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Code of Practice for Microbiological Work

1. Introduction

Very little of the microbiological and biochemical work in the University laboratories involves dangerous pathogens, but even many micro-organisms of relatively low pathogenicity are capable of causing significant disease, especially in particularly susceptible individuals, or may cause problems of contamination if released into the general environment. Thus, whether working with known pathogens or supposedly harmless species, correct techniques and attention to good microbiological practice are essential.

The principal hazards of microbiological work arise from inhalation, ingestion or inoculation of micro-organisms. The following rules and guidelines are designed to minimise such risks. Inexperienced persons must not work in a microbiology laboratory until the safety principles have been explained and understood, and then only under the supervision of an experienced laboratory worker.

2. Risk Assessments

Under the Control of Substances Hazardous to Health (CoSHH) Regulations 1994, all work involving biological agents (which includes the general class of micro-organisms, as well as cell cultures and human endoparasites) must be prefaced by a suitable and satisfactory risk assessment. This must take into account the nature, associated hazards, minimum containment level, and any control measures necessary to minimise the risk.

CoSHH assessments must be written down and reviewed, preferably annually, to ensure that all potential risks are being controlled adequately.

3. Facilities for Microbiological Work

Microbiological work may only be carried out in laboratories designated for such purposes and with appropriate containment facilities (see section 7). The laboratories must be under the supervision of a designated Departmental Biological Safety Officer who must be satisfied that facilities, training and supervision are adequate.

4. General Items of Good Laboratory Practice

1. Personal items irrelevant to the microbiological work (sports gear, items of shopping, etc) should not be taken into the laboratory.
2. Protective clothing must be worn for all microbiological work.
3. Scrupulous personal hygiene must be observed in the laboratory. Eating, drinking, smoking and the application of cosmetics in the laboratory and before washing after a period of work are forbidden.

4. Mouth pipetting is forbidden in microbiology laboratories.
5. Minor cuts, scratches and abrasions on the hands should be sealed with waterproof dressings before entering the laboratory.
6. Used "sharps" (scalpel blades, syringes and needles, etc) must be disposed of into approved "sharps" containers to be taken for incineration. Containers must be exchanged regularly and not allowed to become over-full.

5. Protective Clothing and Changing Facilities

1. All persons working in a microbiology laboratory must wear a protective coat which should be removed before leaving and left on a convenient hook for "in-use" clothing. A basic garment should be recommended by the Department; if work includes potential pathogens, such a coat should preferably be side or back closing with long sleeves and close fitting cuffs made of material that will minimise the risks of contaminating personal clothing worn beneath.
2. Pegs for "in-use" clothing should be close to the exit door and the wash-hand basins.
3. Wash-hand basins must be provided close to the exit door and all persons leaving the laboratory suite, having removed their protective coat, must wash their hands. Taps must be of a type operated without touching by hand and disposable paper towels must be used for drying.

6. Categories of Pathogens

The Advisory Committee on Dangerous Pathogens (ACDP) assigns microbiological agents to one of 4 hazards groups (1-4) and designates the standards of laboratory containment (containment levels 1-4) needed for work with each group. ("Categorisation of Pathogens According to Hazard and Categories of Containment", Second Edition 1990).

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| GROUP 1 | An organism that is most unlikely to cause human disease. |
| GROUP 2 | An organism that may cause human disease and which might be a hazard to laboratory workers but is unlikely to spread to the community. Laboratory exposure rarely produces infection and effective prophylaxis or effective treatment are usually available. |
| GROUP 3 | An organism that may cause severe human disease and present a serious hazard to laboratory workers. It may present a risk of spread in the community but there is usually effective prophylaxis or treatment available. |
| GROUP 4 | An organism that causes severe human disease and is a serious hazard to laboratory workers. It may present a high risk of spread in the community and there is usually no effective prophylaxis or treatment |

Most work in the University is concerned with organisms of Groups 1 and 2. Some of the organisms in common use throughout the University and most of those used in the Medical School are in Group 2. Therefore, the minimum standard for all microbiological

work in the University is Containment Level 2, as it is for hospital laboratories. Micro-organisms in Group 3 may be handled only in designated Containment Level 3 laboratories. This Code of Practice specifically excludes work with Group 4 materials which is not permitted in the University. Researchers considering using Group 4 organisms must refer to Containment Level 4 Conditions as defined in ACDP (1990).

7. Containment Level Facilities

(a) Containment Level 2

1. The laboratory should be easy to clean. Bench surfaces should be impervious to water and resistant to acids, alkalis, solvents and disinfectants.
2. Access to the laboratory should be limited to laboratory personnel and other specified persons.
3. There should be adequate space (24 m³) in the laboratory for each worker.
4. If the laboratory is mechanically ventilated, an inward airflow into the laboratory must be maintained by extracting room air to atmosphere.
5. The laboratory must contain a wash-hand basin which should be located near the laboratory exit. Taps must be of a type which can be operated without being touched by hand.
6. An autoclave for the sterilisation of waste materials must be readily accessible, normally in the same building as the laboratory.
7. The laboratory door should be closed when work is in progress.
8. Laboratory coats or gowns, preferably side or back fastening, must be worn in the laboratory and removed when leaving the laboratory suite. Separate storage (e.g. pegs) must be provided in the laboratory suite for this clothing.
9. Eating, chewing, drinking, smoking, storing of food and applying cosmetics must not take place in the laboratory.
10. Mouth pipetting must not take place.
11. Hands must be disinfected or washed immediately when contamination is suspected, after handling infective materials, and also before leaving the laboratory.
12. In general, work may be conducted on the open bench, but care must be taken to minimise the production of aerosols. For manipulations such as vigorous shaking or mixing and ultrasonic disruption etc, a microbiological safety cabinet (class 1; BS5726: 1979) or equipment which is designed to contain the aerosol must be used. The cabinet must exhaust to the outside air or to the laboratory air extract system.
13. Effective disinfectants must be available for routine disinfection and immediate use in the event of spillage.
14. Bench tops must be disinfected after use.

15. Used laboratory glassware and other materials awaiting sterilisation must be stored in a safe manner. Pipettes, if placed in disinfectant, must be totally immersed.
16. Material for autoclaving must be transported to the autoclave without spillage in robust containers.
17. All waste materials must be made safe before disposal or removal to the incinerator.
18. All accidents and incidents of contamination must be immediately reported to and recorded by the person responsible for the work, and reported to Safety Services on a University Accident/Dangerous Occurrence form by the Biological Safety Officer.

(b) Containment Level 3

In addition to the Level 2 facilities

1. The laboratory must be sealable to permit fumigation.
2. The laboratory should be sited in an area away from general circulation. Access to the laboratory must be limited to authorised personnel. The laboratory door must be locked when the room is unoccupied.
3. A specific biohazard sign must be posted at the entry to the laboratory and the door must contain a glass panel so that the occupants can be seen.
4. A continuous airflow into the laboratory must be maintained during work with pathogens by one of the following means:
 1. extracting the laboratory air through independent ducting to the outside air through a HEPA filter;
 2. extracting the laboratory air to the outside air with a fan and HEPA filter sited in a wall or window of the laboratory;
 3. ducting the exhaust air from a microbiological safety cabinet to the outside through a HEPA filter;
 4. a safe variation of these provisions. Provisions should also be made for comfort factors, e.g. fresh-air, temperature control. In laboratories which have a mechanical air supply system, the supply and extract airflow must be interlocked to prevent positive pressurisation of the room in the event of failure of the extract fan. The ventilation system must also incorporate a means of preventing reverse airflows.
5. An autoclave for sterilisation of waste materials should be situated preferably within the laboratory, but one must be readily accessible in the laboratory suite.
6. Laboratory doors must be kept closed when work is in progress.
7. Side or back fastening gowns must be used in the laboratory and they must be autoclaved before removal for laundering. These gowns must not be used outside the laboratory suites.
8. Gloves must be worn for all work with infective materials and the hands must be washed before leaving the laboratory.

9. (a) All laboratory procedures with infective materials must be conducted in a microbiological safety cabinet (class I or class III BS 5726: 1979, or unit with equivalent protection factor or performance) except where the equipment to be used provides containment of the potential aerosol. Cabinets must be serviced regularly according to recommended maintenance schedules and the airflow must be checked at regular intervals between servicing. The equipment must be decontaminated before service personnel are asked to handle it (see below).
- (b) The cabinet must exhaust through a HEPA filter to the outside air or to the laboratory air extract system, and in other respects such as siting, performance, protection factor and air filtration, it must comply with the specifications detailed in BS 5726: 1979. When laboratories are faced with a major problem because of difficulties in arranging for the cabinet to exhaust to the open air, recirculation of exhaust air through two HEPA filters in series may in exceptional circumstances be considered as an alternative. In these cases the maintenance of a continuous airflow into the laboratory during work with pathogens will be of particular importance and such an option must not be adopted without prior consultation with the HSE.

Decontamination of Equipment.

Contaminated equipment (e.g. cabinets, centrifuges, etc) must be rendered safe before maintenance inspection, servicing or repair. Anyone who inspects, services or repairs laboratory equipment has a right to expect that the equipment has been cleaned and properly treated so as to remove or minimise the risk of infection. Equipment which is visibly soiled must never be presented or sent to third parties for inspection, maintenance or repair. This is a legal requirement under the Health and Safety at Work Act, 1974.

Manufacturer's recommendations should be consulted for appropriate methods of decontamination for specific items and each Department must have a written procedure for such items.

10. The laboratory should contain its own equipment, e.g. centrifuge in which sealed buckets must be used, incubator, refrigerator, deep-freeze, vapour phase liquid nitrogen chest, etc so that all infective group 3 pathogenic materials are held within the laboratory and nowhere else. Where this is not reasonably practicable materials must be transported and stored without spillage in properly labelled containers which must be opened only in containment level 3 accommodation.

All accidents, spills and exposures to infective materials must be immediately reported to and recorded by the person responsible for the work.

8. Special Hazards

The following are examples of particular hazards for which special care is needed and specialist advice should be followed.

1. Work with known human or animal pathogens, or material from cases of disease or from autopsy.

2. Harvesting bulk growth of any micro-organisms.
3. Handling vessels containing large volumes of microbiological cultures; these should not be left unattended
4. Procedures that generate aerosols.
5. Transfer of infective material from one container to another.

9. Accidents – however trivial, these must be reported

A record must be kept of all accidents, incidents of contamination, and infections that occur in the laboratory and regularly scrutinised by the Departmental Biological Safety Officer. Safety Services must be informed on an Accident/Dangerous Occurrence form. Measures must be taken to prevent recurrence. Laboratories must have written procedures for dealing with accidents and spillages, copies of which must be sent to Safety Services, indicating the people responsible for the action, who must be experienced laboratory workers and have received appropriate training.

1. Accidents

In the case of accidental skin puncture, contamination of abraded skin or mucosae (eyes, mouth, nose): any wound should be encouraged to bleed freely, the contaminated part should be washed gently (not scrubbed) under running water; the Head of Department or Biological Safety Officer must be informed and Safety Services notified on the forms as above. Every care must be taken to avoid needlestick injuries. Needles should only be removed from syringes by use of "needle remover" systems. Needles should not be re-sheathed unless the sheath is held in a protective holder – NOT in the hand. Syringes and needles should be discarded directly into a sharps container for incineration. When expelling air from a syringe, do not discharge into the atmosphere but into an alcohol-soaked swab.

2. Spillages

The spill or debris should be covered with cotton wool or absorbent paper towels and a suitable disinfectant (see section 13) should be poured on to the absorbent material to soak it thoroughly; the area should be cordoned off until decontaminated. After leaving for a designated period (depending upon type of spillage and the disinfectant), the debris and covering material should be cleared by an experienced and fully trained member of the laboratory staff. He/she must wear disposable gloves and must sweep the material into a suitable impervious container (e.g. plastic bag or box – NB broken glass should not be put into plastic bags) using disposable cloths or paper towels or a strong piece of card. Dust pans and brushes must not be used unless they can be autoclaved. All debris and materials used to clear up the spillage must be placed in a laboratory discard receptacle.

10. Transport of Micro-organisms

Viable micro-organisms of Group 2 and above (see section 6) may be transported outside the laboratory complex only when properly packed. The primary container must be closed securely (e.g. screw cap) and placed inside a sealable plastic bag; any letters or forms must be attached to the bag (NOT with staples) and NOT placed inside it. The bag should be transported in a closed, impervious box that will contain any spillages and can be disinfected. The handle should not be part of the lid. For postal transmission, the Post Office regulations must be followed.

11. Health Records of Laboratory Workers

Health records must be kept for all workers dealing with known pathogens. For workers exposed to primary specimens from cases of human or animal infection (i.e. in medical laboratories dealing with material taken directly from patients), a baseline chest x-ray must be taken and an assessment of immunisation status should be recorded. Appropriate immunisation should be recommended. Advice should be sought from the Occupational Health Unit, 40 Victoria Street.

12. (i) Disposal of Waste Materials

Waste material must not be removed from the laboratory until either it has been sterilised by autoclaving or has been placed in an appropriate container for sending for autoclaving within the general laboratory area or for incineration.

Fluids known or suspected to be contaminated must be sterilised by autoclaving before disposal. Infectious fluid waste may not be discharged to the sewer. Cultures must be autoclaved before disposal; immersion in disinfectant is not sufficient.

(ii) Cleaning

Hard surfaces (benches and other work surfaces) should be cleaned at the end of each working day with a prescribed disinfectant containing a compatible surfactant. When contamination with blood or body fluids, or with cultures has occurred, immediate action to disinfect and clean the area must be taken by staff wearing appropriate protective clothing.

13. Disinfectants

1. Containers of fresh disinfectant at in-use dilutions must be readily available. Containers must be regularly exchanged to ensure activity.
2. Disinfectants must be available for discard of contaminated materials and equipment (which must be completely immersed). Discarded items should be left in disinfectant for a specified period, usually overnight.
3. Separate supplies of disinfectants must be readily available to deal with accidents and spillages, they must be renewed with fresh supplies daily.

4. Suitable disinfectants are:
 - a phenolic disinfectant (e.g. stericol) at 1% in-use dilution for general bacteriological hazards;
 - 1% hypochlorite for general virology (or similar solution containing 1000 ppm available chlorine);
 - a solution containing 10000 ppm available chlorine (e.g. 10% hypochlorite);for blood spillages or suspected hepatitis B or HIV contamination, Precept granules or sodium dichloroisocyanurate tablets are recommended.

(D Coates, Journal of Hospital Infection, Jan 1988 11 (1): 95–96)

14. Autoclaves

Autoclaves nominated for disposal purposes must have a regular maintenance schedule including monitoring by an engineer as recommended by the manufacturers. This must include trial cycles with thermocouples inside disposal containers at least on an annual basis. The performance of other autoclaves must be monitored regularly by including an easily-read temperature indicator (e.g. Brown's tubes, or spore strips) in each cycle. Boxes, bins or bags for material to be sterilised must be suitable. They should not restrict removal of air and penetration of steam. They should be impervious to prevent spillages.

15. Stocks of Micro-organisms

As far as is practicable, stocks of micro-organisms should be kept to a minimum. Unwanted stocks should be disposed of safely. Stocks should, where possible, be protected in fire-resistant containers and freezers/refrigerators containing stocks of micro-organisms should be labelled with the biohazard sign. All containers must be labelled with identity/details of micro-organisms:

Name of Researcher

Date

Nature of Material