MICROBIOLOGICAL SAFETY RULES

Revised October 2008

These rules were approved by the
Biological Hazards and Genetic Modification Committee

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1. APPLICABILITY

These rules shall apply to:

(i) all University laboratories and other rooms where micro-organisms are cultured, examined or otherwise handled.

(ii) all persons working, receiving instruction or visiting these rooms.

(iii) all procedures where exposure to biological agents may arise out of the work activity e.g. environmental sampling, tissue handling, etc..

These Microbiological Safety Rules do not apply to cell cultures (defined by COSHH regulations as *in vitro* growth of cells obtained from multicellular organisms). However, some cell cultures are defined as "biological agents" in the COSHH regulations. Appendix 13 of the ACDP guidance "Categorisation of biological agents according to hazard and categories of containment" deals with the hazards of cell cultures. All work with cell cultures must be supported by a written risk assessment. The ACDP publication gives guidance on assigning hazard categories to cell cultures from different sources. In general all work with cell cultures should be carried out in Containment Level 2 laboratories or the Containment Level 3 laboratory suite. In the case of cell cultures from tissues of human origin, additional precautions are necessary and are covered by the UEA Rules for work with Human Body Tissues and Fluids.

Environmental work needs to undergo microbiological risk assessment if:

a) any samples are cultivated within a laboratory

or

b) the work involves potential exposure to materials that have a significant risk of containing pathogens which cause human disease.

2. MANDATORY REQUIREMENTS

The Control of Substances Hazardous to Health Regulations 1999 (the "COSHH" regulations) require that an assessment of risks to health is made by the Project Leader before any micro-organism is used in teaching or research. The assessment must be recorded in writing (on form USS/BH/F01, unless it

involves genetic modification (see below)). If advice is required from the Occupational Health Service, then a draft copy of the proposed assessment should be sent to them.

Assessments for all HG1 and HG2 pathogens must be submitted to the Biological Hazards and Genetic Manipulation Committee (via the Director of Safety Services) for approval before a start can be made. The assessment shall be reviewed annually by the Project Leader or whenever there has been a significant change in the work to which the assessment relates.

Assessments for HG3 pathogens must be approved in compliance with the 'Code of Practice for Working with Hazard Group 3 biological agents' and the General Standing Operating Procedures of the Containment Level 3 Suites in the Biomedical Research Centre (BMRC).

Work with genetically modified micro-organisms (including those in which the genetic modification has been carried out elsewhere) is subject to the Genetically Modified Organisms (Contained Use) Regulations 2000 and to the UEA Rules which covers work of this kind.

3. CATEGORISATION OF MICRO-ORGANISMS

The ACDP has classified biological agents into four hazard groups:

Hazard Group 1 agents are unlikely to cause human disease

Hazard Group 2 agents can cause human disease and may be a hazard to employees, but are unlikely to spread to the community and effective treatment is usually available

Agents in **Hazard Groups 3 and 4** can cause severe human disease and present a serious hazard to employees.

USE OF BIOLOGICAL AGENTS IN HAZARD GROUP 4 IS NOT PERMITTED IN THE UNIVERSITY.

Work with Hazard Group 3 agents can only be undertaken in Containment Level 3 laboratory suites, within the Biomedical Research Centre (BMRC).

Before work is started with any micro-organism, it shall be classified in accordance with the ACDP guidance. Where the microorganism of interest is not covered by the ACDP guidance the Project Leader shall include in the risk assessment a justification for the classification that will be adopted.

4. OTHER STATUTORY REQUIREMENTS

Work with specified animal pathogens requires a licence, under the Specified Animal Pathogens Order 1998, from the Department for Environment, Food and Rural Affairs (DEFRA) and shall not be started unless the licence has been obtained. Further details are available from the DEFRA website (animal health guidance) and the Safety Services website (national legislation).

Pathogens listed under Schedule 5 of the Anti-terrorism, Crime and Security Act 2001 (Modification) Order 2007 can not be kept or used without formal notification to the Home Office (see Safety Services website – national legislation). Approval to use such pathogens must be sought from University Safety Services prior to ordering.

If there is any intention to transport such a pathogen or HG3 pathogen from UEA to any other organisation for any reason, checks must be made, by the Principal Investigator, on the notification status of the premises prior to despatch. Any dispatch of HG3 or Schedule 5 pathogen to an organisation outside of the United Kingdom shall only occur after the Principal Investigator has received written confirmation that the organisation is authorised, competent and suitably equipped to hold such material (the confirmation shall be guaranteed by a signatory at institutional level). Documented records of these checks shall be retained.

The import, movement and keeping of certain plant pathogens require licences under the Plant Health (Great Britain) Order 1993. Further details are available from the DEFRA website (plant health guidance).

5. PROJECTS THAT REQUIRE A DEFRA LICENCE FOR WORK WITH PLANT PATHOGENS OR SPECIFIED ANIMAL PATHOGENS

The approval of such projects by the Biohazards and Genetic Manipulation Committee will be provisional until a final risk assessment quoting the DEFRA licence number has been submitted.

6. TRAINING OF PERSONNEL

All staff and graduate students involved in the experimental work must be adequately trained in microbiological or other appropriate laboratory practice and must be familiar with the requirements of the Regulations and the guidance from the ACDP. All new entrants must be given a copy of these Rules.

7. WORK WITH HAZARD GROUP 1 MICRO-ORGANISMS

Micro-organisms in hazard group 1 may be used in Containment Level 1 laboratories. The basic requirements for these laboratories are described in the ACDP guidance, "The management, design and operation of microbiological containment laboratories". In particular there is a need for good containment and appropriate written procedures. Hazard group 1 micro-organisms may be used in teaching laboratories provided the same requirements are met and the risk assessment has been approved. Conditions of "Good Laboratory Practice" shall be observed - see Appendix 1 to this Code.

8. WORK WITH HAZARD GROUP 2 MICRO-ORGANISMS

Work with these organisms will require the provision of laboratory facilities and operating procedures to Containment Level 2 standard, as described in the ACDP Guidance, "The management, design and operation of microbiological containment laboratories". The work shall be supervised by a person who has experience and competence in microbiological techniques.

A "Biohazard" sign shall be displayed on the entrance door and access to the laboratory shall be restricted to authorised persons. Special arrangements must be made for cleaning or maintenance work in Containment Level 2 laboratories, in order to ensure adequate safety of the employees concerned.

All Group 2 micro-organisms in storage must be kept in containers which are appropriately labelled (including a biohazard label) to give information about the contents and to include a statement that the organism may cause disease. All such containers shall be kept in a secure location.

Microbiological safety cabinets shall conform to BS EN 12469 2000 and shall be tested as required by Regulation 9 of the COSHH Regulations. Guidance given in Appendix 6 of the ACDP guidance, "The management, design and operation of microbiological containment laboratories" and in the Approved Code of Practice for the COSHH Regulations shall be followed.

9. WORK WITH HAZARD GROUP 3 MICRO-ORGANISMS

Work with Hazard Group 3 biological agents can only be undertaken in the Containment 3 Laboratory suites, within the Biomedical Research Centre (BMRC), and under the management arrangements, and in compliance with the local rules and Standard Operating Procedures, for those laboratory suites.

Hazard group 3 agent projects will need to be notified to the Health and Safety Executive (HSE). The Director of Safety Services will carry out this notification on behalf of the University. This notification has to be done at least 20 working days before work, on the project, is due to start.

10. LARGE-SCALE USE OF BIOLOGICAL AGENTS

The ACDP publication 'The large-scale contained use of biological agents' provides advice & good practice on the use of fermenter systems. All large-scale operations should follow written standard operating procedures and conform to this guidance.

11. DISINFECTION, SPILLS AND WASTE DISPOSAL

All work with biological agents shall take account of the procedures which need to be followed by workers in order to ensure that apparatus and laboratory fixtures are effectively disinfected. These procedures shall be described in the written risk assessment and a copy shall be displayed in the laboratory for the information of all users.

Provision should be made for dealing promptly and effectively with accidental spills. It is essential that written instructions are prepared and are readily available in all laboratories involved in the work. In all cases, it is necessary to ensure that an appropriately equipped "spill kit" is readily available. A written report shall be made of the circumstances of any spillage and any actions subsequently taken.

Disposal of waste material must take account of the pathogenicity of the organisms involved. In the case of hazard group 2 and 3 agents it will be necessary to demonstrate that effective destruction of viable organisms is achieved through autoclaving or other means.

12. REPORTING ACCIDENTS OR POSSIBLE INFECTION

The Reporting of Injuries, Diseases and Dangerous Occurrences Regulations require a report to be made to the Health and Safety Executive in the case of any acute illness requiring medical treatment where there is reason to believe that this resulted from exposure to a biological agent. A report shall also be made about any accidental release of a biological agent likely to cause severe human illness. Within the University, any incidents of this kind shall first be reported to the University Safety Services for onward transmission to HSE.

APPENDIX

GOOD LABORATORY PRACTICE

General

1. Hands should be washed frequently. Workers must avoid touching their mouth and eyes.

2. Food and drink must not be stored or consumed in the laboratory.

3. There must be no smoking or gum-chewing in the laboratory.

4. Cosmetics must not be applied in the laboratory.

5. The face and eyes must be shielded or otherwise protected during any operation that might result in the splashing of microbiological material.

6. A microbiological safety cabinet must be used for all procedures that may generate aerosols

Microbiological Safety Cabinets

1. Only workers who have been trained in their use shall use microbiological safety cabinets. Written procedures shall be provided to workers.

2. The cabinet must never be used unless the fan is switched on and the air flow indicator is in the "safe" position.

3. The viewing panel must not be raised or removed when the cabinet is in use.

4. Apparatus and materials in the cabinet during operation should be kept to a minimum and at the rear of the working area.

5. Bunsen burners must not be used in the cabinet. The heat produced will distort the air flow and the filters might be burned. A microincinerator is permissible, but disposable plastic loops are preferable. Use of other flames in the cabinet should only be allowed after ‘in-use’ operator protection factor testing has established that protection is not compromised in any way.

6. All work should be done in the middle of the rear of the cabinet and be visible through the viewing panel.

7. Traffic behind the operator should be minimised.

8. The cabinet fan should be run for at least 5 minutes after completion of work in the cabinet.

Pipetting

1. A pipetting aid must be used. Mouth pipetting is prohibited.

2. During pipetting, air must never be blown through a liquid containing biological agents.

3. Infectious material must not be mixed by alternate suction and expulsion through a pipette.

4. Biological material must not be expelled forcibly from a pipette.

5. Where serological pipettes are used, a disinfectant-soaked cloth should be placed on the work surface to trap spilled drops and be autoclaved after use.

6. Fluids should be discharged down the inner wall of the tube or bottle or beneath the surface of the liquid in the container.

7. Contaminated pipettes or pipette tips must be completely immersed in a suitable disinfectant before being autoclaved.

8. The discard container for pipettes or pipette tips must be placed within the microbiological safety cabinet.

9. A syringe fitted with a sharp hypodermic needle must not be used as a pipetting device. Blunt cannulas should be substituted for needles. There are devices for opening septum-capped bottles which avoid the use of hypodermic needles and syringes.

Ampoules

1. Ampoules of freeze-dried cultures must always be opened in microbiological safety cabinets. This is because the contents are in a vacuum and the sudden inrush of air may disperse the contents into the atmosphere.

2. The outside of ampoules should be decontaminated before use.

3. Procedure for opening ampoules:

    i) make a file mark on the tube near the middle of the cotton-wool plug.

    ii) use commercially-available ampoule breakers which contain the broken ends of the ampoule and/or hold the ampoule in a wad of cotton to protect the hands.

    iii) apply a red-hot glass rod to the file mark to crack the glass.

    iv) gently remove the top and treat the top as contaminated material.

    v) remove, with sterile forceps, the cotton-wool plug, if it is still above the contents of the ampoule.

    vi) add liquid for resuspension slowly to the ampoule, to avoid frothing.

4. Glass ampoules must never be immersed in liquid nitrogen because cracked or imperfectly sealed ampoules may break or explode on removal. If very low temperatures are required, plastic ampoules designed for cryogenic storage may be stored in the vapour phase only, i.e. above the level of the liquid nitrogen. Whenever possible, biological agents should be stored in mechanical deep-freeze cabinets or on dry-ice rather than in liquid nitrogen.

5. Hand and face protection must be worn by workers when removing ampoules from cold storage.

6. The outsides of ampoules should be decontaminated when they are removed from storage.

Centrifuges

1. Centrifuge rotors and buckets must be inspected before use for signs of corrosion and for hair-line cracks. The interior of centrifuge bowls should be checked for evidence of bad techniques, indicated by staining or soiling at the level of the rotor. If this occurs revise centrifugation protocols.

2. Centrifuge tubes and specimen containers to be used in the centrifuge should be made of plastic or other suitable material, designed for the purpose and should be inspected for defects before use.

3. Tubes and specimen containers must always be securely capped.

4.. Except in ultracentrifuges, sufficient headspace should be left above the fluid level to allow for the angle of rotation of the rotor, usually equivalent to one-third of the capacity of the tube.

Homogenizers and Shakers

1. Sonicators, homogenizers and shakers must be used in microbiological safety cabinets because of the potential for aerosol production. Containers must be purpose-made and of suitable material to withstand such procedures.

2. Machines should be suitably shielded & disinfected after use.

3. After shaking or homogenization, all containers must be opened in a microbiological safety cabinet.

4. Hearing protection must be worn by people using sonicators.

Refrigerators and Freezers

1. Refrigerators, deep-freeze and dry-ice chests must be cleaned out and defrosted periodically and any ampoules, tubes, etc. containing hazardous materials that may have broken during storage removed. Face protection and rubber gloves must be worn. After cleaning, the inner surfaces of the cabinet must be disinfected.

2. All containers stored in refrigerators or deep-freezes must be clearly labelled with the scientific name of the material, the date stored, and the name of the individual storing the material. Unlabelled and obsolete materials must be autoclaved.

3. Flammable solutions must not be stored in non-explosion-proof refrigerators. Notices to this effect must be placed on refrigerator doors.