Health and Safety Office

CODE OF PRACTICE

Laboratory Biosafety Levels 1 and 2

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1. General Introduction

This code of practice lists the essential laboratory facilities, practices and procedures that are central to good (safe) microbiological technique when handling biological agents (BAs). It is given as minimum requirements for laboratories where BAs in Hazard Groups/Classes 1 and 2 are used. This code will be useful when preparing risk assessments of BAs and when designing new laboratories or refurbishing existing laboratories. Some of the precautions may appear unnecessary for biological agents in Risk Group 1, but they are desirable not least for training purposes to promote good microbiological technique; this aspect is of particular relevance to NUI Galway as a teaching and research institution.

Biological agents of hazard group 1 involve little or no hazard to workers, and no special design features beyond those suitable for a well designed and functional laboratory are required. Biological safety cabinets are not required. Work may be done on an open bench top and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

Figure 1. A typical biosafety Level 1 Containment laboratory

Biological agents of hazard group 2 may cause infectious diseases in humans. Agents requiring containment level 2 facilities are not usually transmitted by airborne routes.

Care must be taken to avoid the generation of aerosols or splashes as these can settle onto bench tops and become an ingestion hazard through contamination of the hands. Containment devices such as biological safety cabinets and centrifuges with sealed rotors or safety cups are to be used as well as appropriate personal protective equipment. Environmental contamination must be minimised by the use of hand washing sinks and decontamination facilities (such as autoclaves).

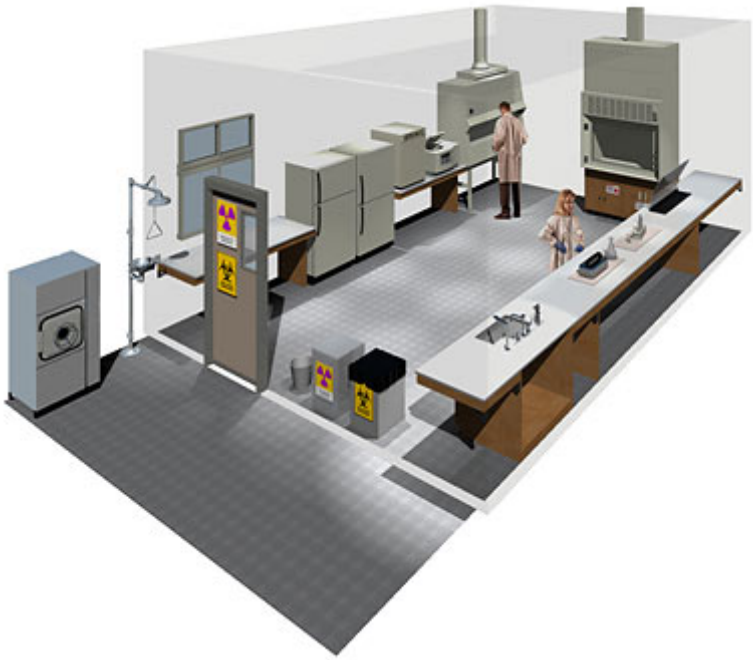

Figure 2. A typical biosafety Level 2 Containment laboratory

2. Biosafety management

1. It is the responsibility of the laboratory director (the principal investigator who has immediate responsibility for the laboratory) to ensure the development and adoption of a biosafety management plan and a safety or operations manual.
2. The laboratory director must ensure that the relevant provisions of the Safety Health and Welfare at Work (Biological Agents) Regulations 2013 are adhered to.
3. The laboratory supervisor (reporting to the laboratory director) should ensure that regular training in laboratory safety is provided.
4. Personnel should be advised of special hazards, and required to read the safety or operations manual and follow standard practices and procedures. The

laboratory supervisor should make sure that all personnel understand these. A copy of the safety or operations manual should be available in the laboratory.

5. There should be an arthropod and rodent control programme.
6. Appropriate medical evaluation, surveillance and treatment should be provided for all personnel in case of need, and adequate medical records should be maintained.

3. Laboratory design and facilities

In designing a laboratory and assigning certain types of work to it, special attention should be paid to conditions that are known to pose safety problems. These include:

- Formation of aerosols
- Work with large volumes and/or high concentrations of microorganisms
- Overcrowding and too much equipment
- Infestation with rodents and arthropods
- Unauthorised entrance
- Workflow and ergonomics

Design features should include the following:

1. Ample space must be provided for the safe conduct of laboratory work and for cleaning and maintenance.
2. Walls, ceilings and floors should be smooth, easy to clean, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be slip-resistant.
3. Bench tops should be impervious to water and resistant to disinfectants, acids, alkalis, organic solvents and moderate heat.
4. Illumination should be adequate for all activities. Undesirable reflections and glare should be avoided.
5. Laboratory furniture should be sturdy. Open spaces between and under benches, cabinets and equipment should be accessible for cleaning.
6. Storage space must be adequate to hold supplies for immediate use and thus prevent clutter on bench tops and in aisles. Additional long-term storage space, conveniently located outside the laboratory working areas, should also be provided.

7. Space and facilities should be provided for the safe handling and storage of solvents, radioactive materials, and compressed and liquefied gases.
8. Facilities for storing outer garments and personal items should be provided outside the laboratory working areas.
9. Facilities for eating and drinking and for rest should be provided outside the laboratory working areas.
10. Hand-washing basins with hot and cold running water should be provided in each laboratory room, preferably near the exit door.
11. Doors should have vision panels, appropriate fire ratings, and preferably be self closing.
12. At Biosafety Level 2, an autoclave or other means of decontamination should be available in appropriate proximity to the laboratory.
13. Safety systems should cover fire, electrical emergencies, gas releases and emergency shower and eyewash facilities.
14. First-aid areas or rooms suitably equipped and readily accessible should be available
15. In the planning of new facilities, consideration should be given to the provision of mechanical ventilation systems that provide an inward flow of air without recirculation. If there is no mechanical ventilation, windows should be able to be opened and should be fitted with arthropod-proof screens.
16. A dependable supply of good quality water is essential. There should be no cross connections between sources of laboratory and drinking-water supplies. An anti-backflow device should be fitted to protect the public water system.
17. There should be a reliable and adequate electricity supply and emergency lighting to permit safe exit. A stand-by generator is desirable for the support of essential equipment, such as incubators, biological safety cabinets, freezers, etc., and for the ventilation of animal cages.
18. There should be a reliable and adequate supply of gas. Good maintenance of the installation is mandatory.
19. Laboratories and animal houses are occasionally the targets of vandals. Physical and fire security must be considered. Strong doors, screened windows and restricted issue of keys are compulsory. Other measures should be considered and applied, as appropriate, to augment security.

4. Access

1. Only authorised persons should be allowed in the laboratory working areas.
2. Laboratory doors should be kept closed during working hours and should be locked when the laboratory is not occupied.
3. Lone working and out-of-hours working must be risk assessed and authorised.
4. Children should not be authorised to enter the laboratory.
5. Access to animal houses should be especially authorised.
6. Vermin/pests and other animals, including pets, must be kept away from the laboratory.
7. The international biohazard warning symbol and sign (Figure 1) must be displayed at the entrances to rooms where biological agents in Risk Group 2 or higher are handled.

BIOHAZARD

ADMITTANCE TO AUTHORISED PERSONNEL ONLY

Biosafety Level: _____

Biological Agents in Hazard Group/s_____ is/are in this room

Principal Investigator: _____

In case of emergency call: _____

Daytime phone: _____Other phone: _____

Authorisation for entrance must be obtained from the Principal Investigator named above.

Figure 3. International biohazard symbol and sign for display at entrances to laboratories where class 2 biological agents are present

5. Personal Protective Equipment (PPE)

1. Laboratory coats, coveralls, gowns or uniforms must be worn, as appropriate, at all times for work in the laboratory.
2. Appropriate protective gloves must be worn for all procedures that may involve direct or accidental contact with blood, body fluids and other potentially infectious materials or infected animals. After use, gloves should be removed aseptically and hands must then be washed. Wearing gloves outside the immediate risk area or when not handling infectious material is expressly forbidden.
3. Personnel must wash their hands after handling infectious material and animals, and before they leave the laboratory working areas.
4. Safety glasses, face shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects and sources of artificial ultraviolet radiation.
5. It is prohibited to wear any protective laboratory clothing outside the laboratory, e.g. in canteens, coffee rooms, offices, libraries, staff rooms and toilets.
6. Open-toed footwear must not be worn in laboratories.
7. Eating, drinking, smoking, applying cosmetics and handling contact lenses is prohibited in laboratories.
8. Storing human foods or drinks anywhere in the laboratory is prohibited.
9. Protective laboratory clothing that has been used in the laboratory must not be stored in the same lockers or cupboards as personal clothing.

6. Procedures

1. Pipetting by mouth is strictly forbidden.
2. Items must not be placed in the mouth. Labels must not be licked.
3. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets.
4. The use of hypodermic needles and syringes should be limited. They must not be used as substitutes for pipetting devices or for any purpose other than parenteral injection or aspiration of fluids from laboratory animals, and only after a detailed and recorded risk assessment.

5. All spills, accidents and actual or potential exposures to infectious materials must be reported to the laboratory supervisor. A written record of such accidents and incidents should be maintained.
6. A written procedure for the clean-up of all spills must be developed and followed.
7. Contaminated liquids must be decontaminated (chemically or physically) before discharge to the sanitary sewer. An effluent treatment system may be required, depending on the risk assessment for the agent(s) being handled.
8. Written documents that are expected to be removed from the laboratory need to be protected from contamination while in the laboratory.

7. Laboratory working areas

1. The laboratory should be kept neat, clean and free of materials that are not pertinent to the work.
2. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day.
3. All contaminated materials, specimens and cultures must be decontaminated before disposal or cleaning for reuse.
4. Packing and transportation must follow applicable national and/or international regulations.
5. When windows can be opened, they should be fitted with arthropod-proof screens.

8. Laboratory equipment

Together with good procedures and practices, the safe use of equipment and use of safety equipment will help to reduce risks when dealing with biosafety hazards. Equipment should be selected to take account of certain general principles, i.e. it should be:

- Designed to prevent or limit contact between the operator and the infectious material
- Constructed of materials that are impermeable to liquids, resistant to corrosion and meet structural requirements
- Fabricated to be free of burrs, sharp edges and unguarded moving parts

- Designed, constructed and installed to facilitate simple operation and provide for ease of maintenance, cleaning, decontamination and certification testing; glassware and other breakable materials should be avoided, whenever possible. Detailed performance and construction specifications may need to be consulted to ensure that the equipment possesses the necessary safety features.

Essential biosafety equipment includes:

1. Pipetting aids – to avoid mouth pipetting. Many different designs are available.
2. Biological safety cabinets, regularly validated and certified, to be used whenever:
    a. infectious materials are handled; such materials may be centrifuged in the open laboratory if sealed centrifuge safety cups are used and if they are loaded and unloaded in a biological safety cabinet
    b. there is an increased risk of airborne infection
    c. procedures with a high potential for producing aerosols are used; these may include:
        i. centrifugation
        ii. grinding
        iii. blending
        iv. vigorous shaking or mixing
        v. sonic disruption
        vi. opening of containers of infectious materials whose internal pressure may be different from the ambient pressure
        vii. intranasal inoculation of animals
        viii. harvesting of infectious tissues from animals and eggs.
3. Plastic disposable transfer loops. Alternatively, electric transfer loop incinerators may be used inside the biological safety cabinet to reduce aerosol production.
4. Screw-capped tubes and bottles.
5. Autoclaves, regularly validated and certified, or other appropriate means to decontaminate infectious materials. Frequent use, as appropriate, of spore strips should be considered to ensure that the essential criteria have been achieved by the autoclave to ensure thorough inactivation.

6. Plastic disposable Pasteur pipettes, whenever available, to avoid glass.
7. Equipment such as autoclaves and biological safety cabinets must be validated with appropriate methods before being taken into use. Recertification should take place at regular intervals according to the manufacturer's instructions.

9. Health and medical surveillance

The objective of such surveillance is to monitor for occupationally acquired diseases. Appropriate activities to achieve these objectives are:

- Provision of active or passive immunization where indicated
- Facilitation of the early detection of laboratory-acquired infections
- Exclusion of highly susceptible individuals (e.g. pregnant women or immunocompromised individuals) from highly hazardous laboratory work
- Provision of effective personal protective equipment and procedures.

1. Guidelines for the surveillance of laboratory workers handling microorganisms at Biosafety Level 1
    a. Microorganisms in hazard group 1 and handled at this level are unlikely to cause human disease or animal disease of veterinary importance. However, prompt reporting of illnesses or laboratory accidents is mandatory and all staff members should be made aware of the importance of maintaining good microbiological technique.

2. Guidelines for the surveillance of laboratory workers handling microorganisms at Biosafety Level 2
    a. A pre-employment or pre-placement health check may be necessary. The person's medical history should be recorded and a targeted occupational health assessment performed.
    b. Records of illness and absence should be kept by the laboratory management.
    c. Women of childbearing age should be made aware of the risk to an unborn child of occupational exposure to certain microorganisms, e.g. rubella virus. The precise steps taken to protect the foetus will vary,

depending on the microorganisms to which the women may be exposed.

10. Training

Human error and poor technique can compromise safeguards to protect the laboratory worker. Thus, safety-conscious staff, well informed about the recognition and control of laboratory hazards, are key to the prevention of laboratory acquired infections, incidents and accidents. For this reason, continuous in-service training in safety measures is essential. An effective safety programme begins with the laboratory managers, who should ensure that safe laboratory practices and procedures are integrated into the basic training of employees. Training in safety measures should be an integral part of new employees' introduction to the laboratory. Employees should be introduced to this code of practice and to local guidelines, including the safety or operations manual. Laboratory supervisors play the key role in training their immediate staff in good laboratory techniques. Staff training should always include information on safe methods for highly hazardous procedures that are commonly encountered by all laboratory personnel and which involve:

1. Inhalation risks (i.e. aerosol production) when using loops, streaking agar plates, pipetting, making smears, opening cultures, taking blood/serum samples, centrifuging, etc.
2. Ingestion risks when handling clinical specimens or cultures
3. Risks of percutaneous exposures when using syringes and needles
4. Bites and scratches when handling animals
5. Handling blood and other potentially hazardous pathological materials
6. Decontamination and disposal of infectious material.

11. Waste handling

Waste is anything that is to be discarded. In laboratories, decontamination of wastes and their ultimate disposal are closely interrelated. Most glassware, instruments and laboratory clothing will be reused or recycled. The overriding principle is that all infectious materials should be decontaminated, autoclaved or incinerated within the laboratory. The principal questions to be asked before discharge of any objects or materials from laboratories that deal with potentially infectious microorganisms or animal tissues are:

- Have the objects or materials been effectively decontaminated or disinfected by an approved procedure?
- If not, have they been packaged in an approved manner for immediate on-site incineration or transfer to another facility with incineration capacity?
- Does the disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to those who carry out the immediate disposal procedures or who might come into contact with discarded items outside the facility?
- Decontamination: Steam autoclaving is the preferred method of decontamination. Materials for autoclaving and disposal must be placed in appropriate primary and secondary containers, e.g. autoclaveable plastic bags (double-bagging could be considered) and a solid receptacle to contain and material should bag puncture or failure occur. Alternative decontamination methods may be used only if they are proven to remove and/or kill microorganisms.

An identification and separation system for infectious materials and their containers should be adopted. Categories should include:

1. Non-contaminated (non-infectious) waste that can be reused or recycled or disposed of as general, "household" waste
2. Contaminated (infectious) "sharps" – hypodermic needles, scalpels, knives and broken glass; these should always be collected in puncture-proof containers fitted with covers and treated as infectious
3. Contaminated material for decontamination by autoclaving and thereafter washing and reuse or recycling
4. Contaminated material for autoclaving and disposal
5. Contaminated material for direct incineration.

Specific waste material:

1. Sharps. After use, hypodermic needles should not be recapped, clipped or removed from disposable syringes. The complete assembly should be placed in a sharps disposal container. Disposable syringes, used alone or with needles, should be placed in sharps disposal containers and incinerated, with

prior autoclaving if required. Sharps disposal containers must be puncture-proof/-resistant and must not be filled to capacity. When they are three-quarters full they should be placed in "infectious waste" containers and incinerated, with prior autoclaving if laboratory practice requires it. Sharps disposal containers must not be discarded in landfills.

2. Contaminated (potentially infectious) materials for autoclaving and reuse. No pre-cleaning should be attempted of any contaminated (potentially infectious) materials to be autoclaved and reused. Any necessary cleaning or repair must be done only after autoclaving or disinfection.
3. Contaminated (potentially infectious) materials for disposal. Apart from sharps, which are dealt with above, all contaminated (potentially infectious) materials should be autoclaved in leak proof containers, e.g. autoclaveable plastic bags, before disposal. After autoclaving, the material may be placed in transfer containers for transport to the incinerator. If possible, materials deriving from healthcare activities should not be discarded in landfills even after decontamination. If an incinerator is available on the laboratory site, autoclaving may be omitted: the contaminated waste should be placed in designated containers (e.g. colour-coded bags) and transported directly to the incinerator. Reusable transfer containers should be leak proof and have tight-fitting covers. They should be disinfected and cleaned before they are returned to the laboratory for further use. Discard containers, pans or jars, preferably unbreakable (e.g. plastic), should be placed at every work station. When disinfectants are used, waste materials should remain in intimate contact with the disinfectant (i.e. not protected by air bubbles) for the appropriate time, according to the disinfectant used. The discard containers should be decontaminated and washed before reuse.

12. Chemical, fire, electrical, radiation and equipment safety

A breakdown in the containment of pathogenic organisms may be the indirect result of chemical, fire, electrical or radiation accidents. It is therefore essential to maintain high standards of safety in these fields in all laboratories and other rooms that contain biological agents.

APPENDICES

APPENDIX 1 –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 to be effective, some should be made fresh each working day. The active effective strength required for each disinfectant 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.

Selection of Disinfectants

- **The micro-organism:**

  Disinfectants vary both in the spectrum of microorganisms inactivated (Table 8) and in specific activity for different microorganisms. Disinfectant manufacturers can provide details of the activity of their product for particular microorganisms and recommend appropriate dilutions. When the type(s) of microorganism 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.**

Disinfectants are chemicals or chemical mixtures and so a risk assessment must be made for their safe handling. Most disinfectants are toxic and some are also corrosive The 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.

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 workstation 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 form (e.g. Virkon) 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. 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 microorganisms for sufficient time to achieve disinfection. Manufacturers should recommend appropriate combinations of disinfectant concentrations and contact times for various applications.

- **Validation of disinfectant activity**

The effectiveness of disinfectants for use with all Hazard Group 3 agents and in certain circumstances with Hazard Group 2 agents (for example when working with high titres, or with significant quantities of organic material or where little or no relevant efficacy data is available) 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 microorganisms or new materials are proposed.

- **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.

- **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:

Table 1 Activities of some common classes of disinfectants

	Active Against					
	Vegetative bacteria	Bacterial spores	Fungi	Enveloped viruses	Non-enveloped viruses	Myco-bacteria
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

		Inactivated by			
	Hazard Class	Organic matter	Hard water	Detergent	Corrosive to metals
Phenolic	Toxic	-	+	1	-
Hypo-chlorites	Toxic Corrosive	+	-	1	+
Alcohols	Harmful Flammable	-	-	-	-
Aldehydes	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

Examples of disinfectants 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. Previously the agent of choice for mycobacteria disinfection. Damages the surface of many plastics. Now a banned substance in the EU.

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 } Disinfection of TSE infected material}	20,000 ppm	available chlorine.

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 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. Workplace Exposure Limits (WELs) have been set for both formaldehyde and gluteraldehyde.

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.

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.

APPENDIX 2 – Laboratory Hand Washing Facilities and Hand Wash Technique

Hands must be washed after handling biohazardous materials and animals, and before leaving the laboratory. Despite this requirement many laboratory personnel don't wash their hands properly. Hand washing is the single most important procedure for preventing the spread of biological contamination. Hand washing should be conducted in a dedicated hand wash facility, which should include a sink with hot (and cold) supply, automated tap(s), liquid soap dispenser, exclusive paper-towel supply and waste towel receptacle.

1. Consider the sink, including the taps, as contaminated and avoid direct contact with them.
2. With conventional taps turn water on using a paper towel and then wet your hands and wrists.
3. Work soap into a lather, vigorously rubbing together all surfaces of the lathered hands for 15 seconds. Friction helps remove dirt and micro-organisms. Wash around and under rings, around cuticles, and under fingernails. NOTE: The wearing of hand/wrist jewellery is not recommended in the laboratory as they can harbour infectious organisms.
4. Rinse hands thoroughly under a stream of water. Running water carries away dirt and debris. Point fingers down so water and contamination won't drip toward elbows.
5. Wipe excess water from hands and shake firmly but not wildly.
6. Dry hands completely with a clean dry paper towel.
7. If using conventional taps use a dry paper towel to turn water off.
8. To keep re-fillable soap from becoming a breeding place for micro-organisms, thoroughly clean soap dispensers before refilling with fresh soap. Better still, buy suitable individual, disposable soap dispensers.
9. When hand-washing facilities are not available at a remote work site, use an appropriate antiseptic, alcohol-based hand cleaner or hand rub or antiseptic towelettes, for lightly soiled hands. As soon as possible, re-wash hands with soap and running water.

APPENDIX 3 – Principles of Good Laboratory Hygiene

1. Wash hands (wrists, and arms if necessary) before eating, drinking, smoking, using the telephone, taking medication, applying cosmetics and hand creams, inserting contact lenses.
2. Cover all new and existing cuts and grazes with waterproof dressings and/or gloves before starting work. If cuts and grazes occur, wash immediately with soap and running water and apply waterproof dressing.
3. Take rest breaks and meal breaks away from main work areas.
4. Wear appropriate protective clothing to stop personal contamination, e.g. waterproof/water-resistant protective clothing, plastic aprons, gloves, rubber boots/disposable overshoes. Ensure its safe disposal or cleaning.
5. Avoid hand-mouth and hand-eye contact; don't put pens/pencils in mouths.
6. Dispose safely all contaminated waste.

Supplementary Controls

- If the work activity could result in a skin piercing/cutting injury, the risk of puncture wounds, cuts or grazes should be controlled by avoiding the use of sharp objects, e.g. needles, glass, metal, knives, etc. If this is impossible, safe working practices for handling and disposal of sharps should be used and appropriate protective equipment provided.

- If the work activity could result in the splashing of any body fluid, the eyes and the mouth should be protected with a visor or goggles/safety glasses and a mask.

- If the work activity could generate aerosols of either dust or liquid, you should take steps to avoid their generation, by:
    - Altering the work activity, e.g. using a vacuum rather a brush to clean a dusty workplace;
    - Using a containment facility to contain and capture the aerosols;
    - If these are impossible, then appropriate respiratory personal protective equipment should be used.

APPENDIX 4 – Principles of Good Microbiological Practice

- A laboratory that is easy to clean
- Bench surfaces impervious to water and resistant to chemicals
- Sink for hand washing
- Inward flow of air into laboratory to be maintained
- Door to be closed while work is in progress
- Laboratory coats to be worn in the lab and removed before leaving the laboratory
- Eating, chewing, drinking, smoking, storing food, applying cosmetics and mouth pipetting are forbidden
- Hands must be disinfected and washed as appropriate
- Aerosol production must be minimised
- Effective disinfectants must be available
- Bench tops cleaned after use
- Used equipment awaiting sterilisation must be stored safely. Pipettes in disinfectant must be totally immersed
- Waste material must either be incinerated or rendered non-viable before disposal. It must be transported in robust containers without spillage
- Accidents and incidents must be reported

APPENDIX 5 – Relevant Legislation, Codes and Guidance

- SAFETY HEALTH AND WELFARE AT WORK (BIOLOGICAL AGENTS) REGULATIONS 2013 (S.I. No.572 of 2013).
  http://www.irishstatutebook.ie/2013/en/si/0572.html
- 2013 CODE OF PRACTICE FOR THE SAFETY HEALTH AND WELFARE AT WORK (BIOLOGICAL AGENTS) REGULATIONS 2013 (S.I. No.572 of 2013).
  http://www.hsa.ie/eng/Publications_and_Forms/Publications/Codes_of_Practice/2013_Code_Of_Practice_for_Biological_Agents.html
- GUIDELINES TO THE SAFETY HEALTH AND WELFARE AT WORK (BIOLOGICAL AGENTS) REGULATIONS 2013 (S.I. No.572 of 2013).
  http://www.hsa.ie/eng/Publications_and_Forms/Publications/Codes_of_Practice/Code_of_Practice_Biological_Agents_SI_572.pdf
- GENETICALLY MODIFIED ORGANISMS (CONTAINED USE) REGULATIONS 2001 (S.I. No.73 of 2001)
  http://www.irishstatutebook.ie/2001/en/si/0073.html
- GENETICALLY MODIFIED ORGANISMS (CONTAINED USE) (AMENDMENT) REGULATIONS 2010 (S.I. No. 442 of 2010)
  https://www.epa.ie/pubs/legislation/geneticallymodifiedorganismsgmo/GMO%20Contained%20Use%20SI%20442%20of%202010.pdf
- ENVIRONMENTAL PROTECTION AGENCY
  http://www.epa.ie/licensing/gmo/#d.en.42858
- HEALTH AND SAFETY AUTHORITY
  http://www.hsa.ie/eng/Topics/Biological_Agents/

APPENDIX 6 - Relevant Published Standards

- IS EN 14056:2003. LABORATORY FURNITURE – RECOMMENDATIONS FOR DESIGN AND INSTALLATION.
- IS EN 12128:1998. BIOTECHNOLOGY – LABORATORIES FOR RESEARCH, DEVELOPMENT AND ANALYSIS – CONTAINMENT LEVELS OF MICROBIOLOGY LABORATORIES, AREAS OF RISK, LOCALITIES AND PHYSICAL SAFETY REQUIREMENTS.
- IS EN 12741:1999. LABORATORIES FOR RESEARCH, DEVELOPMENT AND ANALYSIS – GUIDANCE FOR BIOTECHNOLOGY LABORATORY OPERATIONS.
- IS EN 12740:1999. LABORATORIES FOR RESEARCH, DEVELOPMENT AND ANALYSIS – GUIDANCE FOR HANDLING, INACTIVATING AND TESTING OF WASTE.
- IS EN 13150:2001. WORKBENCHES FOR LABORATORIES. DIMENSIONS, SAFETY REQUIREMENTS AND TEST METHODS.
- IS EN 14175:PARTS 1, 2, 3, 4, 6 & 7 (VARIOUS DATES). FUME CUPBOARDS.
- IS EN 12469:2000. BIOTECHNOLOGY – PERFORMANCE CRITERIA FOR MICROBIOLOGICAL SAFETY CABINETS.
- BS 5726:2005. MICROBIOLOGICAL SAFETY CABINETS. INFORMATION TO BE SUPPLIED BY THE PURCHASER TO THE VENDOR AND TO THE INSTALLER, AND SITING AND USE OF CABINETS – RECOMMENDATIONS AND GUIDANCE.
- IS EN 12347:1998. BIOTECHNOLOGY – PERFORMANCE CRITERIA FOR STEAM STERILISERS AND AUTOCLAVES.
- IS EN 13441:2002. LABORATORIES FOR RESEARCH, DEVELOPMENT AND ANALYSIS – GUIDANCE ON CONTAINMENT OF GENETICALLY MODIFIED PLANTS.