- ***Sterilisation*:** A process that renders an object effectively free of viable microorganisms, including those that that may survive disinfection treatments (eg bacterial and fungal spores; Prions). A validated autoclave cycle represents the most effective way to achieve sterilisation of waste or equipment and must reduce the number of viable organisms by six logs.
- ***Activity*:** The effectiveness of a particular disinfectant, which varies with the target microorganism.
- ***Concentration*:** The 'working-concentration' or 'working dilution' refers to the correct dilution for effective disinfection in any given circumstance. This may be dependent on the intended use, for example, the dilution factor needed for tackling spillages, eg spillages may be different than for those required in discard jars. The effective concentration may also depend upon the age of the solution as some diluted disinfectants lose effectiveness over time.
- ***Contact*:** A sufficient period of time where the GMM is exposed to the disinfectant will be necessary to effect the required level of killing. This contact must also be effectively maintained between the disinfectants and the contaminated article, for example air-bubbles should be removed from submerged articles.
- ***Validation*:** Establishing documented evidence that a disinfection process will consistently inactivate target organisms under defined conditions of use.

Physical inactivation methods

4. Physical methods of inactivation are arguably the most reliable way to achieve a high kill rate. For large-scale operations, inactivation will usually involve heat inactivation of cultures. For other operations, the use of an incinerator (for example to dispose of infected animal carcasses) or rendering procedure might be appropriate or sufficient. However, the use of a validated autoclave cycle remains the most effective means of inactivating GMMs.

5. A range of autoclave cycle parameters are suitable for inactivating microorganisms and a typical cycle would be 121 °C, maintained for 15 minutes. Holding-time may need to be increased for work with particular organisms (for example spore-forming bacteria), large amounts of contaminated material or where steam penetration is inefficient. Users should consult the technical specifications for their particular autoclaves. A higher heat setting is recommended for work with TSEs (134-138 °C). Further information on the handling of TSEs can be found in ACDP/SEAC guidance document *Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection*.

6. The Regulations require that an autoclave be available for all laboratory-scale activities with GMMs and those that involve animals. The use of an autoclave should always be an

option for these activities, therefore, and it is recommended that one be used for waste inactivation, either by itself or in combination with chemical methods. Care should be taken, however, to ensure that chemical disinfectants do not damage the autoclave. The autoclave should be serviced regularly and its performance tested frequently in accordance with the manufacturer's recommendations and national standards (BS 2646-3: 1993).

7. Any inactivation method used should be validated or verified under working conditions.

Chemical inactivation methods

8. Chemical inactivation methods are commonly used in laboratory-scale operations to avoid the need to autoclave bulk waste (eg spent media and liquid cultures). Chemical disinfection is an inherently less reliable method of inactivation as there are many factors that can come to bear on the effectiveness of the chosen disinfectant. For example, the presence of organic matter can impede the performance of certain disinfectants. Similarly, the disinfection regime may be compromised if cultures are buffered or proteinaceous. Furthermore, the requirement to validate or verify the effectiveness of the procedure stands, and it can be technically difficult or impractical to adequately remove the disinfectant and screen for viable GMMs. For these reasons it is recommended that, for high-risk activities, chemical methods are not relied upon as a sole means of inactivation but are used in combination with physical methods. However, the sole use of chemicals does represent an acceptable means of inactivation for lower-risk activities.

9. When selecting a disinfectant, its toxicity to humans and the environment should be considered. Appropriate safety precautions should be adopted. Different disinfectants must not be mixed together or used in combination unless the possibility of hazardous reactions or the formation of toxic products has been properly assessed. Appropriate procedures should be used to ensure suitable disinfectants at the correct dilution are available at the point of need. Personnel should be trained in the correct use of disinfectants and in the emergency spillage protocols associated with them.

10. There are advantages in limiting the number of different disinfectants available in the workplace to the minimum necessary, in order to avoid confusion and to reduce costs. Once a disinfectant has been selected, in-use tests should be carried out to monitor both the performance of an individual chemical but also the way in which it is used (for example tests to detect incorrect dilution, old solutions and mixtures of incompatible reagents).

11. Disinfectants and chemical inactivation methods in common use are discussed below, including considerations relating to the characteristics, advantages and disadvantages of each.

12. ***Hypochlorites.*** Hypochlorites (eg bleach) have a wide spectrum of antimicrobial activity and are rapid in action. However, hypochlorites are corrosive and may damage equipment and stainless-steel surfaces. Furthermore, they are inactivated by organic matter and decompose in solution. Sodium hypochlorite can be used to inactivate Prions (20,000 ppm for 1 hour). Further information on the handling of TSEs can be found in ACDP/SEAC guidance document *Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection.*

13. ***Chlorine releasing agents.*** Chlorine releasing agents (eg sodium dichloroisocyanurate - NaDCC) are broad-spectrum and rapid acting biocides. Dry forms of NaDCC are stable but can be dissolved in water to generate chlorine, a process that is accelerated in an acidic environment. Chlorine releasing agents are corrosive and easily inactivated by organic matter but are widely recommended in conditions where corrosion or bleaching are not an issue.

14. ***Phenolics.*** Phenolics are non-corrosive and are not readily affected by organic matter. They have a wide range of antimicrobial activity (although they may be ineffective against non-enveloped viruses) but this may be impaired if diluted in hard water. Phenolics should not be stored diluted.

15. ***Alcohols.*** Alcohols (eg 70% ethanol; 60% isopropanol; Industrial methylated spirits) rapidly kill eukaryotic pathogens, bacteria and some viruses due to their dehydrating action. They are particularly useful for low-level contaminations and surface sterilisation. Alcohols are relatively volatile, however, and do not provide a sustained antimicrobial action. Furthermore, they are flammable, requiring appropriate precautions in storage and use.

16. ***Aldehydes.*** Formaldehyde vapour or aqueous solution (formalin) is highly toxic and unsuitable for general purposes. It is used for fumigating microbiological safety cabinets and sealed rooms (eg high-containment laboratories). During fumigation, containers of other disinfectants should be sealed if the disinfectant is incompatible with the fumigant. Glutaraldehyde solutions are also toxic and have a wide range of antimicrobial activity, including against bacterial spores. They are non-corrosive but are unstable once activated and do not readily penetrate organic matter (see Section 3.6). Furthermore, they are potent sensitisers and should not be used for routine laboratory work.

17. ***Surface-active agents*.** Quaternary ammonium compounds (QACs) are often sold in combination with other disinfectants and form the basis of the majority of cationic detergents. Only cationic and amphoteric detergents have any antifungicidal or antibacterial activity, and are regarded as being more bacteriostatic than they are bactericidal. They are less active against Gram-negative bacteria and vary in their antiviral performance. Furthermore, they appear to be inactive against mycobacteria and spore-forming bacteria. Soaps, anionic detergents and organic matter inactivate them, although they are relatively non-toxic and non-irritant.

18. ***Iodophors.*** Iodophors provide a sustained-release of antimicrobially active iodine from an inactive reservoir. They essentially consist of iodine in combination with a solubilising agent or carrier. They are bactericidal, fungicidal and virucidal, but show limited activity against spores. Iodophors are inactivated by organic matter (dependent upon preparation and concentration) and may corrode metals.

19. ***Peroxygen compounds.*** The antimicrobial properties of Peroxygen compounds are due to their oxidising activity, which damages cellular proteins, lipids and nucleic acids functions. They are effective against a wide range of microorganisms although this is impaired in the presence of organic matter. They can be used for routine laboratory disinfection but, before using on equipment, approval should be obtained from the manufacturer since corrosion of some metals and plastics may be a problem.

20. ***Hydrogen peroxide.*** Hydrogen peroxide owes its antimicrobial properties to its potent oxidising activity, which damages cellular proteins, lipids and nucleic acids. It is unstable when diluted and in the presence of microorganisms, this gives rise to highly oxidising hydroxyl radicals (such as those used by macrophages to destroy infectious organisms). Vapour-phase hydrogen peroxide has been developed as a fumigant (see Section 3.6) and diluted forms have also been used as a general disinfectant. The by-products of hydrogen peroxide decomposition are harmless oxygen and water, although concentrated solutions are irritating to the skin, eyes and mucous membranes and therefore care must be taken.

21. ***Sodium hydroxide solutions.*** Sodium hydroxide solutions (1%) may be used under certain circumstances as a laboratory disinfectant. Care must be taken with their use, due to the caustic nature of the chemical. Prions can be inactivated using 1M NaOH, which is less corrosive than the concentrated sodium hypochlorite alternative. This is particularly pertinent when steel or other metal surfaces require disinfection. Further information on the handling of TSEs can be found in ACDP/SEAC guidance document *Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection.*

22. ***Alkaline hydrolysis.*** Alkaline hydrolysis uses high pH (pH14, usually from concentrated sodium or potassium hydroxide) in combination with high pressure and heat to reduce organic matter to liquid waste. The liquid waste, while still very alkaline (pH10.5), contains nothing more hazardous than short peptides, amino acids, sugars, salts and fatty acid residues. Due to the need for pressure and heat, alkaline hydrolysis requires proprietary equipment, and these systems have been developed for healthcare and tissue digestion applications and vary in scale from small laboratory units to those used for the disposal of large animal carcasses. The process can be validated and is effective against all infectious agents, including prions. The use of this system is particularly attractive for animal facilities as carcasses are reduced to harmless 'bone-shadow' powder and liquid waste. However, there are significant hazards associated with handling and storing large amounts of concentrated alkali.

3.6 Fumigation

Overview

1. On occasions it will be necessary to decontaminate laboratories, animal containment facilities and safety cabinets using a fumigation technique. This might be a routine procedure or performed if maintenance work is to be carried out. Alternatively, fumigation might be an emergency procedure if, for example, there has been a spillage of infectious material or an escape of an airborne pathogen. The following guidance offers advice for the safe and effective decontamination of microbiological safety cabinets (MSCs) and rooms using fumigation techniques. Information relating to commonly used fumigant chemicals and technologies is also included.

2. Fumigation should always be a planned exercise with suitable controls in place and with appropriate information and warnings provided for those who need to know. Only designated personnel, who are fully trained, should carry out fumigation operations. A standard procedure for routine decontamination and emergency fumigation plans should be agreed. Only a fumigant and method known to be effective in the circumstances of use should be used.

Fumigation of rooms

3. Where a room in a laboratory or animal containment unit is to be fumigated, the area should be checked to ensure that it is securely sealed so as not to allow the escape of fumigant to other parts of the building. The legal requirement that the room be sealable for fumigation can be interpreted as meaning that only the door should need sealing. It should not be necessary to enter the room in order to seal up gaps in the integrity of the laboratory prior to fumigation. Suspended ceilings can present a special difficulty as there may be a void above connecting with other rooms nearby. Careful thought also needs to be given to fumigation of any ductwork. Users should consult the HSE guidance *Sealability of Microbiological Containment Level 3 and 4 Facilities* (available at: www.hse.gov.uk/biosafety/gmo/guidance/sealability.pdf) for further information.

4. Fumigant concentrations can become stratified and may not penetrate completely to all parts of the room. It may be necessary to stir the air in the room to ensure even distribution of the fumigant. This can be achieved using desktop fans or, for newly-built facilities, integral fans can be fitted. The normal air-handling or air-conditioning systems should not be used as these could positively-pressurise the room and breach the door/vent seals.

5. Exposure to the residual effect of the fumigant after generation should take place for a time period appropriate to the chemical or system being used. Fumigant may be extracted from the area by the air handling system but only when that is a total loss system with no possibility of fumigant being conducted to other areas. More commonly, use is made of a microbiological safety cabinet or a fume cupboard as a means of extraction if one is situated within the area under treatment and if it exhausts to atmosphere. In all cases, an external switch should operate the ventilation system or equipment extracting the air so as to avoid entering the room.

6. Where possible, there should be a thorough check of the level of residual fumigant before anyone enters following extraction. This may be done most conveniently by, for example, sampling the air through a small port fitted in the door for this purpose. For example, meters and other assay devices are available to indicate the concentration of formaldehyde vapour remaining in the air.

Emergency room fumigation

7. Fumigation is an important component of the procedures used to treat spillages or escapes of GMMs in Containment Level 3 or 4 facilities. In the event of a major spillage, the safest and most logical procedure would be to:

    1. Evacuate the laboratory.
    2. Fumigate the laboratory.
    3. Disinfect the bulk spillage and remove any debris.
    4. Fumigate again, if deemed to be necessary.

8. Personnel should not enter an area when a major spillage of microorganisms has taken place, as they may remain suspended in the air for some time and there may be a great risk of exposure. The laboratory air handling system should be allowed to clear airborne particles before re-entering the laboratory. Users may wish to consult the ACDP publication *The management, design and operation of microbiological containment laboratories* Guidance HSE Books 2001 ISBN 978 0 7176 2034 0 for further information on how to calculate the appropriate time interval. Upon re-entry, the use of respiratory protective equipment may be necessary for the process of disinfecting the bulk spillage.

9. Emergency fumigation should be viewed as a way of inactivating small deposits of an infectious agent that may have been generated as a consequence of the splashes and aerosols that occur during a spillage. Fumigant will not penetrate throughout the entire volume of a large spillage and fumigation should not be seen as a way of inactivating the

bulk of the main spill. Thus, the main spill should be treated with an appropriate disinfectant and it may be necessary to fumigate again if the disinfection could have generated further infectious aerosols.

10. Personnel should not enter an area after the fumigant has been generated unless suitable breathing apparatus is worn. Only those trained in the use of breathing apparatus should use it. Respirators are not appropriate for use in the concentrations of formaldehyde vapour achieved when carrying out these procedures. Full breathing apparatus with an independent air source must be worn.

Fumigation of microbiological safety cabinets

11. Microbiological safety cabinets should be fumigated:

- Following a small spillage of infectious material within the cabinet.
- Before filters are changed.
- Before any maintenance work that requires access to the interior of the cabinet.

12. In the event of a large spillage, however, consideration should be given to the fumigation of the whole room (see above).

13. Fumigant should only be generated within a MSC with the sash/night door securely sealed and the non-return valve closed. Passive migration of the fumigant through the filter can occur but an alternative is to leave the valve open and the fan running for 10 to 15 seconds to ensure penetration of the filter medium. The valve should then be closed and the fan switched off while the remainder of the fumigant is left to disperse within the cabinet. After a time period appropriate for the chemical or system in use to be effective, the fumigant should be exhausted to atmosphere by switching on the fan. Air from the room must be allowed to enter the cabinet (for example through a large bunghole in the night door).

14. Before venting a hazardous fumigant to atmosphere (for example formaldehyde), it is essential to ensure that no personnel (for example engineers or contractors) are in the vicinity of the exhaust outlet and that the exhaust air does not enter nearby windows or ventilation air intakes.

15. If filters are to be changed after fumigation, the discarded filter unit should be bagged and disposed of. There are special difficulties if the cabinet is used with the agents causing transmissible spongiform encephalopathies, as they are resistant to fumigants.

Validation of fumigation

16. A test of the effectiveness of fumigation may be carried out by placing spore strips/discs carrying *Bacillus subtilis* var. *globigii* (filter paper inoculated with a suspension of the organism) at various points in the room to test penetration of the fumigant. Similarly, a standardised spore suspension may be painted onto small marked areas on surfaces that are later swabbed to recover any surviving organisms. As fumigant concentrations can often become stratified or not penetrate completely to all parts of the room, the positioning of spore strips/discs should be carefully considered and placed at a point where fumigant concentration is likely to be lowest or where contact time will be the least (for example, the floor, or beneath a piece of equipment.

Fumigants and fumigation methods

17. There are several different fumigant chemicals and fumigation technologies available which vary in their effectiveness and relative safety from a human health or environmental perspective. These are listed below:

- Formaldehyde.
- Hydrogen peroxide.
- Ethylene oxide.
- Propylene oxide.
- Peracetic acid.
- Chlorine dioxide.
- Ozone (O_3).

18. To date, the most commonly used fumigant in UK laboratories has been formaldehyde vapour. While it is a highly effective biocidal agent and is relatively easy to generate, it is also a Schedule 1 chemical under the COSHH Regulations and is both a sensitiser and a carcinogen. The harmful effects of formaldehyde have provided strong motivation for the development of alternative, less toxic gaseous fumigants. The requirement by COSHH to prevent or control exposure to hazardous chemicals as far as reasonable practicable confers a responsibility to replace formaldehyde with a less toxic alternative. Most technologies have struggled to match formaldehyde vapour for efficacy, economy and ease of use, although hydrogen peroxide now represents a realistic alternative and should be considered. The properties and limitations of formaldehyde vapour and hydrogen peroxide are discussed below.

19. ***Formaldehyde.*** Formaldehyde (CH_2O) acts as an alkylating agent, reacting with proteins and nucleic acids to inactivate a broad spectrum of microorganisms, including viruses and bacterial spores. There is more than one way of generating formaldehyde vapour, however, effective fumigation is typically achieved by heat-initiated vaporisation of a 40% solution of formaldehyde vapour in water (Formalin), usually performed at 70-80^{o}C (note that formaldehyde vapour is also explosive at 7.75% in dry air with an ignition point of 430 °C). Formaldehyde vapour penetrates slowly into air spaces and porous surfaces. Therefore, a prolonged contact time of at least 12 hours is required.

20. A number of factors affect the efficiency of fumigation:

- The ratio of formalin to water used (and thereby the relative humidity created).
- The volume of the space to be fumigated (Typically, 100ml of formalin diluted in 900ml of water is vaporised for every 28.3m^3 of space).
- The surface area exposed in that space and the presence of absorbent materials such as cardboard boxes.
- Temperature - below 18 °C formaldehyde fumigation is less effective and below 9 °C formaldehyde sublimes and is less easy to vaporise.

21. Pre-cleaning will increase efficiency (provided it can be done without jeopardising safety) and it must be able to dissolve at adequate concentrations in the film of moisture in the immediate vicinity of the organisms to be inactivated. Water vapour generated in the process of dispersing formaldehyde provides is essential to achieve this (ie relative humidity between 35% and 80% is optimum). Too much formaldehyde will result in sticky deposits of paraformaldehyde.

22. It should be noted that any hydrochloric acid and chlorinated disinfectants should be removed from the room before fumigating with formaldehyde. This is to prevent the possibility of forming potentially carcinogenic bis (chlormethyl) ether. In high containment facilities, care must be taken where double-ended dunk tanks are present.

23. Formaldehyde fumigant is potentially harmful to health and will require strict controls to ensure the workplace exposure limit is not exceeded (see Table 3.6.1). The fumigant must therefore be fully evacuated from the room using the ventilation system before anyone re-enters the room unless suitable breathing apparatus or respiratory protective equipment (RPE) is worn.

24. Independent formaldehyde removal units are available and could be used to remove fumigant in large volume rooms or from a microbiological safety cabinet. Typically, such

equipment draws contaminated air over reusable carbon filter where chemical residues become bound are therefore immobilised for safe removal.

25. Formaldehyde vapour can be neutralised following the fumigation with either liquid ammonia in an open vessel or with carefully controlled ammonia vapour generation. The use of liquid ammonia is likely to be most appropriate for low-volume cabinet fumigation, where 300ml of ammonia is used in an open vessel for every 28.3m^3 of treated space. Ammonia vapour, however, can be applicable to both whole room and cabinet fumigation. Chemical neutralisation procedures such as this might be particularly applicable to the fumigation of recirculating class II MSCs that cannot be vented externally to the atmosphere. This means that the exhaust of a fumigated cabinet can be recirculated back into the room. This will usually require charcoal filtration of the exhaust air to scrub any excess fumigant or neutraliser. Complete fumigation/neutralisation systems are available commercially.

26. ***Hydrogen peroxide*.** The broad antimicrobial activity of hydrogen peroxide is attributable to its powerful oxidising ability resulting in damage to proteins, lipids and nucleic acids. Several sterilisation systems that employ Vapour-phase hydrogen peroxide vapour (VPHP) and hydrogen peroxide plasma (HPP) technology is now commercially available and can be applied to whole room or cabinet sterilisation applications.

27. The use of VHP and HPP technology offers several advantages over traditional formaldehyde fumigation approaches:

- Hydrogen peroxide vapour is non-carcinogenic and environmentally friendly.
- A much lower contact time is required (around 1-3 hours).
- It is less sensitive to conditions (vapour phase temperature range is 4-80 °C).
- Various hydrogen peroxide concentrations can be effective (0.3-12 mg/l).

28. Hydrogen peroxide decomposes when diluted into harmless by-products, oxygen and water. In the presence of metal transition ions (for example iron, copper, chromium, cobalt or manganese salts), highly toxic hydroxyl radical is generated, which is believed to be the strongest known oxidising agent. The microorganisms targeted for destruction provide these ions and therefore hydrogen peroxide vapour is only toxic at the specific point of action. HPP systems use various methods to generate plasma (for example direct current, radio- and microwaves) from hydrogen peroxide gas, this is thought to result in the generation of biocidal hydroxyl radicals and UV light without the need of transition ions.

29. Since hydrogen peroxide decomposes into harmless by-products, these systems should be considered to be a much safer alternative to the use of formaldehyde vapour.

However, like formaldehyde, hydrogen peroxide itself is also a hazardous chemical (see Table 3.6.1). Exposure to the vapour must be controlled and the laboratory must be sealed during fumigation.

30. Concentrated solutions of hydrogen peroxide are irritating to the skin, eyes and mucous membranes. Furthermore, VPHP can cause inflammation of the respiratory tract. Exposure to these substances is usually minimised through the use of cassette-loading systems when using commercially available equipment. However, gloves must be worn and rooms/cabinets will still require sealing during the application.

31. Currently, effective hydrogen peroxide fumigation systems are only available commercially. However, given the advantages over formaldehyde, their use may represent a safer option.

	WORKPLACE EXPOSURE LIMIT	
	Long-term exposure limit (8-hour reference period)	Short-term exposure limit (15-minute reference period)
FORMALDEHYDE VAPOUR	2 ppm 2.5 mg.m^{-3}	2 ppm 2.5 mg.m^{-3}
HYDROGEN PEROXIDE VAPOUR	1 ppm 1.4 mg.m^{-3}	2 ppm 2.8 mg.m^{-3}

Table 3.6.1: Workplace exposure limits for formaldehyde and hydrogen peroxide vapour. Long-term exposure is an 8 hour time-weighted average exposure limit.

3.7 Microbiological safety cabinets

Overview

1. The following guidance provides a brief summary of the essentials of the design and function of Microbiological Safety Cabinets (MSCs) and offers practical recommendations for their safe use and operation. For full descriptions of the different types of MSC, reference should be made to British Standards BS EN 12469: 2000 and BS 5726: 2005. These define specifications for design, construction and minimum performance criteria. They also specify the test procedures that are required with respect to protecting the worker and cover aspects of installation and environmental factors that affect performance. For older equipment, reference should be made to BS 5726: 1992.

2. An MSC is a ventilated enclosure intended to offer protection to the user and the environment from infected and hazardous biological materials. They use a combination of airflow and filtration to contain airborne droplets and particles generated during handling, thus preventing their escape and exposure of workers and the local environment. All exhaust air discharged to atmosphere from an MSC is HEPA filtered. The use of an MSC is a regulatory requirement for laboratory activities involving genetically modified microorganisms (GMMs) at Containment Levels 3 and 4 and they are often required at lower containment levels, if not to protect the worker and environment, then to maintain sterility of the materials themselves. It should be remembered, however, that MSCs can only supplement containment afforded by the room/building and procedures in place that minimise exposure. It is not only vital that MSCs are correctly installed, but that attention is paid to the arrangement of the room and facilities present to ensure that all aspects of the MSC operation and maintenance are accounted for. Furthermore an MSC's performance can be affected by incorrect operation and users should be appropriately trained.

Choosing the correct equipment

3. MSCs vary in design, but they fall into three different classifications, each offering different levels of protection to workers and materials.

4. ***Class I*** cabinets have an aperture at the front through which the operator can carry out manipulations on potentially hazardous materials. They provide operator protection by maintaining an inward flow of air past the operator and over the work surface within. Exhaust air is HEPA filtered, although incoming air is unfiltered and, therefore, this type of cabinet is not designed to offer protection to material being handled (see Figure 3.7.1).

Hence, there is a risk that airborne organisms in the working environment will contaminate the work. Class I cabinets are appropriate for use with GMMs in activity classes 1 to 3.

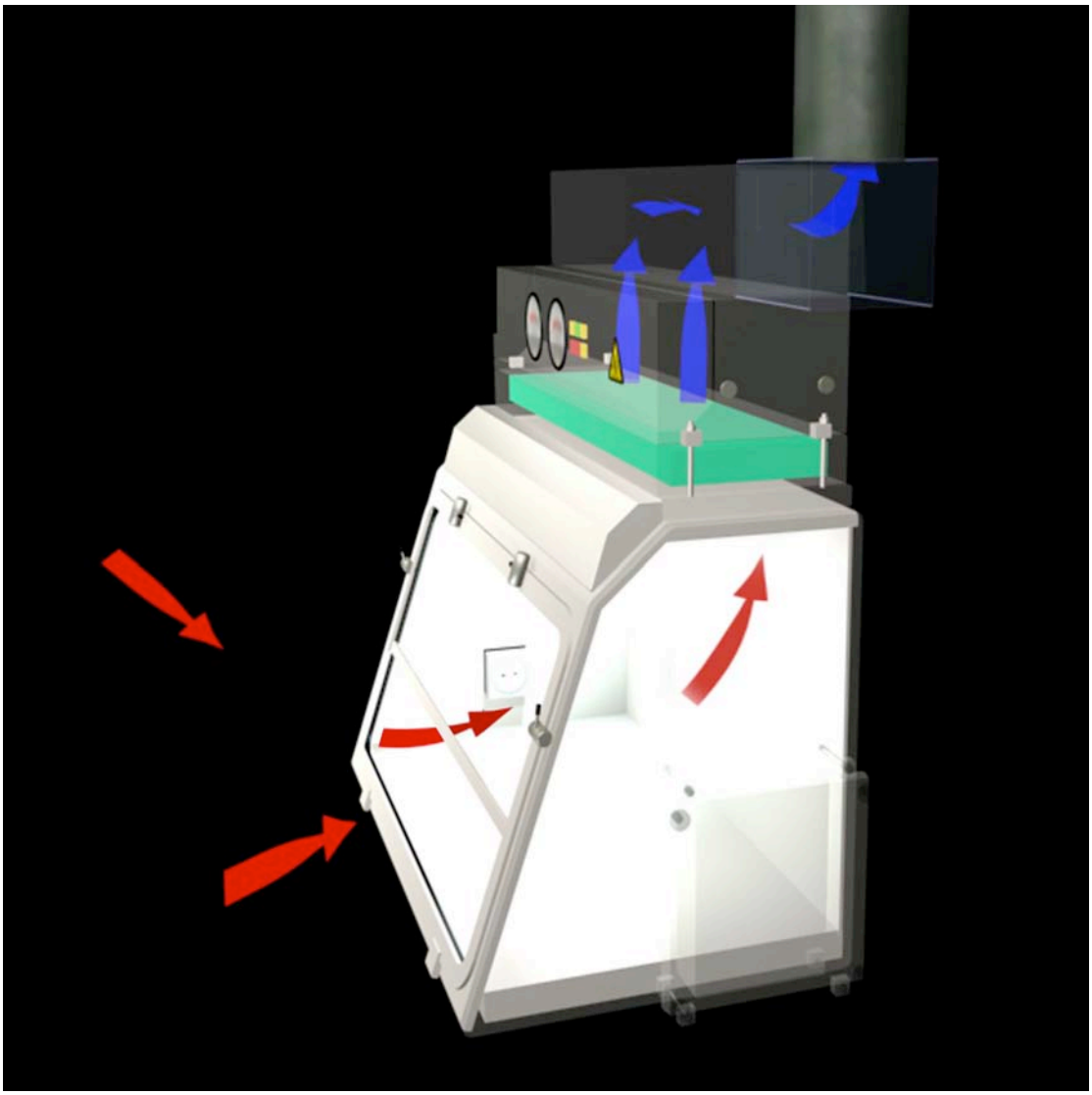

Figure 3.7.1 A class I microbiological safety cabinet. Coloured arrows indicate airflow – red arrows denote 'dirty', unfiltered air; blue arrows denote 'clean', filtered air.

5. ***Class II*** cabinets have an aperture at the front through which the operator can carry out manipulations on potentially hazardous materials. They provide protection to both the operator and the materials being handled as the inward airflow is diverted beneath the work surface and is HEPA filtered prior to circulation within the work area (see Figure 3.7.2). The downward airflow onto the work surface also minimises the possibility of cross-contamination within the cabinet. Exhaust air is HEPA filtered and this sort of cabinet is suitable for work with all GMMs, except those that are activity class 4 (unless derogation is successfully obtained from the competent authority).

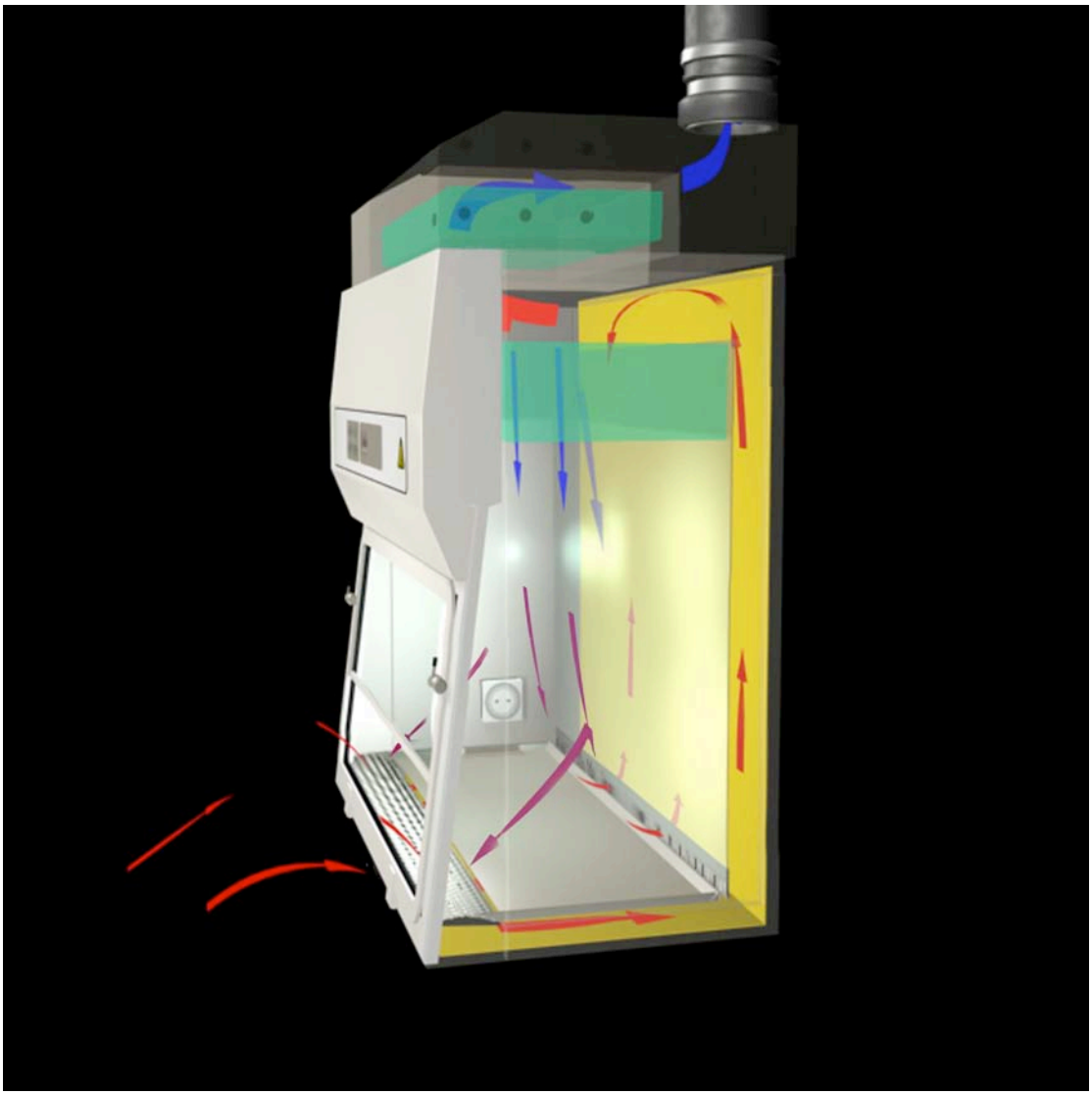

Figure 3.7.2 A class II microbiological safety cabinet. Coloured arrows indicate airflow – red arrows denote 'dirty', unfiltered air; blue arrows denote 'clean', filtered air.

Class I or Class II? **Modern Class II MSCs will offer similar operator protection to a Class I MSC. However, Class I MSCs are less affected by external factors and internal flow rates than Class II. Users should consider the needs of the work and be aware of the limitations of the equipment before selection.**

6. ***Class III*** cabinets are totally enclosed, providing maximum protection for the operator, the work and the environment. All inward and exhaust air is HEPA filtered and access to the work area is provided via the use of full arm-length gloves that are sealed to ports in the front of the cabinet (see Figure 3.7.3). Use of Class III cabinets is usually restricted to work with hazard group 4 biological agents or GM activity class 4 GMMs, but they may also be appropriate for work where there is a risk of generating large aerosols or where there is uncertainty as to the route of transmission of a highly hazardous virus, for example, following modification of viral tropism.

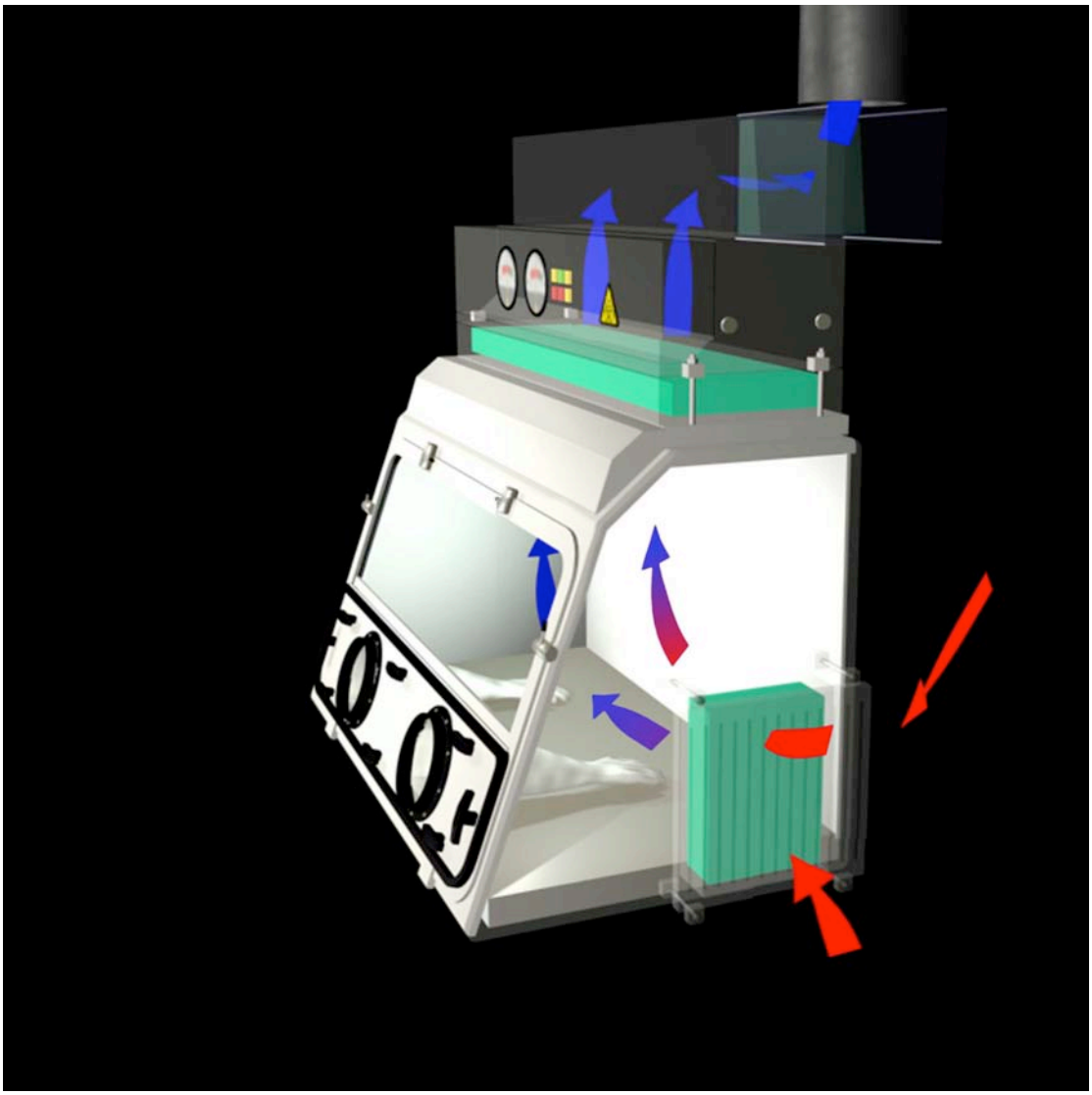

Figure 3.7.3 A class III microbiological safety cabinet. Coloured arrows indicate airflow – red arrows denote 'dirty', unfiltered air; blue arrows denote 'clean', filtered air.

Laminar flow cabinets or 'clean air' systems are NOT MSCs and should never be used when handling potentially infectious materials. These units are designed to deliver a stream of HEPA filtered 'clean' air over a working surface so as to prevent sterile materials such as culture media or drug preparations becoming contaminated. Any airborne droplets are actively directed at the operator and therefore the use of laminar flow cabinets with any material that is potentially infectious for humans is hazardous.

Installation

7. The effectiveness of an MSC will depend upon (1) its design; (2) correct use/maintenance and (3) suitable installation. All newly purchased and supplied cabinets should be compliant with the standards set by BS EN 12469: 2000 and BS 5726: 2005 for design,

construction and installation. Older cabinets should comply with the standards set by BS 5726: 1992. It is important to choose a cabinet that is appropriate for the work. Class I and II cabinets should not be used for class 4 GMMs unless the competent authority agrees derogation.

8. Under normal operational circumstances, it is considered good practice for filtered exhaust air to be discharged to the atmosphere via a dedicated extraction system. This is not always reasonably practicable or feasible and the recirculation of discharge air back into the laboratory can be considered. If being used in recirculation mode at Containment Level 3, a double HEPA filter should be used. It is also important in these cases to consider factors such as cabinet fumigation and how the fumigant is to be safely extracted from the room. For instance, temporary ducting could be used to extract fumigant to an air outlet or vent. Alternatively, the fumigant could be neutralised inside the sealed cabinet. For example, formaldehyde fumigant can be neutralised using ammonia vapour prior to recirculating the contents of the cabinet back into the room. This will usually require charcoal filtration of the exhaust air to scrub any excess neutraliser. Complete fumigation/neutralisation systems are available commercially (see Section 3.6).

9. It is important to consider the optimum position of the cabinet within the room, taking into account any factors that may affect performance (the installer of a newly purchased MSC should discuss these issues with the customer). Factors to be considered are the proximity of the MSC to doors, windows ventilation ducts and personnel movement routes. Standards relating to installation and positioning of an MSC are set out in BS 5726: 2005 and BS 5726: 1992.

10. Once installed, commissioning tests should be conducted to verify the performance of the cabinet *in situ*. This includes verification of the operator protection factor (OPF - this is referred to in the standard as the aperture protection factor). which is related to the inward airflow through the front aperture of a Class I or Class II cabinet and is defined in BS EN 12469: 2000. The importance of these tests cannot be over-emphasised as they demonstrate the cabinet's performance and the level of protection in practice rather than on paper. Commissioning tests should also be performed if the MSC is moved within the room, moved to a new location or where changes have been made to the laboratory that may impact upon factors that affect the cabinet's performance.

Factors affecting MSC performance

11. The OPF should be verified at installation and thereafter on a regular basis (see Table 3.7.1). The inward airflow, and therefore the level of protection afforded by a Class I or Class II MSC, can be disturbed by a number of factors. These include:

- Sudden movement at the aperture, eg sudden movements of operator's arms.
- Turbulence generated by air movement around items on the work-surface.
- Movement of people in the vicinity of the cabinet.
- Changes in pressure or air movements in the room, eg opening doors or switching on another cabinet.

12. Modern Class II MSCs built to the correct standards will offer a similar level of operator protection as a Class I MSC. Tests have shown, however, that Class I MSCs are less affected by external factors and internal flow rates than Class II. Centrifuges should not be placed inside the cabinet, unless 'in use' tests show that the overall protection factor is unaffected. Similarly, the use of bunsen burners is not recommended as localised heat sources may affect airflow patterns and should only be used following verification from an 'in use test'. If used, they should be placed toward the back of the work surface.

'In use' operator protection factor testing

13. To test containment afforded by a Class I or Class II MSC *in situ*, it may be necessary to carry out 'in use' operator protection factor tests. This may be particularly appropriate when working with GM activity class 3 GMMs, particularly if there are local factors that may affect the cabinet's performance. The tests should be performed under conditions that are as representative as possible to the working conditions expected.

14. The ventilation system, laboratory equipment and working practices should be normal. The laboratory should not be modified for the tests. It may be that two or more scenarios should be tested where use of the room will vary.

Maintenance

15. Fumigation and decontamination procedures (see Section 3.6; Section 3.5) should be in place. Where the unit has been used to handle an organism that may be hazardous to human health, full decontamination should take place before engineers carry out maintenance. Recommendations as to the type and frequency of the tests can be found in Table 3.7.1. It should be noted that MSCs are considered to be Local Exhaust Ventilation (LEV) systems under the COSHH Regulations and are subject to a statutory requirement for maintenance and testing annually.

3.8 Containment and control measures for work with DNA encoding oncogenic sequences

Overview

1. DNA sequences are regarded as oncogenic if they are able to make cells tumorigenic. Potentially oncogenic sequences, particularly where they are handled as preparations of naked DNA or in viral vectors with a human host range, may be carcinogens as defined under the COSHH Regulations. Further guidance relating to oncogenic sequences vectored by GMMs can be found in Part 2, Sections 2.2 and 2.3. The guidance given here should not preclude assignment of a particular experiment to a higher standard of containment where that is appropriate.

2. It is prudent to adopt a precautionary approach to work with potentially oncogenic sequences, irrespective of whether they are COSHH carcinogens or not. It is arguably a substance hazardous to health and the general provisions of COSHH will therefore apply. This approach is recommended in the COSHH General Approved Codes of Practice (ACOP), and is endorsed by SACGM.

Naked DNA: possible hazards and routes of transmission

3. Handling naked oncogenic DNA may involve a potential risk to the laboratory worker. Although there is no direct evidence as yet that contact with DNA can lead to tumours in humans, this possibility cannot be discounted, as evidence does exist for animals. Possible routes of transmission of naked DNA sequences to laboratory workers will primarily be inoculation or entry through broken skin. Other possible routes of transmission such as inhalation, ingestion and eye splashes may be less likely to lead to tumourigenesis.

4. Workers with unprotected skin lesions on the hands or forearms should have their suitability for work reviewed. Where a worker has active eczema, chapping or sepsis, they should consult a competent person (eg a medical professional or occupational health provider) before embarking upon, or continuing activities. The use of suitable personal protective clothing such as gloves and laboratory clothing may be sufficient to prevent exposure.

5. GMSCs should consider any risk to workers in handling oncogenes and potentially oncogenic sequences as naked DNA, particularly if linked to strong promoters or enhancer sequences that function in mammalian cells. The GMSC should be satisfied that

local rules and standard procedures give effective guidance on laboratory discipline and on avoiding accidental inoculation of workers.

Control measures

6. COSHH requires that exposure to any substance hazardous to health is prevented, or where this is not reasonably practicable, adequately controlled. In cases where the oncogenic DNA is clearly a COSHH carcinogen and it is not reasonably practicable to prevent exposure (eg by substitution with a less hazardous substance), COSHH sets out a series of control measures that must be applied.

7. Given the uncertainty about the hazards of most potentially oncogenic sequences and the small quantities used, prevention of exposure or total enclosure will rarely be 'reasonably practicable' because the costs will outweigh any benefits such as a reduction in exposure. The minimum requirements for handling such sequences should therefore include:

   - ***The wearing of protective gloves*****.** Gloves should be worn for all work with naked oncogenic DNA sequences. The use of gloves should not preclude the covering of cuts by suitable dressings.
   - ***Avoiding the use of sharps.*** Sharps should be not be used for work with naked oncogenic DNA, unless it is essential (eg for animal inoculation). Glassware should not be used where plastic alternatives exist.

8. All experimental procedures involving naked oncogenic DNA should be performed so as to minimise aerosol production. Procedures likely to generate aerosols such as the use of blenders, sonicators, vigorous shaking and mixing etc, must be conducted under effective containment. This might include the containment of the equipment in a sealed box or the use of a microbiological safety cabinet. The suitability of such systems should be decided after a risk assessment as required under the COSHH Regulations, although the control measures implemented must not increase risk in other workplaces or the environment.

Further information

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