Guidance notes

These Notes provide guidance on aspects of work with biological agents. Several contain **MRC Codes of Practice (CoP)**. Those containing a CoP are marked with ***** in the index below. Where a **CoP** is specified, establishments are expected to achieve and maintain those standards, which are set as the minimum acceptable for carrying out the related work. The associated guidance will aid the achievement of MRC standards and ensure the work meets all related legal requirements.

In all **MRC CoPs**, **'must'** indicates a mandatory requirement whereas **'should'** reflects a strong recommendation. Statements using **should** relate to the required standard and any modification must be justified through the process of a suitable and sufficient risk assessment.

Where appropriate the content of these notes can be adopted as local codes of practice and referred to by risk assessments. Establishments are encouraged however to adapt the content for local needs, with the strict provision that local standards are at least as high as those stipulated in this guidance.

Part A The management of work with biological material

Guidance note 1 a) The appointment and duties of a Biological Safety Officer: b) The formation and remit of Biological Safety Committees

Guidance note 2 Laboratory management

Guidance note 3 Risk assessments and related procedures - overview

Guidance note 4 Use of blood from volunteer staff, including use of 'own' cells*****

Part B Working with biological hazards

Guidance note 5 Working with human pathogens*****

Guidance note 6 Working with transmissible spongiform encephalopathies*****

Guidance note 7 Working with genetically modified organisms

Guidance Note 8 Working with cell cultures

Guidance Note 9 Working with human material:
1. Code of practice and guidance for laboratory work*****
2. Guidance for the taking of blood from volunteers in the community.

Guidance Note 10 Working with animals

Guidance Note 11 Working with animal and plant pathogens

Part C Key laboratory equipment and associated practices

Guidance Note 12 Microbiological safety cabinets*****

Guidance Note 13 The Use of Centrifuges in biological containment laboratories

Guidance Note 14 Decontamination and disinfection*****

Guidance Note 15 Disposal of biological waste*****

Guidance notes

Part A

The management of work with biological material

MRC Guidance Note 1

(a) The appointment and duties of a Biological Safety Officer:

Appointment of a Biological Safety Officer

The responsibility for safety for work with biological agents rests with the unit Director or ESS team leader. To assist with the discharge of this responsibility, the Director must appoint a Biological Safety Officer (BSO) and, where appropriate, a deputy BSO. In certain circumstances (e.g., where no work with GMOs is done and the amount of work with biological agents is minimal or a low risk work activity) the unit safety co-ordinator or adviser may undertake the duties of the BSO.

Establishments working with genetically modified organisms (GMOs) must appoint a biological safety officer specifically for this work.

These appointments must be made in writing. The letter of appointment should specify the duties required by selection from or referral to the duties listed here. Where it is not wholly or part of a full-time appointment, it is recommended that it be made for a fixed period, say three to five years. This allows both parties to review their position at the end of this period.

Whatever arrangements are made locally, the BSO is appointed as a 'competent person' and therefore must be provided with sufficient resources, including time, to carry out their duties satisfactorily.

Larger ESS teams may wish to make similar arrangements as Units. It is anticipated however that whereas the responsibility for assessing risks remains with the team leader, ESS teams will make arrangements with their host institution for their work with GMOs to be approved by the host's biological safety committee.

Duties of the Biological Safety Officer

Health and safety management guide

An outline of the duties of a BSO can be found in the Management Guide, alongside similar sections relating to other health and safety appointments.

Main purpose

To advise the Unit Director on all matters relating to the use of biological agents within that establishment.

Training and competence requirements

The BSO should have experience in working with biological agents in a containment laboratory and, where applicable, a working knowledge of the assessment of risks for work with genetically modified organisms. The BSO must be conversant with MRC policy and guidance on work with biological agents and where appropriate with that of the host

institution. Newly appointed BSOs are expected to attend the 2-day training course provided by the MRC.

Duties

The duties listed can be divided for convenience into executive and advisory functions. The letters of appointment should make clear where any executive authority is given to a named individual member of staff.

- To ensure, in collaboration with line managers, that MRC policy and, where applicable, the rules and procedures of the host institution relating to work with biological agents are fully implemented in the establishment;
- To ensure that consent as required by the relevant legislation is obtained from the Health and Safety Executive for work proposed with genetically modified organisms;
- To ensure that licences as required by the relevant legislation are obtained from the proper authorities for work proposed with specified animal or plant pathogens;
- To ensure, with the other members of the local biological safety committee, that all notifications of work with genetically modified organisms are made as required.
- To advise on risk assessment for all proposed work with biological agents and the development of codes of practice;
- To advise on waste disposal policy and arrangements;
- To advise on disinfection policy;
- To prepare contingency plans for action following accidents and incidents involving biological agents;
- To advise and assist management in investigations following accidents and incidents involving biological agents;
- To carry out periodic inspections of containment facilities;
- To assist team leaders in assessing training needs for those working with biological agents.

(b) The formation and remit of biological safety committees

Biological Safety Committee set up under the Genetically Modified Organisms (Contained Use) Regulations

Remit

Each Unit that intends to carry out any activity involving genetic modification is required to set up a Genetic Modification Safety Committee (GMSC). The only statutory purpose of GMSC is to advise on the suitability of risk assessments associated with proposals made by those who will be doing the work (the project proposer). The Compendium of Guidance (CoG) from the Advisory Committee on Genetic Modification (ACGM) does suggest certain duties for the committee but these are beyond the strict legal requirements.

It is likely therefore that the role of the GMSC will be to help ensure that all of the issues relating to carrying out the proposed work safely are properly considered in order to determine safe working practices.

To achieve that aim the duties of the committee may include:

- To ensure that the facilities are adequate for the purpose
- To ensure that the equipment is adequate and properly maintained
- To provide correct training
- To draw up local rules and procedures
- To draw up emergency plans

Constitution

Neither the regulations themselves nor the ACGM CoG note on safety committees specify precisely the constitution of a GMSC. We expect however each GMSC to contain sufficient expertise and representation of staff members to enable proposed work to be suitably assessed for possible risks to health and to the environment and to meet legal obligations.

The composition of local GMSCs will thus vary from centre to centre but may include representatives with some or all of the following functions:

1. A Chair preferably elected by the committee and carrying the Director's authority.
2. A representative of management with responsibility for work involving genetic modification.
3. A representative of other staff involved in such work or having access to the genetic modification facilities.
4. The Biological Safety Officer
5. A representative of the General Safety Committee to act as liaison.
6. Co-opted members to supplement internal expertise where necessary. These may be from another department, laboratory or outside body.

The membership structure of the GMSC should be notified to the HSE as part of the notification of first use of the premises for genetic modification activities. Actual names do not have to be forwarded.

Relationship with the host

Where small units are located within a department of a host institution it may be preferable to process risk assessments and notifications through the host GMSC. It remains the responsibility of the Unit Director or the ESS Team Leader to ensure that assessments are made for all MRC projects and approved by the host committee. To that end it is expected that the Unit or team would seek for a member of MRC staff to sit on the committee or, as a minimum, be invited to attend all meetings where MRC proposals are discussed.

General biological safety committee

Larger institutes and units may wish to set up a biological safety committee (BSC) to oversee all work with biological material. This is not a legal requirement, but would enable the monitoring of work with, for example, human pathogens, clinical material and cell and tissue culture. The membership may be the same as or drawn from the GMSC or be independent of it.

Duties could include:

- Ensure that all risk assessments are in place as required
- Ensure facilities are suitable for the work
- Ensure personnel are trained
- Monitor working standards

It is recommended that reports of the work of any committee reviewing work with biological material, both the GMSC and general BSC, are made to the general safety committee.

MRC Guidance Note 2

Laboratory management

Laboratory design and function

Details on laboratory design and function for laboratories handling biological agents can be found in the Health and Safety Commission/Department of Health (HSC/DH) publication **'The management, design and operation of microbiological containment laboratories'** [1].

Important considerations are:

- The quality of the flooring and other main surfaces
- The quality of the work surfaces
- The location of the equipment within the laboratory or laboratory suite
- The provision of services
- The control of temperature and lighting
- Appropriate access to decontamination, including autoclaving

For containment level 3 facilities, full guidance for designers and engineers is contained in the joint MRC/Imperial College London document **'Standards for Containment Level 3 Facilities: New Builds and Refurbishments'**.

All projects involving new building, refurbishments or change of use must be referred to the MRC Health, Safety and Security Section. For major projects this will be done through the Estates Management Section. For those units with full-time qualified unit safety co-ordinators, those appointees will provide immediate advice with further input from the corporate section as is appropriate.

Biohazard signs

Biohazard signs should warn of genuine infectious hazards. These must always be placed at the entrance to level 3 facilities, and should be used at level 2, either where a whole room is dedicated for work at that level, or to mark an item of equipment used for storage of biohazards within an otherwise level 1 area.

Access control

The control of access to the laboratory and the security of samples are of prime importance to the safe management of laboratories. The higher the level of containment the greater the control should be. Management of access control is, like any aspect of working with hazardous substances, subject to the hierarchy of control. Thus the first control measure applied to work that may expose workers to hazardous biological material is to reduce the number of people exposed to as low as is practicable.

At containment level 1 no formal system of authorising personnel is required due to the fact that by definition group 1 organisms are unlikely to cause human disease. However, it would be prudent to limit access to laboratory areas to laboratory staff so as to reduce the

risk of an accident occurring due to a non-biological hazard. Administrative and other non-laboratory staff should not normally access any laboratory.

Restricted access should be operated at containment level 2. Doors must always be kept closed while work is in progress; visitors should be given protective clothing and safety instructions as necessary; cleaners and service engineers must only be allowed to enter if safe to do so.

At containment level 3 access must be only for authorised competent persons who have written protocols for their tasks which have been approved. When not in use the facility must remain locked. Cleaning must be done by trained staff who have need to use the facility for their Hazard Group 3 work. Servicing of equipment may only take place by authorised personnel and only when safe to do so, after appropriate decontamination measures have been taken and the area certified safe. Items taken out of containment level 3 facilities must be suitably decontaminated first and a certificate signed for any equipment to be sent for repair.

Control of access to containment level 2 and 3 for maintenance and service personnel should be through a permit to work system. Under the new Anti-terrorism, Crime and Security Act 2001 the Police have powers to insist on additional security measures for work with pathogens as defined in Schedule 5 of the Act.

Care should also be taken to ensure that adequate control is taken over contractors who may be required to enter the laboratory or break into 'services' such as water or waste. It is important to remember the person in charge of the laboratory has responsibility to check that an appropriate risk assessment has been carried out, that adequate supervision is provided and that safety precautions are taken.

Security of samples is also an issue that should not be overlooked and samples should therefore be capable of being secured.

Standards of work

Hygiene

High standards must always be maintained. In addition to the no eating, drinking, smoking or chewing rule for work carried out at all levels of containment, the licking of labels and sucking of pencils must be avoided at containment levels 2 and 3. Touching the face with unwashed hands must also be avoided and sitting or leaning on work surfaces is discouraged.

Hands must be washed after handling microbiological material and always before leaving the facility. If necessary, for protection from other hazards such as toxic chemicals or radioactive materials that are required to be taken out of the laboratory (where permitted after decontamination), then a clean pair of gloves should be used. Any lesions on hands must be protected with waterproof plasters and gloves.

Managers should check that all working surfaces (and equipment, if appropriate) is cleaned and disinfected as soon as possible after the work session has been completed. Effective disinfectants must be available at all times and staff should be instructed in the correct disinfectant, its concentration and method of use for the micro-organisms they are using. Spillages must be dealt with promptly.

Protective clothing

Protective clothing must be worn in containment 2 or 3 facilities. The laboratory coat/gown should have a high neck and close fitting cuffs with either back or side fastenings. They

should be changed frequently and always immediately after any spill on to them. These coats/gowns must not be worn outside the facility, but changed for another as necessary.

Gloves of adequate resistance should be worn and the wearing of two pairs should be considered if there is a risk of gloves being torn or punctured when high-risk samples are handled and where the loss of dexterity does not prejudice personal safety.

A decision on the need for eye protection is dependent upon the risk assessment, taking into account the possibility of splashing and the possible routes of transmission.

Training

All staff working with biological agents should be trained to the required level of competence. Specific training requirements should be identified as part of the risk assessment process. Thus the prime responsibility for ensuring staff are trained remains with the team leader.

The important issue however is that personnel should only work in containment facilities if they have received training on all aspects of the work. For convenience the duty for training can often be delegated to the person responsible for the laboratory or equipment.

There should also be an appropriate system for assessing the competence of visiting workers (see also MRC Health and Safety Policy and Guidance on **Visitors to Council Establishments**).

Further details on general and specific training requirements can be found in the appropriate guidance notes.

Record keeping

Full details on record keeping can be found in the MRC Policy and Guidance document on **Record Keeping**.

Specifically for biological containment laboratories, the following requirements should be noted.

Premises

Records of notifications to HSE relating to use of pathogens, genetically modified organisms and animals.

Plant and equipment

Records of maintenance and testing of key equipment, e.g., centrifuges, autoclaves and safety cabinets.

Procedures and arrangements

Risk assessments, Codes of Practice, Standard Operating Procedures. Records of safety inspections and audits.

Personnel

Training records, health surveillance and monitoring, records of blood donations by staff, accident reports.

Occupational health

Unit arrangements for occupational health service provision will have been set up by unit administration and the advice of the occupational health service provider should always be followed. The laboratory manager should however be aware of specific requirements.

- All laboratory staff should be advised to ensure they are immunised against tetanus.
- All staff working with animals should be included in a surveillance programme, monitoring for sensitivity to animal allergens.
- Specific immunisations may be required, e.g., against Hepatitis B for those working with human blood. Immunisation however provides secondary protection, the primary protection being afforded through the correct use of equipment and through adherence to safe working practices. Thus, although the Occupational Health Provider may recommend that the efficacy of any immunisation should be checked, it does not necessarily follow that a persistent negative result will preclude the affected individual from doing the work.
- Records of exposure to certain agents are required; some for 10 years and some for 40 years (see later guidance for details)

Audits and inspections

Full guidance on local inspections can be found in MRC Health and Safety Policy and Guidance on **Local Safety Inspection and Monitoring**. This section summarises key points relating to audits and inspections.

Audits

Aims

An audit is a systematic review that paints a picture of how things are at a particular point in time. The picture can be compared against standards and also against previous reports as a measure of progress.

The aims of an audit are therefore to establish that:

- Appropriate management arrangements are in place
- Adequate controls exist, are implemented and are consistent with the risk
- Appropriate workplace precautions are in place

Biological laboratories should be subject to periodic audits to ensure that the systems put in place are actually working satisfactorily and areas for improvement are identified. It necessarily involves examination of records, risk assessments and procedural documents, e.g., codes of practice, as well as inspection and personal questioning.

Time-scale

The frequency of audits depends upon the technical complexity of the work process and the level of perceived risk of the process. Audits of high-risk processes would tend to be done more frequently than of management systems. An audit of overall management systems is only likely once every two to three years, whereas an annual audit of a containment level 3 facility may be appropriate.

Audit team

The better structured an audit team is, the better and more comprehensive the end result. A team approach involving managers, safety representatives and other employees is an effective way to widen involvement and co-operation with the scheme.

The audit team will need sufficient skills to collect and collate information on health and safety systems and procedures. They should also have an ability to make critical judgements on the adequacy of such information and the effectiveness of any safety procedures encountered during the audit.

Evaluating performance

The report should not aim to apportion blame to any failings that it may find as it could lead to the audit being seen as a 'fault finding activity'. It is important to dispel such perceptions from the very beginning and for the audit to be seen as a valuable tool that will help everyone in achieving the required standards. The audit should not only point out weaknesses but also strengths, allowing those audited to see in which areas they need to concentrate their efforts so that performance and efficiency can be raised.

Inspections

A system for inspecting workplace precautions is important in any active monitoring programme. The inspection programme should take into account any statutory obligations (such as COSHH) and the risk involved in conducting the work. The greater the hazard the more frequent the inspection rate should be. However, it is accepted that in most laboratories an inspection rate of twice per annum would be adequate. This may need to be increased for high-risk areas such as containment level 3 laboratories.

Checklists are a great help when conducting inspections as they act as an aide-memoir. Such checklists should not just cover the high-risk activities but all workplace risks including welfare items.

Inspection teams should comprise of both management and staff. Where there are safety representatives they should be part of the team. The inspection report should be seen by all involved including the staff and it should also be discussed at the relevant safety committee. Remedial action should be agreed and a deadline set for the work to be completed. The safety committee should see follow-up reports so as to monitor progress.

Emergency procedures

Risk assessment must include consideration of foreseeable emergencies. Where such an occurrence could lead to harmful exposure within the facility or unplanned release of a biological agent into the environment, the risk assessment must include the procedures to be adopted or refer to a code of practice. Examples are the procedures to be adopted for spillages inside and outside microbiological safety cabinets (MSC) and for the failure of equipment that may lead to loss of containment (e.g., the failure of an MSC or a centrifuge rotor or bucket).

References

1. The management, design and operation of microbiological containment laboratories. HSC/DH 2001. ISBN 0 7176 2034 4.

MRC Guidance Note 3

Risk assessments and related procedures – overview

Policy review

All work activities are subject to risk assessment. The MRC requires its team leaders to ensure that risk assessments are properly made and are suitable and sufficient. All risk assessments for work with biological agents should thus be made following this guidance read in conjunction with the **MRC Health and Safety Management Guide** and the Policy and Guidance on **'Risk assessment of work with chemical, biological and ionising radiation hazards'**. Specifically, all work with biological agents is subject to risk assessment under the COSHH Regulations[1]. Where a risk assessment is made under the GMO (Contained Use) Regulations[2] however, that part of the assessment does not need to be duplicated. It must be recognised however that the GMO risk assessment may not address all relevant issues, for example the toxicity of chemical reagents used in either the production or subsequent use of a genetically modified organism or the potential hazards of host cell cultures.

Review of risk assessment process

Principles of risk assessment relating to work with biological agents

Hazard identification

The definition of biological agent includes all pathogenic (i.e., capable of producing human disease) micro-organisms, cell cultures, or human endoparasites, as well as those agents that may cause allergy, toxicity or any other risk to human health. In practice, the list may extend to include animals (including transgenics) and the associated allergens, and macromolecules, including naked nucleic acid and prion agents.

Who will do the work and where is it proposed they do it?

An early stage of carrying out the risk assessment must be the consideration of who will actually do the work. In making the assessment you have to consider the competence of the staff and take into account relevant factors that may affect safe working. This may relate to physical features such as size and strength; or pregnancy; or a member of staff possessing a particular disability. All these factors may affect the risk assessment, the overall objective being competent staff able to do the work in a safe manner.

Consideration of the basic facilities required for the work must be done at a very early stage. At the extreme, work may be proposed when there is simply no possibility of providing the proper facility. Examples may include work with a hazard group 3 organism when there is no prospect of providing a proper laboratory, or work with volatile toxic substances when total enclosure is not practicable and no fume cupboard is available.

Risk evaluation

This is the process of estimating the likelihood of an event occurring, such as exposure to a micro-organism, plus identifying the likely consequence of exposure, in this example the

possibilities of acquiring a progressive infection, becoming sensitised to an allergen or coming in contact with a toxin.

The major consideration of the assessment is likely to be the risks to human health. However, risks to the environment, including to animal, fish and plant life, must be included. The risk to human health may be through infection, toxicity or allergy and thus will focus upon the staff doing the work within the workplace. The threat to the environment is likely to be via unintentional release, either through a breakdown in containment or through uncontrolled disposal or discharge of waste.

Risk control

All precautions or measures that are reasonably practicable to eliminate, minimise or control exposure to risk must be taken. The term 'reasonably practicable' incorporates consideration of cost against benefit, but there is an order, or 'hierarchy', which must be followed. The first question that must be asked for each significant hazard identified is "Do I have to use it?" or "Do I have to do the task at all?" coupled with the question "Is there any less hazardous substitute?" relating to a substance or method. This can be an important question when working with biological agents.

If the decision is to proceed with the project and the need for further risk control measures has been identified, those measures must be taken.

Those working with biological agents are greatly assisted however by the requirements set out in law and the associated guidance. The law specifies levels of containment required once the basic hazards have been identified and the guidance interprets those legal requirements thus setting the expected standard. The standards for containment level 2 and 3 laboratories are set out in the publication '*The management, design and operation of microbiological containment laboratories*' [3].

Personal protective equipment (PPE) is considered a 'last resort' but where its use either supplements the protection given through engineering controls, or where cost benefit analysis identifies PPE as being reasonably practicable, then PPE should be used. The definition of PPE would include laboratory coats or gowns, gloves, eye protection and airflow helmets or visors.

Monitoring and review

A good management system that provides for regular appropriate monitoring coupled with a review of risk assessments is essential to make risk assessments 'live', accurately reflecting and, where appropriate, modifying current work practices.

Thus, monitoring the work for compliance with set procedures also enables judgements to be made on the procedures themselves, which may in turn lead to modifications being made. Regular review of risk assessments will identify any changes made within the review period, which again in turn may lead to changes in the assessments themselves.

Summary note on legal background

'Management' regulations

The **Management of Health and Safety at Work Regulations**[4] require employers to identify **all** of the significant hazards of the workplace and to conduct proper risk assessments. Where other regulations have specific requirements for risk assessment, those requirements take precedence and no additional assessment under the Management Regulations is needed.

COSHH

The hazard identification may include work with hazardous substances. **Hazardous substances**, in this context, are defined within **COSHH**[1]. Within the definition of a 'hazardous substance' is the category **biological agent**.

Genetic modification

Within the definition of a 'biological agent' is the phrase **whether or not genetically modified**. Thus risk assessments made under the **Genetically Modified Organisms (Contained Use) Regulations**[2] need not be repeated for COSHH, but may constitute only part of the overall COSHH risk assessment.

Codes of practice

Generic Codes of Practice (CoPs) provide an effective means for ensuring safe working. Similar working practices can be developed for most organisms at each hazard group level. The major variable that may lead to differences in working practices is the route of transmission, or likely route of infection.

The following guidance notes include examples of CoPs, which can be used as models for local working practice.

Standard operating procedures

On occasion there may be a need to be prescriptive in how specific work is to be done. The decision on introducing a Standard Operating Procedure (SOP) will be based on either a need for absolute consistency, i.e. part of quality control, or through perceived high risk, or a combination of both. Divergence from the set procedure is likely to put the work or worker at risk. Examples may include the preparation, freezing and thawing of samples stored in liquid nitrogen or the opening of received packages known or presumed to contain Hazard Group 3 pathogens.

References

1. Control of Substances Hazardous to Health Regulations 2002. Approved Codes of Practice, L5. HSE Books. ISBN 0 7176 2534 6.

2. A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000. L29 HSE Books. ISBN 0 7176 1758 0 and the Compendium of Guidance from the Health and Safety Commission's Advisory Committee on Genetic Modification. HSE 2000

3. The management, design and operation of microbiological containment laboratories. HSC/DH 2001. ISBN 0 7176 2034 4.

4. Management of health and safety at work: Management of Health and Safety at Work Regulations1999: Approved Code of Practice and Guidance. L21 Second Edition. HSE. ISBN 0 7176 2488 9.

MRC Guidance Note 4

Use of blood from volunteer staff, including use of 'own' cells

Introduction

The Council's booklets "Personal information in medical research" and "Human tissue and biological samples for use in research" (MRC Ethics series, October 2000 and April 2001 respectively) provide guidelines for staff who undertake research involving the use of human volunteers in experimental protocols. This includes guidance on relevant aspects of the 1998 Data Protection and Human Rights Acts.

This statement supplements these guidelines by addressing the specific issue of MRC staff members who act as volunteers. The purpose is not to inhibit staff from volunteering but to allow most existing practices to continue within a framework which provides safeguards for the individual volunteer, the scientific worker, the director and Council.

We recognise that no single policy can address the unique demands of each individual research project. Accordingly, this statement only seeks to outline fundamental principles around which units may wish to frame local policies designed to satisfy their particular needs.

Principles

1. Project approval.

All projects which involve the use of staff as volunteers must have the prior approval of the Unit director or a delegated nominee. This includes non-invasive projects that would not normally be subject to ethical committee approval. When projects have been approved by a local or national research ethics committee, directors will nevertheless need to consider the specific issues arising from the involvement of their staff in the project before giving consent.

2. Recruitment.

Members of staff must never be coerced, or placed in a position where they may feel under obligation, to volunteer for studies. For this reason staff should not be approached by reporting officers, team leaders or other senior members of staff to whom they may feel compelled to volunteer for career reasons. We recommend that recruitment is undertaken by indirect rather than direct means, e.g., by way of a notice board announcement calling for volunteers or from a group gathering in which the value of volunteers to a particular study is explained by the project leader.

Staff should only participate in one experiment at a time and should not be paid for volunteering their services except on the same basis as other volunteers in the same study. In order to monitor staff involvement a central list should be kept of staff participation, independent of the research records.

3. Recording informed consent.

Although many of the procedures and techniques used in experiments on human subjects will be familiar to staff volunteers, investigators should not assume that it is unnecessary to explain fully the precise nature, purpose and possible effects of any experiment. A staff volunteer should be treated the same way as any other volunteer and should be asked to sign a consent form only after a briefing has been given.

It is left to the discretion of units to devise consent forms best suited to their particular requirements, however in some cases these will be prescribed by the Local Research Ethics Committee. We nevertheless advise that the issues outlined in the appendix are given full consideration before a consent form is drawn up.

4. Confidentiality

Information recorded relating to staff volunteers must be treated in the strictest confidence. Arrangements for keeping study information and access to it by other members of staff may need to be modified to accommodate this level of confidentiality.

Specific areas of guidance

1. Blood donation for laboratory purposes.

Blood samples should only be taken by a registered clinician, phlebotomist or other person trained and certified by a registered medical practitioner as competent. Samples should be taken in a suitable area set aside for this purpose and should never be taken in laboratories or non-clinical offices. When large quantities of blood are required, (e.g. >50 ml) clearance should be obtained from a medical practitioner. In addition, in these circumstances, the approval of a local ethics committee may be required.

Staff who donate blood for experimental procedures should be asked to sign a consent form prior to doing so. A record should be kept of all significant donations giving the date and time of donation and the quantity of blood taken per individual. These should be reviewed at regular intervals to ensure that excessive frequency or volume of donation by an individual is avoided.

Staff must not culture their own or should not culture close colleagues' blood cells. (See below)

2. Culture of own or close colleagues' cells or tissues.

It is inadvisable to culture one's own or close colleagues' cells or tissues and unacceptable to use such cells in genetic modification experiments or if there is any risk of the cells becoming transformed in culture. The concern stems from the potential failure of the immune system to recognise as foreign a cell that has been deliberately or inadvertently transformed or modified in vitro if those cells are then accidentally re-introduced into the original donor.

(Additional notes:

1. Transformation of one's own cells is dangerous and must not be done.

2. When cells are put into culture and in particular when they are deliberately immortalised then the risk to the donor of those cells subsequently being recognised as "self" in the event of a needlestick accident should be recognised. Donors are therefore not permitted to handle their own immortalised cells or cells in long term culture where there is the risk of spontaneous transformation.

3. A donor must not be present in the laboratory at any time when their cells are being handled by others and preferably should not have any access to these laboratories.

4. A similar restriction applies to the use of host cells capable of colonising workers, for example the workers own cells or those from other workers having access to the laboratory, in genetic modification activities involving the use of eukaryotic viral vectors

5. Records of primary cell cultures and the individuals from whom they were isolated should be kept.)

3. Work with ionising radiation.

Classified radiation workers and other staff whose work involves regular exposure to occupational radiation should not act as subjects for radiation studies without first consulting the local Radiation Protection Adviser for advice and review of their dose records.

4. Drug trials.

Consideration should be given to ensuring that a reasonable time period elapses before a volunteer engaged in a drug trial is allowed to volunteer for other studies. We advise that a volunteer is given a written protocol at the end of a trial detailing the treatment or drugs which have been administered.

5. Pre-trial experiments.

The same standard of care must be applied to pilot experiments designed to verify procedures or methods as those that will be applied to the trial itself.

6. Self-experimentation.

The principles set out in this paper, including seeking the director's and ethical committee approval, apply equally to MRC staff that wish to engage in self-experimentation.

Guidance notes for drafting consent forms.

A) Informing the volunteer.

- Does the consent form record whether the volunteer is fully satisfied with the information given about the nature, purpose, supervision and possible effects of the experiment or the significance of any results that will be obtained e.g., the identification of genetic markers predicting future health outcome?
- Is it made clear to the volunteer that he/she may withdraw from the study at any time without giving a reason?

B) Data recording.

- Is it possible to record on the consent form data about the volunteer's participation in the experiment - e.g., details of blood donation, drugs administered etc.?
- Does the consent form request personal information from the volunteer - e.g., about recent illnesses, habits or lifestyle? If so, does it state whether this information will be kept confidential?
- Is the volunteer asked about participation in any other studies over the preceding 12 months?

C) Results.

- Does the consent form address the question of confidentiality of results?
- Has the volunteer been asked to indicate his consent for any abnormal results to be disclosed to him or his GP? Is there a formal system in place locally to administer this?

D) Consent.

- Is the volunteer asked to give his signature to an explicit statement recording his consent to participate in the experiment?

Guidance notes

Part B

Working with biological hazards

MRC Guidance Note 5

Working with human pathogens

Classification

Hazard grouping

Biological agents are classified into four hazard groups according to the answers to four key questions: (a) is it pathogenic for humans; (b) is it a hazard to employees; (c) is it transmissible to the community and (d) is effective prophylaxis or treatment available? The classification scheme is illustrated in the following table, taken from Schedule 3 of the COSHH Regulations[1].

Hazard Group 1	Unlikely to cause human disease
Hazard Group 2	Can cause human disease and may be a hazard to employees; it is unlikely to spread to the community and there is usually effective prophylaxis or treatment available.
Hazard Group 3	Can cause severe human disease and may be a serious hazard to employees; it may spread to the community, but there is usually effective prophylaxis or treatment available.
Hazard Group 4	Causes severe human disease and is a serious hazard to employees; it is likely to spread to the community and there is usually no effective prophylaxis or treatment available.

Related factors

The basic classification system is confined to the answers to the four key assuming the average person is immunocompetent. Thus a biological agent will be assigned to Hazard Group 1 if it is non-pathogenic to the majority of the population, without taking consideration of individuals or groups that might be especially vulnerable. Neither does the hazard grouping take into account the possible effects on the environment, e.g., on animal or plant life. The Health and Safety Commission publishes an Approved List of known pathogens (reference **2, -** also available at http://www.hse.gov.uk/hthdir/noframes/agent1.pdf).

A proper risk assessment will therefore consider the individual workers that may be affected by any exposure to the agent. Those that carry an existing disease or infection, those whose immunity is compromised either temporarily or more permanently, those on certain medication that may affect the individual's capacity to resist infection and the unborn child in pregnant women or those women who are breast-feeding may all be more susceptible to biological agents, in particular to opportunist infection. The assessment will also consider any potential threat to the external environment, should the agent be released into it.

Safe workplace

Physical containment

The relationship between Hazard Grouping and the requirements for physical containment are set out in detail in three key publications[1,2,3]. Briefly, four different levels of laboratory

containment are described, directly related to each Hazard Group. Work must be done at the containment level appropriate to the Hazard Group, **as a minimum**, e.g., with Hazard Group 3 agents at Containment Level 3.

This works well wherever the identity, and thus the pathogenicity, of the agent is known. Containment requirements in those situations where the identity or presence of a biological agent is not known are now included within the law and amongst the requirements are the following:

Where uncertainty exists over the presence of pathogenic biological agents	Minimum of Containment Level 2
Where the presence of a pathogenic biological agent is known or suspected	Minimum of Containment Level appropriate to the agent
Where the assessment is inconclusive but where the activity might involve serious risk	Minimum of Containment Level 3

Schedule 3 of COSHH states: "The Health and Safety Commission may approve guidelines specifying the minimum containment measures which are to apply in any particular case". Thus, for example, in serology, clinical chemistry, haematology or forensic science laboratories, where materials liable to be contaminated with a particular Group 3 virus are handled, uprated level 2 containment may be appropriate. In reality, however, the rule of thumb is "if in doubt, go up" (rather than down).

MRC laboratories are now constructed to meet the physical requirements for Containment Level 2. In terms of basic construction, a Level 2 laboratory requires a sealed coved flooring, easily washable work surfaces that are chemically resistant, separate facilities for handwashing (including hands-free operated taps) and the laboratory door to be closed during the work.

Suitable construction does not necessarily mean suitable for use. The authorities are unlikely to approve the routine setting-aside of bays in Level 1 laboratories for Level 2 work and will argue for dedicated facilities at this level. This of course will be supplemented with requirements for good management and appropriate staff training, plus in many cases specialist equipment.

Equipment

It is good practice in each and every laboratory that essential equipment is readily accessible. Requirements for 'self-contained' equipment however go up with the level of containment. For example, for work with pathogens, an autoclave should be (a) in the same building (and preferably the same suite) for containment level 2; (b) in the same suite (but preferably in the same laboratory) for containment level 3; and (c) double-ended with interlocked doors, with entry in the laboratory and exit into a clean area for containment level 4. There are stricter requirements for work with genetically modified micro-organisms at containment level 3 in respect of the location of autoclaves. Specific derogation is required to locate an autoclave outside the level 3 area.

In more general terms, it is always beneficial to have as much of the key equipment within the laboratory as possible as long as crowding does not compromise safety. It is not however absolutely necessary at containment level 2, should be provided so far as is reasonably practicable at containment level 3 and is mandatory at level 4.

Safe procedures

The development of generic codes of practice (systems of work, standard operating procedures) is an important measure toward ensuring safe working in containment facilities. A basic code can be developed for each level, the requirements being more stringent and

more demanding as the level of containment goes up. A simple code to cover work at containment level 1 can be based upon accepted good microbiological practice (GMP). The fundamental hygiene measures are designed to eliminate or at least minimise the risk of exposure to biological hazards and the risk of transporting potentially hazardous material outside the laboratory. The requirements for levels 2, 3 and 4 can then be built onto the basic framework. MRC CoPs can be seen in **Appendix 1**.

Safe people

Appropriate training programmes should be developed for working with biological agents. The programme may identify key competencies to be achieved and demonstrated. Achievements should be verified in writing at least for work at levels 3 and 4. The key competencies should relate to the generic codes of practice and examples of these are illustrated in Table 1.

Table 1 Training and competence requirements

Containment level	Key competencies
1 and 2	Awareness and understanding of all procedures and risk assessments Technical competence for all aspects of the work* Knowledge and understanding of disinfection policy Knowledge and understanding of waste disposal arrangements† Knowledge and understanding of emergency spillage procedures
3	All those included for containment levels 1 and 2 plus - Full understanding of emergency procedures, including – safe evacuation and sealing of the facility prior to emergency room fumigation securing and sealing a cabinet prior to emergency cabinet fumigation Full training, where applicable, on fumigation procedures.

* Where this includes the use of a microbiological safety cabinet, specific training should be given

† Where local arrangements require the trainee to operate an autoclave, specific training should be given

Note: Where competence in the use of key equipment is required for work at containment level 3, e.g., the use of a microbiological safety cabinet, recovering samples from liquid nitrogen storage, the use of automatic pipetting aids, the use of centrifuges, etc., and other specialised equipment, competence should first be demonstrated at a lower level of containment.

Notifications

Establishments wishing to **intentionally** work with biological agents in hazard groups 2, 3 and 4 for the first time must notify the HSE. The legislation, introduced in COSHH in 1994, was not retrospective, so those laboratories already working with agents on 16 January 1995 were not required to inform the HSE. However, under COSHH 2002, further notifications are required as shown in the table. Establishments that were already using or holding biological agents prior to 1995 are therefore recommended to contact HSE to ensure HSE are aware of the work. Certainly an establishment that worked with biological agents before 1995 but which has stopped the work since that date, would be required to notify the HSE of any resumption of that work or new work as 'first use'.

Hazard Group	First use on premises	Subsequent use of new agents in same group
2	Yes	No*
3	Yes	Yes*
4	Yes	Yes*

* **Notification is required for all agents included in COSHH Schedule 3 Part V. This is all agents in Groups 3 and 4, plus Group 2 agents *Bordetella pertussis, Corynebacterium diphtheriae and Neisseria meningitides*.**

The notification must be made 20 working days in advance of the work. Where the intended use of more than one agent is intended, a single notification will suffice.

The full particulars required in the notification are set out in Schedule 3 of COSHH and include name, address, and identity of local safety personnel, the agents, risk assessments and control measures.

Further notification is **not** required if the work has already been notified to HSE under the Contained Use (CU) regulations (see **Guidance Note 7**). The reverse is however not the case. Further notification **may** be required however if the original work notified under the CU regulations related to a, for example, disabled strain and further work not covered by the CU regulations was planned with a wild-type or other strain of the same organism assigned to a higher Hazard Group.

Record keeping

Each employer must keep a list of all those exposed to a Group 3 or Group 4 biological agent. The record must list the agents and periods of exposure. The guidance states that where partial enclosure is used, i.e., Class I and Class II safety cabinets, then protection is not complete and thus exposure must be assumed.

COSHH 2002 requires the records to be kept for 40 years following the last entry in the book. This is a significant change from COSHH 1999, where the record keeping requirements were related to the individual worker and most individual records were only kept for 10 years.

Although there is no absolute requirement to keep written risk assessments, it would however stand an employer in good stead if assessments were retained for a certain period, particularly in situations where exposure records have also been kept.

Derogation

The general rule, as stated earlier, is that the minimum containment required for working with specified agents is that level equivalent to the hazard grouping, e.g., containment level 3 for working with Hazard Group 3 agents. In certain circumstances however for work with specified Group 3 agents, the employer is permitted to use less stringent conditions.

The mechanism for this 'derogation' was set down in a Certificate of Exemption included within the Approved List of Biological Agents in the ACDP guidance document[2]. The Certificate is now replaced by the inclusion of paragraph 3(5) in Schedule 3 of COSHH which states (see also earlier page) "The Health and Safety Commission may approve guidelines specifying the minimum containment measures which are to apply in any particular case". Thus at present derogation is only permitted by applying the guidance contained in related HSC approved publications[2,4,5]. Derogation is permitted is specified circumstances for certain mycobacteria and enteric bacteria, parasites (including Plasmodium falciparum and some Leishmania spp.), certain blood-borne viruses and the agents for human Transmissible Spongiform Encephalopathies.

The derogations permit some relaxation of the physical requirements for containment level 3, e.g. for laboratory work with human TSE agents subject to risk assessment and for diagnostic work with enteric pathogens. Specifically these relate to protection from airborne infection, including the requirements for:

- A net inward flow of air
- The use of microbiological safety cabinets
- The laboratory to be sealable for fumigation.

They do **not** however permit any derogation from management requirements, e.g., record keeping, staff training (including knowledge of emergency procedures) or access control.

References

1. Control of Substances Hazardous to Health Regulations 2002. Approved Codes of Practice, L5. HSE Books. ISBN 0 7176 2534 6.

2. The 2000 supplement to the Categorisation of biological agents according to hazard and categories of containment, incorporating Appendix 24 and the 4th edition of the Approved List. C40. MISC208.

3. The management, design and operation of microbiological containment laboratories. HSC/DH 2001. ISBN 0 7176 2034 4.

4. Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection. HMSO 1998. ISBN 0 11 322166 5.

5. Protection against blood-borne infections in the workplace: HIV and hepatitis. HMSO 1996. ISBN 0 11 321953 9.

6. **Appendix 1**

Code of Practice: Standards of laboratory practice:

1. Good microbiological practice;

2. ACDP containment level 2;

3. ACDP containment level 3

The standards set out here are the minimum required for working with biological material assessed as Hazard Groups 1, 2 and 3. For some criteria, MRC standards are more rigorous than that strictly required within the ACDP guidance.

1. Good microbiological practice (GMP) (Containment Level 1)

1. The room should be easy to clean. Bench surfaces should be impervious to water and resistant to chemicals. There must be a sink for handwashing.
2. If the room is mechanically ventilated, the airflow should preferably be inward, at least while the work is in progress.
3. The door should be closed while work is in progress.
4. Laboratory coats, preferably side or back fastening, should be worn in the room and removed when leaving for non-laboratory/animal areas.
5. Eating, chewing, drinking, smoking, storing food and medicines, applying cosmetics and mouth pipetting are forbidden.
6. Hands must be decontaminated and washed when contamination is suspected and before leaving the room.
7. Effective disinfectants must always be available (see **Guidance Note 14**).
8. Aerosol production must be minimised.
9. Bench tops should be cleaned and disinfected as necessary after use.
10. Used equipment awaiting disinfection/sterilisation must be stored safely. Pipettes in disinfectant must be completely immersed.
11. All waste material should be disposed of safely by appropriate means. Contaminated material for disposal or recycling should be transported in robust containers without spillage (see **Guidance Note 15**).
12. Animal cages must be rendered non-infective after use.
13. Accidents and untoward incidents, including animal bites, must be reported.

2. Containment Level 2: additional requirements over level 1

1. The handwash basin must be fitted with taps operable without being touched by hand and sited near the exit to the laboratory.

2. If the room is mechanically ventilated, it must be maintained at negative pressure at least while the work is in progress.

3. Access should be restricted and space should be adequate for the tasks.

4. Laboratory coats or gowns which are side or back fastening, must be worn in the room and removed when leaving.

5. Damaged skin must be covered with a protective waterproof dressing. The wearing of gloves is recommended.

6. There must be specified decontamination procedures, including those for disinfection and for the safe handling and disposal of waste.

7. There must be safe storage of biological agents.

8. Work likely to generate significant aerosols must take place in a safety cabinet or within equivalent containment.

9. Within animal accommodation there must be effective vector control for rodents and insects and avian pests.

10. There must be an incinerator accessible for disposal of infected animal carcasses.

11. A biohazard sign should be displayed outside the entrance to the room.

Training and competence requirements for work at containment levels 1 and 2

- Training requirements of each individual worker must be assessed by the team leader or nominated supervisor
- Training programmes based on risk-based training needs analysis must be developed for each individual
- Key competencies will include all of the following:

  awareness and understanding of all procedures and risk assessments for the work (including local and corporate MRC Codes of Practice);

  technical competence for all aspects of the work*;

  knowledge and understanding of disinfection policy;

  knowledge and understanding of waste disposal arrangements †;

  knowledge and understanding of emergency spillage procedures.

* Where this includes the use of a microbiological safety cabinet, specific training must be given (see **Guidance Note 12**)

† Where local arrangements require the trainee to operate an autoclave, formal training must be given (see **Guidance Note 14**)

Note: It is likely that much of the training for work at containment levels 1 and 2 can be given 'at the bench' under supervision which reduces as competence is acquired.

3. Containment Level 3: additional requirements over levels 1 and 2

1. The room must be sealable for fumigation (except facilities for work subject to specific derogation under the Certificate of Exemption as included in the ACDP document on the categorisation of pathogens).

2. The laboratory/animal room should be sited away from the general access and separated from other activities in the same building.

3. Floors and bench surfaces (and walls in animal facilities) must be impervious to water and easy to clean.

4. Access must be for authorised competent personnel only.

5. The laboratory must be maintained under negative pressure, and exhaust air must pass through a high efficiency particulate air (HEPA) filter (except as qualified in 1. above).

6. The laboratory door must be closed whilst work is in progress and locked at all times when the room is unoccupied. A biohazard sign must be displayed by the entrance to the room or suite. The door should contain an observation window to enable occupants to be seen.

7. Side or back fastening coats/gowns must be worn. These must not be worn outside the containment facility and be either disposable (autoclaved, then sent out via appropriate waste arrangements when discarded) or re-usable (autoclaved before washing and re-use).

8. Gloves must be worn. Other items for personal protection such as aprons, caps, overshoes, masks and safety glasses may be necessary for some operations.

9. As far as is reasonably practicable the room should have its own equipment, and a strict policy for movement of items in and out of the room.

10. Infected material should be transported to and from the room in sealed containers.

11. Work should be handled inside a suitable safety cabinet as much as possible and whenever there is any possibility of the generation of aerosols. All centrifugation must be done in sealed buckets or rotors, opened only within the safety cabinet.

Training and competence requirements for work at containment level 3

Working at containment level 3 is subject to formal competence assurance by local management.

The achievement of satisfactory competence must be through a formal verified training programme, the components of which are as follows:

- Technical competence for all aspects of the work.

Note: Where competence in the use of key equipment is required, e.g., the use of a microbiological safety cabinet, recovering samples from liquid nitrogen storage, the use of automatic pipetting aids, the use of centrifuges, etc., and other specialised equipment, competence must first be demonstrated at a lower level of containment.

- Full knowledge of the safe operation of a microbiological safety cabinet (following the Code of Practice included in **Guidance Note 12**) where applicable.

- Full understanding of all risk assessments, procedures and Codes of Practice relevant to the work and the use of the containment level 3 facility.

Working with biological agents

- Full understanding of the use of disinfectants, including the working concentrations, the frequency of making fresh solutions.
- Full understanding of emergency procedures, including safe evacuation and sealing of the facility prior to emergency room fumigation and securing and sealing a cabinet prior to emergency cabinet fumigation.
- Full training, where applicable, on fumigation procedures.

MRC Guidance Note 6

Working with transmissible spongiform encephalopathies

Code of Practice:

Handling precautions for laboratory work with human brain tissue and with human and animal Transmissible Spongiform Encephalopathies (TSEs) and Prions.

Introduction

This code applies to all staff, attached and visiting workers who work under the management control of the MRC. All workers using these tissues and agents should also be familiar with the publications listed at the end of this **Guidance Note** and with **Guidance Notes 5** and **9**.

Risk assessment

A risk assessment must be made for all work involving the handling of brain, cerebrospinal fluid (CSF) and central nervous system (CNS) tissue. The risk assessment should be specific for the procedures involved and take account of the nature and source of the samples to be handled.

The principal risk associated with handling CNS tissue is that of self-inoculation by accidental cuts with instruments, or skin penetration via pre-existing cuts and abrasions. Precautions should also be adopted to prevent tissue having contact with mucous membranes (mouth, eyes) and to avoid inhalation of particles in aerosol form, produced by the mechanical dispersion of tissue.

HIV has been detected in both CSF and brain tissue. Possible HIV contamination should therefore be considered when handling these materials in the laboratory.

In view of the potential hazards which may be associated with handling post-mortem brain and other clinical material, initial and most subsequent manipulations of brain tissue should be carried out in a designated facility. CSF may be handled in other laboratories, subject to full risk assessment.

Hazards and risks

Hazard categorisation of TSE agents

In determining the appropriate hazard grouping of a pathogen, the ACDP has considered the pathogenicity of the organism to man, the hazard to laboratory workers, the potential for transmission to the community and the seriousness of any illness that might result after taking into account the availability of prophylaxis or effective treatment (see **Guidance Note 5** for definitions of Hazard Groups).

The human TSE agents

These agents are invariably fatal, at least for those where the disease has been diagnosed clinically, and there is no effective prophylaxis or treatment available. This suggests that the highest of the ACDP's Hazard Groups would be appropriate for the human TSEs. Apart from the rare examples of iatrogenic transmission however, there is no evidence that Creutzfeld-Jakob disease (CJD) or Gerstmann-Straussler-Scheinker syndrome (GSS) has been or can spread to the community, or has occurred in workers through occupational exposure. There is also no evidence that nvCJD patients acquire their disease through occupational exposure. This would suggest a hazard grouping of no higher than 2. In fact, human TSE agents are placed in Hazard Group 3 because of the severity of infection. Derogation from full Containment Level 3 however is permitted as it is recognised that transmission is unlikely via the aerosol route. Thus, full Containment level 3 may not always be necessary for work with these agents. **Table 1** describes the categorisation of the agents, linked to the containment measures required.

Table 1. Summary of categorisation of the agents of TSEs

	Agent	**Hazard Group**
Human	Creutzfeldt-Jakob disease (CJD, including nvCJD)	3
	Gerstmann-Sträussler-Scheinker syndrome (GSS)	3
	Kuru	3
	Fatal familial insomnia (FFI)	3
Animal	Bovine spongiform encephalopathy (BSE) and other related animal TSEs	3**
	Scrapie	1†
	Others	1

** there is some evidence, albeit inconclusive, that BSE agents can be transmissible to man. For that reason the agents are considered as properly belonging in Hazard Group 3 with derogation similar to that for the human TSEs.

† there is currently no definitive evidence that these are pathogenic for man, but until the position is clarified some additional precautions are recommended. These are outlined in later paragraphs.

The animal TSE agents

Animal TSEs, like human TSEs, appear to be invariably fatal in the animals they affect. For most animal TSEs however there is no definitive or conclusive evidence of transmission to humans and therefore Hazard Group 1 is generally indicated. In consequence, Containment Level 1 is appropriate when handling intact animals or tissue perceived to be of low risk to man.

Nevertheless, any work with lymphoid or neural tissues from, or extracts derived from known or suspected cases of animal TSE other than BSE, should be conducted using

procedures that limit exposure and, in particular, reduce the risk of accidental parenteral inoculation.

There is however some evidence that BSE agents can be transmissible to man, requiring the reclassification of these agents to Hazard Group 3.

Control of Exposure

Containment

Schedule 3 of the COSHH Regulations states that the containment level at which a pathogen is to be handled must correspond with its hazard grouping. The only exception to this is where, through the use of an exemption, a specific derogation from the full containment level specification is approved. Although the human TSE agents responsible for CJD, GSS, Kuru, FFI and the BSE agents are to be placed in Group 3, there is HSE-approved derogation from full level 3 containment for these agents. Given that they are likely to be transmitted only by parenteral routes, the requirements of Containment Level 3 are therefore applied with the exception of those requirements concerning fumigation and mandatory inward airflow as set out in **Guidance Note 5**. The use of a microbiological safety cabinet can be restricted to work where there is a significant risk of aerosol production. The laboratory should be kept sharp-free as far as is reasonably practicable with emphasis placed on the precautions shown in **Table 2** and the requirements for containment in **Table 3**. These reflect, in part, the incomplete state of knowledge of the TSE agents and the need to protect workers from potential risk. The requirement for formal competence assurance for work at Containment Level 3 set out in **Guidance Note 5** applies for all work with TSEs categorised as Hazard Group 3.

The extent of use of such precautions should be determined by a local risk assessment that would need to take into account, amongst other factors; the agent(s) under investigation, the tissues being manipulated, the type of experimental or other work to be performed and the concentrations of the agents likely to be encountered. In particular, experimental work involving concentration or modification of agents may require special consideration.

Containment for genetic modification work with prion genes has been considered by the Advisory Committee on Genetic Modification (ACGM). For transgenic animals, in general those engineered for the human prion protein have been housed at containment level 1. The occurrence of a single case of spontaneous conversion of PrP, however, has led to some centres handling all such animals as if they were infected, i.e., at Containment Level 3.

Workers should be aware that samples of neurological origin may contain slow viruses. This should be taken into account in the risk assessment and particular care taken when samples are handled.

Table 2 Summary of specific precautions for experimental work with the TSE agents

(These are found in detail in the published Guidance from ACDP)

- Practise good hygiene, including wearing of protective clothing (but removing protective clothing on leaving laboratory)
- Cover all damaged skin and wear gloves
- Wear eye protection where appropriate
- Avoid splashing and use containment measures (sealed centrifuge buckets, MSCs)
- Avoid or minimise use of sharps
- Record all accidents

Notes: *For some equipment only parts may need to be dedicated: for example with electron microscopes and ultracentrifuges only the grid holders and rotors respectively.

Factors to be considered when performing a risk assessment to decide whether the precautions detailed in **Table 2** are required will include:

- nature of the TSE
- type of task (e.g. concentration/purification)
- if work is likely to result in a high titre of infectivity
- frequency of contact with the agents or materials likely to contain them
- genetic modification
- possible routes of exposure
- knowledge of expression of agent in experimental model
- the potential for inoculation injury.

Table 3 Containment Levels recommended for experimental work with the TSE agents

Type of work	Overall laboratory containment level	Animal containment level
Work with human TSEs and BSE agents[(a)] BSE and other related animal TSE agents	3[(b)]	3 for small animal work 1 for large animal work[(c)]
Work with scrapie material[(d)]	2	2 for small animal work 1 for large animal work
Work with other neurological material identified as low risk	1	1

Note:

(a) This should include passaging animal TSE material in primates or GM mice carrying the human PrP gene
(b) Derogation may be applied if risk assessment indicates
(c) Smaller animals generally present a higher risk of biting and scratching and so should be handled at containment level equivalent to the hazard group of the agent
(d) Despite no evidence that infections by other animal TSEs can occur to man, the derogated Level 3 containment measures are recommended, except for known strains of Scrapie which can be handled at Containment Level 2.

Safe working practices and sample reception

The criteria for safe working practices and sample handling outlined in **Guidance Note 9** for the handling of blood also apply to this work.

Decontamination and waste disposal procedures for TSE agents

The agents of TSE exhibit unusual resistance to conventional decontamination methods used in clinical and laboratory practice. As the longest recognised and most intensively studied of the TSEs, scrapie is the acknowledged model for the group. Much of what is recommended here about decontamination in general is derived from studies involving scrapie. Where findings are paralleled by work on the agent of CJD, this is stated specifically.

Ineffective methods of decontamination

The agents of TSE are well recognised as being particularly resistant to all standard physical and chemical methods of laboratory decontamination. They are not greatly affected by boiling. The standard laboratory autoclaving regime of 121°C for 15 minutes, which is known to inactivate the hardiest pathogenic bacterial spores, is ineffective with TSE agents. Similarly, the cycle normally used for the sterilisation of surgical equipment (134°C for 3 min.) cannot be relied upon. In addition the TSE agents are not significantly affected by a number of common chemical disinfecting agents.

The doses of ionising or UV irradiation required to produce significant reductions in TSE infectivity are too great to be of practical value. CJD agent can survive at room temperature for at least 28 months and residual scrapie infectivity has been found after burial for 3 years. Therefore, unless appropriate chemical or physical decontamination methods are used there is the potential for the inapparent survival and accumulation of infectivity on work surfaces and equipment.

Effective methods of decontamination

Autoclaving

Autoclaving infected material at 134°C for 18 minutes has been recommended. Although this treatment will reduce the infectivity considerably, it may not achieve complete sterilisation. Autoclaved material should therefore still be considered infected and must be sent for incineration.

Dry heat

Dry heat treatment of macerated infected tissue at 160°C for 24 hours, leaves some residual infectivity. Lyophilised (freeze-dried) tissue homogenates exposed to 360°C for one hour also remain infectious. As the water content of material to be heat-treated has an influence, desiccation confers a particular resistance to inactivation. The infectivity of moist tissue is destroyed in 60 minutes at 200°C. Substantial, but not complete, inactivation of both CJD and scrapie is attained after one minute at 240°C.

Chemical decontamination

The disinfectant of choice is sodium hypochlorite. A one hour treatment with sodium hypochlorite at 20,000 ppm available chlorine is appropriate for TSE agents.

Specific decontamination procedures

Treatment of work surfaces and equipment

Hypochlorite

A one hour exposure to sodium hypochlorite containing 20,000 ppm available chlorine is effective in destroying infectivity on open surfaces. Repeated wetting with the disinfectant is necessary over the treatment period. The concentration of available chlorine in hypochlorite solutions may be significantly affected by the presence of organic matter, especially blood. As this concentration of hypochlorite can be corrosive for metals and some commonly used surface finishes, work that involves the handling of infected materials should be conducted only on chemically resistant surfaces or work benches shielded by disposable absorbent plastic-backed temporary covering. The use of enamel, heat-stable plastic or disposable trays is recommended to confine contamination. These should be autoclaved after use (but see above). Temporary bench coverings and disposable trays should be bagged for incineration.

Sodium hydroxide

Sodium hydroxide solution 2M is also active against scrapie but may not completely inactivate high concentrations of agents. Constant rewetting during the treatment of surfaces is necessary.

Decontamination and disposal of liquids

TSE contaminated fluids should be disposed of regularly to limit the volumes to be dealt with at one time. Large volumes of organic solvents should be disposed of by the commercial controlled incineration techniques generally used for these materials. Small volumes of fluid may be conveniently absorbed in containers carrying sawdust ready for incineration. Water-based fluids may be autoclaved (see above) or preferably treated with hypochlorite to achieve a final concentration of at least 20,000 ppm of available chlorine allowing for the effects of any organic matter present (see above).

Formaldehyde solutions must not be autoclaved or mixed with other chemical disinfectants. Contaminated formalin solutions should be disposed of by incineration. Workers must ensure that alternative methods of formalin disposal such as discard to the sewerage system are in accordance with local Water Authority rules.

Decontamination of microbiological safety cabinets

As indicated, formalin or vaporised formaldehyde, which is the conventional medium for the fumigation of safety cabinets, is not effective against the TSE agents and it may in fact stabilise them. Nonetheless, fumigation will need to be carried out as a precaution against other infectious agents that may be impacted on the surface of the cabinet's HEPA filter. Service engineers will require the unit to be decontaminated before changing filters and must be informed (preferably in advance of a visit) where a cabinet has been used for work with TSEs.

Due to the difficulties associated with their decontamination, it is recommended that safety cabinets used for work with TSEs should be of the type with the facility for removing HEPA filter units by bagging. Whether or not bagging of the filter as it is withdrawn is possible, spraying the filter face after fumigation and before removal with a fixative (e.g. hair spray) will help to limit the shedding of particulate matter. Where a Class II cabinet (BS:5726 or

BS EN: 12469) is to be used, a model that has the main HEPA filter immediately below the work surface is preferred as this will prevent contamination of the plenum of the cabinet. With the filter in this position, use may be made of liquid latex to seal the filter surface before removal. Pre-filters (dust filters) are generally easily removed and after immersion treatment with 2M sodium hydroxide solution (see above) to limit dust dispersal they should be contained securely for incineration. If made of durable but not heat stable material, they may alternatively be treated with hypochlorite solution containing 20,000 ppm available chlorine.

Working in a shallow tray in the cabinet will limit dispersal onto work surfaces by splashing but it is essential to ascertain by testing the cabinet with the tray in situ that containment for operator protection is not affected. Another option is to tape disposable plastic backed absorbent paper to the working surface in order to minimise contamination. The covering must be renewed regularly (preferably after each period of work) and incinerated.

Fixation for histology

Conventional methods of tissue fixation employing formalin or glutaraldehyde are known to be ineffective in destroying CJD and scrapie infectivity. It is likely that the other TSE agents are similarly resistant. Exposure to 96% formic acid for one hour after formalin fixation has been shown to be effective in reducing scrapie and CJD infectivity substantially (see below).

Formic acid treatment of formalin-fixed tissue for human and animal neuropathology

i. Fix tissues* in 4% formaldehyde solution (10% formol-saline) for minimum time necessary for optimal tissue preservation

ii. Immerse in 96% formic acid for one hour

iii. If tissues are to be processed by hand, they may be taken directly from formic acid into ascending alcohol solutions

iv. If tissues are to be processed by machine, they should be washed again with formalin (formic acid may damage plastic containers).

(*blocks should be of a size to ensure adequate penetration)

Notes:

1. Formic acid should not be used on brain tissue that has been exposed to phenol as well as formalin as this causes a deleterious tissue reaction.

2. Phenol has been shown to be ineffective in decontaminating formalin treated tissue and should not now be used. Some archive material is likely to have been exposed to phenol in the past, and care should be exercised when handling these tissues.

3. To control noxious fumes, it may be appropriate to conduct this work in a fume cupboard.

Disposal of waste

Guidance on the disposal of clinical and laboratory waste is given in **Guidance Note 15**. There are also statutory requirements under the Controlled Waste Regulations 1992. Carcasses of animals that have been experimentally infected with TSE must be disposed of by incineration.

Final disposal of processed tissue should be by incineration using appropriate precautions during handling and packaging for carriage. Stored fixed material (bulk tissue, blocks and stained or unstained slides) from known or suspected cases of TSE must be handled as though it were infectious, and attention paid to the possibility of sharps exposure.

References

1. Transmissible Spongiform Encephalopathy Agents: Safe working and the prevention of infection. 1998. ACDP/TSO. ISBN 0 11 322 166 5.

2. Protection against blood-borne infections in the workplace: HIV and hepatitis. 1995. ACDP/HMSO. ISBN 0 11 321 953 9.

3. The 2000 supplement to the Categorisation of biological agents according to hazard and categories of containment, incorporating Appendix 24 and the 4th edition of the Approved List. C40. MISC208.

4. The management, design, and operation of microbiological containment laboratories. 2001. HSC/DH. ISBN 0 7176 2034 4.

5. Control of Substances Hazardous to Health Regulations 2002, Approved Codes of Practice (L5). HSC. ISBN 0 7176 2534 6.

6. Safety in Health Service Laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2003. HSAC/HSC. ISBN 0 7176 2513 3.

7. Safe Disposal of Clinical Waste. 1999. HSAC/HSC. ISBN 0 7176 2492 7.

MRC Guidance Note 7

Working with genetically modified organisms

Introduction

This guidance summarises the main requirements of the Genetically Modified Organisms (Contained Use) Regulations 2000, referred to for the remainder of the document as the GMO(CU) Regulations. All units working with genetically modified organisms should have access to these Regulations with the associated Guidance and the Compendium of Guidance[1] from the Advisory Committee on Genetic Modification (ACGM). They must also be registered with the Health and Safety Executive (HSE) either (as in the majority of circumstances) as an independent Centre, or within the registration of the host institution.

This **Guidance Note** applies to all staff, attached and visiting workers who work under the management of the MRC.

Definitions

Genetic modification

The GMO(CU) Regulations define genetic modification in relation to any organism as "the altering of the genetic material in that organism by a way that does not occur naturally by mating or natural recombination or both".

Examples of techniques covered by this definition are:

- Recombinant DNA techniques using viral or bacterial vectors;
- The direct introduction of heritable material into an organism, e.g. by micro-injection;
- Cell fusion or hybridisation;
- Transgenic animals or plants.

The GMO Regulations do not apply to certain techniques of genetic modification (GM) if they do not involve the use of genetically modified organisms/micro-organisms (GMO/GMM) as recipient or parental organisms. Examples of these exempt techniques are:

- In vitro fertilisation
- Natural processes including conjugation, transduction or transformation
- Somatic hybridoma cells used for the production of monoclonal antibodies
- Cell fusion of plant cells where the resulting organisms can be produced by traditional breeding methods
- Mutagenesis
- Self-cloning of organisms that are unlikely to cause harm to humans, animals or plants

There can be some exceptional circumstances where it is possible that neither nucleic acid is apparently involved in the transfer of new or altered hereditary information that results in phenotypic change. One such example may be the so-called prion proteins. In this situation

changes to the expressed phenotype in certain cell types appear to come about through modifications of naturally occurring protein molecules.

The creation of a GMO does not necessarily mean the direct alteration of nucleic acids. It could be the addition of another genome into a cell. For example, the creation of a novel hybrid virus by two viral genomes fusing within a cell could constitute the formation of a GMO.

Contained use

The GMO(CU) Regulations define 'contained use' not only as any operation in which the organisms are genetically modified but also any operation in which the GMOs are cultured, stored, used, transported, destroyed or disposed of where measures are required to prevent or limit their contact with the general population and the environment.

Notification

Premises

Establishments must notify HSE before any work with GMOs starts. No work regardless of class of activity for the GMO being used can be started until HSE has approved the site by issuing a GM centre number. Each centre number is unique for that place of work. A number of different laboratories with a single employer doing contained use work within a site or complex may well be registered as one contained use centre. This may also be true where different employers occupy laboratories on the same site or share the same suite of facilities. The HSE is more likely to agree that the work can be done under a single centre registration if there is a formal agreement on health and safety management in place between the respective employers.

Notification for GMMs

The risk assessment procedure (see below) will place the work with a GMM into a particular class of activity. The class will then determine both the notification period required before work can commence and whether written permission from HSE is required before work commences.

Notification for GMOs other than GMMs

This is for work with genetically modified (GM or transgenic) animals and plants or their cellular constituents.

The risk assessment procedure, outlined below, will be used to determine whether the GMO poses an increased hazard to human health than the parental organism. If it does then this places the organism into the category of a notifiable GMO.

Notification for first time GMO activities

Regardless of class (for GMMs) or whether a GMO (other than a GMM) is regarded as a notifiable GMO or not HSE must be informed of all first time activities. The risk assessment and how waste material will be dealt with must be included with the first time risk assessment notification.

The table below gives more detail on notification periods.

Type of Notification	Notification period	HSE consent required?
First use of premises	None. Work can start on receipt of HSE acknowledgement.	No. Work can begin after HSE confirms receipt of application.
GMMs class 1	First activity only requires HSE notification (see above).	No.
GMMs class 2 first activity	HSE must be notified unless previous work in a higher class has already been sanctioned.	No. Activity can begin 45 days after HSE confirms receipt of application. Work can begin earlier if HSE consents.
GMMs class 2 subsequent	HSE must be notified.	No Work can commence after HSE confirms receipt of application
GMMs class 3 and 4 First activity	90 days notification before work commences.	Yes. HSE must give consent. This will be 30 – 90 days after HSE confirms receipt of application.
GMMs class 3 and 4 Subsequent activities	45 days notification before work commences.	Yes. HSE must give consent 30 - 45 days after HSE confirms receipt of application, or say why consent is refused
Non-notifiable GMOs (excluding GMMs) Work with plant and animals not likely to harm humans.	No, except for first activity	No.
Notifiable GMOs (excluding GMMs) Work with plant and animals likely to harm human health.	45 days before work commences	No Activity can start 45 days after HSE confirms receipt of application. No work can be done if the application is refused. HSE must give reasons why consent is refused.

For all of the above HSE will acknowledge an application within 10 days of receiving it. The waiting time can be reduced but HSE must give written consent. The minimum waiting time will be 30 days for first applications. A fee is also payable to the HSE for each application. Information on current fees is available from the HSE on their web-site.

Disclosure of information

The competent authority maintains a public register of all GM activities. Thus outline information relating to the premises and the nature of the activity is accessible by the public. The regulations allow for some information to be withheld from the register subject to the responsible person making an application which satisfies certain conditions. Similar application can be made to withhold information from the public that relates to a notifiable accident. These could include matters that would affect public security or national defence, affect the confidentiality of any relevant person or affects commercial confidentiality or intellectual property, but these could not prevent basic information being placed on the register. In 2002 the GMO(CU) (Amendment) Regulations were introduced to allow the Secretary of State to authorise any information to be kept confidential if it is in the interests of national security.

Risk assessment for work with a GMM

A risk assessment for a GM experiment must address certain prescribed key matters and guidance is given in the ACGM Compendium of Guidance. The information gained is used in the risk assessment process, which must include consideration of key questions. In turn this process will determine the control measures necessary for the experiment and ultimately determine the class appropriate for the project. An outline of the matters and questions is set out below.

The main purpose of the risk assessment process is to classify activities involving GMMs into one of four classes. These classes (1-4) equate in level of assessed risk to those of the hazard groups for known human pathogens with class 1 relating to work with non-hazardous GMMs and class 4 as the highest hazard. The classification does however also include an assessment of the risks to the environment. The containment measures necessary to ensure that the activity presents no harm to man or to the environment will determine the class for any particular GMM activity. Biological containment is always considered during the risk assessment process. In some instances, the biological factors may constitute the principal containment measure, whereas other examples may require more physical containment measures to be in place for necessary control of the hazard.

The containment levels, which also range from 1-4, may be physical in nature and the appendices in the GMO(CU) Regulations and the tables in the Compendium of Guidance go into the detailed requirement for each level.

The risk assessment process

Key matters

First, you must look at the properties of the genetically modified micro-organism (GMM).

1. Does it have properties that differ from the parental organism?
2. If yes, then is there a potential for this change to present a hazard to human health?

   In answering this question, you should also consider the recipient organism, the inserted genetic material, the vector and in some circumstances the donor organism, as well as the GMM itself.
3. What is the likelihood that the GMM could cause harm to human health?
4. What type of work is going to be undertaken?
5. What possible environmental hazards are there?
6. What are the waste products and how are they rendered safe?

Key questions to be included in the risk assessment process

1. Hazard identification in respect of human health and environmental safety

If the parental micro-organism of the GMM is a known pathogen then the Approved List included in the ACGM guidance document may give an initial starting point for the containment level necessary for any activity.

Risk assessments on genetically modified organisms can be assisted by initially referring to the Approved List, to published scientific papers or information from commercial suppliers of vectors. These may provide guidance on the recommended containment levels for bacteria, viruses, parasites and fungi. In many experiments however the host organism is selected because of its own inability to infect or replicate.

Even at this initial stage, the question of whether the micro-organism is a plant or animal pathogen must be borne in mind for consideration later in the assessment.

As well as the host, the properties of the vector, insert and final GMM must be considered.

If you are applying the Brenner scheme (see **Annex**) to work with bacterial host/vector or tissue culture systems, the Compendium gives details of the numerical **access** values for host/vector combinations, relative values for the **expression** factor and recommended values for **damage** factors. This information leads to the provisional containment levels the numerical values allow you to assign. It must be realised that the Brenner scheme alone is not sufficient for a full risk assessment since it only looks at the hazard to human health and does not consider other factors. Thus the use of the Brenner scheme must be complemented by the addition of a detailed environmental assessment.

The eukaryotic and invertebrate viral vectors are considered to be a useful tool because they have the advantage of producing expressed proteins in abundance. The Brenner scheme cannot be applied to this work.

In most instances the Brenner method is longer viewed as a suitable way of doing a risk assessment on GMOs because of these limitations.

Use of viral vectors

Viruses are grouped on the basis of size and shape, chemical composition and structure of the genome, and mode of replication. Helical morphology is seen in nucleocapsids of many filamentous and pleomorphic viruses. Helical nucleocapsids consist of a helical array of capsid proteins (protomers) wrapped around a helical filament of nucleic acid. Icosahedral morphology is characteristic of the nucleocapsids of many "spherical" viruses. The number and arrangement of the capsomeres (morphologic subunits of the icosahedron) are useful in identification and classification. Many viruses also have an outer envelope.

The genome of a virus may consist of DNA or RNA, which may be single stranded (ss) or double stranded (ds), linear or circular. The entire genome may occupy either one nucleic acid molecule (monopartite genome) or several nucleic acid segments (multipartite genome). The different types of genome necessitate different replication strategies.

Retroviruses have proved to be very useful tools for the genetic engineer. Infection by these endogenous viruses is dependent upon the presence of cellular receptors on the membrane of cells. In most instances the presence or absence of cellular receptors will determine the host range of the viruses.

Retroviruses are generally classified as **Ecotropic**, **Xenotropic** or **Amphotropic**.

Ecotropic viruses are presently used more frequently than viruses in the other two classes. The murine ecotropic leukaemia virus (MuLVe) is commonly used with rodent cells. Amphotropic viruses are being developed and clearly the host range of the virus will be a major factor in determining the containment and ultimate class for any recombinant work.

The use of viruses as vectors for recombinant work should lead to the research worker asking a number of questions during the risk assessment process in order to classify the work properly. Some examples are listed below.

Could the recombinant virus result in an alteration of tissue tropism or host range?

Could there be an increase in infectivity or pathogenicity of the recombinant virus?

Could there be additional effects on other organisms?

What effect will the recombinant virus have on the environment? Is it, for example, better equipped for survival?

Can potentially harmful exposure to the recombinant virus be effectively controlled through vaccination programmes?

Viral vectors which do not infect human cells in culture or for which there is no evidence of human infection, e.g. Baculoviruses, represent a minimal risk to human health and can be assessed provisionally as being suitable, in most cases, to be worked with at containment level 1. Many of these vectors however can be an environmental hazard and a further environmental risk assessment may indicate that additional containment measures are necessary. Their attraction to the genetic engineer is the fact that they contain non-essential genes that can be replaced by genetic modification. These viruses also contain powerful promoters making them useful as expression vectors for protein molecules.

Any Hazard Group 2 pathogen which has been disabled in such a way as to render it "unlikely to cause human disease" may also be assigned to containment level 1, unless they would be a risk to other species. For example, a great deal of work has been undertaken with viral vectors based on non-primate retroviruses, e.g. MuLVe, which are replication defective. There may be instances where mobilisation of replication incompetent viruses can occur by a replication competent wild type virus, but this generally requires deliberate superinfection. The risk assessment will consider this possibility.

The Compendium gives information on assessments of commonly used viral vectors.

The natural host organism for the recipient micro-organism will influence the risk assessment: these could be humans, wild animals, domestic animals insects or plants. You should ask if there are any other host organisms which the recipient micro-organism is capable of infecting and if adequate controls can be put in place to prevent this.

When disabled micro-organisms are used, the assessment must examine whether there is any possibility of the disabling mutations being overcome due to reversion, complementation or recombination. For example, in the case of viral work the latter process could occur in a number of ways: *in vitro* cross contamination within the laboratory; recombination with helper sequences in the packaging cell line; or recombination *in vivo* as the result of a laboratory-acquired infection in a persistently infected individual.

2. Assignment of provisional containment level

On the basis of answers related to the previous section, a provisional containment level is assigned to the project. In most situations this will relate to the level of containment that is appropriate for the recipient micro-organism, but occasionally, for example where the GMM is adjudged to be more hazardous than the recipient, it may be appropriate to assign the work to a higher class.

3. The likelihood of possible adverse effects within the contained area

The main purpose here is to examine whether the control measures that relate to the provisional containment level are sufficient or appropriate for the work.

Examples of factors that may influence this process are:

- The scale and type of the activity (for example, particular consideration should be given to work likely to generate aerosols or to high risk operations such as the use of glass flasks in orbital shakers or the handling of infected animals);
- The extent to which the work can be contained within safety cabinets or sealed vessels (particular consideration should be given to those experimental manipulations where physical containment may be limited);
- The nature, duration and degree of possible human exposure (particular consideration should be paid to the route(s) of transmission and the infective dose);
- The possibility of disabled vectors deliberately infected into laboratory animals recombining with pre-existing infectious agents, e.g., endogenous retro-viruses.

4. The likelihood of environmental adverse effects

The possible adverse effects on the environment must be considered and these may depend on the following kinds of factors:

- Is there a requirement for special methods for waste decontamination?
- Is the GMM able to survive and persist or disseminate in the environment?
- Do the route(s) of transmission of the GMM indicate an additional risk?
- Does the nature, degree and/or site(s) of possible exposure of the environment pose an additional risk?
- Are there any possible adverse affects on the non-living environment (one example could be research using engineered soil bacterium. In this instance the risk assessment would consider whether the recombinant if released could alter the structure of the soil)?
- Are there possible non-direct adverse effects (e.g an engineered marine bacterium which could encourage overgrowth of ponds with weed and so make them unsuitable for fish)?

5. Adjustment of level of risk and selection of appropriate containment measures

In the light of the final assessment of risk, appropriate containment measures are selected using the tables in the GMO(CU) Regulations.

The containment levels are essentially the same as those described for work with human pathogens, but the GMO(CU) Regulations allow specific measures to be taken that relate directly to the risk.

6. Selection of final class

This is dependent upon the containment measures required and equates to the highest containment level at which a necessary control measure has been identified. Thus work predominantly requiring level 1 containment but needing one or more measures appropriate to level 2, must be classified as a Class 2 project. There is provision within the regulations to apply to HSE for derogation where for example the additional measures required are needed to protect the experimental sample from environmental contamination rather than a risk control measure for the GMM. An example here could be the use of class II microbiological safety cabinets when using cell cultures infected with a class 1 recombinant GMM. This of course has ramifications with respect to notification to HSE. The following table summarises the process for GMM work.

Containment levels required to control the risk	Classification of Activity
Level 1 (or less)	Class 1
Level 1 plus some measures from level 2 Level 2 measures	Class 2
Level 2 plus some measures from level 3 Level 3 measures	Class 3
Level 3 plus some measures from level 4 Level 4 measures	Class 4

It should also be noted that the highest Class in any experiment will determine the management requirements. If the example in the previous paragraph is used, then full containment level 2 management is required, for example in respect of staff training, access control and hygiene measures. This is even more significant if measures that equate to

containment level 3 are required. In practice it will usually be simpler to choose to work at the containment level that equates to the class of the project, especially when other work requiring the same containment level is being done in the same facility.

7. Waste disposal

It is important to note that there is also a requirement to state how the GMM and waste and by-products from any such activity are made safe. There is a requirement to validate the procedure used and also to place estimates of effectiveness of the process. For example, if all waste products are autoclaved then 100% effectiveness can be quoted. However, this can only be done when the autoclave has been validated for the conditions used in the decontamination of GMMs.

A record of the validation must be kept.

Chemical treatment may be less effective and again a figure for the efficiency of the decontamination procedure must be kept. This figure may come from documented and published protocols in the literature.

The above must be done irrespective of GMM class.

Genetically modified animals and plants

Introduction

The procedures used for proposals within this field have always relied upon the risk assessment approach. The risk assessment for such proposals will look at the following:

- Properties of the genetically modified organism
- Hazard group of the micro-organism (using the approved list in the ACDP supplement)
- Potential to cause harm to man
- Potential to cause harm to the environment.

Answers to the above points will determine the control measures which need to be in place. The control measures required will ultimately determine the appropriate level for the proposal.

In some fields it is difficult to get precise answers to some of the questions. This is particularly so when dealing with genetically modified plants. The risks to man, or more likely the environment, from genetically modified animals are in many instances easier to assess.

Definition

A genetically modified (transgenic) animal or plant is an animal or plant that has had a gene of a different species added to its normal nuclear gene complement. The new gene or transgene is usually integrated into a chromosome within the genome of the animal or plant. It may or may not be transmissible through the germ line.

Organisms with homozygous deletions (e.g., gene knock-outs) derived by experimental recombination should also be classified as transgenic organisms.

The experimental manipulation of whole animals and plants can be grouped into three main categories.

1. Genetically Modified (GM) animals or plants
2. GMM animals or plants defined as an animal or plant infected with a genetically modified micro-organism (GMM)
3. A GM animal or plant infected by a GMM

For all of the above categories the risk assessment will determine the properties of the genetically modified animal or plant as outlined above.

Genetically modified plants

The potential to transfer genetic information from genetically engineered plants to indigenous microbes in the soil has been looked at experimentally.

It is known that naked duplex DNA is stable under different physical conditions. In soils much depends upon the cationic content and thus the type of soil in which the DNA finds itself. It is clear, however, from such studies that decomposition of organic matter can and does result in duplex molecules potentially capable of binding to bacterial cell walls followed by movement into the bacterial cell.

If this occurred then the potential to transmit modified genes to other species either directly via bacteria or via a plasmid could become a reality. Active transformation of bacteria by free transgenic plant DNA in the environment is the most likely route for gene transfer to other plant species.

Experiments of this nature have recently been carried out using plants modified to carry resistance to kanamycin. Possible horizontal gene transfer was looked at in the soil bacterium *Acinetobacter calcoaceticus* BD413. This particular bacterial isolate is known to be closely associated with plant roots.

Even in the most favourable conditions the transformants per recipient were less than 10^{-13}. The conclusion from these experiments was that *A. calcoaceticus* did not take up non-homologous plant DNA at appreciable frequencies under natural conditions.

Genetically modified plants

Work on GM plants can itself be placed into one of two categories.

Containment level A

Plants within this category are those unlikely to cause environmental harm. In terms of risk to man then a comparison will be made to see if the modified plant poses a greater risk to human health than the non-modified plant. For example, is the GM plant more immuno- or aller-genic? Providing the risk to man and the environment is assessed to be negligible or low then GM plants can be grown in level A premises. A major condition is whether the GM plant is native to the country in question. Can it survive and compete with the endogenous plants? If the answer is no then containment level A facilities will normally be used.

Containment level B

If the GM plant is identified as being a hazard to either man or the environment and the risk cannot be reduced to an acceptable level by containment level A premises then additional measures must be put in place.

Containment level B premises will normally be designed to prevent the escape of GM plant material (e.g. pollen) into the environment. When this is the case, records of environmental risk assessments for level B premises must be kept for 10 years.

DEFRA, the Scottish Executive on Rural Affairs for Scotland (SERAD) or the Forestry Commission Secretariat (FCS) may require to be notified if level B work is being done.

GMM plants

In this situation the risk assessment will determine the level of containment and hence class required for the GMM experiment. Levels 1-4 and class 1-4 will be determined.

GM plants infected with a GMM

A combination of GM and GMM guidelines will be necessary.

Genetically modified animals

Genetically modified (transgenic) animals are those animals where the genetic material has been altered using an approach that does not occur naturally. Mosaic animals are included in this category. The term animal in this context is used in the broadest sense to include vertebrates and invertebrates.

The introduction of pathogenic or non-pathogenic recombinants into embryo stem cells followed by *in vitro* implantation and inbreeding to raise animals with homogeneous transgenes is rapidly expanding. The GMO(CU) Regulations and the Compendium will give aid in determining the control levels required and in the production of a relevant risk assessment. The control measures required to protect both man and the environment ultimately determine the containment level necessary for a particular experiment.

During this risk assessment process care must be taken to look at what the consequences would be to the natural population if a transgenic animal escaped. The risk assessment in the case of genetically modified animals might for example consider the long or short term effect of introducing a foreign gene into the native population.

The containment levels may not necessarily be physical, they could be biological. For example, the work may involve fish which do not survive in waters below a certain temperature (e.g. *Tilapia)*. This species does not survive below 27^{0}C so escape into UK waters means death. Although for many experiments it seems somewhat far-fetched that animals in the wild would be affected one must consider this as a possibility. For example, transgenes affecting the fecundity or the natural ageing process could be introduced into the wild affecting population dynamics.

Containment levels for animals

There are two containment levels for GM animals,

Containment level A and Containment level B.

Animals in containment level A facilities are those that are unlikely to survive in the local environment, unlikely to transfer genetic material to endemic species and the GM modification does not present an additional hazard to man than the non modified parental organism. Finally, the GM animal does not contain or carry a GMM or known pathogen. One such example could be the use of the octopus.

Containment level B animals on the other hand will present a risk both to the environment and to man. This is in terms of transmission of genetic material to endemic species with a possible health risk to the population. Work on rodents can come into this category of containment.

The HSE must be notified if the risk assessment determines that the genetically modified animal poses a greater risk of harm to human health and safety than the non-modified animal.

GMM animals

The risk assessment will determine the containment levels and hence the class of GMM in use: levels 1-4 and classes 1-4.

GM animals infected with a GMM

A combination of GM and GMM guidelines will be necessary.

The Animals (Scientific Procedures) Act 1986 will be the major piece of legislation responsible for ensuring the welfare of animals used in any of the above conditions.

Emergency plans

The risk assessment will also determine the need or otherwise for an emergency plan to be actioned in the event of a spillage, accident or accidental release of the GMO into the environment. This is particularly so for GMM experiments in class 4 but it could be the case for some class 3 experiments. Remember that the emergency services should be fully updated with GMM experiments that require emergency planning procedures to be in place.

HSE will also have to be notified if a spillage, accident or unintentional release of a GMO into the environment occurred where man or the environment was placed at risk by this occurrence.

References

1. A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000. L29 HSE Books. ISBN 0 7176 1758 0 *and* the Compendium of Guidance from the Health and Safety Commission's Advisory Committee on Genetic Modification. HSE 2000.

Annex

The Brenner scheme

Initially the classification of GMMs was done through the use of the "Brenner" scheme. In certain restricted circumstances, primarily for work with specific prokaryotic micro-organisms, this is still operational and pertinent. It can however only be seen as part of, or support for, a full risk assessment, since it only considers potential effects on human health and not the environment. Historically this scheme was based on cloning into the bacterium *Escherichia coli* (*E. coli*). Under the Brenner scheme hazard identification was determined using three independent parameters; "Access, Expression and Damage". The process of hazard identification is illustrated in the Tables 1 to 4.

Table 1 Access factors for host/vector combinations

Vector	Especially disabled[1]	Disabled or non-colonising[2]	Pathogenic, colonising or wild type[3]
Non- mobilisable[4]	10^{-12}	10^{-9}	10^{-3}/1
Mobilisation-defective[5]	10^{-9}	10^{-6}	10^{-3}/1
Self mobilising	10^{-6}	10^{-3}	1

[1]**Especially disabled** host means one whose growth requires the addition of specific nutrients not available in humans or outside of the culture media and is sensitive to physical conditions or chemical agents present in man or the environment. This definition applies to certain specific organisms with an extended history of safe use, as well as some strains of *E. coli* K12 and cell and tissue culture systems where the vector does not have the ability to infect or transfer DNA to other cells.

[2]**Disabled or non-colonising** hosts means a multiple auxotroph or other host which is unlikely to persist in the gut, lung, or survive outside of the culture media, e.g. this includes most strains of *E. coli* K12 and other related species.

[3]**Pathogenic or colonising hosts** include all other hosts. A value of 1 applies if it is pathogenic or non-pathogenic but able to colonise humans. A value of 10^{-3} is appropriate if it is wild type and capable of survival outside of cultures.

[4]**Non-mobilisable** vectors are Bom$^-$, (Nic$^-$), Mob$^-$ and Tra$^-$. They include *E.coli* plasmid vectors such pUC, pAT153, pACYC184, pBR327 and pBR328 and their derivatives.

[5]**Mobilisation defective** vectors are usually Bom$^+$ but Mob$^-$ and Tra$^-$. They include *E.coli* vectors such as pBR322, pBR325, RP4DI, pACYC177 and p154 and their derivatives.

Conjugative or self-transmissible plasmids possess, in addition to information required for autonomous replication, a set of genes for bacterial mating and conjugal transfer of the plasmid to another bacterium. Non-conjugative plasmids lack this set of mating or transfer genes. Non-conjugative plasmids can be mobilised by the presence of another conjugative plasmid if the latter contains mobilisation genes such as "Mob" plus an additional element called Bom. Both the Nic and Mob sites are required for mobilisation of recombinant viruses such as ColK. The tra gene is required for the conjugated transfer of the F factor. Derivatives of single-stranded DNA bacteriophages containing bacterial genes have been developed as cloning vectors. The most widely used are based upon the filamentous coliphage M13 into which the *E.coli lac* promoter, operator and lacZα coding sequence has been introduced. On an appropriate LacZα$^-$ host strain and in the presence of an inducer for the *lac* operon these phages give rise to a Lac$^+$ phenotype and appear as intensely blue plaques containing the chromogenic substrate, 5-bromo-4-chloro-indole-β-D-galactoside (X-gal). Derivatives containing the polylinker cloning site within the lacZα coding sequence allow detection of recombinant phages as colourless (white) plaques.

Table 2 Relative values for the Expression factor for an initial cloning experiment

Deliberate in-frame insertion of expressible DNA downstream of a strong promoter with the intention of maximising expression.	1
Insertion of expressible DNA downstream of a strong promoter with no attempt to maximise expression.	10^{-3}
Insertion of expressible DNA at a site of limited promoter activity.	10^{-6}
Insertion of expressible DNA at a site specifically engineered to prevent expression.	10^{-9}
Non-expressible DNA e.g. DNA with non-foreseeable biological effect or gene containing Introns which the host is unable to process.	10^{-12}

Table 3 Recommended values for damage factors

A toxic substance or pathogenic determinant that is likely to have a significant biological effect.	1
A biologically active substance which might have a deleterious effect if delivered to a target tissue, or a biologically inactive form of a toxic substance which, if active, might have a significant biological effect.	10^{-3}
A biologically active substance which is very unlikely to have a deleterious effect or, for example where it could not approach the normal body level (e.g. less than 10% of the normal body level).	10^{-6}
A gene sequence where any biological effect is considered highly unlikely either because of the known properties of the protein or because of the high levels encountered in nature.	10^{-9}
No foreseeable biological effect (e.g. non-coding DNA sequence).	10^{-12}

Under the "Brenner scheme" the Access, Expression and Damage factors were then multiplied together to give a value which could then allocate the proposed work into an appropriate containment level. This is shown in Table 4.

Table 4 Provisional containment levels for human health

Overall value (Access x Expression x Damage)	Containment level	Class[1]
10^{-15} or lower	1	1
10^{-12} or lower	2	2
10^{-9} or lower	3	3
10^{-6} or lower	3 or 4*	4
greater than 10^{-6}	4	4

- * -This is where an application for derogation can be sought.
- 1 -The new class for activity has been added to show how the Brenner scheme might fit into the activity class bearing in mind the limitations of the scheme (see below).

In GMM work it is always the **risk assessment** which will ultimately determine which containment level is required. It is important therefore to note two things.

1. The **risk assessment** may raise or lower the containment level and from this it follows that the final containment level for GMM experiments may well differ from that determined initially from the ACDP supplement.
2. One of the drawbacks in the case of the "Brenner scheme" was that the risk assessment considered only the level of containment appropriate to protect human health. It did not consider the broader issues such the effects on the environment which may result from a GMM experiment.
3. If you decide to use the Brenner scheme the risk assessment must also include the environmental impact and treatment for the waste or by-products as mentioned previously.
4. Thus although it can be applied in some circumstances to contribute to the risk assessment using prokaryotic vectors, it cannot be applied in all situations. Brenner cannot be used for viral vectors.

MRC Guidance Note 8

Working with cell cultures

Cell culture includes continuous cell lines, finite-life cell strains, and cultures propagated from freshly disaggregated tissue or body fluids (primary cell cultures). Cell cultures may either harbour infectious agents or possibly themselves may be or create a risk to human health. Cell cultures that may cause such harm are included in the definition of a 'biological agent' found within the COSHH Regulations and thus work with them is subject to risk assessment. Within COSHH, Schedule 3 specifically covers all work with biological agents. Further guidance can be found in the associated Approved Code of Practice.

Basic ground rules to follow when culturing cells

The safe use of tissue culture requires that staff are trained in "Good Microbiological Practice" (see **Guidance note 5**), are aware of the potential hazards and meet the conditions imposed by working at the various levels of containment. A guide to appropriate containment levels for tissue culture is given below.

It is important that laboratories adopt appropriate quarantine and segregation procedures to prevent both cross contamination of cell cultures and/or accidental infection with an infectious agent.

The following paragraphs address key issues of working with cell cultures.

A) - What type of cells are you working with?

The hazards and therefore the risk to personnel will vary depending upon the nature of the cells being handled. Many well-characterised cell lines present little or no hazard to the worker and therefore can be handled at containment level 1. However, it is well worth remembering that The Catalogue of Cell Lines from the National Institute of Health or from the American Type Culture Collection state that although their cells are well characterised they still constitute a possible biohazard. These cells still have the potential to carry viruses or latent viral genomes or other infectious agents in a non-apparent state. Users should therefore not assume they are free from contamination. Cells from such companies however will normally have been karyotyped for properties such as the modal chromosome number, chromosome aberrations or breaks, or aneuploidy and translocations. Procedures using fluorescent antibodies, molecular hybridisation and the Polymerase Chain Reaction (PCR) as well as *in vivo* tests on animals may well have been employed to screen cells for adventitious agents. Some may also have been screened using the electron microscope for viral-like particles. The company will supply this information.

The risk assessment may determine that containment level 1 is appropriate, but the work is often carried out using class II microbiological safety cabinets and even at containment level 2. This is primarily to protect the cells from infection from the surrounding environment. In these circumstances it important to note down why the additional precautions are being taken (i.e. not through risk assessment but to protect the cells).

Unscreened cells or cells with a less well defined history however should be handled at a minimum of containment level 2 and in some circumstances there may be a requirement for containment level 3.

Viruses are used to infect cell cultures deliberately, particularly to transform specific cell types. One of the most common in use is the Epstein-Barr virus (EBV). Many B-lymphocyte cells have been transformed in this manner and it is possible that some may harbour residual virus activity with the release of low-level but competent viral particles. A risk assessment on the transformed cell itself may conclude it is non-hazardous (but see **'B'**

below). The possibility that competent viral particles may be shed (i.e., the presence of an 'adventitious agent') however may result in a requirement for the work to be done at containment level 2. In addition portions of viral genomes have also been used to transform cells. The SV40 large T-antigen has been used frequently to transform cells. One such example is the COS-1 cell.

In both of these examples, a risk assessment would conclude that for transformations done 'in-house', or for lines with little or no evidence for non-shedding, etc., Containment Level 2 is required. Transformed cell lines with a long history of safe handling can however be worked with at Level 1.

Other cell lines have been derived from the 292 cells, which are used for recombinant replication-defective adenoviral vectors and NIH 3T3 cells, used for recombinant replication-defective retroviral vectors. New viral vectors are also being introduced particularly in the field of gene therapy. Included amongst these are viruses derived from herpes and from alpha viral genomes.

Thus in general when handling any cell line it is important to be aware of its history and to ensure that appropriate precautionary measures are adopted.

B) - Which species are we looking at?

This is an important question to ask when risk assessments are carried out. In all probability the risk to the worker will be that much higher if he/she is dealing with human or primate cells rather than, for example, using mouse or Chinese hamster ovary (CHO) cells. The reason for this is that there is always the possibility that human or primate cells will be harbouring natural viruses. Simian herpes B virus or Simian immunodeficiency virus (SIV) are two such examples of viruses which can infect humans.

C) - Where does it come from in the world?

With human material such as blood cells or tissue contaminated by human blood components an important question to ask is whether the sample has come from a high-risk environment harbouring the HIV or hepatitis viruses. Samples from these areas must always be handled as high risk and appropriate control methods introduced. Particular care should be taken when handling cells or cell lines recently derived from human tissue. Soft tissues such as spleen, liver and kidney may well harbour adventitious agents such as bacterial or viral pathogens. A clinical history is always valuable if it can be obtained. The human retroviruses are not easily transmissible but when dealing with blood cells suspected of harbouring HIV or similar retroviral particles then needle-stick or puncture wounds are major areas of concern. This is particularly so for samples suspected of carrying a hepatitis virus. The introduction of small amounts of hepatitis-infected blood ($<0.1\mu l$) directly into the blood stream of the operator can be sufficient for viral infection to occur. Emphasis must therefore be placed on procedures that do not use sharps and care must be taken to ensure that pre-existing cuts and scratches are adequately protected.

In the case of animal cells it is pertinent to know if the samples came from a certified laboratory or from a less reputable source. Again the risk assessment should take these factors into account.

Primary cultures

One of the major concerns with primary cultures comes from the likelihood that they may undergo spontaneous transformation. This happens relatively frequently with rodent cells and although not so frequent an occurrence with primate or human cells, this event cannot be dismissed entirely. It is important because of this to follow some simple guidelines, which introduce elementary precautionary measures for the protection of staff.

Transformation of cells isolated from self or a colleague or indeed a fellow worker not necessarily within the same laboratory can pose a hazard to that person and this is discussed in more detail in **Guidance Note 4**. In summary, the key points are: -

A Never culture your own cells and if possible avoid culturing cells from your immediate colleagues.

B Never transform your own or your immediate colleagues' cells.

C Wherever practicable, culture primary cells for short-term periods. In the case of blood cultures then this will normally be for a maximum of between 48-72h. This reduces the probability of spontaneous transformation to practically zero.

D Are the samples screened or unscreened for HIV or infective Hepatitis viral particles? If unscreened does the work require extra precautions? Some issues to consider could be the following:

(1) How many samples are being dealt with at the one time?

(2) How many samples a week?

(3) Screened or unscreened samples?

(4) Experience of the worker?

(5) Are the facilities adequate?

Further culture of cells

Risk control measures

Table 1, together with the accompanying notes, gives advice on the selection of appropriate risk control measures.

Table 1 Containment levels appropriate for handling of cell cultures

Hazard	Cell Type	Containment
LOW	Non-human, non-primate cell lines that have been authenticated and have a low risk of endogenous infection with a human pathogen and present no apparent hazard to laboratory workers.	Containment level 1
	Well-characterised, screened and/or authenticated finite-life cell lines of human or primate origin.	Containment level 1
MEDIUM	Cell lines or cell strains that have not been fully characterised.	Containment level 2
HIGH	Primary cells from blood, lymphoid cell, neural tissue of human or simian origin.	Containment appropriate to the potential risk.*
	Primary cell lines cultured for more than 100 hours.	Containment appropriate to the potential risk.*
	Cell cultures known, or strongly suspected, to harbour an endogenous pathogen.	Containment appropriate to the pathogen.*
	Cell cultures deliberately infected with a human pathogen.	Containment appropriate to the pathogen.*

* see **Note 2** following

Notes to table.

1. General comments.

Whenever practicable, only cell strains that have been authenticated and/or have a documented provenance should be used. These are best obtained from the originator of the cell culture or from a culture collection. To avoid cross contamination of cell cultures only a single cell line should be handled at a time with appropriate decontamination between operations. The culture medium, however, may contain components (e.g. serum or antibiotics) that have sensitising activities. The presence of allergenic products in the cell culture requires stringent primary containment and/or personal protection to avoid inhalation or contact with skin, eyes, or the mucosal membranes. Sharps should not be used, and rapid pipetting, scraping and pouring should be avoided to limit the risk of exposure to aerosols or splashes. Microbiological safety cabinets should be used where appropriate.

2. Cell cultures, deliberately infected or potentially contaminated with an adventitious agent

Where cell cultures are deliberately infected the level of containment required will be that appropriate for handling of the particular agent. All procedures involving **propagation** of infected cell cultures should be conducted at a minimum of containment level 2 with all manipulations performed in a microbiological safety cabinet. Where the volume and number of cells are high (e.g. large-scale cultures in a single large vessel), or where the level of exposure is increased because of unavoidable production of aerosols, then containment conditions should be upgraded.

If at any time there is knowledge, or suspicion, that a cell culture contains a hazard group 3 pathogen then the work must immediately be transferred to containment level 3.

Signs to look for.

1. Changes in the phenotype of the cell for example, alterations in its shape, size and cell confluence and the loss of contact inhibition;

2. Speed of cell division (has this changed and if so what does it or could it mean?)

If some of the above parameters have altered then they can be possible indicators that a natural transformation of a primary cell culture has occurred. Alternatively the presence of virus particles may be detected. In these situations the level of containment must be re-assessed.

3. Genetically modified cell cultures

Well characterised eukaryotic cells and tissue culture systems can be considered to be especially disabled hosts provided that the cells are unable to colonise the worker (not own cells) and contain no adventitious agent. A risk assessment under the COSHH regulations should be done on these cells to ensure the safety of the worker. Similarly a COSHH risk assessment should be carried out on any other type of cell in use to ensure safe operations and procedures. If it is proposed to use such cells as a vehicle for GMM (including viruses) experiments then a second risk assessment must be carried out on the GMM under the Contained Use Regulations. The GM risk assessment will solely be concerned with the properties of the GMM and will determine the containment and class level for that GMM. It may well be that when the risk assessments are completed the containment levels are different. The higher containment level is the one adopted.

It is acceptable to do work at a higher containment level. For example, the risk assessments may determine the work to be Class 1/Containment Level 1 but to prevent infections of the cells from outside agents a Class II microbiological safety cabinet is used. The risk assessment should state this fact.

4. Disinfection and waste disposal

An effective decontamination policy is required for all wastes generated by cell cultures including discarded cell culture fluids, cells and glass or plasticware. Decontamination procedures should be capable of inactivating viruses and other contaminating agents in the presence of fluids that are often heavily loaded with organic material. The risk of infection at the various stages of disposal should be assessed and appropriate measures taken. The procedures and disinfectant to use in the event of accidental spillage should be part of disinfection policy.

Culture work

Use of a Class II microbiological cabinet.

When used properly this provides protection both to the cells and to the users.

Ensure that:

(a) The correct waste disposal facilities are on hand;

(b) You have the use of an autoclave to sterilise biological waste;

(c) You use of the correct type of disinfectant and that it is at the correct concentration;

(d) You use the correct type of personnel protective equipment.

Cell storage

You should have a formal system for storage of cells, including a details of location for each sample and records of removal for use.

Where samples are stored in or above liquid nitrogen staff must be instructed in its proper use, including what is the correct protective equipment to wear (visor, gloves, suitable footwear, laboratory coat).

Ethical issues

These can be extremely important. Questions of confidentiality often arise. These broader issues should be examined before experiments are started. It is important that guidance and ground rules on these issues are firmly established and understood. Fuller guidance can be found in **Guidance note 4**.

MRC Guidance Note 9

Working with human material

1. Code of practice and guidance for laboratory work

Introduction

This code applies to all staff, attached and visiting workers who work under the management control of the MRC. All workers using these tissues and agents should also be familiar with the publications listed at the end of this **Guidance note**.

Risk assessment

A risk assessment must be made for all work involving the handling of any human material. The risk assessment should be specific for the procedures involved and take account of the nature and source of the samples to be handled. Work may not begin prior to the assessment and confirmation that the correct facilities required are available.

All human tissue will be contaminated with blood. Therefore it should be regarded as potentially infected material. Of particular concern is the possible presence in the material of blood borne pathogens, most notably HBV and HIV. Specifically, where it is known or strongly suspected that Hazard Group 3 pathogens are present then samples must be handled at the corresponding containment level.

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, saliva and both CSF and brain tissue. Possible HIV contamination should therefore be considered when handling materials of these types in the laboratory.

Any unscreened samples should be regarded as potentially infected. Wherever possible material should be used that can be shown by screening to be pathogen free at source. This may not however guarantee the sample is HIV negative because of the window between infection and sero-conversion. 'In-house' screening of samples should not be done unless it has full approval of local ethical committees and full informed consent of all donors.

All work on unscreened samples must be undertaken at a minimum of Containment Level 2 with the additional precautions given in this guidance note. 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 must immediately be transferred to Containment Level 3. Where such facilities are not provided, work on these samples must cease.

In general, work at Containment Level 2 does not need to be confined to a safety cabinet unless there is reason to believe the specimen contains other pathogens that do require such containment. There is no substantive evidence which supports aerosol transmission of HBV and HIV. However where handling or processing may generate large droplets or splashes containment control measures must be adopted. At containment Level 3 all handling of material must be undertaken in a microbiological safety cabinet.

The onus is on the researcher to assess the risks associated with the work and specify appropriate working practices. A table to aid with risk assessment for the different types of

samples and the procedures required to control exposure is provided in Table 1. This will apply to most samples in most circumstances. Additional precautions for handling unscreened samples in the laboratory are also given in the notes to the table.

Table 1. Containment levels for laboratory work with human blood and other human tissues.

Question	Answer	Action
Is the sample from the Blood Transfusion Service or known to be negative for any pathogen, Hepatitis &/or HIV?	YES	Use Containment **L 1**
	NO	Use Containment **L 2** and **additional precautions if required** by risk assessment
If the samples are from the Blood Transfusion Service or known to be negative for any pathogen, Hepatitis &/or HIV are they to be grown in tissue culture for more than 100 hours?	YES	Use Containment **L 2** and **additional precautions if required** by risk assessment
Is the sample from a patient suspected or known to be positive for any pathogen, Hepatitis &/or HIV?	NO	Use Containment **L 2** and **additional precautions if required** by risk assessment
	YES	Handle at **appropriate CL** corresponding to **hazard group of pathogen**, with **additional control measures if required** by risk assessment*
Are the suspect or known positive samples to be grown in tissue culture for more than 100 hours?	NO	Handle at **appropriate CL** corresponding to **hazard group of pathogen**, with **additional control measures if required** by risk assessment*
	YES	Use Containment **L 3***

Note:
*Detailed risk assessment may exceptionally justify undertaking the work at a lower level of containment to that described here. Derogation of containment in this way is subject to consultation with the Health and Safety Section and approval by the local safety committee. Help and advice on risk assessment can be given on a case by case basis and researchers are encouraged to contact the Section at an early stage.

Additional precautions for handling unscreened samples in the laboratory:

1. The use of sharps is only permitted where there is no alternative. If sharps are used then they must be placed directly in sharps bins for disposal.

2. Sharps bins should be autoclaved wherever possible before being incinerated.

3. Gloves must be worn at all times when handling samples and be removed before leaving the laboratory.

4. Single use (disposable) gloves must not be re-used. Multiuse gloves must be regularly checked for integrity.

5. In the event of gloves becoming damaged or significantly contaminated the gloves must be discarded, hands washed and new gloves put on.

6. Eye protection (goggles or safety glasses) and a plastic apron should be worn if the work activity could cause splashing.

7. Materials must only be handled at clearly identified, designated work stations.

8. On completion of work the work station and all equipment must be disinfected.

9. Samples must be centrifuged in sealed safety buckets or rotors. For work at containment level 2 or 3 the buckets or rotors must only be opened within a microbiological safety cabinet.

10. All waste materials must be made safe before disposal.

Hazards and risks

Hazard categorisation

In determining the appropriate hazard grouping of a pathogen, the ACDP has considered the pathogenicity (disease-producing capability) of the organism to man, the hazard to laboratory workers, the potential for transmission to the community and the seriousness of any illness that might result after taking into account the availability of prophylaxis or effective treatment (see COSHH Schedule 3 for definitions of Hazard Groups).

Control of exposure

Safe working practices

Good working practice standards must be maintained. Those who work with potentially contaminated samples can be protected by the consistent application of simple precautions which will protect against the transmission of all blood borne pathogens by the percutaneous route. Most importantly the use of sharps should be avoided wherever practicable.

The following guidance on control of exposure is directed to work that does not require level 3 containment (see Table 1).

- Work must be assigned to the appropriate containment level and the necessary requirements with respect to the facilities and working practices met.
- Staff must be trained and proficient in safe working practices and techniques for the safety of themselves and other persons in the laboratory. Workers must be able to recognise how exposure can occur and how it can be prevented.
- Local rules, based upon those included in **Guidance Note 5**, should be drawn up to ensure that working practices ensure high standards of hygiene and the correct wearing and use of protective clothing.

Additional notes on the wearing of protective clothing

For work included in this appendix, properly designed laboratory coats (see **Guidance Note 5**) should always be used, be kept apart from other laboratory clothing and never stored with other clothing.

It is recommended that, wherever dexterity is not prejudiced or loss of some dexterity is not prejudicial to safety, two pairs of single use (disposable) gloves be worn when handling high risk samples. Minor damage to thin gloves often goes undetected until skin contamination is noticed.

In these circumstances, if during use the outer glove becomes punctured or significantly contaminated it should be removed and disposed of immediately. If the inner glove is also damaged or contaminated it should also be disposed of and hands should be washed thoroughly before new gloves are put on.

Single use (disposable) gloves should not be re-used.

Eye protection (goggles or safety glasses) and a plastic overall should also be worn if splashing is possible.

On completion of work gloves should be removed and discarded, hands should be washed and protective clothing changed.

Sample reception

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

Decontamination and disinfection

Disinfection

All specimen containers, glassware and used equipment should be immersed in a suitable disinfectant before cleaning or disposal.

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. Permanent cleaning or maintenance staff should be trained in the correct procedures and non-technical visitors should be instructed in the work to be carried out and, where any hazard may be present, suitably supervised.

Disinfectants should be used in accordance with local policy developed from the guidance included in **Guidance Note 14**.

Disposal of waste

All contaminated waste must be disposed of safely. Local Rules, based on the recommendations of **Guidance Note 15**, must specifically state laboratory procedures and arrangements for disposal of contaminated materials. All contaminated equipment, surfaces, protective clothing, etc. should be decontaminated after use.

Safety and competence of staff

Training and competence

Appropriate training in the techniques to be adopted and the safety precautions to be followed must be given to all workers. The degree of training required will depend on the expertise of the person being trained but supervisors should not assume competence until it has been demonstrated. Particular care must be taken in the training of undergraduates and in their supervision. Any work involving persons on youth employment schemes must be subject to an in depth assessment of potential risks and these staff will also require close supervision. Young persons on work experience schemes should not work with blood, blood products or pathogens. They must at all times be closely supervised when working in any hazardous area. Line managers should note there may be a requirement to supply schools, parents or guardians with a statement on the hazards and risks of the work.

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 nominated supervisor or team leader and addressed immediately.

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 of sero-conversion and (although the likelihood still remains difficult to predict) that the person will go on to develop AIDS. Whilst the length of the illness may vary considerably, often interspersed with periods of remission, the causally related outcome is usually death. The consequence of infection dictates that all effort must be made to prevent accidental exposure.

Formal competence assurance is required for all personnel working at containment level 3.

Accident reporting and investigation

Full details on the requirements and procedures for reporting and investigating all accidents and incidents are included in specific MRC Health and Safety Policy and Guidance. The following paragraphs complement that document.

In the event of an accident any resulting wound should be encouraged to bleed and the area washed with soap and water. Scrubbing should be avoided. The wound should be covered with a waterproof dressing. Any contaminated skin, conjunctivae or mucous membranes should be washed immediately.

Particular care should be taken to ensure that others do not help with the clear-up of an accidental spillage (especially where there has been an accident that involves broken glass) if they are not aware of the potential risks and trained in safe working practices.

Accidents should be reported to and recorded by the person responsible for the work. A full accident record should be completed as soon as possible. The source of any contamination (specimen, sample, material, etc.) should be clearly identified and retained for testing if necessary. Every effort should be made to ensure the confidentiality of persons potentially exposed to HIV as a result of an accident. In the event of potential exposure to any blood borne pathogen the injured party should be referred to the Occupational Health Service Provider immediately.

The Occupational Health Provider may request a serum sample for storage, and testing if indicated. Staff 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. The taking of further samples for storage and possible testing may be required.

Safe workplace and equipment

Designated workstations

Work should be conducted at a delineated and secluded work station which is clearly identified. Systems of work should be in place to ensure that the person carrying out the work is free from the risk of disturbance or accidental contact with others.

There should be sufficient room to work safely. There should be enough bench space to ensure the workstation is not cluttered and working practices are not compromised due to lack of space.

The work station 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.

Use of sharps

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 must be replaced with plastic alternatives.

Used sharps should be placed directly into a sharps bin. Unless safe means have been introduced needles should not be re-sheathed. Sharps bins should not be overfilled, as used sharps protruding from bins are very dangerous for those who have to handle them. DO NOT DISPOSE OF ANY SHARPS, PARTICULARLY HYPODERMIC NEEDLES VIA NON-HAZARDOUS NORMAL WASTE DISPOSAL SERVICES. DO NOT DISPOSE OF ANY SHARPS IN PLASTIC BAGS.

All sharps and hypodermic needles must be disposed of directly to sharps containers which conform to the British Standard 7320: 1990 and UN packaging requirements. All sharps which may be contaminated with pathogenic organisms should, wherever possible, be autoclaved in their boxes before collection for incineration.

The term sharp should be taken to refer to any item that is sharp and not be restricted to needles and scalpels. Commonly used items that could easily cause damage to the skin include 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. This list is not exhaustive and all items should be assessed for sharp edges.

Laboratory equipment

All equipment should be cleaned and disinfected at the end of the working day (or after each use if it is used for other purposes). If the operation of the equipment results in the production of droplets or splashes these must be contained to avoid spread of contamination. Equipment must be decontaminated prior to maintenance work. A signed statement should be issued to this effect before maintenance work is allowed.

Samples should be centrifuged only in sealed buckets or rotors. These should be cleaned and disinfected regularly and immediately following leakage. Rotors or buckets 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.

Summary of requirements

The main requirements are to:

- Assess the risk of work activities involving the handling of blood, blood products and other human tissues;
- Provide information and training prior to starting work and thereafter monitor standards;
- Control exposure to pathogenic organisms. Handling precautions are given, and should be incorporated into local rules;
- Ensure staff are strongly recommended that they be vaccinated against hepatitis B according to risk assessment and local policy;
- Notify the Headquarters Office Safety Section of any work knowingly involving HBV, HIV or other hazard group 3 pathogens; and
- Ensure where appropriate, approval has been sought and received from the local research ethical committee.

2. Guidance for the taking of blood from volunteers in the community

Introduction

Several establishments undertake work in the community as a principal or supporting research activity. In some studies, there is a requirement to acquire tissue samples from volunteers. These samples are often taken at locations outside the main unit or team premises, usually in the home of the volunteer. This guidance provides an overview of the significant health and safety issues related to this work. It is written for work with blood, but the principles also apply to taking other samples, e.g. mouth swabs.

Ethical approval and consent

All studies involving the taking of blood from volunteers are subject to approval by the local ethical committee. Formal consent will also be required from each volunteer.

Counselling

As part the process of acquiring informed consent, consideration should be given to the possible need for counselling of study subjects. This may arise where subsequent tests made on the blood sample may reveal factors potentially harmful to the individual or their future children: for example, the presence of a genetic marker indicating heritable disease, or a latent infection, e.g. HIV seropositivity. These issues may be less important in studies where samples are anonymised prior to testing.

Risk assessment

Overview

A risk assessment must be made for all work conducted outside the permanent workplace. Additional guidance on working in the community can be found in MRC Policy and Guidance on 'Working alone'. The risk assessment should take account of the nature and source of the samples to be handled.

As a starting point, human blood should be regarded as potentially infected material. Of particular concern is the possible presence in the material of blood borne pathogens, most notably HBV and HIV. Thus the risk assessment should consider the individuals from whom the samples are being taken, in respect of whether or not they should be assigned to a high, medium or low risk group.

Any unscreened samples should be regarded as potentially infected. Thus the precautions taken should mimic as closely as is practicable those required for working with Hazard Group 2 micro-organisms. Consideration may have to given to including additional precautions, or even the exclusion of some subjects from the study if, during the testing process, the presence of blood-borne viruses is confirmed.

The onus is on the researcher to assess the risks associated with the work and specify appropriate working practices.

Hazards and risks

Hazard categorisation

The researcher should assume that any one sample may carry an infectious agent, assigned to Hazard Group 2 as a minimum.

Control of exposure

Safe working practices

Good working practice standards must be maintained. Simple standard precautions should protect against the transmission of all blood borne pathogens by the percutaneous route.

- Staff must be trained and proficient in safe working practices and techniques for the safety of themselves and the study subjects. Workers must be able to recognise how exposure can occur, how it can be prevented and what to do in an emergency.

- Local rules should be drawn up to ensure that working practices ensure high standards of hygiene and the correct wearing and use of protective clothing. The following section provides base-line guidance.

Precautions for taking blood samples in the community:

1. The process will involve the use of sharps. Staff must ensure that they carry with them approved sharps bins for safe disposal on site. Sharps containers should conform to the British Standard 7320: 1990 and UN packaging requirements

2. Staff are advised that they should keep all sharps containers and other yellow bags out of sight both in their vehicles and when carrying equipment to and from the vehicle. It is recommended that all such containers are held within a second unidentifiable box or bag.

3. Measures should be in place to ensure that sharps bins are autoclaved wherever possible on return to the workplace before being incinerated.

4. Only sufficient needles, syringes and/or vacutainers should be taken to each premises for the number of samples required.

5. Gloves should be worn at all times when taking samples and be removed into an appropriate waste bag on completion of the task before leaving the subject's home.

6. Single use (disposable) gloves must not be re-used.

7. In the event of gloves becoming damaged or grossly contaminated the gloves must be discarded, hands washed and new gloves put on.

8. On completion of sample collection, staff should ensure that all swabs, syringes and other packaging are collected, made safe and removed from the premises.

Additional notes on the wearing of protective clothing

For work included in this guidance, it may not be appropriate to wear a laboratory coat. It is appropriate, however, to consider any need for wearing protective clothing as part of the risk assessment, for example a plastic apron.

Sample storage

Wherever practicable, samples should be returned to the workplace for refrigeration or frozen storage. If circumstances dictate that samples are taken home first, then storage must be safe. If such use is occasional, then it may be acceptable, according to the risk assessment, for samples to be stored temporarily at home. The primary sample tubes should be placed securely in a secondary container. The container should be wiped with disinfectant and sealed before storage in a defined area of a domestic refrigerator or freezer. If the requirement is regular, consideration should be given to the purchase of a small dedicated refrigerator or freezer.

Decontamination and disinfection

Disinfection

Wherever reasonably practicable, equipment should be single use and disposable. Disinfectants should be used in accordance with local policy developed from the guidance included in **Guidance Note 14**. Wipes impregnated with the appropriate disinfectant should be carried by research staff on visits.

Disposal of waste

All contaminated waste must be disposed of safely. Local rules for taking blood samples in the community must specifically include procedures and arrangements for disposal of contaminated materials

Safety and competence of staff

Training and competence

Appropriate training in the techniques to be adopted and the safety precautions to be followed must be given to all workers. The degree of training required will depend on the expertise of the person being trained but staff are referred to **Guidance Note 4** for competence requirements for those taking blood.

The HIV virus is not highly infectious nor particularly hardy and studies have shown that the occupational risk of exposure is low. The risk assessment will have made a suitable judgement on the likelihood of the subjects being HIV-positive and hence the possibility of sero-conversion following accidental inoculation. Staff must be fully aware of the risk assessment carried out for the study and of any contingency measures in place should a needlestick injury occur.

Immunisation

All staff that take blood samples should be offered immunisation against Hepatitis B virus.

Accident reporting and investigation

Full details on the requirements and procedures for reporting and investigating all accidents and incidents are included in specific MRC Health and Safety Policy and Guidance. The following paragraphs complement that document.

In the very unlikely event of an accident occurring in a subject's home related to the study process, the researcher should ensure as far as possible that any wounds are cleaned and appropriately dressed. Action should be limited to immediate first-aid. If further action is required, appropriate medical assistance should be summoned, e.g. an ambulance or general practitioner.

Accidents to researchers or study subjects should be reported to and recorded by the person responsible for the work. A full accident record should be completed as soon as possible. In the event of potential exposure of a researcher to any blood-borne pathogen the injured party should be referred to the Occupational Health Service Provider immediately.

The Occupational Health Provider may request a serum sample for storage, and testing if indicated. Staff 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. The taking of further samples for storage and possible testing may be required.

Safe workplace

Designated workstations

In fieldwork, there is limited control over the workplace, so greater reliance has to be placed on the competence of the individual worker, supplemented by good work practice. There should however be sufficient room to work safely. As far as possible the sample should be taken in an area free from the risk of disturbance or accidental contact with others.

Summary of requirements

The main requirements are to:

- Assess the risks of handling blood in domestic premises;
- Develop safe handling procedures and where appropriate local rules;
- Ensure all staff taking blood are competent and aware of emergency procedure;
- Ensure staff are strongly recommended that they be vaccinated against hepatitis B according to risk assessment and local policy - and
- Ensure where appropriate, approval has been sought and received from the local research ethical committee.

References

1. Protection against blood-borne infections in the workplace: HIV and hepatitis. 1995. ACDP/HMSO. ISBN 0 11 321 953 9.
2. The 2000 supplement to the Categorisation of biological agents according to hazard and categories of containment, incorporating Appendix 24 and the 4th edition of the Approved List. C40. MISC208.
3. Control of Substances Hazardous to Health Regulations 2002, Approved Codes of Practice (L5). HSC. ISBN 0 7176 2534 6.
4. Safety in Health Service Laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2003. HSAC/HSC. ISBN 0 7176 2513 3.
5. Safe disposal of clinical waste. 1999. HSAC/HSE. 0 7176 2492 7.

MRC Guidance Note 10

Working with animals

Introduction

There are four major subject areas when considering working with animals. First, there is the welfare of the animal. This area is governed by Home Office requirements and is not covered here.

Secondly, there are issues relating specifically to transgenic animals. These issues are included in **Guidance note 7.**

Thirdly, there are the hazards and risks to human health of animal husbandry, the most significant being exposure to animal allergens. This is covered fully in the MRC Health and Safety Policy and Guidance on **allergy** (Version 1, 1999). That guidance is complemented here with Frequently Asked Questions (FAQs) for those working with animals.

Finally there are the specific issues that relate to work with animals deliberately infected with biological agents hazardous to man. The second part of this Guidance Note focuses on this aspect of the work.

Animal allergens

Background

These notes are intended to assist those working with laboratory animals. They address specific concerns about Laboratory Animal Allergy (LAA) and what you need to do. They should be used in conjunction with the Policy and Guidance on **allergy**. In addition helpful guidance on avoiding and controlling exposure to animal allergens can be found in HSE Guidance Note EH76[1]. An Approved Code of Practice on Occupational Asthma is associated with the COSHH Regulations[2].

What Is laboratory animal allergy?

Laboratory animal allergy (LAA) is an allergic response which may develop as a result of repeated exposure to animal allergens. In some cases specific allergens have been identified, e.g. proteins in rat urine, but in general, fur, saliva, serum and urine are all regarded as sources of unidentified allergens.

What are the symptoms?

The main symptoms are: -

- Rhinitis (blocked or running nose and sneezing)
- Conjunctivitis (irritation and watering of the eyes)
- Skin rashes (usually nettle rash or eczema)
- Asthma (cough, chest tightness, wheezing).

The symptoms are usually immediate but in the case of asthma may occur several hours after exposure has ceased.

The commonest symptoms are rhinitis and conjunctivitis. The most important health problem from LAA is allergic asthma.

Allergic asthma

Allergic asthma arises from an increased responsiveness of the respiratory system as a result of breathing animal allergens. The process is known as sensitisation.

- Sensitisation is allergen specific and probably irreversible;
- Only some of the individuals at risk will become sensitised;
- Symptoms do not occur on first exposure to the allergen so it may be difficult to identify when sensitisation occurred;
- It is thought that the onset of sensitisation is particularly associated with short-term peak exposures;
- Further exposure of a sensitised person may lead to respiratory symptoms at concentrations which do not produce symptoms in unsensitised individuals. Sensitised employees may suffer acute effects on their health when exposed to concentrations of a sensitiser that have no evident effect on others doing identical work. In rare cases, exposure of a sensitised individual to allergens can lead to anaphylactic shock.

When do symptoms occur?

LAA symptoms commonly develop within six months of first starting work with animals and in most cases within a two year period. However, they can occasionally occur for the first time after many years working with animals.

Prevalence

Recent data showed that of those people working with laboratory animals in Britain, between 15% and 35% may experience some symptoms of LAA. Up to 3% may have occupationally induced asthma.

Who may suffer?

It is not possible to predict who will develop LAA. Any person exposed to animal allergens at work may develop LAA. Individuals with allergic symptoms from exposure to animals, including pets, may be particularly susceptible. There is an association between individuals who suffer from atopy (e.g. allergenic reactions such as hay fever) and the development of symptoms to LAA, in particular, asthma. However, a third of the population is atopic if judged by skin testing and it would not be justified to exclude all these people from working with animals as LAA will only develop in a minority.

Which animals may induce allergy?

Insects, birds and most mammalian species may induce allergy. However, most cases relate to work with rats, mice, guinea-pigs and rabbits and in particular rat urine is known to be a major sensitiser.

Routes of exposure

Allergens may be deposited on animal food, litter or dust particles and become airborne as a result of both human and animal activity. Inhalation of these allergens in the form of aerosols or particles is the primary route of sensitisation. However, reaction can occur by entry through damaged skin.

Can it be measured?

Although analytical techniques do exist to measure airborne allergen concentration they are still in the developmental stages and are not yet suitable for practical use.

Total dust measurement is not suitable either as the allergen levels only make up a small proportion of total dust levels.

Risk assessment - Is there a safe level of exposure?

The levels of animal allergen to which individuals have to be exposed before sensitisation occurs or symptoms of LAA appear are not known. It is known that people who become sensitised can experience symptoms after exposure to very low allergen concentrations.

As there is no known safe level of Laboratory Animal Allergen and it is impractical to conduct routine monitoring it is essential that risk assessments are carried out to identify the length and concentration of exposure to these allergens.

The assessment should be conducted as part of the project proposal and take into account the following:

1. Length of exposure.
2. Likely concentration of allergens, e.g. high, medium or low.
3. Ventilation changes and airflow patterns within the rooms.
4. Other protective equipment (local extract, filter top cages).
5. Respirator protection.

The aim of the assessment is to minimise the risks to health by reducing exposure to the allergens as far as is reasonably practicable.

What should you do - Health surveillance?

If you work with animals you should be undergoing health surveillance to monitor and protect your health. Current best practice includes a lung function test on appointment along with a questionnaire. The recommendation is that the questionnaire and lung function testing is repeated at twice more in the first six months and at twelve months. Thereafter the testing is done on an annual basis. If you are working with animals and you are not currently undergoing health surveillance you should contact your Occupational Health Unit.

What should you do - Protective clothing?

When undertaking work with animals **dedicated** protective clothing must be worn. This should include overshoes and a laboratory coat or overalls. This is to help reduce the spread of allergens outside the animal unit. Disposable gloves should be worn as appropriate to protect the skin against urine and contaminated bedding.

What should you do - Respiratory protective equipment (RPE)?

The use of suitable RPE has been shown to reduce effectively the exposure to airborne animal allergens. These must be worn when the procedure or risk assessment warrants their use.

Two types of RPE are generally used within animal units. Disposable (Shift Masks) and ventilated powered Respirators.

Disposable

These must comply with European Standard EN 149 and have a minimum rating of FFP 2(S).

Ventilated

Ventilated respirators provide positive pressure protection to the user and pass filtered air over the wearer's face. These must comply with the European Standard EN 146 and have a minimum rating of P2(S).

What should you do - Transport of animals (outside of animal unit)?

- Animals should be transferred in clearly labelled filter top boxes containing suitable temporary bedding.
- Used bedding should not be removed from the animal unit.
- Racking should not be removed from the animal unit. Animals should be transported either on trolleys or carried securely.

What should you do - Working with animal tissue?

- An assessment will be required to ensure that suitable and sufficient precautions have been undertaken.

Working with infected animals

Background

Guidance on working with infected animals previously contained within the ACDP Guidance on biological agents, has now been extracted and expanded into specific guidance, 'Working safely with research animals: management of infection risks'[3]. Staff requiring more detailed guidance should refer to that document.

Animal pathogens

In addition to the concerns for human health arising from LAA, there is also the potential problem of zoonoses. As animals can pass on zoonoses to staff all animals should have a health check prior to arriving at the laboratory. Staff should also be aware of which diseases animals can carry, how they can be transmitted and what the early diagnostic signs in humans are if infection occurs.

Other pathogens

If the animals are to be deliberately infected with pathogens appropriate containment measures will be required. These are listed in the ACDP document[3]. As with research laboratories there are four levels of containment, with one being the lowest and four the highest.

Thus animals infected with human pathogens in Hazard Group 2 must be housed, as a minimum, in animal containment level 2 housing, and similarly for work with Hazard Group 3 and 4 pathogens.

Those working with animal or plant pathogens will have to satisfy the requirements of other authorities such as the Department for Environment, Food and Rural Affairs (DEFRA, see also **Guidance note 11**).

Most animal holding and procedure rooms meet the requirements of containment for level 2, due to Home Office standards of housing.

Ventilation and containment

As with research laboratories, the requirement for containment level 2 is that where mechanical ventilation is installed, then the room should be under negative pressure. Since Home Office requirements include the need for significant air changes per hour, it follows that containment level 2 animal accommodation should be maintained at negative pressure. Occasionally this may be balanced by animal welfare requirements, but positive pressure is only permitted where there is no significant risk of airborne infection or exposure to animal allergens. All procedures involving handling of pathogens should be conducted either in a safety cabinet or isolator.

At containment level 3 the room must be extracted through HEPA filters and all infected animals must be contained within primary containment, such as an isolator. Independently ventilated cages, maintained at negative pressure, have been developed but their use at Containment Level 3 for airborne pathogens can only be considered following a rigorous risk assessment. If they are selected for use, it is likely that additional respiratory protective equipment (RPE) will be required. If, exceptionally, open cages are used, then staff must wear high performance RPE at all times while in the room.

Environment

Waste disposal

The disposal of animal waste and carcasses is covered by the Environmental Protection Act 1990. The definition of hazardous waste includes: -

"any waste which consists wholly or partly of tissue, blood, excretions, etc., being waste which unless rendered safe may prove hazardous to any person coming into contact with it".

The above definition would include all quarantine waste, bedding and carcasses. It would also include all waste, bedding and carcasses from animals infected with pathogens. It is possible that animals undergoing drug studies may also come under the above.

More details can be found in **Guidance note 15**.

References

1. Control of laboratory animal allergy. Guidance Note EH76. HSE 2002. ISBN 0 7176 2450 1.

2. Control of Substances Hazardous to Health Regulations 2002. Approved Codes of Practice, L5. HSE Books. ISBN 0 7176 2534 6.

3. Working safely with research animals: management of infection risks. ACDP. HSE 1997. ISBN 0 7176 1377 1.

MRC Guidance Note 11

Working with animal and plant pathogens

The importation and holding of animal and plant pathogens is subject to legislation enforced by National Agriculture and Fisheries Departments (NAFDs - for addresses see the last page of this note).

Importation of animal and poultry pathogens

Within the European Union (EU)

No licence is required to import from another country within the community, but approval must still be obtained via a Specified Animal Pathogens Order (SAPO) 1998[1] to transport within the UK from the point of entry. Notice has to be given 24 hours in advance to the Divisional Veterinary Officer (DVO) of any such imports. The DVO is contactable through NAFDs.

From outside the EU

A licence is required to import any animal or poultry pathogen from outside the EU. The licence is authorised under the Importation of Animal Pathogens Order 1980[2] and is issued in writing by the appropriate NAFD. No pathogen covered by the Order can be imported without a licence, and in accordance with the conditions of that licence.

Handling of specified animal pathogens in Great Britain

Anyone wishing to possess, handle, or transport dangerous animal pathogens or carriers which contain them requires a licence issued under SAPO 1998. The 1998 Order, which replaced the 1993 Order, extended the definition of "specified animal pathogen" to include nucleic acid derived from a pathogen listed in the Schedule and which may be capable of producing that pathogen. Copies of the Order can be obtained from the Stationery Office. A licence is required whether or not the pathogen or carrier is to be imported or is already present in Great Britain and is issued only after the laboratory is inspected to ensure suitable facilities and procedures are in place. The licence stipulates the conditions of holding, use and transportation and includes a requirement to keep records. Transportation is only permitted (again subject to approval) to another licensed laboratory.

Application for a licence must be made at least 6 weeks in advance to the NAFD on the approved form also obtainable from that office. A list of the specified animal pathogens is included on the form but is also included in Schedule 1 of the 1998 Order.

Applications for licences should only be made by Directors or authorised team leaders.

Guidance on holding pathogens, including the application form, can be found at
http://www.defra.gov.uk/corporate/regulat/forms/ahealth/.

Pathogens of other animals, bees and fish

There are specific Orders that govern the handling of pathogens of other animals, bees and fish. Details, including lists of pathogens, can be found in the ACDP publication and advice

can be obtained through the appropriate NAFD. Further assistance can be obtained from the Regional Safety Co-ordinator.

Plant pathogens

The Plant Health (Great Britain) Order 1993[3] prohibits the importation from non-EU countries of any plant pathogen or pest that is not already established in Great Britain. The controlled pathogens and pests can only be imported for experimental purposes under licence and the Order includes control of genetically modified plant and tree pests.

A full list of controlled plant and tree pests is included in the ACDP publication (currently under revision).

Details of the requirements of the 1993 Order can be obtained from the appropriate specialist office within the NAFD (see below). DEFRA works closely with the Welsh National Assembly and can deal with enquiries about imports to Wales (see note on next page).

Any person who intends to work with a plant pathogen, even if it is resident in Great Britain, is advised to consult with the local enforcing authority, since certain strains may have differing pathogenicity from the resident strain. The local authority will advise on selection of lower risk organisms wherever practicable.

Guidance on importing plant pathogens, including application forms, can be found at http://www.defra.gov.uk/corporate/regulat/forms/phealth/index.htm.

References

1. Specified Animal Pathogens Order 1998. SI 1998/463. HMSO. ISBN 0 11 065801 9.

2. Importation of Animal Pathogens Order 1980. SI 1980/1212. HMSO.

3. Plant Health (Great Britain) Order 1993. SI 1993/1320. ISBN 0 11 034320 4.

National agriculture and fisheries departments

Animal pathogens

ENGLAND

Department for Environment, Food and Rural Affairs
Area 104, Diseases Prevention Branch
1A Page Street
London, SW1P 4PQ

Switchboard: 020 7904 6000
Telephone: 020 7094 6144/6151
Fax: 020 7904 6128

Helpline: **08459 33 55 77**

SCOTLAND

Scottish Executive for Environment and Rural Affairs
Department of Animal Health and Welfare
Room 350
Pentlands House
47 Robbs Loan
Edinburgh, EH14 1TY

Telephone: 0131 244 6405
Fax: 0131 244 6616

WALES

Welsh Assembly Government
Animal Health Divison
Crown Buildings
Cathays Park
Cardiff, CF1 3NQ

Switchboard: 02920 825111
Direct line: 02920 823593

Plant pathogens

ENGLAND

Department for Environment, Food and Rural Affairs
Plant Health and PHSI
Room 340
Foss House
1 - 2 Peasholme Green
York, YO1 7PX

Telephone: 01904 455192
Fax: 01904 455199

Helpline: **08459 33 55 77**

SCOTLAND

Scottish Executive for Environment and Rural Affairs
Horticulture Unit

Pentland House
47 Robbs Loan
Edinburgh, EH14 1TW

Telephone: 0131 244 6303
Fax: 0131 244 6449

WALES

Welsh Assembly Government
Agriculture Department
Crown Buildings
Cathays Park
Cardiff, CF1 3NQ

Switchboard: 02920 825111*

* If the Welsh Assembly receive a request concerning a plant pathogen, they refer the enquiry to the British Importers Association, at Suite 8, Castle House, 25 Castlereagh St, London, W1H 5YR. Telephone: 020 7258 3999; Fax 020 7724 5055. Since DEFRA works closely with the Assembly it may be preferable to contact DEFRA in York directly.

Guidance notes

Part C

Key laboratory equipment and associated practices

MRC Guidance Note 12

Microbiological safety cabinets

Selection, installation, use and maintenance

The microbiological safety cabinet (MSC) is a basic tool in the laboratory for those who work with biological agents; these include pathogenic micro-organisms (including genetically modified micro-organisms), cell cultures and human and animal tissues and fluids.

There are four major components that contribute to ensuring safe working with MSCs:

- the design, construction and function of the cabinet itself;
- good laboratory design (specifically with respect to cabinet location and room ventilation)
- safe systems of work that incorporate good operational technique
- regular appropriate testing and maintenance.

These Notes discuss the types of MSC in the context of their intended use and provides a sample **Code Of Practice** for the proper use of MSCs, with recommendations for the level of competence required for operators.

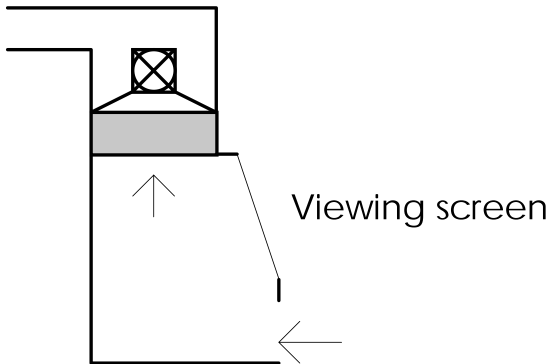
What is a microbiological safety cabinet?

This is defined in the European Standard on Microbiological Safety Cabinets as:

> "Ventilated enclosure intended to offer protection to the user and the environment from the aerosols arising from the handling of potentially hazardous and hazardous micro-organisms, with air discharged to the atmosphere being filtered".

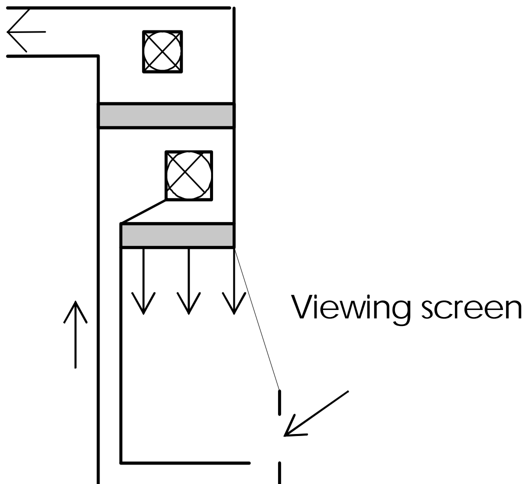
The three basic types of cabinet

Class I cabinet



An open fronted cabinet designed to protect the operator by continuously drawing air into the front of the cabinet.

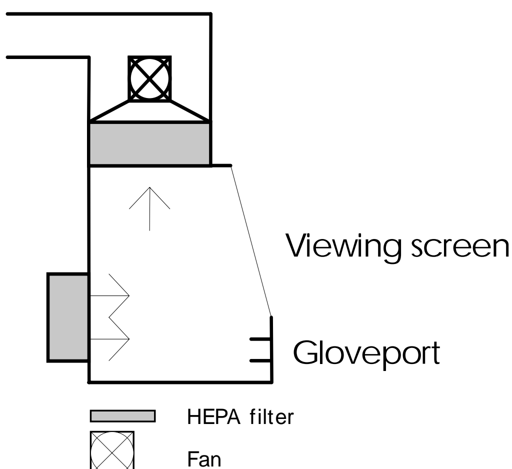
Class II cabinet



An open fronted cabinet designed to protect the operator from exposure and the work from external contamination.

Inward air is directed downwards into a plenum below the work surface and is filtered before being redirected into the work area as a laminar down flow of clean air. The balance of this laminar down flow with the incoming air provides an air curtain at the open front which provides the operator protection. The split between exhausted and recycled air on each cycle is normally in the range 20/80 to 30/70.

Class III cabinet



A totally enclosed cabinet in which operations are conducted through gloves attached to glove ports.

Air enters the cabinet through a HEPA filter at the side or rear of the cabinet and is exhausted in a similar way to a class I cabinet.

Making the right choice

Class I

This is the traditional cabinet of microbiology and clinical diagnostic laboratories. Its construction is simpler than the other types and is therefore cheaper to produce and buy. It is the cabinet of choice when the key requirement is the protection of the worker.

Class II

These cabinets are designed so that the work area is kept clean by a down-flow of HEPA-filtered air across the work. With good working technique it is possible to achieve a high degree of product protection whilst at the same time achieving a degree of operator protection of the same order as that achieved by Class I cabinets. They are typically used for cell and tissue culture applications and for other work where product protection from contamination is important.

Cabinets that meet the performance requirements of the British Standard should be able to afford the worker a degree of protection equivalent to that from a Class I cabinet. Their performance is however more affected by other factors, such as operator movement and air movements outside the cabinet.

Class III

Class III cabinets are used mainly for work with hazard group 4 organisms or work with hazard group 3 organisms deemed to be at high risk, for example where highly concentrated samples are being handled. They offer the greatest protection to the worker and work, but movements are more restricted and this may affect dexterity.

Laminar flow hoods

MSCs should not be confused with laminar flow hoods. These hoods provide a filtered air-flow which is intended for product protection only. The air can be directed either vertically or horizontally toward the operator. In the former orientation, the cabinets must not be confused with Class II cabinets as they have no inflow and thus afford no operator protection. Horizontal laminar flow hoods direct the air across the work toward the operator and thus offer no protection at all. They must not be used therefore with any biological material of human origin. Other animal-derived material or tissue may only be used after a full risk assessment that confirms the total non-pathogenic and non-allergenic status of the material.

Conditions of purchase

The cabinet manufacturer or supplier must be able to provide a test certificate demonstrating type compliance with BS EN 12469:2000.

Poorly sited cabinets can be adversely affected by disruptive airflows from obstacles, passing traffic or excessive airflows from poorly located room ventilation ducts. As a condition of purchase, the supplier should inspect the proposed location for the cabinet and confirm that it is an appropriate site (see the recommendations for siting and installation in part 4 of the BS 5276 and the Annex to this Guidance).

The purchase contract should include a clause requiring in situ tests to confirm the performance after installation. These tests should include demonstration of meeting the

Standard requirements for airflow and operator protection and the demonstration of filter integrity, both of the filter itself and of the filter housing.

Although the performance of Class II cabinets can be as good as Class I cabinets with respect to operator protection, they are more susceptible to the effects of air movements outside the cabinet. The use of a Class II cabinet, rather than a Class I or III, at containment level 3 must therefore always be justified. For Class II cabinets used for work with biological agents, additional appropriate 'in use' tests which reflect normal operating procedures in the laboratory may be required where work is proposed with hazard group 2 agents and must be done if the cabinet is to be used with hazard group 3 agents.

Two examples of additional 'in use' tests are:

1. the four-headed test as specified in the British Standard (but without the artificial arm) with an operator in position with both hands and lower arms within the cabinet throughout the test. The operator should perform repetitive and continuous procedures (e.g., pipetting to and from a multi-well plate and a bottle with a suitable automatic pipette);

2. the same test but with a second person wearing a properly fastened laboratory coat or gown walking backwards and forwards behind the operator at approximately 100 paces per minute at the rate of 3 to 5 passes per minute.

It should be a condition of your purchase contract that satisfactory performance in the post installation tests is demonstrated before any payment is made.

Choosing the right configuration and location

Arrangements for exhaust air

The preferred option for venting safety cabinets is to duct the exhaust air to the exterior of the building. This option confers some significant advantages: first, the safety cabinet exhaust can provide a cheap and simple method of keeping a containment laboratory under negative pressure; secondly, the discharge of fumigant gas after decontamination of the room or the cabinet is made significantly easier; thirdly, there may be occasions when a worker needs to treat cultures with small amounts of volatile toxic chemicals in which circumstances discharging the exhaust air back into the laboratory would not be acceptable.

Where it is clear that the only risk in the work is microbiological, it is acceptable for cabinets to discharge the exhaust air back into the work room. It is acceptable in most circumstances at containment level 2 but should only be permitted in exceptional circumstances at containment level 3. In the latter case the exhaust must be filtered through two HEPA filters in series according to the Standard specification.

For cabinets with integral fans the exhaust ductwork should be kept below 2 metres in length and be air tight. Where the duct run has to be longer, the exhaust fan should be located as near the discharge end of the duct as is possible.

Siting

Cabinets should be sited so as to minimise disturbance of the airflow at the front of the cabinet. Part 2 of BS 5726 (which is still operational) gives recommendations on siting. Particular care must be taken in locating recirculating cabinets where the exhausted air may cause air disturbance at the front of the cabinet, adversely affecting containment performance. The key issues are a) that the cabinet is located with sufficient clearance from walls, corners and doorways; b) that no obstacles are placed where they may interfere with the airflow and c) that sufficient room is provided for the operator to avoid interference with other workers.

Once the key requirements are met, the location is fixed and the cabinet passes the in situ tests, the position of the cabinet must not be changed unless full repeat tests are carried out. Some examples of the BS recommendations on siting are given in the Annex.

The provision of gas and ultra-violet (UV) lighting

Gas

Bunsen burners or other naked flames should not be used in microbiological safety cabinets for the following reasons:

- Disturbance of air-flow
- Fire risk
- Potential damage to the filters

With open-fronted cabinets the use of Bunsen burners may reduce the operator protection by disturbing the air-flows. They should never be used in Class II cabinets. Exceptionally they may be provided for Class I cabinets but if so they should be low profile microburners equipped with a lever control to give full flame only as required, in order to minimise disturbance. To minimise the risk of fire and heat damage, gas provision must be controlled via a solenoid valve, interlocked positively with the operation of the cabinet fan(s).

UV light

UV light is generally ineffective for the disinfection of safety cabinets. Radiation is directional and therefore for it to have any effect, the cabinet must be totally empty. UV lamps are active microbicidally for a relatively short period of time that is a fraction of their total lifetime as a source of visible (blue) light. If its installation is insisted upon by the purchaser, the following conditions should be met:

- UV lighting must be installed in a manner that cannot affect the performance or durability of the cabinet. Thus only materials that are unaffected by UV rays should be used for the construction and coating of the cabinet

- Electrical interlocking must be fitted and operational to prevent direct operator exposure to UV light

- The efficacy of the microbicidal activity of the light must be monitored regularly

- The lamp must be changed whenever its efficacy is reduced (or regularly at a pre-determined frequency that ensures the light is still effective).

The proper use of safety cabinets

Code of practice

Preparation for work

Before starting the work or placing any biological material in the cabinet the following tasks should be done:

- Put on the appropriate protective clothing according to the level of containment and the risk assessment for the work

- Remove the night door (Class I and II) or inward filter cover (Class III)

- Ensure that the cabinet is switched on and running for sufficient time to allow air-flow stability before starting the work
- Do not use unless the airflow indicator is registering in the 'safe' zone
- Prepare thoroughly for the work, e.g., number or code tubes, flasks, dishes etc., organise media, solutions, etc. (a check list may be useful, and/or referral to protocols, codes)
- Ensure active solutions of appropriate disinfectants (and granules or powders as required) are available according to local codes
- Ensure the inside of the cabinet is clean and free of clutter
- Ensure the laboratory door is closed
- Ensure any equipment required for the work is available and ready for use (e.g., centrifuge, microscope, etc. - but note following section re use of equipment within the cabinet)
- Place work in the cabinet, ensuring clean and dirty materials are kept separate.

Use of cabinets

Whilst working at the cabinet the following precautions should be taken:

- Do not overcrowd the cabinet
- For open-fronted cabinets, always work as near to the centre of the work area as possible, but at least 15 cm from its front
- For Class II cabinets, never obstruct the air in-flow grille or any exhaust grilles. Obstructions will adversely affect performance, in particular operator protection. Large equipment (e.g. centrifuges, especially air-cooled models) should not be used within an open fronted cabinet unless appropriate testing has been done to establish that containment performance is maintained
- Do not mix sterile with infected materials and avoid passing potentially infected material over clean material
- Dispose of equipment and contaminated material appropriately after use. Wherever practicable at containment level 2, this means disposal into appropriate containers or disinfectants within the safety cabinet. This must <u>always</u> be done at containment level 3
- Do not allow any casual visitors. Other authorised users of the facility should be discouraged from making movements that may affect the performance of the cabinet
- Never use a cabinet if its operational safety is in doubt. If the alarm sounds, make the work secure, for open-fronted cabinets place the front on the cabinet, and inform the appropriate people according to local arrangements
- Do not rely upon a safety cabinet to cover up for poor work. A GOOD CABINET CAN NEVER BE A SUBSTITUTE FOR GOOD PRACTICE OR GOOD MICROBIOLOGICAL TECHNIQUE.

Clearing the cabinets after use

After completion of the work the following actions are required:

- Check the performance of the cabinet
- Remove samples for incubation, etc., after wiping down flasks, containers, etc. as appropriate with disinfectant
- Ensure that all containers for autoclaving and incineration are marked correctly and secured. Only remove contaminated materials from the cabinet as directed by local protocols. Normally this will mean only when the material can be taken directly to the autoclave, although exceptionally it may be permitted to place containers in a holding area within the containment area
- Wipe all surfaces with disinfectant
- Leave fan(s) on for 5 to 10 minutes
- Where local arrangements require, turn off cabinet and replace front of open-fronted cabinet or inward filter cover (Class III).

Precautions to be adopted for cleaning the interior of MSCs used for work with dangerous pathogens

The interior of a MSC should be wiped down with appropriate disinfectant or fumigated after use (see previous section). Normally it will not be necessary to remove the working surface grilles and indeed this should be avoided wherever possible. If it is absolutely necessary the following precautions should be taken:

- Wear heavy duty PVC or rubber gloves, that can be disinfected after use, over normal surgical gloves to provide adequate protection for hands and wrists
- Spray the appropriate disinfectant at the prescribed dilution for the pathogens in use onto all exposed surfaces and allow sufficient time before proceeding
- Do not attempt to lift the grilles by placing the fingers through the holes or slots, which may have sharp edges. If no handles are provided, use an implement to hook or lever the grilles up from their housing so that the edges may be grasped safely. Warning: the edges may have sharp corners or edges
- Spray the appropriate disinfectant at the prescribed dilution onto all newly exposed surfaces. Allow sufficient time before proceeding
- For cleaning use a thick wad of material to protect your gloved hand from sharp edges
- Disinfect the outer gloves before removal
- Autoclave the cleaning material before disposal.

Training and competence

No person should be allowed to work at a microbiological safety cabinet unless proper training has been given and the person is competent to do the work. Training programmes should be set up and where a supervisor has identified a requirement for training this should be provided before work can commence.

The requirements for competence to use a MSC should include full instruction in the following, as stated in Part 4 of the relevant British Standard (BS5726:1992):

- Classification of cabinets
- Appropriate and inappropriate use of cabinets
- Mode of operation and function of all controls and indicators
- Limitations of performance
- How to work at cabinets safely
- How to decontaminate after use
- Principles of airflow and operator protection tests.

These criteria provide the framework for the MRC computer –based training programme available to all units from 2003.

Maintenance and testing

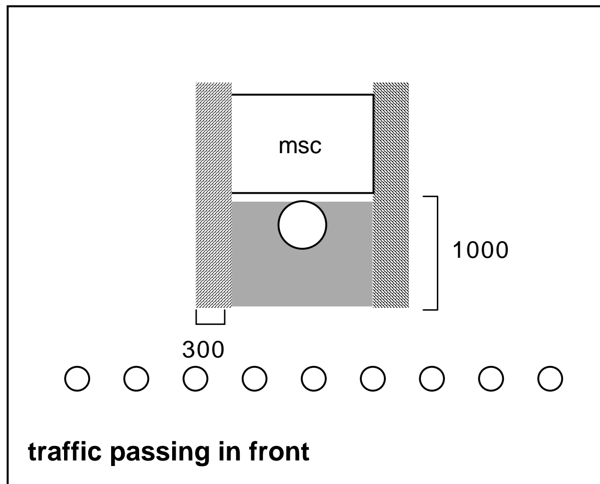
All microbiological safety cabinets must be regularly serviced and tested to ensure their continued safe performance and thereby to satisfy legislative requirements for risk control equipment. The recommended maintenance and testing programme is subject to local circumstances but should normally be regarded as a minimum. In exceptional circumstances however, in low-risk areas (i.e. containment level 1 or equivalent) an annual frequency for all operations may be acceptable but is subject to regular risk assessment review. The expected frequency for maintenance and testing is set out below:

Operation		Frequency	
Air-flows	(contractor)	6-monthly	
	(user)	Monthly	
Filter integrity		6-monthly	
Mechanical and electrical function		6-monthly	
Mechanical integrity (including visible ductwork)		Annual	
Operator protection		Level 2	annual
		Level 3	6-monthly

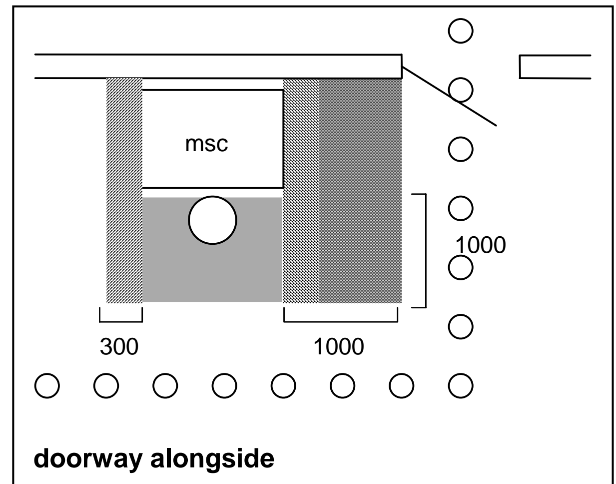
Annex

Siting microbiological safety cabinets to limit disturbance

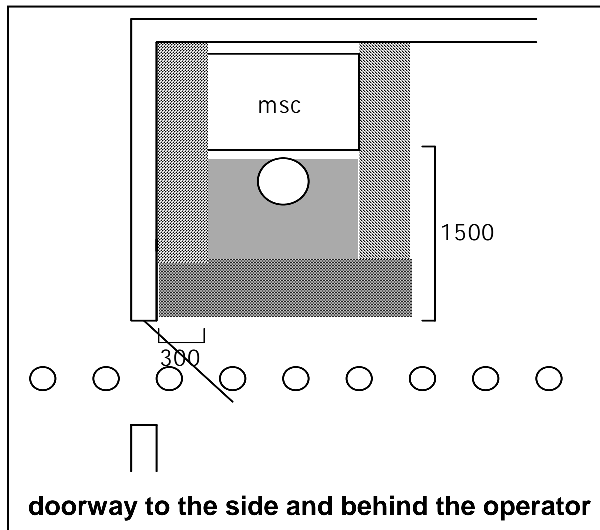
1. Separation from traffic flows and doorways



traffic passing in front



doorway alongside

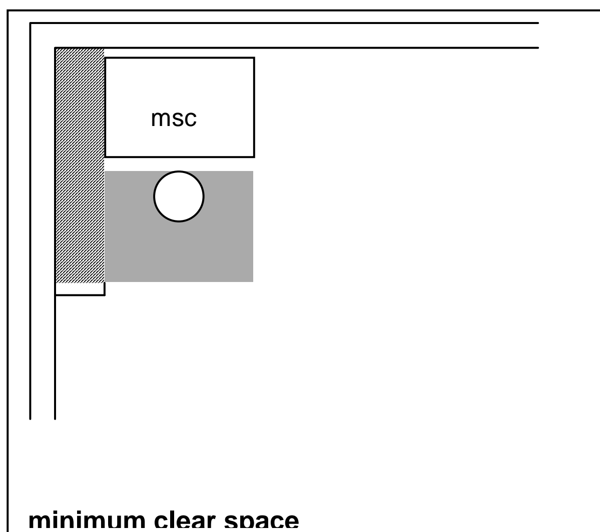


doorway to the side and behind the operator

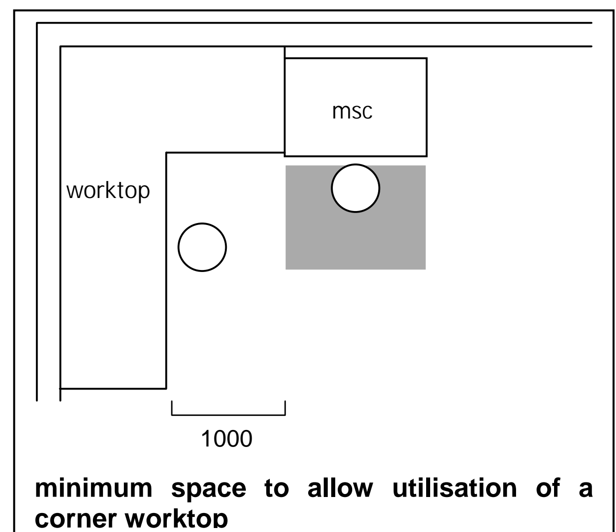
Note:

Although a cabinet may be sited 300 mm from any corner for it to perform satisfactorily, it may be preferable in the interests of efficient use of space, to locate it further away. This is because a small gap is frequently unusable and also difficult to keep clean.

2. Separation in corners

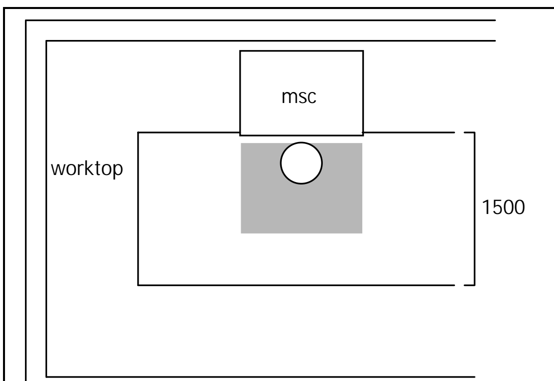


minimum clear space

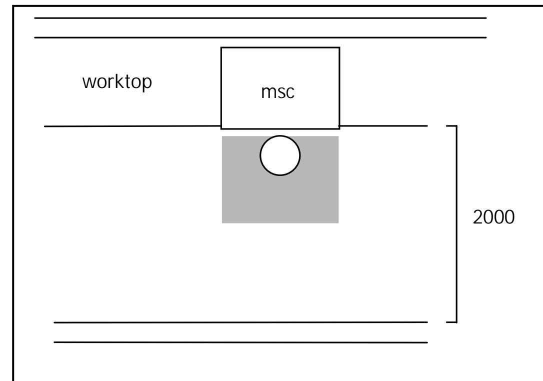


minimum space to allow utilisation of a corner worktop

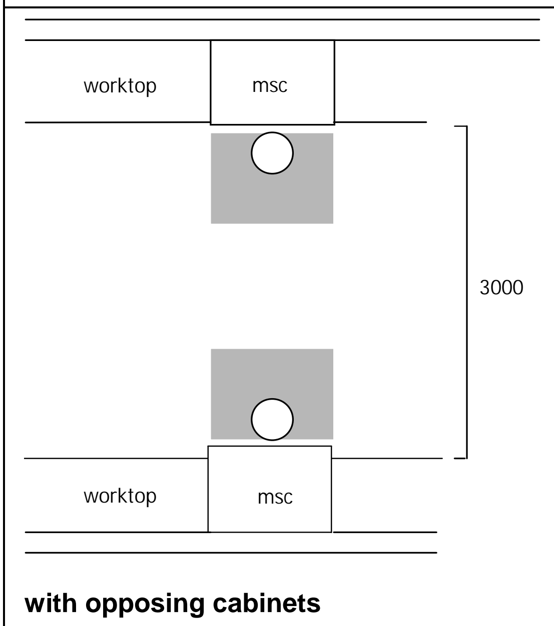
3. Recommended spacing in front of cabinets



same operator using worktop opposite



with an opposing wall



with opposing cabinets

References

British Standard BS 5726:1992

Part 2. Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation

Part 4. Recommendations for selection, use and maintenance

British Standard BS EN 12469:2000 (Replaces Part 1 and 3 of BS 5726)

Biotechnology. Performance criteria for microbiological safety cabinets

MRC Guidance Note 13

The use of centrifuges in biological containment laboratories

Specification

All centrifuges used in biological containment laboratories should conform to the British/European Standard BS EN 61010-2-020: 1995, 'Safety requirements for electrical equipment for measurement, control and laboratory use: Particular requirements for laboratory centrifuges'. It should be read in conjunction with BS EN 61010-1: 1993 (amended in 1995), 'Safety requirements for electrical equipment for measurement, control and laboratory use: Part 1. General requirements'.

The Standard includes sections on Marking and documentation, Protection against electric shock and mechanical hazards, Protection against, for example, escape of microbiological materials and Protection by interlocks.

Documentation

The instructions supplied with the centrifuge should include the precautions to be taken when centrifuging materials contaminated with biological agents, with respect to the operator and service personnel.

Protection against mechanical hazards

Part of this section specifies that the lid must remain locked whilst the rotor is in motion. The lid lock mechanism must be strong enough to ensure that any fragments produced by any disruption are contained.

Protection against escape of microbiological materials

The Standard states "Bioseals in rotors and buckets which a manufacturer specifies to contain microbiological specimens during centrifuging shall be proof against escape of droplets and aerosols, when used according to the manufacturer's instructions".

Location

The Standard specifies that centrifuges should not be able to move more than 300mm as a consequence of a disruption. Although in reality centrifuges are unlikely to move significantly users should consider leaving a clear area around the centrifuge or advising operators to remain outside that area while the centrifuge is operational.

For all heavier permanently sited centrifuges there should be an emergency switch for disconnection of the mains. This should be located away from the centrifuge either outside the room or close to the exit from the room.

The requirements for the location of equipment for each level of containment specify that at containment level 2 it is not necessary for key equipment to be located within the laboratory. At containment level 3 it becomes a requirement, 'so far as is reasonably practicable' and at level 4 it is mandatory.

Work practices

Samples should be centrifuged in sealed buckets or rotors. For work at containment level 2 or 3 the buckets or rotors must be opened only within a microbiological safety cabinet. They should be cleaned and disinfected regularly, and immediately following any leakage. Seals on buckets and enclosed rotors should be checked regularly for wear and damage and be replaced as necessary.

Centrifuges and rotors should be cleaned and disinfected at the end of the working day. They must be decontaminated prior to maintenance work. A signed clearance statement should be issued to this effect before any service or repair work is allowed.

When choosing the disinfectant it is important to remember that some disinfectants can damage surfaces and materials. For example, disinfectants containing electrolytes, strong acids, alkalis, or hypochlorites can chemically attack stainless steel and other metals resulting in corrosion or pitting of surfaces. Thus these disinfectants should not be used with metal rotors or buckets.

MRC Guidance Note

Decontamination and disinfection

1. Laboratory Autoclaves; selection, use and maintenance

Introduction

Detailed information on laboratory autoclaves can be found in British Standard BS2646 'Autoclaves for sterilisation in laboratories' and in the HSE publication PM73, 'Safety at autoclaves'. The following notes provide additional practical guidance.

Selection of autoclave

Specification

The choice of autoclave will primarily depend upon its intended use. Small bench-top or floor-standing autoclaves are adequate for use in most containment level 2 laboratories generating limited quantities of material, either preparing solutions or instruments or decontaminating laboratory discards. These autoclaves are normally supplied with integral heaters for generating steam.

For laboratories at containment level 2 requiring larger capacity and all containment level 3 laboratories a larger machine will probably be required and, especially for the latter, should be equipped with the capability of vacuum pulsing loads to increase the efficiency of penetration. These machines are likely to require steam to be provided either from a steam line or by an independent generator.

For containment level 3 laboratories, the use of a two-ended front loading autoclave can provide a 'dirty' and 'clean' end (also see next paragraph). They are however only mandatory at containment level 4. If one is being considered for level 3 use, serious consideration must be given to the significant additional purchase and maintenance costs. Generally, except for the very smallest autoclaves, front-loading types rather than top-loading are preferred (see section on proper use).

Location

For containment level 2 laboratories the autoclave should be readily accessible in the same building, preferably in the same suite. At level 3 it should preferably be situated within the laboratory, but where this is not reasonably practicable it should be in close proximity to, or readily accessible to the containment level 3 area. Formal derogation is required however under the Genetically Modified Organisms (Contained Use) Regulations 2000 to permit the use of an autoclave sited outside the containment suite for decontaminating waste generated from the containment level 3 laboratories.

The area directly in front of the door(s) should be adequate to enable safe loading and unloading. At containment level 3, the provision of a double-ended autoclave should be considered. The use of these machines enables dirty material to be loaded from within the laboratory area(s) and decontaminated material or prepared media or instruments, etc. to be unloaded or loaded respectively from the outside the facility.

Installation

Smaller autoclaves will not require specialised installation other than an adequate power supply. They should be located however to allow proper venting of escaping steam. Small top-loading machines should be located at a height enabling the operator to load and unload safely.

Larger machines must be properly installed by a competent person. There must be sufficient space around the machine to allow for access during servicing and maintenance. The drainage system must be designed and installed to avoid dispersion of steam into the working area. In particular it should be recognised that the vapour and condensate emitted from the autoclave during the initial heating and pulsing stage may not be free of contamination. Thus the condensate should be trapped either in a chamber held in close proximity to the pressure vessel to achieve as high a temperature as practicable (close to 100°C) or in appropriate disinfectant. Vapour emissions may need to be passed through a HEPA filter prior to release into the environment. These measures will be particularly important at containment levels 3 and 4.

To avoid unnecessary manual handling it is recommended that a trolley be used to facilitate loading and unloading. If possible the trolley should be equipped with runners to enable loads to be slid in and out of the chamber. Sufficient space will therefore be required in front of the autoclave to allow for the positioning of the trolley.

Servicing and maintenance

Legal requirements

The Pressure Systems Safety Regulations 2000 apply to laboratory autoclaves. These place duties on all parties including suppliers, installers, service engineers and users. No autoclave should be operated unless a written scheme for the periodic examination, by a competent person, of specified parts of the pressure system has been drawn up (also by a competent person as defined in the Regulations). Included in the scheme must be specifications for the nature and frequency of the periodic examination.

Provision of information

The supplier must provide comprehensive information on the specification of the autoclave sufficient to enable safe installation and to enable the competent person to draw up the written scheme. The information must include the maximum operating limits of pressure and temperature. These parameters must be confirmed or revised following each inspection and full servicing.

Validation

It is essential that each autoclave is properly validated before use and the performance tested at prescribed intervals. This should be done using calibrated thermocouples placed strategically within the chamber for each type of load. The number of thermocouples used will depend on the size of autoclave. Typically, for a larger front-loading machine, at least 5 will be required, placed at the front and rear of the chamber, in central free space, at the place in the chamber that is least efficiently heated (the ‘cold spot’) and within the load. A validation cycle should be carried out for each condition of use, e.g. gowns, instruments and liquids both for preparation and discard operations. A probe inserted in the largest volume of liquid anticipated for use will enable a calculation to be made of the time necessary for heating up the liquid to the required temperature.

In addition to the thermocouple testing, other regular performance checks should be made. Each run should be monitored for temperature and pressure. In addition steam penetration

can be measured using the Bowie-Dick test. In this test, two strips of autoclave tape are placed in the shape of a St Andrew's cross on to a piece of paper which is then placed in the centre of a standard test pack of towels. The test pack is autoclaved on its own and the efficiency of total steam penetration (only achieved through complete air removal) is measured by observing the change in colour of the tape. Total steam penetration will result in an even change in colour of the tape. Where total air removal is not achieved, a pocket will remain at the centre of the pack resulting in an uneven change of colour.

The frequency of validation will in part depend on the hazardous materials. For guidance, full validation annually where only hazard group 2 material is treated would be appropriate and six-monthly for hazard group 3 material supplemented with monthly monitoring of temperature and pressure.

Servicing

Autoclaves should be examined weekly for the proper function of all safety devices. A record should be kept of these examinations. Particular attention should be given to the door seals. Any leak should be attended to as soon as practicable.

All autoclaves should be serviced regularly by a competent person according to the written scheme. Typically this will mean a six-monthly service. In addition the pressure system must be inspected regularly to ascertain it is fit for continued use. Since the autoclave is considered a device to control exposure to hazardous substances the frequency should be at least every 14 months. No service engineer should be allowed to work on an autoclave unless it is decontaminated (a formal permit to work scheme is recommended).

Full records of all servicing and testing must be kept according to MRC Policy and Guidance on Record Keeping.

Proper use

Training

All autoclave operators should be fully trained in correct procedures and be fully aware of the purpose and function of all controls and safety devices. Manufacturers' instructions should readily accessible to all operators and should be followed. Operators should be instructed to report any apparent faults immediately to a responsible person.

Routine checks

Operators should be satisfied as far as is reasonably practicable that the autoclave is fit for use before each cycle. Where steam is provided via a steam line or generator the operator should check the steam pressure is adequate and that there are no significant leaks. The seating and condition of the door seal should be examined on a daily basis.

Procedures

Proper protective clothing must be worn. Specifically the requirements are a properly designed laboratory coat (back or side fastening), an impervious apron, suitable heavy-duty gloves (gauntlet type), a face visor and suitable footwear (sandals and any open-toed footwear are forbidden).

All materials awaiting autoclaving must be kept safely. 'Clean' materials for sterilisation prior to use should be placed within robust containers. The weight of each container should be restricted to enable safe lifting. Contaminated materials must be stored safely awaiting autoclaving. In containment level 2 laboratories this should preferably be within the laboratory but may be in a safe holding location within the suite, ensuring that each

container is secure. No material should be removed from a containment level 3 laboratory other than to move it directly into the autoclave.

Clear succinct operating instructions, including instructions on procedure in the event of an emergency, must be available to and understood by each operator. A copy should be displayed prominently near to the autoclave.

Loading the autoclave

- All materials should be in robust secure containers. Each item should be affixed with autoclave tape. Autoclave bags should be supported in a robust container and the necks loosened for steam penetration during autoclaving;
- Each container should allow sufficient steam penetration (as established by thermocouple testing);
- No item should be placed in the autoclave unless and until a risk assessment has established the materials will not adversely affect the autoclave and that the materials can be handled safely on removal (e.g. corrosive disinfectants, or radioactive liquid or solid waste);
- Unnecessary manual handling procedures should be eliminated. Where the need remains, then operators should be trained in handling procedures to minimise risk of injury.

Unloading the autoclave

- Ensure the cycle is complete;
- Do not open the door until it is safe to do so (temperature lowered and pressure to atmospheric). Most autoclaves are equipped with safety locks to prevent premature opening of the door;
- Only open the door when wearing protective clothing, especially the face visor;
- Minimise manual handling of containers on removal;
- Ensure safe disposal of autoclaved discards.

Operating cycles

Normal recommended autoclaving conditions for typical loads

Process	Sterilising temperature (range, ^oC)	Sterilisation time (minutes)
Liquids sterilisation*	121 - 124	15
Equipment/glassware sterilisation	121 - 124 134	15 3
Make-safe cycles (decontamination of materials for discard or for re-use)	121 - 124 134	15 3†

* Some media are sensitive to heating at higher temperatures. In some circumstances it may be possible to use lower temperatures (i.e., 115°C) but the time is then extended to 30 minutes.

† This time is not sufficient for autoclaving material known or presumed to be infected with agents causing Transmissible Spongiform Encephalopathies. The recommended time is 18 minutes. It is now acknowledged however that although these conditions will reduce the infectivity of the samples they may not enable full sterilisation. This material must still be considered infective therefore and must be sent for incineration.

Hazards and precautions

The major hazards from operating autoclaves are:

- Pressurised steam
- Incomplete sterilisation due to cycle failure
- Physical injury during loading and unloading

The risk of exposure to the steam is minimised through the provision of proper safety devices in the machine and associated pipework, and proper maintenance and staff training programmes. Most autoclaves are equipped with door-interlocking safety devices which both prevent pressurisation of the chamber unless the door is properly secured and prevent opening before safe opening conditions are achieved. The training programme will include instruction on checking that each cycle is satisfactorily completed and on the procedures to be adopted should the cycle not achieve sterilising conditions or not achieve safe opening conditions.

The principal risks of personal injury are from scalding, cuts and abrasions and musculo-skeletal injury. Scalding should be preventable through the wearing of appropriate protective clothing. Local management should monitor the standards of preparing loads to ensure that sharps are not allowed to project from loads and in addition operators should be alerted to the dangers of this occurring.

Loading and unloading of autoclaves can cause musculo-skeletal injuries through incorrect manual handling. A manual handling risk assessment should eliminate unnecessary lifting and for front loading machines this can be done through the use of trolleys that are of the same height as the chamber to allow containers to be slid in and out of the chamber. The weight of containers should never exceed the comfortable lifting capability of the operators. Except for the smallest bench top models, the purchase of top-loading machines should be avoided, since stretching over these machines to lift containers in and out of them can cause injury.

2. The selection and use of disinfectants.

Chemical disinfectants reduce the number of viable micro-organisms to a level below which infectivity is destroyed and the disinfected object rendered safe to handle. Disinfection is not an alternative to sterilisation, but chemical disinfection may, where appropriate, be followed by autoclave treatment or by incineration.

Many disinfectants remain active in solution for relatively short periods and indeed to be effective some should be made fresh each working day. In addition the active strength required for each disinfectant to be effective will vary according to the operation. To ensure that effective solutions of disinfectant are always available for use each container should be marked with its identity, concentration and date of preparation.

Choosing an appropriate disinfectant

The following factors should be considered:

The micro-organism:

Disinfectants vary both in the spectrum of micro-organisms inactivated (Table 1) and in specific activity for different micro-organisms. Disinfectant manufacturers can provide details of the activity of their product for particular micro-organisms and recommend appropriate dilutions. When the type(s) of micro-organism present in a sample(s) is not known a wide-spectrum disinfectant must be used.

The circumstances under which the disinfectant will be used.

The optimal concentration of disinfectant will vary depending on whether it is used under "dirty" or "clean" conditions. The efficacy of a disinfectant against viruses may be altered if the virus is intracellular. The presence of other chemicals, or organic materials in liquid wastes or on the surface to be decontaminated can inhibit disinfection activity. Disinfectant activity may also be affected by temperature, pH, or even by the "hardness" of the water used to dilute the product. Further advice on appropriate dilutions of disinfectants for use under particular conditions should be available from manufacturers.

The nature of the surfaces to be disinfected.

Disinfectants containing electrolytes, strong acids, alkalis, or hypochlorites can chemically attack stainless steel and other metals resulting in corrosion or pitting of surfaces. Disinfectants containing organic solvents may similarly affect the integrity of plastic surfaces. In contrast to these unwanted effects, some disinfectants have a surface-active (detergent) component which allows for simultaneous cleaning and disinfection of contaminated surfaces. Such disinfectants are especially useful in decontamination where blood or other body fluids have been spilt. Manufacturers will provide advice on the suitability of using their product on particular surface materials.

The hazard to health posed by disinfectants.

Under the requirements of the COSHH regulations a risk assessment must be made for the safe handling of disinfectants. Most disinfectants are toxic and some are also corrosive (Table 2). The COSHH risk assessment must state the controls and personal safety measures to be taken when handling concentrated and working dilutions of disinfectant. Gluteraldehyde and hypochlorites release vapours that are sensitising and irritant respectively to the lungs and should be used only in well ventilated areas. Some disinfectants release hazardous gases when mixed with other chemicals or with organic materials. Safety data sheets can be obtained from manufacturers. Different disinfectants must not be mixed together, or used in combination unless the possibility of the formation of toxic products has been properly assessed.

Table 1 Activities of some common classes of disinfectants

Disinfectant type	Active against: Vegetative bacteria	Bacterial spores	Fungi	Enveloped viruses	Non-enveloped viruses	Myco-bacteria	TSE and prion agents
Phenolic	+	-	+	+	2	+	-
Hypo-chlorites	+	+	1	+	+	1	+
Alcohols	+	-	-	+	+	+	-
Aldehydes	+	+	+	+	+	+	-
Surface-active agents	+	-	1	2	2	-	-
Peroxygen compounds	+	+	+	+	+	+	-

+ Generally effective
- Generally ineffective
1 Limited activity
2 Depends on the virus

Note : the specific activity of a particular disinfectant must be assessed on a case by case basis.

Table 2 Characteristics of some common classes of disinfectants

Disinfectant type	Hazard Class	Inactivated by: Organic matter	Hard water	Detergent	Corrosive to metals	Flammable
Phenolic	Toxic	-	+	1	-	-
Hypo-chlorites	Toxic Corrosive	+	-	1	+	-
Alcohols	Harmful Flammable	-	-	-	-	+
Aldehydes	Very Toxic Irritant	-	-	-	-	-
Surface active agents		+	+	2	-	-
Peroxygen compounds	Irritant (dust)	-	-	-	3	-

1. Inactivated by cationic detergents
2. Inactivated by anionic detergents
3. Can corrode lower quality steel on prolonged contact

Disinfection and spills of biological agents.

Procedures for dealing with accidental spills of biological agents should be part of safety policy. Containers of disinfectant at an appropriate concentration should be available at each work station where biological agents are handled. In the case of an accidental spillage a disinfectant that gives a rapid kill is required. Disinfectants supplied in powder or granular form (e.g. Virkon and Presept) and gelating agents that contain disinfectant are especially useful for sprinkling over spills. In contrast, liquid disinfectant added to a spill necessarily increases the surface area of the spill and may also result in splashing. This can be minimised however by covering the spill with tissues before pouring on the liquid disinfectant.

Use of disinfectants

Stability of working dilutions

Once diluted the activity of disinfectants decays with time. Even 'stock' concentrated hypochlorite will lose half the available chlorine in three months, with decay much more rapid in more dilute solutions. Some products (e.g. Virkon) contain a coloured indicator to show effective disinfection capacity. Manufacturers will recommend an active-life for their products in concentrated form and when diluted to working strength. If the disinfectant does not contain a colour indicator the expiry date should be clearly marked on the container when the working strength solution is prepared.

Contact time

Disinfectants must remain in contact with micro-organisms for a period of time sufficient to achieve disinfection. Manufacturers should recommend appropriate combinations of disinfectant concentrations and contact times for various applications.

Validation of disinfectant activity

Where little or no relevant efficacy data is available, (e.g. when working with high titres or with significant quantities of organic material), the effectiveness of disinfectants for use with specific biological agents should be determined experimentally to identify the optimal combination of disinfectant concentration and contact time. Thereafter, standards should be established for local use to enable the performance of the disinfectant to be critically assessed, especially when changes in working practices, new micro-organisms or new materials are proposed.

Discard jars

Containers of working strength disinfectant must be placed at, or close to, each work-station where waste is generated. Items placed in discard containers should be completely immersed in the disinfectant and care taken to ensure that air bubbles do not prevent contact with surfaces to be disinfected. If liquid waste is to be decanted to a discard jar the amount of concentrated disinfectant in the jar must allow for dilution to the final working strength.

Decontamination of working surfaces

Benches and other working surfaces should be cleaned with disinfectant at the end of each working day as a matter of routine. Work surfaces that are contaminated with blood or other body fluids must immediately be treated with disinfectant. Control measures to avoid the hazard to health posed by the disinfectant must be taken during disinfection procedures.

Types of disinfectant

The activities and characteristics of the common classes of disinfectant are shown in Tables 1 and 2. Examples of commonly used disinfectants and additional comments are given below:

Examples and additional comments:

Phenolics (e.g. Hycolin, Stericol, Clearsol.)

Phenol is an effective protein denaturant which, when in contact with membranes, results in lysis of the micro-organism. Some phenolic disinfectants also contain detergents that result in synergistic activity. Concentrates are stable but stability is reduced on dilution. The agent of choice for mycobacteria disinfection. Damages the surface of many plastics.

Chlorine-containing or -generating compounds (e.g. Hypochlorite, Chloros, Presept.)

Rapid action probably due to protein denaturation. Chlorine gas is released when mixed with strong acids and some other chemicals. Carcinogens may be produced when mixed with formaldehyde.

Common dilutions of hypochlorite for the following applications are:

General use,		1-2,500 ppm available chlorine;
Discard containers,		5-10,000 ppm available chlorine;
Treatment of accidental spillages	}	20,000 ppm available chlorine.
Disinfection of TSE infected material	}	

These compounds generally decompose more rapidly once diluted. In addition some commercial products contain a perfume (analogous to that added to natural gas to enable its detection by smell) which can persist beyond the active life of the solution. Organic chlorine-releasing compounds, e.g. Chloramine, have the advantage that chlorine is not liberated so readily and so exert a more prolonged disinfectant effect. Hypochlorites (not Presept) are the only disinfectants known to inactivate the prions that are presumed to cause transmissible spongiform encephalopathies.

Alcohols (e.g. Ethanol, Propanol, Industrial methylated spirits)

The efficacy of alcohols as disinfectants is generally poor and highly susceptible to interference. They produce a very rapid kill of bacteria and some viruses probably by denaturation of protein, but should only be used on physically clean surfaces as alcohols have poor penetration of organic matter. Alcohols must be diluted (70% ethanol; 60% propanol) before use (100% alcohol is not an effective disinfectant). Due to their flammability alcohols require appropriate precautions during storage and in use. They should not be used in microbiological safety cabinets or on large areas.

Aldehydes (e.g. Cidex, Gluteraldehyde, Formaldehyde)

These chemicals have irritant and toxic properties and are extremely hazardous. They are not suitable as general disinfectants but may have a place in specialised usage although even here their use should be regularly reviewed and consideration given to using alternative compounds. Their use therefore must be justified through proper risk assessment, starting with the option of using a less toxic alternative wherever practicable. The use of formaldehyde should be limited to gaseous fumigation for disinfection of microbiological safety cabinets or for rare occasions when a laboratory requires fumigation. Occupational Exposure Limits (OELs) have been set for both formaldehyde and gluteraldehyde (see HSE Guidance note EH40 Occupational Exposure Limits).

Surface-active agents (e.g. Cetrimide, Tego)

These are relatively non-toxic and non-irritant but are inactivated by organic matter and anionic detergents, e.g. soap.

Peroxygen compounds (Virkon)

Virkon has a wide range of bactericidal, virucidal, fungicidal and sporocidal activities. Representative viruses from all the major virus families are inactivated by Virkon. Working solutions of 1% w/v have low toxicity and no irritancy. In powder form it is moderately irritant for eyes and the respiratory tract. It has a built-in colour indicator for effective disinfection capacity and contains detergent properties that combine cleaning with disinfection. Virkon is stable for seven days in solution.

There is some evidence however that Virkon can be corrosive for lower quality steel surfaces, and can attack certain plastics.

Other disinfectants (skin disinfectants, e.g. Hibiscrub, Hibitane, Betadine, pHisomed, Cidal etc., or household disinfectants, e.g. bleach) are not suitable for use as general laboratory disinfectants.

Guidance on fumigation with formaldehyde (incorporating code of practice)

Introduction

In certain prescribed circumstances it is necessary to fumigate microbiological safety cabinets and occasionally rooms for the purposes of decontamination. The fumigant of choice is formaldehyde, although vapourised hydrogen peroxide, which is less toxic, can now be used in strictly defined circumstances where its efficacy can be demonstrated. Formaldehyde is a hazardous chemical and this guidance describes its main properties, and how and in what circumstances it should be used.

Properties of formaldehyde

Toxic properties

Formaldehyde has a Maximum Exposure Limit (MEL) of 2 ppm (or 2.5 $mg.ml^{-3}$; EH40 – Occupational Exposure Limits). It is thus mandatory to ensure that exposure to formaldehyde is kept below this limit and indeed as low as practicable. During fumigation the concentration of fumigant far exceeds the MEL and so measures must be taken to prevent exposure.

Physical properties

Formaldehyde is explosive at 7.75 percent in dry air. It penetrates very poorly however in dry conditions and so both for safety and efficacy it should be used in humid, warm conditions, i.e., at or above a relative humidity of 65 percent (optimally at 80 percent) and above $20^{o}C$.

Chemical interaction

Under certain circumstances formaldehyde can react with hydrochloric acid and chlorine-containing chemicals such as hypochlorite (e.g. Chloros) to form bis-(chlormethyl)-ether, a known lung carcinogen. These chlorine-containing compounds should therefore be removed from all cabinets or rooms before fumigation. In containment level 3 areas this may require proper safe disposal.

Generation of fumigant

Formaldehyde vapour can be generated by a number of methods:

- heating a mixture of formalin (a commercially available 40% solution of formaldehyde in water) and water in a thermostatically controlled heating unit (such as an electric frying pan or electric kettle);
- using commercially available formaldehyde generating kits;
- heating formalin in a purpose-built vaporising unit (for safety cabinets only).

Code of Practice

Competent persons and safe systems of work

Fumigations must only be carried out by identified personnel, properly trained in the properties and safe use of formaldehyde. Fumigations must be carried out according to a written protocol and sufficient warnings must be displayed to ensure that no-one is exposed inadvertently to the vapour.

Fumigation of microbiological safety cabinets

When cabinets must be fumigated

Microbiological safety cabinets, whenever they have been used for handling biological agents (i.e., for all work requiring containment level 2 or above), must be fumigated in the following circumstances:

- Before any maintenance work on the cabinet where access is required to potentially contaminated parts (including filter and pre-filter changes);
- Before carrying out filter penetration tests;
- When there are any significant changes to the nature of the work carried out (e.g. the use of a significantly different pathogen);
- After any major spillage or any spillage resulting in contamination of inaccessible surfaces.

Method of fumigation

Preparation

The cabinet fans must first be switched off. Fill the vaporiser with the appropriate amount of formalin plus water. Where the unit is not attached to the side of the cabinet, place it inside the cabinet.

The cabinet must then be sealed before fumigation to prevent leakage of formaldehyde vapour. All of the cabinet's own seals (front window, night door) must be properly seated and secured and it will probably be necessary to use sticky tape to ensure complete sealing. When using Class III cabinets or Class I/III hybrids in the Class III mode a blank plate should be fitted over the inlet filter housing.

For recirculating Class II cabinets, flexible ducting must be attached (usually with an adaptor kit) to the cabinet exhaust to allow discharge through a dedicated duct, ducted cabinet, fume cupboard or through a window. Where the latter is used, consideration must be taken of factors that might cause others to be exposed to the discharged vapour, such as the location of the window, wind direction, proximity of other people, etc. Alternatively a commercially available unit may be used which absorbs the formaldehyde vapour onto an activated impregnated charcoal filter. The unit also contains a fan and can be attached to the cabinet via flexible ducting connected to the cabinet exhaust. The manufacturer's instructions on correct use must be followed. Warning signs should be affixed to the cabinet and to the door of the laboratory indicating that fumigation is taking place.

Quantity of formalin used

Wherever available, the cabinet manufacturer's recommendations should be followed. As general guidance the following quantities are recommended following the formula of 60 ml of formalin plus 60 ml of water vaporised per cubic metre:

Class I, Class III and Class I/III hybrid	20 ml (plus equal volume of water)
Class II (900 mm)	20 ml
Class II (1200 mm)	25 ml
Class II (1800 mm)	30 ml

Procedure

Many cabinets are now equipped with an automatic fumigation cycle and in these cases the manufacturer's instructions must be followed. These instructions will not differ significantly however from the steps below.

- Switch off the cabinet;
- For recirculating Class II cabinets, affix ducting to cabinet exhaust;
- Fill the vaporiser with the appropriate formalin/water mixture;
- If separate, place vaporiser in cabinet;
- Seal cabinet, including positioning of night door or filter-housing plate;
- Affix warning signs to cabinet and to outside of the laboratory door;
- Switch on the vaporiser;
- After about 15 minutes, turn on the fans for about 30 seconds to allow full filter penetration and access of fumigant to all potentially contaminated internal areas;
- Turn off fans and ensure that all dampers are shut off;
- Leave the cabinet for a minimum of 6 hours, but optimally overnight;
- Open dampers and ensure the external environment is safe for discharge (especially where fumigant is discharged out of a window);
- Switch on cabinet fans then allow limited flow of air into cabinet by removing the bung (where fitted) in the night door or partially opening the night door (or cover plate for Class III and Class I/III hybrids);
- After 5 minutes, remove night door completely and allow cabinet to run for at least 20 minutes.

Note: Cabinets, in order to comply with the European Standard, should be type-tested to ensure they do not leak during fumigations. It would be prudent however to take care when first fumigating a cabinet to check if leakage has occurred.

Filter handling and disposal

The recommendation of the British Standard for Microbiological Safety Cabinets is that filters and pre-filters should not be regarded as sterile after fumigation but merely 'safe to handle' using proper precautions. The filters should be removed using suitable protective clothing and should be packaged for autoclaving or incineration after removal. (**Note:** the agents causing transmissible spongiform encephalopathies are resistant to formaldehyde and autoclaving may not result in complete decontamination. Filters removed from cabinets

that have been used for work with these agents or with material that is known to be or has a high probability of being infected with these agents must be packaged securely and incinerated).

Fumigation of rooms

There may be occasions on which it is considered necessary to fumigate a room. An example would be a spillage of material being handled outside a cabinet in a containment level 3 laboratory, although it must be emphasised that the development of protocols that minimise the use of breakable (e.g. glass) containers will considerably reduce the probability of this happening. In some circumstances it may be necessary to fumigate animal holding rooms following certain work or following an outbreak of infection in the stock.

A full risk assessment will determine when a room should be fumigated in other circumstances (e.g. for visits by service personnel). It may be necessary to link the assessment with the operation of a 'permit-to-work' system.

Method of fumigation

Validation

The efficacy of fumigation should be validated at commissioning of a new or refurbished facility. Further validations would be required if significant changes are made to the facility. It is sensible to initially carry out a smoke test to ensure the room is sealable before actually doing the fumigation. Validation is usually done by placing spore strips at the most 'inaccessible' points in the room.

Preparation

All safety cabinet fans and room ventilation systems must first be switched off. Fill the vaporiser with the appropriate amount of formalin plus water. For larger rooms it may be preferable to locate more than one unit spaced out in the room to increase efficiency of fumigation.

The room must be sealed before fumigation to prevent leakage of formaldehyde vapour. If an externally ducted safety cabinet is to be used at the end of fumigation to remove the formaldehyde vapour the night door (or filter covering plate) must be left off. In these situations controls to operate the cabinet should be located outside the room. Suitable arrangements must be made if a recirculating Class II cabinet is to be used (see preceding section).

A room ventilation system can only be used to extract the fumigant where it is a total loss system extracting to atmosphere. Thus in exceptional circumstances at containment level 2 there may be no practicable alternative to opening the windows. This must only be done by a trained operative wearing full breathing apparatus. Cartridge respirators are not adequate. Alternatively a unit incorporating a centrifugal fan and an impregnated charcoal filter that continuously recirculates the room air through the filter may be used to absorb the formaldehyde vapour. The recommendations of the manufacturer on the time required based on the capacities of the filter and the fan must be followed.

Containment level 3 facilities will be constructed to be sealable for fumigation, but sticky tape may still be required to seal doors effectively. Other rooms may require more extensive preparation, including the sealing of service ducts, windows, etc. Suspended ceilings often present difficulties, in particular in relation to connections with adjoining rooms and corridors. Smoke detectors should be covered or, if practicable, isolated from the system.

Warning signs should be affixed to the door of the laboratory indicating that fumigation is taking place.

Quantity of formalin

The volume of the room should be calculated and 100 ml of formalin (plus 1 litre water) is required per 30 cubic metres. The amount of formalin must be increased where significant quantities of absorbent material are present (e.g. gowns, large volumes of liquid).

Procedure

- Switch of all room ventilation systems and safety cabinets;
- Seal the room where necessary with tape;
- Ensure fronts or filter-housing cover plates are removed from cabinets;
- Attach ducting between cabinet exhaust of any recirculating Class II cabinets in room and the appropriate extraction system;
- Affix warning signs to the outside of the laboratory door;
- Fill vaporiser(s) with formalin/water mixture;
- Switch on the vaporiser(s);
- Leave the room and seal the door to the room if necessary;
- After about 15 minutes, turn on any safety cabinet fans (if operable from outside the room) for about 30 seconds to allow full filter penetration and access of fumigant to all potentially contaminated internal areas;
- Turn off fans;
- Leave for a minimum of 6 hours, but optimally overnight;
- Ensure external environment is safe for discharge (especially where fumigant is discharged out of a window);
- Switch on air extract system (and/or cabinet fans) remotely and allow to run for sufficient time to remove the formaldehyde vapour;

  Calculations can be made to estimate the time allowed to purge the room of fumigant but it is recommended that sufficient air is extracted to allow for 20 to 25 complete air changes. Knowledge is therefore required of the extract fan capacity.

  For example, a typical Class II cabinet exhausts approximately 125 litres per second (equivalent to 7.5 cubic metres per minute). To purge a single occupancy room of 30 cubic metres the cabinet should be run for a minimum of:

  $$\frac{30}{7.5} \times 20 \text{ (or 25) minutes} = 80 \text{ to } 100 \text{ minutes}$$

The level of residual vapour should be checked before entering the room. This is done most conveniently by inserting a probe or detection tube through an access port. No-one may enter the room until vapour levels have been reduced to as low as practicable or at least to below 2 ppm.

MRC Guidance Note 15

Disposal of biological waste

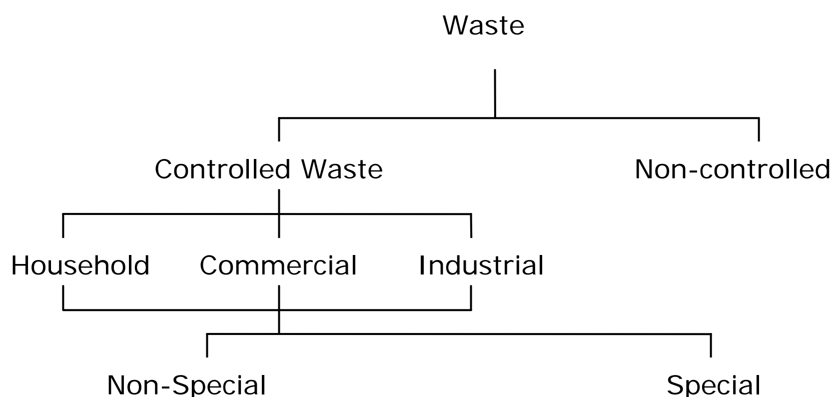
Introduction and legal background

The proper, safe disposal of waste is an important aspect of safeguarding the health and safety of employees and others. A significant proportion of the waste generated by MRC research activities is of biological origin. This waste is categorised in law[1,2] and is also subject to legislation relating to transport of hazardous materials[3]. The generation and disposal of biological waste are also subject to risk assessment under the COSHH regulations[4]. Landfill Regulations[5] ban the consignment of infectious clinical waste to landfill.

This guidance should be read in conjunction with MRC policy and guidance on the safe transport of hazardous material.

Waste categories

Classification of waste



Laboratory waste and Clinical waste are mainly included within the definition of Industrial waste, itself a category of Controlled waste. In addition, some clinical waste is regarded in law as Special Waste[2]. It is unlikely that this will apply in the MRC, other than in the disposal of prescription only medicines.

Controlled waste

Household waste

Local authority arrangements will determine the categorisation and collection of waste.

Commercial waste

The definition of commercial waste includes waste from offices.

Industrial waste

Industrial waste includes all laboratory waste, waste generated from an approved scientific research association and clinical waste. Industrial waste therefore includes all non-clinical and clinical biological waste except that categorised as Special Waste.

Clinical waste

Under the Controlled Waste Regulations 1992, clinical waste is a separate waste category within the umbrella of industrial waste. Clinical waste is defined as:

a *'any waste which consists wholly or partly of human or animal tissue, blood or other body fluids, excretions, drugs or other pharmaceutical products, swabs or dressings, or syringes, needles or other sharp instruments, being waste which unless rendered safe may prove hazardous to any person coming into contact with it';* and,

b *'any other waste arising from medical, nursing, dental veterinary, pharmaceutical or similar practice, investigation, treatment, care, teaching or research, or the collection of blood for transfusion, being waste which may cause infection to any person coming into contact with it'.*

The Health Services Advisory Committee (HSAC) document 'Safe disposal of clinical waste'[6] divides clinical waste into five groups according to hazard. These groups include (see the document for comprehensive definitions):

Group A All human tissue, including blood (infected or uninfected), animal carcasses, and tissue from veterinary centres, hospitals or laboratories, and all related swabs and dressings.

Group B Discarded syringe needles, cartridges, broken glass and any other contaminated disposable sharp instruments or items.

Group C Microbiological cultures and potentially infected waste from pathology departments and other clinical or research laboratories.

Group D Certain pharmaceutical products and chemical wastes.

Group E Items used for disposal assessed as presenting low risk but may be of an offensive nature (e.g., urine containers, bed pan liners, etc.).

Non-clinical biological waste

The Clinical Waste categories Groups A-E above do not specify comprehensively all categories of biological waste that are generated in the laboratory environment. Others include:

- Clinical samples
- Cultures
- Biological agents
- Disposable gloves
- Materials potentially contaminated with biological agents
- Sharps, including scalpels, scissors, needles, Pasteur pipettes, slides, ampoules, vials
- Animal carcasses
- Bedding/litter from animal cages

In general however, the above categories can be considered in practice within the five groups, e.g., clinical samples within Group A.

Non-hazardous biological waste

For biological waste to be considered non-hazardous it must either have been treated so as to render it safe and non-infectious, or be healthcare waste of negligible intrinsic risk (i.e. not included in Waste Groups A-E).

Risk management – human health

COSHH

Waste disposal of biological material is subject to full risk assessment under these regulations. Thus Units must assess the risks to employees and others who might be at risk through exposure to the waste. Suitable control measures, covering decontamination, packaging and disposal must be implemented and staff fully trained in handling the waste.

Management of health and safety at work

These regulations place responsibility on the waste producer to consider vulnerable groups, such as the immuno-compromised, the young, and new or expectant mothers and their unborn or breastfeeding children. In addition, there is the duty to co-operate with other employers. Thus where a host agreement specifies that the unit follows the arrangements put into place by the host, the unit must ensure it does so.

Transport regulations

Where infectious biological material is transported off-site, regulations control and specify categorisation, packaging and labelling requirements, to ensure safe transport.

Risk management – environmental factors

National environment agencies are responsible for waste policy and implementing European Directives. Environmental law places a 'duty of care' upon all waste handlers, from the producer to the final disposal point. Waste producers must provide a written description of all waste with sufficient information to allow the safe handling of the waste. The producer also has to be able to keep track of the waste.

Controlled waste must be kept, treated and disposed of according to a waste management licence. Temporary waste stores (i.e. those where waste is stored awaiting collection) and laboratory autoclaves are exempt from that requirement. Under new legislation licences are being replaced by the permit scheme.

The Environmental Protection Act also covers 'offensive waste'. Most clinical waste could be construed as offensive and must be incinerated if it cannot be rendered 'inoffensive' by other routes. Care must therefore be taken when considering whether or not autoclaved laboratory waste could still be regarded as offensive. This would include any article which remains identifiable as potentially from an infectious source after autoclaving (e.g. human tissue, hypodermic syringes).

All waste generated through activities involving genetic modification must be fully inactivated by a validated procedure before leaving the site, whatever the Class of activity.

Landfill regulations

The following paragraphs summarise some of the key points included in the regulations[5].

Application

The regulations apply to all landfill sites, whatever the size.

Classification

All sites must be classified by the Environment Agency (EA: in England) as sites for either:

- Hazardous waste
- Non-hazardous waste
- Inert waste

No mixing of the three types of waste is permitted.

Permits

Between 2002 and 2007, every landfill site classified by the EA will be issued with a Pollution Prevention and Control (PPC) permit. Amongst several parameters, the permit includes the types and quantities of waste that can be accepted.

Prohibitions from landfill

These include:

- Liquid waste (prohibition to be phased in)
- Reactive chemical waste and those whose effects on man and the environment are unknown
- Infectious clinical waste

Acceptable waste for landfill

From 16 July 2004, all mixing hazardous and non-hazardous waste at landfill will be banned. Hazardous waste cannot be accepted in a non-hazardous waste site unless it is rendered stable and non-reactive (e.g. by solidification), and is then kept separate from biodegradable non-hazardous waste. All waste must be pre-treated prior to landfill unless it is not technically feasible and treatment would not reduce the quantity or hazards of the waste.

Discharge of liquid waste

The discharge of any clinical waste to a sewer requires a discharge consent from the local sewerage undertaker. Liquid waste rendered non-hazardous through the use of a disinfectant will also be subject to local conditions that regulate disposal of chemicals via the sewer.

References

1. Controlled Waste Regulations 1992. SI 1992/588
2. Special Waste Regulations. 1996. SI 1996/972
3. Carriage of Dangerous Goods (Classification, Packaging and Labelling) and Use of Transportable Pressure Receptacles Regulations. 1996. SI 1996/2092
4. Control of Substances Hazardous to Health Regulations. 2002. SI 2002/2677
5. Landfill (England and Wales) Regulations. 2002. SI 2002/1559
6. Safe disposal of clinical waste. 1999. HSE/HSAC. ISBN 0 7176 2492 7

Waste management policy and code of practice

Principles

Consistent with a strategy to protect the environment, local waste management policy should strive toward a) minimising the generation of waste b) encouraging re-use and recycling and c) reducing the proportion of controlled waste going to landfill. The prime strategy should be to minimise the generation of all waste. All waste of biological origin, treated or untreated, is controlled waste.

Thus in line with that strategy, disposal to landfill is discouraged and to incineration encouraged for waste of biological origin especially for that waste originating from work at higher levels of containment.

This strategy is also set out in the following table.

Table 1: Legislative Requirements and 'Best Practice' Expectations for solid and liquid biological waste

Containment Level	Inactivation regimes	Disposal routes
1	Disinfection	Landfill (solid) Sewage systems (liquid)
2	Disinfection Sterilisation	Landfill (solid) **but** Incineration of solids as best environmental option Sewage systems (liquid)
3	Disinfection Sterilisation	Incineration (solid) Sterilised liquids to sewage
4	Disinfection Effluents subject to heat/chemical treatment Sterilisation of solids	Incineration (solid) as Special Waste Sewage systems (for liquid treated by validated means)

Segregation

Waste should be segregated into easily recognisable colour-coded containers according to their final route of disposal. The scheme set out in the HSAC guidance is widely accepted and strongly recommended. For biological and clinical waste the two main colours are YELLOW, for waste for incineration only and LIGHT BLUE or TRANSPARENT, for waste for autoclaving before ultimate disposal. BLACK bags are used for non-hazardous waste. In limited circumstances the use of BLACK bags with YELLOW stripes is permissible for the transport of low hazard biological waste accepted for landfill. It is most important that proper segregation ensures that waste for incineration is kept separate from other waste.

Routes of disposal

In addition to the main strategy, the following scheme should be followed in all but exceptional circumstances. It should be noted that the decision on treatment and final disposal of waste is subject to full risk assessment and is related both to the ACTUAL risks and to the PERCEIVED risks.

Actual risk — The **likelihood** of transmission of an infectious agent.

The **consequences** of infection.

Perceived risk — Based on the perception of third parties, including the general public.

The decontamination process, through autoclaving or disinfection, may be judged as thorough by the waste producer and render the waste non-hazardous with respect to the transport legislation. The appearance of human-derived, or labelled biological waste, or items associated with medical use (e.g., syringes, petri-dishes, etc.), however at a refuse site may cause justifiable concern and lead to repercussions for the producer. Moreover, under the Duty of Care, the nature, origin, and validated treatment of the waste must be recorded and given to the waste disposal contractor. In addition, although a properly maintained and tested autoclave (see **Guidance Note 14**) should decontaminate thoroughly, incomplete penetration of steam can occur and susceptibility to heat varies from agent to agent. **Solid waste known to be, or possibly infected with TSE agents must always be incinerated**. **Liquid waste from similar sources should be treated with hypochlorite then neutralised before disposal via the drains** (see **Guidance Note 6**).

Wherever solid waste is transported off-site, an audit trail should be conducted, at least initially, to determine, under the duty of care, that the waste is disposed of correctly. This would include examination of all relevant licences (e.g. for GM waste).

Other hazards

In addition to consideration of the biological and physical hazards and risks considered above, an assessment must be made of the presence of chemical or radiation hazards that may be present. The assessment may affect the categorisation of the waste and thus determine the conditions of carriage and ultimate route of disposal.

If hazardous chemicals are present, then even if the decontamination process renders the waste non-hazardous biologically, the waste will still categorised as controlled hazardous waste. Thus the packaging and disposal routes applied normally for hazardous chemicals must be used.

Waste from work with genetically modified micro-organisms

The Contained Use Regulations (see **Guidance Note 7**) require all waste to be decontaminated by fully validated means. This includes waste from work from Class 1 projects.

Animal waste

All carcasses or tissues should be disposed of by incineration, whether rendered non-infectious or not. It is recommended that bedding from animals infected with human and animal pathogens be handled as if it were infectious biological waste. Bedding from non-infected animals can be regarded in other than exceptional circumstances (via appropriate risk assessment) as non-hazardous. However waste from animals transfected even with a Class 1 GMM is subject to the Contained Use Regulations.

Final route for biological waste

Incineration

- All items in Groups A and B above and all animal tissue and remains.

- All items presumed or known to contain hazard group 2, 3 or 4 biological agents.
- **As first option**, all laboratory biological waste, treated or untreated.

Landfill

- It is strongly recommended that biological waste should not normally go to landfill (but see section below).
- Hazardous and non-hazardous waste must be segregated at source. The Landfill Regulations do not permit the landfill of non-hazardous and hazardous materials at the same site. Nor do they permit the landfill of any infectious waste. Only non-hazardous waste can be sent to landfill.

Discharge to drains

- Liquid infected waste should be autoclaved prior to disposal via the drains. Where such waste has been pre-treated with disinfectant, it may (through a suitable risk assessment) be appropriate to discharge to the drains after the prescribed period of contact. Users must be aware (through the assessment) however that the use of certain disinfectants can give rise to corrosion problems.

  Hypochlorite should be released to the drains with copious amounts of running water (or pre-neutralised as for TSE agents).

Pre-treatment

Autoclaving

- All items in Groups A to C above.

Exceptional circumstances (but not recommended)

Final route

Landfill

- The recommended procedures should only be varied with the full consent and agreement of the waste contractor. Each unit must also ensure that the waste contractor uses only fully and appropriately licensed sites. The decision to send to landfill can only be reached after a full risk assessment.
- Subject to the preceding paragraph, biological waste for landfill must be kept separate from all other waste while awaiting collection.
- This route can only be taken for low risk material (e.g. Group E) or material that has been rendered non-hazardous through autoclaving. It must never contain material that could be re- or mis-used (e.g., syringe barrels, needles, etc.).

Note: Government guidance states that some materials can be sent for landfill. For environmental reasons, the MRC does not recommend this route for laboratory waste.

In addition some waste authorities are willing to regard autoclaved waste from any source as 'non-hazardous' to be transferred to black sacks for disposal as household waste. Again for environmental reasons, the MRC does not recommend this route. If, following full risk assessment and consideration of all options, landfill is selected, under the Duty of Care a label must be affixed to the sack stating the origin of the waste and that it has been rendered non-hazardous through validated autoclaving.

HSAC and waste regulatory bodies advocate the incineration of all clinical waste and strongly recommend that landfill is not used for any clinical laboratory waste.

HSAC also states that all waste in Groups A and B must be incinerated even after autoclaving. The MRC supports these views.

Pre-treatment

Autoclaving

- All biological waste identified for landfill (subject to the above strategy) should be autoclaved prior to disposal.

Packaging of waste

Autoclaving

Waste material for autoclaving (Group A and Group C) should be placed in blue or transparent biohazard bags suitable for autoclaving. Wherever practicable and according to risk, and the means and route of transport, the bags should be placed within a robust secondary container. Plastics should not be placed directly in a solid container. All containers (inner and outer) should be marked with autoclave tape. This measure will indicate if the container has been autoclaved but is not a guarantee of sterility. Sharps (Group B waste) must be placed in sharps containers complying with the approved British Standard (BS 7320:1990).

Incineration

All material for incineration, whether or not pre-treated, should be placed in yellow containers clearly marked 'for incineration only'. Robust rigid UN approved containers must be used. The use of yellow bags as final containment is no longer permitted for transport off-site, but they can be placed within UN containers. Even for internal transport the use of external rigid containers is recommended. Individual small BS sharps containers may be placed within secondary containers. These containers however should also be yellow, be marked 'for incineration only' and comply with UN packaging requirements.

Landfill

All low risk untreated biological waste acceptable for landfill must be placed in yellow bags with black stripes and clearly labelled. Transport regulations deem this waste as being non-hazardous and therefore not subject to packaging requirements for hazardous materials. Appropriate labels must however be attached (see Notes on Landfill above). The use of sacks is thus still permitted. Whilst awaiting collection it must be stored apart from both waste for incineration (yellow) and non-hazardous waste (black).

Instruction and training of staff

Risk assessments

All staff working on a project must be familiar with the risk assessments and correct procedures, including contingency plans if things go wrong (e.g., the breaking open of a container, the failure of an autoclave).

Procedures

All those producing or handling biological waste must receive instruction and training on the correct packaging, labelling and handling of waste. Training programmes should include as appropriate:

- proper categorisation of waste
- colour coding
- proper labelling of bags and containers
- the safe disposal of sharps
- the use of disinfectants
- segregation criteria and procedures
- requirements for protective clothing and other personal protective equipment
- manual handling instruction
- loading and unloading procedures for the autoclave
- autoclave operation
- emergency procedures following accidents or spillage.

Accident and incident reporting

All staff must be aware of reporting procedures and criteria for accidents involving personal injury, incidents involving near misses and incidents relating to spillages or breakages and non-adherence to working procedures (e.g., wrongly packaged or labelled containers). Any incident involving injury from a sharp should be investigated by management to establish the cause and revise procedures where necessary. Such injuries may require sending the injured party for treatment.