and other naked flames should never be used inside MSCs as they disturb airflows, damage filters and create a fire hazard.

Training Requirements

25. MSCs, users must be fully instructed in the following:

- a) classification of cabinets;
- b) appropriate and inappropriate use of cabinets;
- c) mode of operation and function of all controls and indicators;
- d) limitations of performance;
- e) how to work at cabinets safely;
- f) how to decontaminate cabinets after use;
- g) principles of airflow and operator protection tests.

26. This may be achieved by:

- Medical Research Council (MRC) training CD
- Training by supplier/manufacturer.
- Local training for specific MSCs

27. A record of that training must be kept by the manager (Principal Investigator) or supervisor.

28. Training may need to be refreshed in the light of observations of poor practices, incidents or accidents, routine checks and monitoring exercises.

Further Reading

The management, design and operation of microbiological containment laboratories. HSE Books 2001. ISBN 0 7176 2034 4

Safe working and the prevention of infection in clinical laboratories and similar facilities. HSE Books 2003. ISBN 0 7176 2513 3

BS EN 12469 Biotechnology-Performance criteria for microbiological safety cabinets

BS 5726:2005 Microbiological safety cabinets-Information to be supplied to the vendor and to the installer, and siting and use of cabinets-Recommendations and guidance

The approved list of biological agents. Advisory Committee on Dangerous Pathogens.

Control of substances hazardous to health (Fifth edition). The Control of Substances Hazardous to Health Regulations 2002(as amended). Approved Code of Practice and guidance. HSE Books 2005. ISBN0 7176 2981 3

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