Guidance on the use of (non-GM) Microbiological Organisms in Research

1. Introduction

Work with microbiological organisms plays a significant role in the research portfolio of many science and engineering departments in the University. Few Dangerous Pathogens (see Section 2) are used but even microorganisms of relatively low pathogenicity are capable of causing disease in susceptible individuals, or problems of contamination if released into the environment. Therefore it is necessary to employ correct laboratory techniques and good microbiological practices whether working with known or unknown pathogens.

The principal risks of work with pathogens arise from the potential for inhalation, ingestion or inoculation of the organisms. The following rules and guidance are intended to minimise these risks. Inexperienced workers must not work in microbiological laboratories until the safety principles and practices have been fully explained and understood, and then only under the supervision of an experienced microbiological laboratory worker.

2. Categories of Pathogens

The Advisory Committee on Dangerous Pathogens (ACDP) assigns all microbiological organisms to one of four Hazard Groups and specifies the standards of laboratory containment (Containment Levels 1 – 4) required to work with each Hazard Group. A list of the organisms in each Hazard Group can be found on Safety Services web site at: http://safety.dept.shef.ac.uk/guidance/AppListBiolAgen.pdf. *For further information on Containment Levels see Appendix 1*

Hazard Group 1 (HG 1)	Organisms unlikely to cause human disease
Hazard Group 2 (HG 2)	Organisms able to cause human disease and which may be a hazard to laboratory workers but unlikely to be able to spread to the community. Laboratory exposure rarely produces infection and effective prophylaxis or treatments are usually available.
Hazard Group 3 (HG 3)	Organisms able to cause severe human disease and present a serious hazard to laboratory workers. They may present a risk of spread in the community but there are effective prophylaxes or treatments available.
Hazard Group 4 (HG 4)	Organisms causing severe human disease and are a serious hazard to laboratory workers. They could present a high risk of spread in the community and there are usually NO effective prophylaxes or treatments.

Most microbiological research work in the Science, Engineering and Medical Faculties involves work with microorganisms in HG 1 & 2. All microbiological laboratories in the Faculties are designed to Containment Level 2 standard in order to facilitate work with organisms in HG 1 & 2. Research with microorganisms in HG 3 may only be undertaken in designated Containment Level 3 facilities in the School of Medicine and Biomedical Science on Beechill Road. Research with HG 4 microorganisms must not be undertaken at the University of Sheffield as no facilities exist in the University to deal with them safely and they will not be considered further in this document.

Control of Substance Hazardous to Health Regulations 2002 (CoSHH 2002) requires the University to notify (and receive acknowledgement of receipt from) HSE of the first use of biological agents in HG 2 or 3 at a particular premises 20 working days in advance of the commencement of work; also that we notify the subsequent use of any of agent listed in Part V of Schedule 3 (of CoSHH 2002) at a particular premises. If the agent does not appear on the Approved List, this does not necessarily mean it should be classified as a HG 1 agent; the agent's hazardous properties (if any) still need to be considered to provisionally classify the agent in accordance with CoSHH. If HSE have already been notified of the use of HG 2 or 3 organisms under GM Regulations, there is no further requirement to notify HSE.

3. Risk assessments

CoSHH 2002 require all work involving biological agents (includes microorganisms, cell cultures and human endoparasites) to be assessed for the risks of harm to the health of workers and all others likely to be affected. This must take into account the Hazard Group of the microorganisms, the required Containment Level and all other relevant control measures to minimise the risks to workers and all others likely to be affected under normal work patterns and in the event of spillage or loss of containment.

The assessments must be documented and reviewed when there is reason to believe they are no longer valid, or following an incident which identifies that the control measures implemented are inadequate. However, it is good practice to review annually to ensure that assessments are still current and take account of any new data about the pathogen and technological advances.

4. Special Hazards

Some research work may pose particular hazards for which specialist advice will be required and precautions implemented. Examples are: -

a) Work with known human or animal pathogens or material from cases of disease or from autopsy
b) Harvesting bulk growth of any microorganism

c) Handling vessels containing large volumes of microbiological cultures; these should not be left unattended.
d) Procedures that generate aerosols.
e) Transfer of infective material from one container to another.
f) Work with Transmissible Spongiform Encephalopathies (TSE's) e.g. BSE etc.

5. Facilities for Microbiological Work

Microbiological work must only be undertaken in laboratories designated for this purpose with appropriate containment facilities. Microbiological laboratories must be supervised by a nominated Biological Safety Officer who must be satisfied that facilities, training and supervision are adequate.

6. Protective Clothing and Changing Facilities

a) All workers in microbiological laboratories must wear a protective coat which should be removed before leaving the laboratory and hung on a convenient hook for "in-use" clothing within the laboratory. Hooks should be sited close to the exit door and wash-hand basins. A basic garment should be recommended by the Department; if work involved potential pathogens the coat should preferably fasten at the side or back, have long sleeves with close fitting cuffs and made of a material that will minimise the risks of contaminating personal clothing worn beneath.
b) 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 that can be operated without touching by hand and disposable paper towels must be used for drying the hands, and the towels disposed of properly.
c) Protective gloves will be provided and must be worn when working with biological agents. Check that they are suitable for the intended use. You will be shown how to remove these to avoid contaminating your skin. Do not reuse gloves. Dispose of used gloves in the appropriate waste container.

7. Laboratory Safety Practices and Good Housekeeping

a) Personal items not relevant to microbiological work should not be taken into laboratories. This will include sports equipment and clothing, shopping etc.
b) Eating and drinking in laboratories is not permitted and food items and drinks must not be taken into laboratories or kept in fridges and freezers within laboratories.
c) Wash hands thoroughly after working with biological agents and before touching any personal items such as handbags, cosmetics, work bags etc.
d) Mouth pipetting is forbidden in microbiological laboratories
e) Minor cuts, scratches and abrasions on the hands should be covered with water-proof dressings before entering laboratories.

f) Every care must be taken to avoid needle-stick injuries. Needles should only be removed from syringe bodies 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.
g) When dispelling air from a contaminated syringe, do not discharge to atmosphere but into an alcohol-soaked swab.
h) Used “sharps”, e.g. scalpel blades, syringes and needles, broken ampoules, must be disposed of into approved “Sharps containers” and disposed of according to University procedures. Containers must be sealed and replaced when they are 75% full.
i) 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 up the affected area must be taken by staff wearing suitable protective clothing and according to safe working procedures.
j) All work with Respirable Pathogens should be undertaken in a Microbiological Safety Cabinet which is ON at all times. In the event of spillage, leave the Cabinet ON.

8. Health Surveillance and Records of Laboratory Workers

Health surveillance for biological risks may not be appropriate in all cases but may be useful where the agent causes serious disease which might have an insidious onset, and for which there is effective treatment available. A high level of personal vigilance by workers is required so that prompt medical attention is sought if they develop early signs of infection.

If the risk assessment shows there to be a risk of exposure to biological agents for which effective vaccines exist, then these should be offered if the employee is not already immune. This could be carried out as part of pre-employment screening, or else by making checks on immunity following a course of vaccination, e.g. Hepatitis B. If checks are performed on employees’ health, an up to date record for each individual must be kept.

Health records must be kept for all workers dealing with HG 3 pathogens. For workers exposed to primary specimens from cases of human or animal infections, i.e. with material taken directly from patients in medical laboratories.

Further advice should be sought from the Staff Occupational Health Unit at Brunswick House.

9. Emergency Procedures

All accidents, incidents of contamination and infections that occur in a laboratory must be recorded in the Departments Accident and Incident Report Book and the completed report must be forwarded to Safety Services. Reported accidents should be regularly scrutinised by the Department's Biological Safety Officer and, where necessary, risk assessments and procedures reviewed to prevent recurrence. Laboratories must have documented Procedures for dealing with foreseeable accidents and spillages which must be available to all laboratory workers and all new workers should receive the information and instruction as part of their Induction Training for working in microbiological laboratories. The procedures should indicate the people responsible for specific actions who should be experienced laboratory workers who have received appropriate training in dealing with such incidents.

a) Accidents. In the case of accidental skin puncture, contamination of braded skin or the eyes, mouth or nose; any wound should be encouraged to bleed freely and the contaminated parts should be washed gently (not scrubbed) under running water. The Head of Department or Biological Safety Officer must be informed and Safety Services notified as stated above. Procedures must be established for dealing with incidents of known exposures to HG 2 & 3 biological agents.

b) Spillages of Non-Respirable Pathogens outside a Microbiological Safety Cabinet

This procedure should be supervised or undertaken by fully trained members of laboratory staff wearing appropriate protective clothing and gloves.

1. Surround spill area with suitable disinfectant or absorbent material (e.g. paper towels) soaked in a suitable disinfectant (see Section 11),
2. Place paper towels soaked in disinfectant over the entire spill area,
3. Cordon off the area and allow at least 20-minute contact time with the disinfectant to ensure adequate germicidal action,
4. Wipe down non-autoclavable materials with germicidal disinfectant,
5. Place contaminated, used sharps in a designated sharps container.
6. Dispose of contaminated waste materials in a suitable impervious container, e.g. plastic bag or box using disposable cloths or paper towels or strong piece of cardboard. If broken glass is present do NOT use a plastic bag for disposal. Dust pans and brushes must not be used unless they can be autoclaved.
7. Remove and dispose of disposable protective clothing and gloves used in the cleanup process in accordance with University procedures for the Disposal of Biological Wastes.
8. Place contaminated re-usable items in biohazard bags, autoclavable containers with lids or wrap them in newspaper. Sterilise, preferably by

autoclaving and then clean for re-use. Remove protective clothing used during cleanup then place in a biohazard bag for autoclaving.

9. Wash hands when gloves are removed.
10. Notify Principal Investigator or Biological Safety Officer of the incident.

c) Spillage of Respirable Pathogens outside a Microbiological Safety Cabinet

The following action should be taken before cleaning up the spillage as described in "Spillage of Non-Respirable Pathogens outside a Microbiological Safety Cabinet" above:

1. Inform all laboratory occupants to immediately evacuate the laboratory - do NOT switch off Microbiological Safety Cabinets,
2. Close the laboratory door as soon as everyone has left the laboratory,
3. Place signs on the door(s) to the laboratory stating "Spillage – Do Not Enter" or similar,
4. Immediately remove contaminated clothing, turn contaminated parts inwards) and place in an autoclave bag,
5. Wash all exposed or contaminated skin,
6. Allow 30 minutes for aerosols to settle before re-entering the laboratory,
7. Assemble supplies necessary for dealing with the spillage before entry – disinfectant, towels, tongs, sharps container, autoclave bags etc – if necessary obtain these from an adjacent laboratory,
8. Two or three trained members of laboratory staff should be nominated to deal with the spillage. They should don appropriate PPE prior to entry i.e. disposable gown or overall, eye protection, disposable gloves, disposable shoe covering and disposable face masks (FFP3 minimum standard),
9. Enter the laboratory and follow the procedure outlined in "Spillage of Non-Respirable Pathogens outside a Microbiological Safety Cabinet" above.

10. Inactivation and Disposal of Biological Wastes

All wastes arising from biological laboratories must be disposed of in accordance with current University Procedures for the disposal of Biological Wastes. This may involve inactivation (by validated techniques) in advance of disposal (by autoclave or chemical means; see 11 & 12 below) or disposal in the appropriately coloured containers for the category of waste. Ask the Biological Safety Officer for a copy of the Procedures if you have not previously seen them.

11. Disinfectants

a) Containers of fresh disinfectant at in-use dilutions must be readily available throughout the working day while working with microorganisms. Containers must be refilled daily with fresh disinfectant to ensure activity.

b) 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.
c) Separate supplies of fresh disinfectants must be readily available to deal with accidents and spillages.
d) Suitable disinfectants are: -
    - For **general use** a solution containing 1,000ppm available Chlorine (1% Sodium Hypochlorite (Chloros) solution)
    - For **Discard Jars** a solution containing 2,500ppm available Chlorine (2.5% Sodium Hypochlorite (Chloros) solution).
    - For **spillages** a solution containing 10,000ppm available Chlorine (10% Sodium Hypochlorite (Chloros) solution), especially if blood or suspected Hepatitis B or HIV contamination is present.
    - Precept granules or Sodium Dichloroisocyanurate tablets should be used as recommended by the suppliers. (D Coates, Journal of Hospital Infection January 1988 11 (1): 95-96)
    - "Virkon" and / or other commercially available disinfectants, should be used at concentrations recommended by the supplier, for general use, discard jars and spillages.

12. Use of Autoclaves

Autoclaves designated for inactivating biological wastes must have a regular maintenance schedule including monitoring by a Service Engineer as recommended by the manufacturer. The servicing must include trial cycles with thermocouples inside disposal containers annually (minimum).

The performance of all autoclaves should be monitored regularly by methods such as "spore strips" or Brown's Tubes", in each cycle in order to "validate" the process, i.e. prove that the process is inactivating as intended. Boxes, bins or bags for material to be sterilised must be suitable for the task and must not restrict movement of air or penetration of steam.

Autoclaves require annual statutory examination under the *Pressure System Regulations 2000.* Contact the Department of Estates for further information.

11. Storage of Microorganisms

As far as practicable, stocks of microorganisms must be kept to a minimum, and unwanted stocks should be disposed of in accordance with university Procedures. Stocks should, where practicable be kept in fire-resistant containers and fridges and freezers containing stocks of microorganisms should be clearly labelled with a "BIOHAZARD" warning sign on the fridge or freezer door. Each

container must be labelled with the identity and details of the microorganism; the name of the researcher and the date.

12. Transport of Microorganisms

Viable microorganisms in HG 2 & 3 may be transported outside the laboratory complex but only if properly packaged to minimise the risk of loss. The samples should be placed in a robust, sealable plastic container which must be closed securely and placed inside a plastic bag which should then be sealed Any forms, risk assessments or letters must be attached to the outside of the bag but NOT with staples. The package should be transported in a closed impervious box that will contain any spillages and can be disinfected. The handles of the box should not be part of the lid. Post Office Regulations should be followed at all times when sending through the post.

APPENDIX 1

Containment Level Facilities

Containment Level 2 specifications for laboratories working with HG 2 biological agents (most biological laboratories are constructed to this standard)

1. The laboratory must be easy to clean; walls and ceilings should be of good quality acrylic emulsion or hard gloss paint. Bench surfaces should be impervious to water and resistant to acids, alkalis, solvents and disinfectants.
2. Floors should be of good quality impervious floor covering with a minimum number of joints, all sealed, with coving around the edges of the room. Benches and fitting should be on top of the floor covering to minimise the number of holes in the flooring.
3. Access to the laboratory should be limited to laboratory personnel and other specified persons
4. There should be adequate space ($24m^3$) in the laboratory for each worker
5. If the laboratory is mechanically ventilated, inward airflow into the laboratory must be maintained by extracting room air to atmosphere.
6. The laboratory must contain a wash-hand basin which should be located near the laboratory exit door. Taps must be of a type which can be operated without being touched by hand. Paper towel dispenser should be provided next to basin with a bin for general waste nearby.
7. An autoclave for the sterilisation of waste materials must be available, normally within the same building.
8. The laboratory door should be closed when work is in progress.
9. Laboratory coats or gowns, preferably side or back fastening, must be worn in the laboratory and removed prior to leaving the laboratory suite. Separate storage

(e.g. pegs or hooks) must be provided in the laboratory suite near to the exit door for this clothing.

10. Eating, drinking, chewing, storing of food or applying cosmetics must not take place in laboratories.
11. Mouth pipetting must not take place.
12. Hands must be disinfected or washed immediately when contamination is suspected or after handling infective materials, and also before leaving the laboratory or touching personal items.
13. In general, work may be conducted on the open bench, but care must be taken to minimise the production of aerosols. Manipulations such as vigorous shaking or mixing, or ultrasonic disruption etc, must be done inside a Microbiological Safety Cabinet (BS EN 12469 Biotechnology – Performance criteria for microbiological safety 2000) designed to contain the aerosol and filter the exhaust must be used whether they exhaust externally or internally. Cabinets must NOT exhaust unfiltered air into the laboratory or its air extract system and must be regularly maintained according to manufacturer's instructions by a qualified engineer. Retain documents received from engineer relating to servicing and maintenance.
14. Effective disinfectants must be available for the routine disinfection and immediate use in the event of spillage.
15. Bench tops must be disinfected after each use.
16. Used laboratory glassware and other materials awaiting sterilisation must be stored in a safe manner. Pipettes, if placed in disinfectant must be totally immersed.
17. Material for autoclaving must be transported to the autoclave without spillage in robust containers.
18. All waste materials must be disposed of according to University Procedures for the disposal of Biological Wastes.
19. All accidents and incidents if contamination must be immediately recorded by the principal Investigator or person responsible for the laboratory and reported to Safety Services on a University Accident and Incident Report Form by the Department's Biological Safety Officer.

<u>Containment Level 3 – specification for laboratories working with HG 3 biological agents (Containment Level 3 facilities are only available on F Floor of the School of Medicine and Biomedical Science) Prof D Dockrell is the Manager of this facility.</u>

In addition to the specifications required to comply with Level 2 Containment, Level 3 Containment must also provide the following protection: -

1. The laboratory must be sealable to facilitate fumigation
2. The laboratory must be sited in an area away from general circulation.
3. Access to the laboratory must be limited to authorised personnel only and the laboratory door closed and locked when the room is being used for Containment Level 3 work.

4. A specific “BIOHAZARD” sign must be posted at the entry to the laboratory and the door must contain a glass panel so that occupants can be seen.
5. A continuous airflow into the laboratory must be maintained during the work with pathogens by means of the following: -
    a. Extracting the laboratory air through independent ducting to the outside through a HEPA filter, or
    b. Extracting the laboratory air to the outside with a fan and HEPA filter sited in a wall or window of the laboratory, or
    c. Ducting the exhausted air from a Microbiological Safety Cabinet to the outside through a HEPA filter, or
    d. A safe variation of these provisions. Provision should also be made for comfort factors e.g. fresh air, temperature control. In laboratories with a mechanical air supply system, the supply and extract airflows 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.
6. An autoclave for sterilisation of waste materials should be sited preferably within the laboratory, or within the laboratory suite.
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 suite.
8. Gloves must be worn for all work with infective materials and the hands must be washed before leaving the laboratory.
9. All laboratory procedures with infective materials must be conducted in a Microbiological Safety Cabinet (Class I or Class III BS EN 12469:2000) except where the equipment to be used provides containment of the potential aerosol. Cabinets must be serviced regularly according to the manufacturers recommended maintenance schedules and the airflow checked at regular intervals between servicing. The equipment must be decontaminated before service personnel are asked to undertake any work (See Appendix 2)
10. The Cabinet must be exhausted 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 EN 12469:2000. When laboratories are unable to design the cabinet to exhaust outside the building, recirculation of exhaust air within the laboratory through 2 HEPA filters in series may be acceptable under some circumstances. Where deemed necessary, maintaining a continuous airflow into the laboratory during work with pathogens will be of particular importance and such an option should only be considered following consultation with HSE.
11. Externally ducted Microbiological Safety Cabinets are deemed to be Local Exhaust ventilation systems and will require annual statutory examination under *CoSHH 2002*.

APPENDIX 2

Decontamination of Containment Level 3 laboratory equipment

All contaminated laboratory equipment must be rendered safe before maintenance inspections, servicing or repair. This will require the equipment to be cleaned and disinfected to eliminate the risk of infection. Equipment which is visibly contaminated must never be presented or sent to third parties for inspection, maintenance or repair. The manufacturer's recommendations should be followed for appropriate methods of decontamination for specific items and Departments must have written procedures for decontaminating each item.

The laboratory should contain its own equipment e.g. centrifuges in which sealed buckets must be used, incubator, fridges, freezers, vapour-phase liquid Nitrogen chests etc, so that all HG 3 materials are held and stored within the laboratory and nowhere else. Where this is not reasonably practicable, material must be transported and stored without spillage in sealed, properly-labelled containers which must be opened only with the Containment Level 3 laboratory.

All accidents, spillages and exposures to infective materials must be immediately reported to and recorded by the Principal Investigator, BSO or other responsible person.

Further information on all the above can be found the HSE booklet "***Biological agents: Managing the risks in laboratories and healthcare premises***" (2005) which can be found on the Safety Services web site in the Biohazard page.

DGT January 2010