



UNIVERSITY OF
CAMBRIDGE

Department of Engineering

Biological Safety

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BIOLOGICAL SAFETY

1 Introduction

The hazard from biological agents is dependent upon the pathogenicity of the organism, its transmissibility and whether there is effective prophylaxis or treatment available. Biological agents have been categorised into four hazards groups by the Advisory Committee on Dangerous Pathogens (ACDP; see Appendix 1).

Increasing levels of containment are required for each hazard group. The Departmental Biological Safety Officer will determine which level is required. All procedures known to involve or is suspected to involve biological organisms must undergo a full risk assessment under the supervision of the Biological Safety Officer Safety Officer before work commences.

Handling of unscreened human samples should be at Biological Containment Level 2 (CL2) or higher. The handling of unscreened non-human primate samples is not permitted within the Department. If a project involves genetically modified organisms, human tissue, or an organism requiring CL2 or higher, several weeks notice should be given as regulatory processes and/or registration will need to be checked or completed before the Department can be authorised to carry out the work.

2 Procedure for Containment Level 1 (CL1)

- 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.
- If the room is mechanically ventilated, the airflow should be inward, at least while the work is in progress.
- The door should be closed while work is in progress.
- Wear laboratory coats, preferably side or back fastening, in the room and remove when leaving biological laboratory areas.
- No eating, chewing, drinking, smoking, storing food, applying of cosmetics, taking of medicines or mouth pipetting.
- Decontaminate and wash hands when contamination is suspected and before leaving the room.
- Effective disinfectants must always be available (see Appendix 2).
- Minimise aerosol production.
- Clean and disinfect bench tops as necessary after use.

- Store used equipment awaiting disinfection/sterilisation safely. Pipettes in disinfectant must be completely immersed.
- Place all solid waste material in dedicated clinical waste bins for removal by approved contractors (currently Vetspeed Ltd. of Royston). Contaminated material for disposal should be transported in robust containers without spillage.
- By agreement with the Biological Safety Officer, CL1 waste may be autoclaved or heated to 180°C for a minimum of 15 minutes before disposal through the general waste.
- Disinfect all liquid waste before disposal.

3 Procedure for Containment Level 2 (CL2)

Additional requirements over CL1.

- The hand washbasin must be fitted with taps operable without being touched by hand and sited near the exit to the laboratory.
- If the room is mechanically ventilated, it must be maintained at negative pressure at least while the work is in progress.
- Restrict access and ensure space is adequate for the tasks.
- Wear laboratory coats or gowns, which are side or back fastening, in the room and remove when leaving.
- Wear appropriate gloves at all times when handling samples. Damaged skin must be covered with a protective waterproof dressing. Remove gloves before leaving room.
- There must be specified decontamination procedures, including those for disinfection and for the safe handling and disposal of waste.
- Work over spill trays and/or Bench-Kote (or similar).
- Transport samples in robust leak-proof secondary containment.
- Store biological agents safely.
- Centrifuge samples in sealed safety buckets or rotors. Open within a safety cabinet whenever possible.
- Work likely to generate significant aerosols (e.g. pipetting) must take place in a safety cabinet or equivalent containment.

- Use sharps only if there is no alternative. Place directly into sharps bins for disposal. Autoclave sharps bins before incineration wherever possible.
- A biohazard sign should be displayed outside the entrance to the room.
- Only trained or fully supervised staff may work in CL2 facilities.

4 Training and competence requirements for work with biohazards

Training requirements for each individual worker must be assessed by the project supervisor in consultation with the Biological Safety Officer. Training should be risk-based.

Key competencies will include all of the following.

- Awareness and understanding of all procedures and risk assessments for the work.
- Technical competence for all aspects of the work (including the use of a microbiological safety cabinet).
- Knowledge and understanding of disinfection procedures.
- Knowledge and understanding of waste disposal arrangements.
- Knowledge and understanding of emergency spillage procedure.

5 Additional guidance for CL2 facilities

5.1 Biohazard sign

Biohazard signs will be placed at the entrance to CL2 facilities or to delineate a work area or an item of equipment used for storage or handling of CL2 hazards within an otherwise CL1 area.

5.2 Access restrictions

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.

Control of access to CL2 facilities for maintenance and service personnel should be through a permit to work system via the Biological Safety Officer, Departmental Safety Office or Bioengineering Technician.

5.3 Emergency procedures

Ensure procedures are in place for foreseeable emergencies. Examples are the procedures to be adopted for spillages inside and outside safety cabinets and for the failure of equipment that may lead to loss of containment (e.g. the failure of a safety cabinet or a centrifuge rotor or bucket).

5.4 Protective clothing

Protective clothing must be worn. 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 contamination. Laboratory coats/gowns must not be worn outside the facility, but changed for another as necessary.

Gloves of adequate resistance should be worn. Two pairs should be worn 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.

Use eye/face protection for work on the open bench.

5.5 Immunisation

For most laboratory work, staff are advised to ensure that they are suitably immunised against tetanus. In addition, all staff handling human blood and tissues are strongly advised to be immunised against hepatitis B virus (ask Departmental Safety Office for details).

5.6 Accident reporting and investigation

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 contamination on skin eye or mucous membranes should be washed immediately.

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.

5.7 Designated workstations

Work at a designated and delineated workstation, which is clearly identified. Ensure that there is sufficient room to work safely and that the work is free from the risk of disturbance or accidental contact with others.

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

5.8 Use of sharps

The use of sharps should be avoided wherever possible. If this is not feasible, then handling procedures should be designated 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. Do not re-sheath needles. Sharps bins should not be overfilled. 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 (including plastic pipettes and pipette tips).

5.9 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). Equipment must be decontaminated prior to maintenance work. Complete and sign an equipment decontamination certificate (see Bioengineering Technician or Departmental Safety Office).

Centrifuge samples only in sealed buckets or rotors (to BSEN 1010-2-020). These should be cleaned and disinfected regularly and immediately following leakage. Rotors or buckets should be opened in a safety cabinet whenever possible. Seals on buckets and enclosed rotors should be regularly checked for wear and damage and be replaced as necessary.

6 **Work with human-derived samples**

6.1 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.

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.

All work on unscreened samples should be undertaken at a minimum of CL2. If at any time changes there is a suspicion or knowledge that samples are HIV (or other hazard group 3 pathogen) – positive then the work must stop immediately and the Biological Safety Officer informed.

6.2 Handling human-derived samples

Human samples may only be handled in the Bio Lab (Inglis Building).

6.3 Access restrictions

Access to the Bio Lab is by swipe card only as authorised by the Biological Safety Officer.

7 Selection of disinfectants

Disinfectants vary in effectiveness depending upon the biological agent. Use Appendix 2 as a guide.

8 Use of disinfectants

8.1 Spills of biological agents

Containers of disinfectant at an appropriate concentration should be available at each workstation where biological agents are handled. In the case of an accidental spillage a disinfectant that gives a rapid kill is required. Disinfectants that are supplied as a powder (eg Virkon) are especially useful for sprinkling over spills. Minimise spread when using liquid disinfectant by first covering the spill with tissues.

8.2 Stability of working dilutions

Once diluted, the activity of disinfectants decays with time.

Follow manufacturers guidelines. Some products (eg Virkon) contain a coloured indicator to show effective disinfection capacity. 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.

8.3 Contact time

Disinfectants must remain in contact with micro-organisms for a period of time sufficient to achieve disinfection. Follow manufacturer's guidelines.

8.4 Discard jars

Containers of working strength disinfectant must be placed at, or close to, each workstation 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.

Alternatively, pipettes may be placed in an autoclavable container within the cabinet, and transferred to an autoclave directly.

8.5 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.

8.6 Wear personal protective equipment

Most disinfectants are at best irritants and some are considerably more hazardous. Wear safety glasses/goggles, lab coat and gloves (preferably thicker 'Marigold' type) when cleaning down surfaces and ensure an eyewash is within the work area.

9 **Safety Cabinets**

The Bio Lab contains a Class II Biological Safety Cabinet. This type of cabinet is designed to give protection to both the user and the sample. Instructions for use can be found in Appendix 3.

9.1 Fumigation

Safety cabinets used for handling biohazards should be decontaminated regularly. This must always be done before servicing and following any spillage. Fumigation by formaldehyde gas or hydrogen peroxide may be specified by service and maintenance providers. **Do not attempt to fumigate without seeking advice from the Departmental Safety Office.**

10 **Biological waste disposal**

10.1 Solid waste

Dispose of solid waste into dedicated clinical waste bins or bags (yellow with biohazard symbol).

Do not tape clinical waste or autoclave bags to benches or place disposable pipettes or pipette tips in bags.

Presently, waste is collected on the first Thursday of each month. If large quantities of waste are being produced, additional collections can be arranged (see the Bioengineering Technician or Departmental Safety Office).

Noxious waste or large amounts of biological sludge (e.g. after large-scale sub-cellular preparations) should be frozen in clinical waste bags and placed in the clinical waste bin just prior to collection.

10.2 Contaminated sharps

Sharps contaminated with biological agents should be placed in dedicated sharps bins indicating that the contents present a biohazard and must be incinerated via an approved contractor.

10.3 Aqueous waste

Aqueous biohazardous waste must be treated with an appropriate disinfectant at the percentage and length of time recommended by the manufacturer, preferably overnight.

Waste may then be disposed of down an appropriate laboratory sink. Flush down with copious amounts of water.

Appendix 1

Advisory Committee on Dangerous Pathogens

Categorisation of Biological Hazards

Group 1: unlikely to cause human disease.

Group 2: can cause human disease and may be a hazard to employees; unlikely to spread to the community; effective prophylaxis or treatment available.

Group 3: can cause severe human disease and may be a serious hazard to employees; may spread to the community; effective prophylaxis or treatment usually available.

Group 4: causes severe human disease and is a serious hazard to employees; likely to spread to the community; no effective prophylaxis or treatment available.

Appendix 2

Virkon

A peroxygen compound. Working solutions of 1% w/v have low toxicity and no irritancy. In powder form it is moderately irritant to eyes and 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. Rinse or wipe off after as can be corrosive to steel and certain plastics. Use to inactivate viruses, bacteria, fungi and spores.

Clearsol

Use at 1% v/v in water for routine purposes (giving ~ 0.4%v/v phenols) and about 5% v/v for spills or heavily contaminated surfaces. Use for E. Coli, other enterobacteria and most organic waste.

Some workers may be sensitive to the strong phenolic odour of Clearsol.

Tegodyne

Use at 0.45% v/v in water for routine purposes (giving ~ 0.005% v/v available I₂) and about 3.0% v/v for spills. For Vacusafe units, add 25ml per collecting vessel. Leave for several hours, preferably overnight, before flushing down sink with copious amounts of water. Use for tissue culture waste.

Some workers may be sensitive to the distinctive odour of Tegodyne.

Hypochlorite

Use at 2% v/v for routine purposes (giving $\geq 1,000$ ppm available Cl₂) and about 3.0% v/v for spills. Diluted solutions are only usable within one day and fresh use dilutions should be made up at the start of each working day.

Stock solutions must be $\geq 10\%$ w/v available Cl₂ and discarded within three months if not used. Do not use household bleach solutions.

Use for spores (or spore-forming bacteria) and infective or potentially oncogenic nucleic acids.

Surface acting agents

Commercial disinfectants (e.g. Trigene) and 70% ethanol can be used as a surface disinfectant in CL1 facilities or in CL2 facilities as a general cleaner where there is no obvious spillage.

Formaldehyde

To be used only for fumigation of laboratories or microbiological safety cabinets, under the direct supervision of the Departmental Safety Officer or Biological Safety Officer. Formaldehyde vapour is toxic, with an occupational exposure limit of 2ppm. Precautions must be taken to prevent exposure during fumigation or the subsequent venting of the vapour.

NOT for general use.

BIOLAB CLASS II SAFETY CABINET

INSTRUCTIONS

Starting the cabinet:

1. Wear gloves and put on a clean long-sleeved laboratory coat.
2. Open the sash window to its maximum height (the alarm will sound).
3. Turn on the FAN.
4. The FAN PIN is needed to disable the alarm.
5. Enter the four-digit PIN. The (default) PIN is 0001.
6. Press SET and the four digit display will blink.
7. Press UP or DOWN arrows button to choose the first digit.
8. Press SET. The second digit will blink.
9. Press UP or DOWN arrows button to choose the second digit.
10. Repeat steps until the fourth-digit display has been chosen.
11. Before the fan is fully functioning, there will be a warm-up period (this is currently set to 3 minutes).
12. Surface-decontaminate the work surface, side walls, back wall, and internal surface of the window using 70% ethanol – 30% distilled water. Do not insert any part of your body into the work zone except hands.
13. Surface-decontaminate all materials before loading them into the work zone.
14. Set the sash to normal working height (READY state).
15. LIGHT will automatically go on (dots should match!).

Cleaning and shutting down the cabinet:

1. Discard all waste.
2. Wipe the work surface, side walls, back wall, and drain pan using 70% ethanol – 30% distilled water.
3. Leave the fan for 3 minutes in order to purge airborne contamination from the work area.
4. Turn off the FAN. It'll ask for the PIN so repeat steps 5 to 10. Fully close the sash window (this will automatically turn off the LIGHT).