Safe Biological Practice (SBP) for Prevention and Control of Exposure to Biological Agents in the Laboratory

UNIVERSITY OF CAMBRIDGE

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Contents

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Safe Biological Practice (SBP) - for Prevention and Control of Exposure to Biological Agents in the Laboratory

1 Introduction

This document outlines the precautions to be taken when working with microorganisms, and carrying out genetic modification (GM) or tissue culture work. A summary of the main requirements relating to biological safety is given below. This document does not deal with the specific requirements of the GM (Contained Use) Regulations 2000, the transport of biological samples, allergy to laboratory animals or higher level management issues and notifications. These topics are covered in other documents available from the Health and Safety Division (HSD).

2 Main Requirements

Each department carrying out biological work must make appropriate arrangements to ensure University policy and guidance is implemented. Individuals responsible for the work are required to:

2.1 Find out about your departmental biological safety committee. If the work involves genetic modification the Committee will have an approved structure [see HSD Document 'How to Comply with the GM (Contained Use) Regulations 2000].

2.2 Your department will have a Departmental Biological Safety Officer (DBSO) and deputy (if appropriate). Find out who they are.

2.3 Assess risks associated with work involving microorganisms and prepare a written assessment.

2.4 Assess risks associated with all genetic modification work and prepare a written assessment in accordance with the approved methods. [See HSD Document 'How to Comply with the GM (Contained Use) Regulations 2000.]

2.5 Ensure that the laboratory facilities and working practices conform to the required containment levels. (See ACDP publication 'The management, design and operation of microbiological containment laboratories'.)

2.6 Ensure the microbiological safety cabinet you are using has a valid test certificate and is in working order.

2.7 Report to the HSD on request, data on microorganisms stored, used or intended to be used in the department.

2.8 Notify the HSD in advance of the start of work involving Hazard Group 3 or 4 pathogens, the intentional release of genetically modified organisms and of any genetic modification work requiring Containment Level 3 or 4. (Currently the University has no facilities for handling Hazard Group 4 organisms, and none are envisaged.)

2.9 Ensure that you have adequate training, instruction and supervision for the biological work you are doing.

2.10 If you are working at Containment Level 3 make sure you are registered with the University Occupational Health Service.

2.11 Ensure licences are obtained as appropriate from DEFRA or the Forestry Commission etc for activities involving micro-organisms or carriers controlled by various orders, and send copies to the HSD.

2.12 You must follow written local codes of practice covering routine procedures, working practices and use of equipment in containment laboratories.

3 Routes of Infection

Microorganisms can gain access to the body by ingestion (mouth), instillation (eyes), inhalation (respiratory tract) or via the percutaneous route (skin). Whilst many chemicals can be absorbed through intact skin, microorganisms cannot and only enter the body through skin that is damaged (cuts and grazes or puncture wounds) or the mucous membranes.

4 Control of Exposure

4.1 There is generally no dose-response relationship, and with many microorganisms able to infect at very small doses, there are no exposure limits.

4.2 The number of the hazard group of a particular organism indicates the level of containment under which it must be handled.

In matching hazard group with containment level the pathogenic potential, route of transmission, epidemiological consequence and host susceptibility have been considered.

4.3 Model rules provided by ACDP for the different containment levels set out the minimum standards for control of exposure. The need for additional precautions should also be considered taking account of pertinent features of the microorganism and its route of transmission.

5 Personal Hygiene

5.1 Basic but very effective ways of avoiding the spread of infection or contamination from containment laboratories are the routine washing of the hands and the wearing of laboratory coats.

Workers at all levels of containment must wash their hands before leaving the laboratory/laboratory suite or unit as appropriate or ASAP when contamination is suspected whilst working.

5.2 Where different aspects of work at Containment Level 2 are carried out in several adjacent rooms, it may not be necessary to wash your hands in each room, provided you wash them before leaving the suite.

5.3 Avoid potential contamination of keyboards, telephones, door handles or outside surfaces.

5.4 If liquid soap is used in a containment laboratory this should contain a bacteriostatic agent and refillable dispensers should not be used unless the refill is packaged ready for use rather than to be used for topping up reservoirs. Paper towels are an acceptable means of drying hands. **Cloth towels must not be used.**

5.5 Smoking, eating, chewing, drinking, putting in contact lenses, storing food and applying cosmetics in laboratories carrying out biological work is strictly forbidden.

5.6 Laboratory coats must be worn in the laboratory and removed before leaving.

5.7 Appropriate systems must be in place for the storage of laboratory coats and for their decontamination and laundry. You must always hang/place your laboratory coat in the designated area provided. Potentially contaminated laboratory coats must never be taken home.

6 Handling Precautions for Blood, Blood Products and Human Material

6.1 This section provides guidance on the handling precautions to be adopted when working with blood, blood products and other human tissue in research laboratories. It does not cover specific work with blood borne pathogens such as the human immunodeficiency virus (HIV) or hepatitis B virus (HBV).

The guidance is not aimed at workers carrying out procedures in clinical settings at the point of contact with patients or volunteers or in any health care situation.

The lower standards, or absence of biological containment encountered within the clinical environment eg hospital wards does not negate the legal requirements for biological containment when blood is handled in a research laboratory.

In the case of fieldwork note should be taken of the guidance in this document and it should be applied as far as is reasonably practicable.

6.2 Risk Assessment

6.2.1 A risk assessment must be carried out for all work involving handling of blood, blood products and other human tissues. The risk assessment should be specific for the procedures involved and take account of the nature and source of the samples to be handled. It should be written down.

6.2.2 Of particular concern is the possible presence in the material of blood borne pathogens, most notably HBV and HIV. Where it is known or suspected that specific hazard group 3 pathogens are present then it is recommended best practice that these be handled at Containment Level 3.

In many cases however, it is not known which pathogens (if any) the samples may contain and therefore stringent handling precautions at a minimum of Containment Level 2 must be used.

6.2.3 HIV has been detected in blood and blood products, in serum, plasma, breast milk, semen, vaginal and cervical secretions, urine, tears,

peritoneal fluid, pleural fluid, pericardial fluid, synovial fluid, amniotic fluid and both cerebrospinal fluid (CSF) and brain tissue. There is also evidence that certain specialised cells lining the gut support the multiplication of HIV. Therefore possible HIV contamination should be taken into account when handling materials of these types.

6.2.4 All human tissues will be contaminated with blood. Therefore they should be regarded as potentially infected material. Other specimens such as faeces and urine are not regarded as posing HBV or HIV infection risk as long as they are not contaminated with blood.

6.2.5 Any samples that have not been screened should be regarded as potentially infected. Wherever possible material should be used that can be shown by screening to be pathogen free at source although this does not guarantee the sample is HIV negative because of the window between infection and seroconversion. Blood obtained from the National Blood Service may be regarded as low risk as it is screened and therefore the additional control measures specified here need not be applied. It should be noted however, that blood is a particularly good medium for supporting the growth of a wide range of pathogens and contamination of the blood due to poor working practices may lead to potentially infective material.

6.2.6 All work on unscreened samples must be undertaken at a minimum of Containment Level 2 with the additional precautions given in this guidance note. Less stringent containment conditions must not be adopted unless a full risk assessment has been made, agreed locally by a representative safety committee and approved by the Sub-committee for Biological Hazards. If at any time information changes and there is the suspicion or knowledge that samples are HIV (or other hazard group 3 pathogen) positive then the work should immediately be transferred to Containment Level 3.

6.2.7 In general, work at Containment Level 2 does not have to be confined to a safety cabinet unless there is reason to believe the specimen contains pathogens that require such containment. However, where handling or processing may generate large droplets or splashes the work must be carried out in an appropriate safety cabinet.

6.2.8 At Containment Level 3 all work must be undertaken in a microbiological safety cabinet.

6.2.9 As this document covers an extremely wide range of research activity throughout the University some generalisations have been made. If a researcher believes the detailed risk assessment of their particular project justifies undertaking the work at a lower level of containment to that described here, the appropriate School Safety Officer or the HSD or should be consulted. Help and advice on risk assessment can be given on a case-by-case basis and researchers are urged to make contact at an early stage.

6.2.10 The onus is on the person doing the work to assess the risks and specify appropriate working practices depending on the level of competence to do so.

NB Sometimes this will be more appropriately undertaken by the work supervisor.

6.2.11 The following paragraphs detail hazards that should be taken into consideration for particular types of work. Further advice may be sought from the School Safety Officers and HSD.

6.2.12 Some human cell culture systems incubated for short periods may support the replication of any HIV that may be present in the starting material. These include cell cultures for immunological and cytogenetic studies especially those involving peripheral white blood cells. Similarly certain continuous lines of cells of human origin may be chronically infected with HIV or the virus may be inadvertently introduced during repeated passaging and use in the laboratory.

Therefore in order to contain work of this type safely, the cultivation of cells from known or suspected cases of HIV infection can be conducted at Containment Level 2 with the additional precautions detailed in this guidance note for work on potentially contaminated material **provided that incubation of any cultures does not exceed 100 hours.**

If incubation of known HIV infected material is to exceed 100 hours then this work should be done at Containment Level 3. [Exceptionally, if it you can show in the risk assessment that this level of containment is excessive for the particular circumstances then the material may remain at Containment Level 2 with additional precautions.]

6.2.13 It is advised that all work on sputum samples and specimens of lung tissues are undertaken in a microbiological safety cabinet. Where samples are known to be from patients suffering from tuberculosis they must be handled at Containment Level 3.

6.2.14 Workers should be aware that samples of neurological origin may inapparently carry infectious agents eg prions or viruses. This must be taken into account in the risk assessment and particular care taken when samples are handled.

6.2.15 Sources of Blood

6.2.15.1 Wherever possible blood should be obtained from screened sources such as the National Blood Service.

6.2.15.2 It is inadvisable to work with blood samples from people with whom you work without taking precautions suitable for working with suspect blood.

6.2.15.3 The transformation of cells of one's own blood may be dangerous and must not be done. Staff should also avoid supplying blood for transformation experiments if it is to be used by students closely supervised by them or by other staff who are involved in their work.

6.2.15.4 Students should not draw blood from one another unless the procedure forms part of their training and is done under medical supervision.

6.2.15.5 If blood is to be obtained from undergraduate or other volunteers the following procedure must be adopted:

(i) Written consent must be obtained beforehand by the person who is to withdraw the blood. This should not be the supervisor of the project to avoid accusations that unfair pressure was exerted on the volunteer. It should be made clear that a volunteer can withdraw his or her consent at any time.

(ii) The blood must be withdrawn only by a registered clinical practitioner or phlebotomist or by a technician who has received the appropriate training and where a registered medical practitioner certifies in writing that the technician is fully trained in the correct procedures. Blood must only be taken in a quiet, discrete, clean area set aside and suitable for this purpose, away from the research or teaching area.

(iii) Records must be maintained of the identity of the person from whom the blood is taken.

(iv) Fresh sterile needles and syringes must be used for each volunteer from stocks held for that purpose.

(v) The needle must be carefully removed before discharging the blood into screw topped specimen containers to prevent aerosol formation. (Unless the Vacutainer system is used.) Do not resheath needles.

(vi) All sharps must be disposed of in a suitable sharps container, autoclaved wherever possible and sent for incineration.

6.3. Control of Exposure

6.3.1 A key feature in control of exposure is maintaining good working practices standards and avoiding the use of sharps. Those who work with potentially contaminated samples can be protected by the consistent

application of simple precautions. These precautions will protect against the transmission of all blood borne pathogens by the percutaneous route.

6.3.2 Work must be assigned to the appropriate containment level and the necessary requirements regards facilities and working practices met.

6.3.3 The following guidance on control of exposure is directed to work that does not require **Containment Level 3.**

For your safety and that of others in the laboratory you must be trained and proficient in safe working practices and techniques. You must be able to recognise how exposure can occur and how it can be prevented.

Local rules should have been drawn up to ensure that working practices take into account the following factors.

6.4 Hygiene

6.4.1 Eating, chewing, drinking, smoking, putting in contact lenses, storing of food and applying cosmetics must not take place in the containment laboratory.

6.4.2 Mouth pipetting must not take place.

6.4.3 Since infections can occur via lesions in the skin all workers in the laboratory should cover cuts and abrasions with waterproof dressings.

6.4.4 Contamination of benches and equipment should be avoided and at the end of each working session (or day) these should be routinely cleaned and disinfected.

6.4.5 In addition to the good basic hygiene practices indicated above, hands must be washed regularly.

6.5 Protective Clothing

6.5.1 Laboratory coats and gloves must be worn at all times whilst undertaking laboratory work.

6.5.2 Laboratory coats should be side or back fastening and worn specifically for this type of work. These laboratory coats should be stored separately from other laboratory coats and never be stored with other clothing.

6.5.3 Wear two pairs of single use (disposable) gloves when handling samples as minor damage to thin gloves often goes undetected until skin contamination is noticed.

Remove, dispose of and replace the outer glove if it becomes punctured or grossly contaminated during use. If you find that the inner glove is also damaged or contaminated you should remove and dispose of it too, wash your hands and put on clean gloves.

Never reuse single use (disposable) gloves.

Where possible always use alternatives to latex (e.g. nitrile or vinyl) due to the possibility of developing an allergy to latex, or exacerbating an existing allergy. However, if having done a risk assessment, you specifically choose to use latex gloves they must be powder free.

6.5.4 Wear eye protection (eg goggles, safety spectacles, or prescription safety spectacles, or face shield) and a plastic overall if splashing is likely to occur.

6.5.5 On completion of work gloves should be removed and put for appropriate disposal, hands should be washed and protective clothing changed. Laboratory coats should be designated for wear when handling these types of samples and should only be worn whilst the work is ongoing.

6.6 Sample Reception

All specimen reception must be undertaken in the laboratory by trained workers. Arrangements must be in place to ensure that untrained workers do not inadvertently handle samples, particularly if these are received in the postal system.

6.7 Workstations

6.7.1 Work should be conducted at a delineated workstation which is clearly identified, and preferably separated from other work areas. Systems of work should be in place to ensure that the person carrying out the work is free

from the risk of physical disturbance by others.

6.7.2 There should be sufficient room to work safely. Ideally, this would be between $4m^2$ - $8m^2$ for each worker. There should be enough bench space to ensure the workstation is not cluttered and working practices are not compromised.

6.7.3 The workstation should be cleared of any unnecessary equipment or apparatus before the work starts. The bench surface and any equipment remaining there must be disinfected immediately on completion of the work.

6.8 Use of Sharps

6.8.1 Sharps should not be used in Containment Level 3 work. For other work the use of sharps should be avoided wherever possible. If this is not feasible then handling procedures should be designed to minimise the likelihood of puncture wounds. Wherever possible glass items should be replaced with plastic alternatives. Glass pipettes must not be used.

6.8.2 Used sharps must be placed directly into a sharps bin. Do not resheath needles. Sharps bins must not be overfilled; used sharps protruding from bins are very dangerous.

Do not dispose of any sharps, particularly hypodermic needles, to the 'domestic waste' disposal services. Do not dispose of any sharps into plastic bags.

6.8.3 Sharps containers must conform to the British Standard 7320: 1990. All sharps which may be contaminated with pathogenic organisms should, wherever possible, be autoclaved in their boxes before collection for incineration.

6.8.4 The term 'sharps' is not restricted to needles and scalpels. It includes commonly used items that could easily cause damage to the skin eg all glass items (including microscope slides and cover slips), ampoules, pointed nose forceps, dissection instruments, scissors, wire loops that are not closed circles and gauze grids used in electron microscopy work.

6.9 Laboratory Equipment

6.9.1 All equipment must be cleaned and disinfected at the end of the working day or after each use. If the operation of the equipment results in the production of droplets or splashes these should be contained in some way to avoid spread of contamination. Equipment must be decontaminated prior to maintenance and a signed statement obtained.

6.9.2 Wherever reasonably practicable the lids of rotors and buckets should be manufactured of a suitable transparent material to allow the contents to be seen and checked for integrity before opening. Samples should be centrifuged only in sealed buckets. These should be cleaned and disinfected regularly and immediately following leakage. If a sample is known to have leaked during centrifugation then the bucket should only be opened in a safety cabinet. Seals on buckets and enclosed rotors should be regularly checked for wear and damage and be replaced as necessary. Records need to be kept in accordance with local rules.

6.10 Disinfection

6.10.1 In the laboratory all specimen containers, glassware and used equipment must be immersed in a suitable disinfectant before cleaning or disposal. (N.B. Disinfection of electrical equipment can present certain problems - seek advice from the manufacturer if unsure whether a certain disinfection method is appropriate for your equipment.)

6.10.2 All surfaces should be disinfected immediately following any spillage, at the end of the working day and before any maintenance or cleaning staff are permitted to work in the area where work with blood or blood products has been carried out.

6.10.3 Where any hazard may be present, non-technical visitors, cleaning and maintenance staff should be trained in the correct procedures. Instruction, supervision and permits to work may be required.

6.10.4 Suitable disinfectants, concentrations and contact times must be specified

for work involving human blood and/or other tissues. Examples of suitable disinfectants include hypochlorites and Virkon. Glutaraldehyde based disinfectants should be avoided. Check for suitability and contra-indications with certain substances and materials before use.

6.11 Waste Disposal

You must dispose of all contaminated waste according to local rules and University policy.

6.12 Accidents and Incidents

6.12.1 In the event of an accident resulting in a wound, it should be allowed to bleed by vigorous irrigation under a running tap and the area washed with soap and water. Wounds should not be squeezed or pressed to promote bleeding since this may cause tissue trauma leading to spread of infection. Wash any contaminated skin, conjunctivae or mucous membrane immediately.

6.12.2 Clear up spillages immediately using the procedures outlined in the risk assessment.

6.12.3 All accidents and incidents must be reported and recorded by the person doing the work. A full report should be prepared and forwarded to the HSD as soon as possible. The source of any contamination (specimen, sample, material etc) should be clearly identified and retained for testing if necessary. If someone has been accidentally exposed to HIV then the department must make every effort to ensure the confidentiality of that person.

6.12.4 In the event of potential exposure to any blood borne pathogen the University Occupational Health Service must be informed immediately. If blood borne pathogen transmission is suspected then the Occupational Physician will seek specialist advice on the value of prophylactic treatment. Workers will be requested to provide a serum sample for storage and testing if indicated. Workers should be assured that appropriate advice will be provided, testing will only be undertaken with their informed written consent and all test results will be confidential. Further samples for storage and possible testing will be taken at three and six months.

6.12.5 Those worried about exposure should be aware that confidential arrangements for counselling and testing can be made through the University Occupational Health Service, their General Practitioner or special clinics.

6.13 Training

6.13.1 You must be trained in the techniques to be adopted and the safety precautions to be followed. The degree of training will depend on your previous experience and expertise. Competence must not be assumed. It has to be demonstrated.

6.13.2 Young persons (defined as those above school leaving age and 18 years) and children on work experience schemes should not work with blood, blood products or pathogens. They must at all times be closely supervised. Any work involving persons on youth employment schemes must be subject to an in depth assessment of potential risks and they will also require close supervision. A suitable training programme should be drawn up locally taking into account the nature of the work concerned. On the job training is important and work practices should be monitored. Any shortfall in standards should be brought to the attention of the laboratory supervisor and be addressed immediately. Advice on placements can be sought from the HSD.

6.14 Health Surveillance and Vaccination

6.14.1 The University requires that all workers involved with handling unscreened blood, blood products and other tissues be registered with the University Occupational Health Service. Such registration is compulsory. Health surveillance will be commenced and continued as indicated.

6.14.2 Those handling material that may be infected with HBV must be vaccinated against the virus, and have their response checked. Where they are non-responders further advice will be given by the University Occupational Physician.

6.15 Notification of Work

6.15.1 The HSE must be notified of ALL work carried out on specimens which are known to be infected with HBV, HIV or other hazard group 3 pathogens.

6.15.2 Where work in the laboratory may lead to the unintentional propagation of HBV or HIV, or where it involves the inoculation of experimental animals the work must be notified to the HSE.

6.16 Additional Comments

6.16.1 Under current guidelines samples can only be tested for HIV infection with the permission of the person from which the samples were taken. Even when continuous cell lines are maintained the cells can only be tested when the donor has given informed consent. The guidance provided in this note has been drawn up on the basis that testing is not feasible or practicable in many areas of research within University departments. It is essential therefore that risk assessments take into account all available information and control measures are rigorously applied.

6.16.2 The HIV virus is not highly infectious nor particularly hardy and studies have shown that the occupational risk of exposure is low. However, following accidental inoculation there is the possibility (although the likelihood still remains difficult to predict) that the person will go on to develop clinical symptoms of infection.

7 Handling Precautions for Work with Naked DNA

7.1 Many experimental protocols include extraction of DNA from microorganisms. The DNA is extracted for various reasons such as characterisation on gels or subsequent manipulation in genetic modification work.

If the DNA is inserted into other microorganisms as in genetic modification work then a risk assessment must be made in accordance with the requirements of the Genetically Modified Organisms (Contained Use) Regulations 2000 and recorded. **Specific guidance on risk assessments is provided by the Scientific Advisory Committee on Genetically Modified Organisms (Contained Use) - SACGM(CU) formerly known as the Advisory Committee on Genetic Modification (ACGM) for work of this type**. This section is directed at providing guidance for work that is not regarded as genetic modification work but involves the handling of DNA.

7.2 To comply with the Control of Substances Hazardous to Health (COSHH) Regulations a risk assessment must be made to determine the handling precautions to be adopted whilst working with the naked DNA. This assessment is particularly important when the DNA is from pathogenic microorganisms. You must not automatically downgrade the containment level required for the original pathogen without due consideration of the potential risks involved.

7.3 The risks associated with handling naked DNA are generally regarded as low since the only occasion DNA is likely to gain access to the body is when the skin is punctured and the material effectively injected (sharps injury). Therefore, of prime importance in the control of exposure is the avoidance of “sharps” in the manipulative procedures.

7.4 The risk assessment must have taken into account of the consequence of expression of the DNA if it were to gain access into the body. The question “what does the extracted DNA encode?” should be addressed. For example, is the DNA a full-length profile of the parent, if specific genes are extracted do these code for potentially hazardous products such as toxins, allergens or growth factors, does it contain potentially oncogenic sequences?

7.5 If the DNA were to contain harmful sequences it may be possible to include a stage in the protocol whereby the DNA is denatured. An example of this type of procedure would be heat treatments in excess of 90ºC. DNA denaturing techniques should not be confused with protein denaturing steps such as phenol chloroform treatment which do not affect the DNA. A denaturing stage will eliminate any potential for expression and therefore, the hazard will be minimal.

7.6 When extracts are taken from microbial cultures consider whether the preparation is likely to contain any intact parent microorganisms and whether the extraction procedure is effective in ensuring there is no contamination with, for example, virus particles. If contamination is likely, use further purification steps, an inactivation procedure or full containment precautions.

8 Handling Precautions for Work with Oncogenes

8.1 Introduction

8.1.1 This outlines the precautions to be taken when handling oncogenic DNA and related DNA sequences. The statutory and legal obligation relating to carcinogenic agents are set out in the Control of Substances Hazardous to Health (COSHH) Regulations.

8.1.2 An oncogene is a sequence of DNA that is capable of progressing cells through a stage in the multistage process of cancer. Oncogenes are defined as DNA sequences which induce tumours in experimental animals or which cause transformation of cells in vitro resulting in immortalisation of cells, escape from normal growth control, or production of tumorigenic cells.

8.1.3 The Scientific Advisory Committee on Genetically Modified Organisms (Contained Use) - SACGM(CU) state "Handling naked oncogenic DNA may involve a potential risk to the operator. Although there is no direct evidence as yet that contact with such DNAs can lead to tumours in humans, its possibility cannot be discounted as evidence does exist for animals."

8.1.4 Risks do however, increase significantly when oncogenic sequences are used in genetic modification work. Packaging of oncogenes in delivery systems with host ranges capable of infecting humans presents the greatest hazard. Detailed assessment of risk, taking into account the full nature of the work is required.

8.2 Risk Assessment

8.2.1 Under COSHH an assessment of the health risk must have been undertaken. Ask to see it.

8.2.2 A detailed risk assessment must be carried out prior to starting work and recorded. If you have to use oncogenic sequences then design your experiment to minimise hazards. Often there are experimental options and selection should give due regard to health and safety considerations. For example, if a virus vector is used it should be replication defective and wherever possible the host range should not include humans.

8.2.3 For genetic modification work there is also the additional requirement to classify genetically modified microorganisms. [See HSD document 'How to Comply with the Genetically Modified Organisms (Contained Use) Regulations 2000]. A replication defective virus can generally be considered to be non-pathogenic, and on that basis may be classified as Class 1. If however, this contained an active oncogene it would be Class 2 or above, because the insert is not "free from known harmful sequences". The SACGM(CU) provides guidance on various aspects of work with oncogenic DNA including risk assessment and classification.

8.2.4 On completion of a risk assessment it must be submitted to the departmental biological safety committee or genetic modification safety committee (GMSC). The committee must be satisfied that the laboratory local rules give effective guidance on the maintenance of laboratory discipline and on avoiding accidental inoculation of workers.

8.2.5 For cloning oncogenic sequences in eukaryotic viruses the Brenner scheme emphasising Access, Expression and Damage (ACGM Compendium of Guidance) must not be used. Reference should be made to ACGM Compendium of Guidance: "Guidance on the use of Eukaryotic Viral Vectors in Genetic Manipulation".

8.3 Control of Exposure

8.3.1 As with all substances, routes of entry into the body are by inhalation, ingestion, penetration of the skin or mucosal surfaces or contamination of the eyes. Currently the effects of exposure to oncogenes are unknown and it thus it must be assumed there is no lower limit of exposure.

8.3.2 The following precautions are a minimum for work with oncogenes and related DNA sequences:

- Access to the laboratory where oncogenic DNA is handled must be limited to authorised personnel and designated workers.
- Laboratory coats must be worn at all times in the laboratory.

- There must be adequate space in the laboratory for each worker.
- Laboratory bench space should be designated for work with oncogenic DNA sequences. All designated workers using this space and those likely to be exposed must follow the general requirements of this guidance and all local rules for work involving carcinogenic agents.
- All designated workers should be trained in good laboratory techniques before commencing work with carcinogenic agents. They should be fully aware of the potential hazards of such work.
- Gloves must be worn for all work with oncogenic DNA sequences. Gloves worn for this work should not be worn elsewhere. Cover cuts with waterproof dressings.
- Sharps must not be used for oncogenic DNA work, except where essential such as for animal inoculation. Glassware must not be used where plastic alternatives exist.
- Care must be taken in diluting oncogenic DNA in solvents which have the ability to penetrate the skin eg ethanol precipitation.
- A hand washbasin must be provided, preferably sited near the exit of the laboratory. The taps should be of a type which can be operated without being touched by hand. Hands must be washed before leaving the laboratory.
- Arrangements must be made for immediate surface decontamination after spillages.
- Glassware should be totally immersed in a laboratory detergent solution.
- Disposables should be placed in suitable containers before transport for incineration or other final disposal.
- All work surfaces must be cleaned after use.
- All experimental procedures involving oncogenic DNA should be performed to minimise aerosol production. Procedures which are likely to generate aerosols such as the use of blenders, sonicators, vigorous shaking and mixing etc. must be conducted using suitable local exhaust ventilation systems or in equipment which is designed to contain the aerosol. The suitability of such systems should be decided after risk assessment as required under the COSHH Regulations. However, the control measures utilised for such work must not accentuate the risk in other workplaces or in the outside environment.
- Oncogenic DNA must be securely stored.

8.4 Handling of Animals Treated with Oncogenic DNA

8.4.1 Protective clothing must be worn at all times when handling treated animals. As a minimum this should include gloves and a laboratory coat or overalls.

8.4.2 Animals treated with oncogenic DNA should be kept in appropriately labelled cages. Any animal carcases, bedding and disposable equipment or clothing must be destroyed by incineration.

8.5 Waste Disposal

The local rules must deal with contaminated waste disposal. Oncogenic DNA should be denatured or destroyed, for example by incineration or autoclaving.

8.6 Exposure Records

8.6.1 An estimate of exposure to oncogenic DNA must be made.

The following information should be included:

i. the period of time (dates) over which the work was undertaken;
ii. where the work was undertaken (identify individual laboratories by room number);
iii. the amount of time spent in the area should be given (hours/month); and
iv. an outline description of the material handled should be given eg. naked DNA, DNA in vector (identify vector), identity of oncogenic sequence where possible including source.

8.6.2 As described previously, work with oncogenic DNA should only be undertaken in designated areas. Each worker directly involved in the work must complete a detailed exposure record as above. All other persons working in the laboratory should be identified and the time spent in the area noted. This should then be cross-referenced to the detailed exposure record of the persons involved.

8.6.3 It is the responsibility of individual supervisors to ensure that the appropriate workers keep detailed exposure records. It is suggested that these be reviewed by the Supervisor on a six monthly basis to ensure that they are properly maintained.

8.7 Health Considerations and Health Surveillance

8.7.1 Work with oncogenes and related sequences may present an increased risk to those with certain pathological conditions. These include:

- non-intact skin
- reduced immune competence
- pregnancy
- pre-existing disease
- the effects of medication

8.7.2 Appropriate health advice will be given on a case by case basis to individuals by the University Occupational Physician and on occasion withdrawal from oncogene work will be advised where the increased risk may be considered unacceptable. As a person directly working with oncogenic DNA or working in laboratory where oncogenes work is being undertaken ensure you have been identified to the University Occupational Health Service where appropriate health surveillance records will be set up and medical advice given.

8.7.3 The requirement for health surveillance does not normally involve physical examination or biological monitoring. However, the University Occupational Physician may wish to discuss with individuals certain aspects of their health profile. In particular the University Occupational Physician will be interested in establishing a past history of cancer or related pathology and may, in these cases, wish to liaise with the individual's medical advisers to establish a risk assessment on a case-by-case basis.

8.7.4 Identification of genetic modification workers handling oncogenes will be made by the department and the information passed to the University Occupational Health Service.

8.7.5 As a genetic modification worker exposed to potential oncogenic material you will receive advice on an individual and collective basis and your head of department will be advised if further exposure is considered to be inappropriate for health reasons.

8.8 Training

8.8.1 Persons undertaking work with oncogenic DNA must receive basic information and training prior to starting work. A suitable training programme should be drawn up locally taking into account the nature of the work concerned.

8.8.2 The provision of adequate training is considered to be of prime importance in ensuring effective control of exposure for work with oncogenic DNA. The following items should be discussed as part of the information and training programme:

- The nature and degree of risk associated with the work
- The importance of good working practice and the nature of the control measures
- The role of health surveillance and duties of employees
- A practical demonstration of laboratory techniques should be included.

8.8.3 A nominated suitable person within each department must keep training records and review training requirements on at least an annual basis.

9 Microbiological Safety Cabinets

9.1 General

Microbiological safety cabinets are intended to offer protection to the user and others in the general laboratory area from the aerosol hazards of handling infected and other hazardous (eg. allergenic) biological material.

Some types of cabinet also protect the materials being handled in them from environmental contamination. Air discharged from the exhaust of the cabinets, which is ducted either to outside or recirculated into the laboratory, is filtered to remove contamination.

9.2 Types of Cabinet

There are three types of microbiological safety cabinet, which differ in the mode of operation

and therefore protection afforded. These are referred to as Class I, Class II and Class III cabinets.

Class I: Class I microbiological safety cabinets are open fronted and there is an inward flow of air through the working aperture.

Class II: Class II microbiological safety cabinets are also open fronted but in addition to the inward flow of air, the workspace is flushed with a unidirectional (so-called laminar) filtered downward airflow.

In both Class I and II microbiological safety cabinets, the inward airflow protects the worker by minimising the escape of any airborne particulate contamination generated within the cabinet. In Class II microbiological safety cabinets, the down flow of filtered air affords protection to the work minimising contamination during manipulations.

Class III: Class III microbiological safety cabinets are totally enclosed. The worker is separated from the work by a physical barrier (into which gloves are fitted) and inlet and exhaust filtration systems provide filtered air and prevent escape of airborne particulate contamination.

Where worker protection is required, the cabinet must meet the requirements of the current British Standard relating to Microbiological Safety Cabinets. (Now referred to as BS(EN) standards.)

There are other types of cabinets available but these do not provide operator protection, they are designed to protect the work only. Examples of such types of cabinet include laminar (horizontal/down or vertical) flow cabinets, and tissue culture cabinets. **These types of cabinet must not be used if operator protection is required.**

Fume cupboards and hoods must not be used to provide protection against any biological hazards.

9.3 Venting Arrangements

It is strongly recommended that microbiological safety cabinets vent to the outside. Justification would need to have been made for using a recirculatory cabinet. Approval from the School Safety Officers or HSD must have been sought before this option was adopted. For Containment Level 3 specific approval from the Health and Safety Executive is required. This approval is only granted in exceptional circumstances.

Consideration must have been given to a safe method of fumigating cabinets. There are particular difficulties with removal of the fumigant to be overcome if it is recirculatory.

9.4 Siting, Installation and Commissioning

9.4.1 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.

If a department chooses to install a cabinet itself then the requirements of the British Standard must still be met. A specialist contractor must be appointed to undertake the operator protection (KI Discus) test prior to use. Similarly, these requirements apply when cabinets are moved or relocated.

9.5 Routine Maintenance, Examination and Test

Microbiological safety cabinets constitute local exhaust ventilation systems in that they offer protection to the worker from airborne hazards. As such there is a requirement for regular maintenance, examination and test under the COSHH Regulations. Workers need to check that this has been done before use. (Usually shown as a signed and dated card placed on the cabinet.)

All microbiological safety cabinets should be serviced on an annual basis and undergo examination and test at that time.

9.6 Correct Siting of Microbiological Safety Cabinets (MSCs)

The following should be taken into account:

- Walkways at least 1000mm from the front of the MSC.
- MSC not positioned with either side closer than 300mm from a wall etc.
- No doors situated within 1500mm of the front of the MSC or within 1000mm of the sides. The only exception is when a door includes air transfer gills. Testing should be carried out to ascertain a suitable distance.
- At least 1500mm clear between front of MSC and any benching opposite.
- At least 2000mm clear between front of MSC and any wall opposite.
- At least 3000mm clear between the front of MSC and any other MSC or fume cupboard.

10 Disinfection of Containment Laboratories

10.1. General

10.1.1 **Disinfection** is a process used to reduce the number of microorganisms to an acceptable level. A particular agent is used to destroy the viability of the microorganisms but it does not necessarily kill or remove ALL of them.

10.1.2 **Disinfection** should not be confused with **sterilisation** which is where the process renders an object free from all living organisms.

10.1.3 **Decontamination** can be applied to both **disinfection** and **sterilisation**, as this is the general term referring to the removal of microbial contamination to render an item “safe”.

10.1.4 **Cleaning** may also be regarded as decontamination as it too can remove microorganisms from a soiled surface.

10.1.5 There are several types of agents that can be used for disinfection, these include chemicals, heat and irradiation.

10.1.6 Some disinfectants may just prevent growth of bacteria (bacteriostatic) rather than kill them (bactericidal).

10.1.7 All laboratories handling microorganisms must routinely use chemical disinfection to decontaminate surfaces and equipment. A chemical disinfectant should be chosen based on the situation to which it is going to be applied. Before a disinfectant is chosen, or if there is a subsequent change in usage, it should be confirmed that the particular disinfectant (and working concentration) is suitable for the intended purpose. **Never assume that the disinfectant is active in a particular application without validation.**

10.1.8 Laboratories must have a clear documented disinfection policy stating suitable working concentrations, contact times and applications for all disinfection requirements. New disinfectants should not be introduced without consulting the School Safety Officer or HSD.

10.1.9 It is recommended the disinfection policy be consistent throughout a department, although certain types of work may need specific disinfectants. Information and instructions (the disinfection policy) must be given to all workers to ensure they know what disinfectant to use and how to use it.

10.2 Factors Affecting the Choice of Disinfectant

A disinfectant should be chosen taking account of the following factors:

10.2.1 **The microorganisms to be destroyed:** Disinfectants do not generally kill all the organisms with which they come into contact and vary in efficacy. A disinfectant which is effective against bacteria may not be as effective against viruses. Some disinfectants are more effective against Gram positive than against Gram-negative bacteria, whilst others have a wide spectrum of performance against many organisms. Manufacturers of disinfectants should provide advice on the activity of their particular products.

If the types of organism in samples or materials handled are unknown then a general-purpose disinfectant should be chosen.

10.2.2 **Whether the disinfectant is going to be used in a clean or dirty situation:** The presence of other materials in or on the surfaces to be disinfected can have inhibitory effects on the disinfectant.

The concentration of disinfectant to be used is likely to vary depending on whether it is used in “dirty” or “clean” conditions, used routinely or in the event of accidents, etc.

10.2.3 **The nature of the surfaces and equipment to be disinfected:** Some disinfectants will chemically attack items being disinfected. Strong acids and halogen active disinfectants can pit stainless steel. Plastics may be affected by disinfectants containing organic solvents. Various metals may be attacked by strong acids or alkalis, halogen active substances, or disinfectants containing electrolytes. Manufacturers should provide advice on the suitability of using their products on particular surfaces or materials.

10.2.4 **The problems in using the product:** Under COSHH Regulations a risk assessment must be made for disinfectants. Whether concentrated or diluted, disinfectants must be handled so as avoid splashing, and goggles and gloves should be worn.

Many disinfectants have toxic properties and some are also highly corrosive causing damage if they come into contact with skin or eyes. Some disinfectants eg. glutaraldehyde and hypochlorites have irritant and allergic properties and so can cause respiratory problems if used in poorly ventilated areas. Some disinfectants may react with other chemicals causing hazardous gases.

Some products have cleaning properties in addition to the disinfecting capacity. When using a disinfectant such as Virkon for disinfecting instruments or equipment that have been in contact with blood and tissue, the cleaning effects allow for better overall disinfection.

Alternatives to glutaraldehyde and glutaraldehyde containing products must always be sought. In addition, glutaraldehyde may fix any tissues present.

10.3 Making Up Working Dilutions

Disinfectants are usually provided in concentrated form and have to be diluted in water to the working strength for use. The manufacturers instructions should be followed to ensure that the required concentrations are achieved. Over dilution will render the disinfectant ineffective. Once made up the disinfecting capacity of diluted products tends to deteriorate. How long made up solution can be stored must be noted in the disinfection policy.

Some products contain coloured indicators to show effective disinfecting capacity. If the disinfectant in use does not contain an indicator then a “use by” or expiry date should be clearly marked on the bottle.

10.4 Contact Times

Manufacturers should recommend contact times (in combination with product concentration) for various applications and this should be clearly stated in the disinfection policy.

10.5 Discard Jars

10.5.1 A discard jar is a container of disinfectant into which contaminated items are placed for disinfection.

10.5.2 A suitable disinfectant should be chosen depending on the nature of the work and appropriate dilutions prepared. It is usually convenient for the discard jar, when freshly made up, to be approximately half full to allow for displacement. If liquid waste is to be added to the disinfectant (eg aspiration) then the initial concentration should be proportionately increased to ensure the final concentration after additional waste liquids are added does not drop below the effective disinfection concentration.

10.5.3 Items placed in discard jars must be completely submerged in the disinfectant. All surfaces of the item should come into contact with the disinfectant. Items must remain in the disinfectant for at least the manufacturer’s recommended contact time and preferably overnight before disposal. The disinfectant can then be washed down a sink (not a hand wash basin) through a sieve or colander. The disinfected solids can then be disposed of for incineration in sealed plastic bags along with other laboratory waste

10.6 Types of Disinfectant

10.6.1 The following types of disinfectants are recommended for use in containment laboratories. Key points to be taken into account when selecting the disinfectant are given. Manufacturers instructions should be consulted for suitable concentration and contact times and further details of applications. COSHH risk assessments should provide clear guidance on handling precautions needed particularly when using concentrates.

10.6.2 **Phenolics**

Examples: Hycolin, Stericol, Clearsol

- Wide range of bactericidal activity
- Good fungicidal activity
- Poor activity against spores
- Variable virucidal activity - usually poor against non-enveloped viruses
- Not readily inactivated by organic matter
- Contain detergents

- Concentrates are stable but stability is reduced on dilution
- Agent of choice for mycobacteria

10.6.3 **Hypochlorites**

Examples: Sodium hypochlorite, Chloros, Presept

Only use HOUSEHOLD BLEACHES if you are sure of the initial concentration of available chlorine.

- Wide range of bactericidal, virucidal, and fungicidal activity
- Sporicidal
- Rapid action
- Inactivated by organic matter, particularly if used in low concentration
- Corrosive to some metals
- Incompatible with cationic detergents
- Chlorine gas released when mixed with strong acids
- Carcinogenic products produced when mixed with formaldehyde
- One of disinfectants of choice for use against HIV and hepatitis B viruses

10.6.4 **Alcohols**

Examples: Ethanol, Methanol, Propanol, Industrial Methylated Spirits (IMS)

- Good bacterial and fungicidal activity
- Not sporicidal
- Ethanol effective against viruses (less so non-enveloped viruses)
- Only recommended for limited use (such as on clean surfaces and for flaming forceps etc) - seek alternative wherever possible
- Poor penetration into tissues
- Propanol not effective against viruses
- Rapid action
- Alcohols must be diluted (ethanol 70%, propanol 60%) before use (100% alcohol is not an effective disinfectant)
- Should only be used on physically clean surfaces as poor penetration of organic matter
- Highly flammable – therefore volumes should be restricted

10.6.5 **Aldehydes**

Examples: Formaldehyde, Glutaraldehyde, Cidex

Formaldehyde and glutaraldehyde have irritant and toxic properties. Therefore these types of chemical disinfectants should not be used as general disinfectant and only be employed for specialised uses when no suitable alternative is available.

Glutaraldehyde based disinfectants were once commonly used for disinfection of instruments that cannot be heat sterilised. Alternatives to glutaraldehyde and glutaraldehyde containing products must always be sought.

The use of formaldehyde should be limited to gaseous fumigation for disinfection of, for example, microbiological safety cabinets and laboratories. Suitable alternatives are available for the majority of other applications.

10.6.6 **Peroxygen compounds**

- Wide range of bactericidal, virucidal and fungicidal activity
- Very low toxicity to humans
- Requires expensive specialist equipment to generate a fumigant

10.6.7 **Virkon**

Due to its wide spectrum of activity, suitability for use in most applications, the pink indicator to show disinfectant capacity and the low toxicity to users, Virkon is recommended as the disinfectant of choice for most University laboratory applications.

However, users should be aware that it might cause surface pitting on low-grade stainless steel.

10.7 Other Disinfectants

There are many proprietary products available that are sold as disinfectants. The ones described above are suitable for general use in the laboratory for disinfecting surfaces and equipment etc. Manufacturers should clearly specify the types of applications their product is useful for. Products sold as skin disinfectants eg. Hibiscrub, Hibitane, Betadine, pHiso-med, Cidal etc should not be used as a general laboratory disinfectant.

10.8 Skin disinfection

There should be no need for workers in laboratories to routinely disinfect their hands. Skin disinfectants are for use in clinical settings. All workers should wash their hands regularly whilst working in the laboratory, and always before leaving. Ordinary soap is suitable for this. If liquid soaps are used in containment laboratories they should contain

a bacteriostatic agent to prevent the multiplication of any contamination (many laboratories choose to use products such as Labguard). Cloth towels should not be used in laboratories. Single use paper towels are recommended.

11 Autoclaves

11.1 Operation of Autoclaves

11.1.1 Do not operate or use any autoclave unless you have been trained and instructed in its specific use.

11.1.2 Check the operator instructions. If there are none, ask. Operation instructions should include details of action to be taken by the operator in the event of a fault or any abnormality in autoclave performance. Check that you know what to do in the event of a fault or malfunction. Simple, easy to understand instructions should be provided in addition to the detailed manual provided by the manufacturer, ideally located beside the autoclave.

11.2 Maintenance

11.2.1 The continuing safe and effective use of the autoclave depends on a programme of planned maintenance throughout its life. Before use ensure the autoclave has been tested within the required period. (Usually indicated by a certificate on the machine.)

11.2.2 When a fault occurs during a make-safe cycle an assessment of risk should be made and appropriate action taken. It may be necessary to disinfect those chamber attachments on which engineering work is to be carried out. During a make-safe process, chamber condensate should be considered to be contaminated with viable microorganisms.

11.2.3 A contaminated laboratory autoclave should never be returned to the manufacturer for servicing or repair.

11.3 Protective Clothing

11.3.1 A laboratory coat of side or back fastening style should be worn in the autoclave loading/unloading area.

11.3.2 Additionally, an impervious apron, heat-resistant gauntlet gloves, suitable heavy-duty footwear or overshoes and a full-face visor should be available for the operator. The hazards on loading include spills of biohazardous material, broken glass and dropped load contents.

The hazards on unloading include splashes and spillage of hot material from the load, hot condensate, hot equipment, broken glass, dropped load contents. Vapours from volatile chemicals may be a hazard if they have been inappropriately put into the autoclave.

11.4 Loading the Autoclave

All materials awaiting autoclaving should be stored safely and securely, particularly biologically hazardous waste awaiting inactivation.

Items must be packed in a way which ensures that steam will penetrate the load.

Ensure racks of items to be autoclaved are of appropriate size and weight to minimise manual handling problems.

11.5 Unloading the Autoclave

11.5.1 Temperature and pressure indicators and warning lights must be checked to ensure that the autoclave has successfully completed the operating cycle. If a fault is indicated, attempts to open the autoclave must only be made with the authority of the person competent to do so.

11.5.2 It is dangerous to attempt to release the autoclave door mechanism before the chamber is vented to atmosphere or whilst the load contents are at high temperature. No attempt should be made by the operator to override door interlocking safety devices.

11.5.3 The operator should stand clear when opening the door as hot liquid or vapour may escape from the chamber. The operator should also be aware that containers of liquid could be pressurised and may explode, volatile liquids may produce harmful vapour and liquids spilled on unloading may cause scalding. After autoclaving, discard containers should be emptied in a safe manner and their contents disposed of or reclaimed as specified by local laboratory rules.

11.6 Discard Containers

11.6.1 Discard containers should be easily transportable, leak-proof and of a robust design with solid sides and bottoms. They should allow adequate steam penetration to the contents.

11.6.2 If autoclave bags are used they should be supported in a discard-container in the laboratory and in the autoclave. The bag should be open during the autoclave cycle so that steam can penetrate its contents. It is recommended that transparent (rather than opaque) autoclave bags are used as this allows the autoclave operator to see if any inappropriate materials have been placed in the bag by mistake.

11.7 Operating Cycles

11.7.1 The operating cycle should take account of heat up times. The time required for the load to reach sterilizing temperature will have been determined during validation tests.

11.7.2 **Liquid sterilization**
When liquids are sterilised the possibility of adverse effects on the liquid caused by the heat treatment must also be taken into account when selecting a cycle. The operating cycle should be selected to ensure that sterilisation is achieved with minimum damage to the liquid. Microbiological culture media are particularly heat sensitive; the degree of deterioration is related to the length of time the medium is maintained at sterilising temperature; the heat-up and cooling stages also contribute significantly to this deterioration.

11.7.3 Heat-up times should be as short as possible, achieved by uniformly filling the chamber with steam at sterilising temperature. Large volumes of fluids will heat up slowly, therefore volumes of liquids should be kept small; a maximum container volume of 500ml is recommended, larger volumes taking considerably longer to heat up (and cool down).

11.7.4 Cooling loads quickly helps to protect heat-sensitive constituents and shortens operating cycle times. Air at high pressure may be admitted to ballast the chamber, minimise boiling and prevent bottles exploding.

11.7.5 Containers should be loosely capped unless they are specifically designed for sealing. However, sealing bottles can increase the likelihood of explosion during autoclaving and slows cooling.

11.8 Autoclave Performance

Validation must have been carried out after commissioning or recommissioning before the autoclave is put into service.

12 Preventing Injuries from Sharps

12.1 In containment laboratories where the microorganisms being handled infect via the percutaneous route the use of sharps and glass items should be avoided wherever possible. Injuries which carry a risk of work-related infection should be notified to the University Occupational Health Service as soon as possible, as well as reported to the HSD in the usual way.

12.2 Examples of common accidents and suggestions for their prevention include:

(i) Glass injuries sustained while inserting pipettes into pipetting aids or Pasteur pipettes into teats; attaching or removing glass to or from rubber or plastic tubing; removing “frozen” stoppers from glass bottles; breaking glass tubing; handling or washing up broken glassware.

These injuries may be largely avoided by instruction in correct technique and by ensuring glassware is in good condition (without chips or cracks) before use. Broken or chipped glassware should be thrown away. Plastic alternatives should be used wherever possible.

(ii) Cuts from scalpel, razor or other blades, sustained while cutting plastic tubes or tubing; opening packages; scraping off adhesive labels.

These injuries frequently occur because of misuse of scalpel or razor blades, which are not designed for any of these tasks. The correct tool should be chosen for the job in hand; easily peeled-off labels are available and avoid the need for scraping.

(iii) Cuts sustained while changing scalpel blades or because of inappropriate disposal.

These injuries may be prevented by use of the correct techniques, so appropriate instruction and training should be given to users.

(iv) Needlestick injuries

These occur because of accidental injection or incorrect disposal. Once again, users should be suitably instructed and trained in the use of syringes and needles. Needles must not be resheathed unless a specific risk assessment has determined that this is the safer alternative.

13 Fumigation of Laboratories and Microbiological Safety Cabinets

13.1 Microbiological safety cabinets must always be fumigated before filters are changed or before any maintenance work is carried out which involves gaining access to the interior of the cabinet (for example air ducts) and when there is spillage of infectious material that results in significant contamination of the cabinet surfaces, filters etc. On occasions it may also be necessary to decontaminate laboratories or animal containment facilities when, for example, there has been a major spillage of infectious material or when certain servicing or maintenance work is to be carried out.

13.1.2 Fumigation with formaldehyde vapour is presently the recognised method for these types of fumigation procedures. Formalin is a commercially available 40% solution of formaldehyde vapour in water. When formalin is heated, formaldehyde vapour is generated in quantity.

The School Safety Officers or HSD must be notified in advance of any fumigation operation other than that of microbiological safety cabinets.

13.1.3 Formaldehyde currently has a Maximum Exposure Limit (MEL) short and long term of 2 ppm or 2.5 mg.m^{-3}. Concentrations encountered during fumigation are many hundreds of times higher than this. Fumigation operations must only be carried out by trained personnel under strictly defined conditions to avoid anyone being exposed at all and in no case to exceed 2ppm. All workers using formaldehyde must be aware of safe handling procedures. In the event of accidental exposure to formaldehyde immediate medical advice should be sought from the University Occupational Health Service.

13.1.4 Under certain conditions formaldehyde can react with hydrochloric acid and chlorine-containing disinfectants such as hypochlorites to form bis (chlormethyl) ether, a potent lung carcinogen. hydrochloric acid and chlorine-containing disinfectants must therefore be removed (or safely disposed of in the case of Containment Level 3 facilities) from rooms and microbiological safety cabinets before fumigation.

13.1.5 Formaldehyde vapour is an extremely effective biocidal agent. A number of factors effect the efficiency of fumigation. For formaldehyde to act to maximum effect it must be able to penetrate (pre-cleaning is helpful if it can be done without jeopardising safety) and it must be able to dissolve at adequate concentrations in a film of moisture in the immediate vicinity of the organisms to be inactivated (water vapour generated in the process of dispersing formaldehyde provides the essential optimum level of relative humidity). Too much formaldehyde results in the deposition of sticky deposits of paraformaldehyde and in cabinets may contribute to filter blockage. The amounts of formalin and water required for fumigation are given in the more detailed sections below. Fumigation is most effective above a temperature of 20ºC and relative humidity of 65%. At temperatures below 18ºC formaldehyde fumigation is less effective. Below 9ºC, formaldehyde sublimes and is less easy to vaporise.

13.1.6 Agents causing the transmissible spongiform encephalopathies are resistant to inactivation by formalin. Workers handling these types of agents must ensure they are aware of the appropriate method of inactivation for prions before starting any work.

13.1.7 There are a number of methods of generating formaldehyde vapour:

i) heating a mixture of formalin and water in a thermostatically

controlled heating unit or a purpose-made vaporising unit;

ii) using commercially available formaldehyde generating kits; or

iii) mixing formalin and water with potassium permanganate crystals*.

*WARNING: the correct relative concentration of these two components is essential to avoid a violent reaction. It is therefore recommended that this method is not used.

13.2 Fumigation of Microbiological Safety Cabinets

13.2.1 Microbiological safety cabinets, if they have been used for hazardous microorganisms, must be fumigated in the following circumstances:

i) after a major spillage or a spillage where inaccessible surfaces have been contaminated;

ii) before any maintenance work on the cabinet where access to potentially contaminated parts is necessary (including filter and pre-filter changes);

iii) before carrying out filter penetration tests; and

iv) when there are any changes in the nature of the work that result in significantly different risks.

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

13.2.3 Where the cabinet has been used for hazardous microorganisms HEPA filters must only be handled with appropriate protective clothing (laboratory coat and heavy duty gloves) even after fumigation. Such filters must either be autoclaved prior to disposal in the general waste stream or be securely wrapped in yellow bags for disposal as clinical waste.

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

13.2.5 The fumigation procedure should ensure inactivation of any microorganisms that have penetrated the HEPA filter by adequate exposure of the downstream side of the HEPA filter and the ductwork to formaldehyde. In the absence of specific recommendations by the cabinet manufacturer the cabinet fans should be turned on for 10-15 seconds after about half the formalin has been evaporated and again after evaporation is complete. Passive migration of the fumigant through the filter can occur but this is not ideal. Some cabinets have automatic fumigation cycles programmed into the controls and in these instances you must follow the manufacturers instructions closely.

13.2.6 The formaldehyde should be left to disperse within the cabinet for at least six hours (or preferably overnight) after which time the fumigant should be exhausted to outside by switching on the fan and allowing air from the room to enter the cabinet. Before venting the formaldehyde in this way it is essential to ensure that no one is in the vicinity of the exhaust outlet and that the exhaust air does not enter nearby windows or ventilation air intakes.

13.2.7 Following decontamination purge the microbiological safety cabinet of all residual formaldehyde. If using a recirculation type cabinet a fumigation adaptor kit should be fixed over the cabinet exhaust to allow discharge of fumigant via the flexible trunking into either a ducted cabinet or fume cupboard. Care must be taken not to discharge formaldehyde back into the laboratory. Under no circumstances should recirculating microbiological safety cabinets be used unless there is a safe means for discharging the formaldehyde after fumigation. Formaldehyde must not be recirculated into the laboratory.

13.2.8 The quantity of formalin required is approximately 2 ml per cubic foot

(0.028 m^3) internal air space. Typical quantities of formalin required for fumigation of the different types of cabinets are:

Cabinet Type Amount of BP Formalin
Class II (1200 wide) 25 ml
Class II (900 wide) 20 ml
Class II (1800 wide) 30 ml
Class I 20 ml
Class III 20 ml
Class I/III Hybrids 20 ml

You must post a large notice on the front of the cabinet to warn that it is being fumigated. This notice must be secured in a suitable way to ensure that it cannot be dislodged or obscured. Sealing the front panel and covering the release catches with tape will further indicate the state of prohibition of use.

13.3 Fumigation Procedure

Follow the manufacturers detailed instructions for fumigation of their particular cabinets. 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. The following is a summary of the main steps:

13.3.1 Switch off the cabinet fans.

13.3.2 If the cabinet is a recirculation type, fit the fumigation adaptor kit to the discharge and close the manual shut-off damper.

13.3.3 Fill the vaporiser with the correct amount of formalin and screw on the cap finger tight, having checked the gasket in the cap is undamaged. Place the vaporiser in the cabinet.

13.3.4 Fit the closure panel/night door and fully seal the front screen and closure panel with suitable tape. (eg 50mm wide PVC black and yellow stripped hazard tape)

13.3.5 Post a notice on the front of the cabinet indicating fumigation is in progress.

13.3.6 Switch the vaporiser on.

13.3.7 After approximately 10 minutes switch the cabinet fans on for 10-15 seconds.

13.3.8 After a further 20-30 minutes switch the cabinet fans on again for 10-15 seconds.

13.3.9 Leave the cabinet in this condition preferably overnight, but for a minimum of 6 hours.

13.3.10 If the cabinet is a recirculation type check the exhaust of the fumigation adaptor kit is in a position to discharge safely and open the manual shut-off damper.

13.3.11 Before venting the formaldehyde check no one is in the vicinity of the exhaust outlet and the gas will not enter any open windows nearby.

13.3.12 Exhaust the formaldehyde from the cabinet by switching on the fans and opening the closure panel/night door slightly (remove bung if fitted or crack open for approximately 5 minutes before removing door completely.

13.3.13 Remove the vaporiser. Any poly-formaldehyde residue in the vaporiser may be removed by heating with water containing a little mild detergent.

13.3.14 Run the cabinet for at least a further 15-20 minutes to remove the last traces of formaldehyde.

13.3.15 If the cabinet is a recirculation type the fumigation adaptor kit must be removed before the cabinet is used again.

13.4. Fumigation of Laboratories

13.4.1 Formaldehyde fumigation of laboratories or other rooms must only be carried out by named trained personnel. The School Safety Officer or HSD MUST be notified in advance of any such fumigation operation.

13.4.2 Before commencing fumigation the laboratory/room must be completely sealed to prevent escape of formaldehyde vapour into other areas. The door must be locked to prevent anyone entering the room until the fumigant has been exhausted.

Under no circumstances should anybody be allowed to enter a room whilst fumigation is in progress unless they are wearing full breathing apparatus which provides air from an independent source.

However, no member of staff is currently equipped for this task and therefore such an event would require the intervention of the emergency services. [N.B. Cartridge respirators are not appropriate for use in the concentrations of formaldehyde generated during fumigation.]

Containment Level 3 laboratories are designed so that they are easily sealable for fumigation with minimal effort. In other laboratories/rooms that have not been designed to facilitate fumigation it will be necessary before commencing fumigation to seal service ducts and other holes through which fumigants may escape. If the laboratory has a false ceiling, the void above must be checked to ensure that there is no interconnecting ducting to other areas and if there is these must be effectively sealed.

13.4.3 As nobody must enter the area, there must be a means of exhausting the formaldehyde vapour from the laboratory/room which avoids the need to enter the area. The extract must be a total loss system with no possibility of formaldehyde being ducted to other areas. Most common is the use of a ducted microbiological safety cabinet to exhaust the fumigant to outside. The recommended method is to have engineering controls (switch) external to the room. The School Safety Officers or HSD must be consulted before any other method is adopted. It should also be possible to operate without entering the room any dampers that may be installed if their opening is necessary to ventilate the room.

13.4.4 Calculations should be undertaken to estimate how long it will take to purge all the formaldehyde from the room after fumigation is complete. This should be based on room volume and the rate of air extraction through the particular exhaust system that will be used.

The calculation should also take account of the initial formaldehyde concentration in the room and the need to reduce it to well below 2 ppm. The following formula can be used:
room volume (m^3)
time (mins) = x 25
extraction rate (m^3/min)

13.4.6 If a microbiological safety cabinet is being used to ventilate the room check the exhaust rate with the particular manufacturer. A class II cabinet typically exhausts approximately 125 l/sec (to convert l/sec to m^3/min multiply by 0.06).

13.4.7 After fumigation the level of formaldehyde remaining in the air MUST be below 2 ppm (2.5 $mg.m^{-3}$) before staff are allowed to re-enter the room. The level of formaldehyde should be checked with either a hand-held direct reading instrument (such as a formaldemeter) or appropriate air sampling tubes. The person entering the room to check the formaldehyde levels MUST wear a laboratory coat, gloves and a full-face cartridge respirator.

13.5 Fumigation Procedure

An outline of the main principles of fumigation has been provided above. The following is a summary of the main steps:

13.5.1 Before commencing fumigation switch off all forced air ventilation systems, extract systems and any fume cupboards and microbiological safety cabinets in the room.

13.5.2 Check that the remote switch that will be used to exhaust the formaldehyde from the room is working (run the extract this way for at least 5 minutes then switch off).

13.5.3 Enter the room, wearing a laboratory coat and disposable gloves, equipped with the sealing materials, the appropriate quantities of formalin (see below) and the heater. A second person must remain outside as an observer in case assistance is required.

13.5.4 The following sequence of steps should be taken where relevant:

i) close the door;

ii) apply the appropriate disinfectant to any obvious spillages;

iii) deactivate fire alarm system smoke detectors by covering with a plastic bag and tape;

iv) seal the window frames with suitable tape (eg 50mm wide PVC black and yellow stripped hazard tape) unless they are known to be well fitting;

v) cover any ventilators with polythene sheets and suitable tape;

vi) close fume cupboard sashes. Safety cabinets should be left open to allow entry of the fumigant in the room;

vii) check that the point from which the formaldehyde will be exhausted is free from obstruction;

viii) place the appropriate quantities of formalin and water mixture into the heater unit. Use 100 ml formalin plus 900 ml water per 28.3 m^3 (1000 ft^3) of space; and

ix) activate the heater and leave the room **immediately**.

13.5.5 **lock the door** and effectively seal around the edges with suitable tape (eg 50mm wide PVC black and yellow stripped hazard tape).

13.5.6 Display a notice in large type on the door such as the following:

"DANGER: ROOM FUMIGATION IN PROGRESS. DO NOT OPEN"

Other warning signs may be required in the surrounding areas.

13.5.7 After a period of not less than 12 hours (the procedure is best carried out overnight), the room must be well ventilated. Purge the space by using the remote switch to activate the extraction system (open dampers if necessary).

13.5.8 Allow the room to purge for at least the time calculated to be necessary to remove the formaldehyde.

13.5.9 Check levels of residual formaldehyde in the room with suitable air monitoring equipment (formaldemeter of air sampling tubes). Enter wearing a laboratory coat, gloves and a full-face cartridge respirator.

13.5.10 Check the room and all surfaces for formaldehyde residues and clean up as necessary.

13.5.11 Remove all sealing materials.

13.5.12 Only allow other staff to enter the room when formaldehyde levels are below 2 ppm (2.5 $mg.m^{-3}$). Levels should be as low as practicable before staff re-enter but must be below 2 ppm.

13.5.13 A record must be kept of all fumigations of rooms/laboratories including date, personnel involved and the results of air monitoring on completion of the procedure.

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