University College Dublin

Biosafety Manual

Rev 1. Issued March 2022

University College Dublin SIRC Office

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Part 1

Introduction To Biosafety

&

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1.1 Introduction

Biological agents include any micro-organisms, including those that have been genetically modified, cell cultures and human endoparasites, which may provoke any infection, allergy or toxicity. This guide does not extend to include non-microbiological biological entities as the specific provisions of biosafety legislation do not apply to their usage / interaction with workers. See https://www.hsa.ie/eng/Topics/Biological_Agents/ for further details.

Within UCD two distinct types of potential exposure to biological agents may be defined. The first is incidental exposure, which is where persons are exposed to biological agents which happen to be present in the workplace or in material being treated, but which are not deliberately handled, utilised, concentrated or propagated e.g. in diagnostic laboratories, when engaged in fieldwork, or when handling material of human or animal origin. The second is where persons are exposed to biological agents which are deliberately utilised and / or propagated, e.g. in research labs growing *E. coli* bacteria on plates.

This manual is designed to provide University staff and students with guidance on the safe handling and usage of biological agents in laboratories and similar settings. It also provides advice on how to ensure one's safety when working with potentially infectious materials and in environments where biological agents may be present.

Whilst the provisions of this document apply to the usage of genetically modified microorganisms and genetically modified cell lines, users of genetically modified biological agents must also refer to the *UCD Use Of Genetically Modified Organisms Manual* for further information on the regulatory requirements that pertain to the licencing and usage of same. No Genetically Modified microorganism, cell line, plant or animal can be used without a consent issued by the *Environmental Protection Agency.*

1.2 Types Of Biological Agent

There are a number of different types of biological agent that may be encountered within the University. These include but are not limited to:

1.2.1 Bacteria

These are single celled organisms that can cause a number of diseases in humans. Many types of bacteria pose no risk to healthy humans and live on and in the human body without causing harm. Diseases caused by bacterial infection include Tuberculosis (*Mycobacterium spp.)*; Tetanus (*Clostridium tetani*); and Cholera (*Vibrio cholerae*). Some bacterial infections are susceptible to antibiotics, whilst in a laboratory setting there are a range of disinfectant agents available which can bs used to destroy bacteria. It is possible to get vaccinations against some of the diseases caused by bacterial agents. With respect to bacterial agents it should be noted an individual may become colonised by bacteria, whereby the bacteria live on or in their body with no discernible negative effect or symptoms; or they may become infected by same, in which case an illness will arise.

1.2.2 Viruses

Viruses are entities that can infect individual human cells causing the infected cell to produce more virus particles. Large scale viral infections in populations can lead to world wide epidemics. Some viruses have an ability to constantly change their characteristics leading to many strains of the same virus e.g. the influenza virus. Common diseases caused by viruses include Influenza (Orthomyxoviridae spp); Chicken Pox (*Varicella simplex*); and Cold Sores (*Herpes simplex*). Viral infections are not treatable with antibiotics although effective disinfectant agents are available for destroying viruses in the laboratory or working environment. It is possible to get vaccinated against some viral agents.

1.2.3 Prions

Prions are very simple biological agents made up of proteins. Prion infections can be very difficult to treat and the prion agent itself can also be very difficult to destroy in the laboratory or working environment. Currently there are no vaccinations available to prevent prion infection. Prions are the causative agents of *Creutzfeldt-Jacob Disease* and *Bovine Spongiform Encephalitis*.

1.2.4 Fungi

Fungi include yeasts, moulds and mushrooms. Some species of fungi have the ability to colonise and to grow on and in the body. These include *Candida spp* which can cause thrush infections and *Cryptococcus neoformans* which can cause lung infections. Fungal infections are usually treatable and fungal material can be destroyed by various disinfectants in the laboratory or working environment. There are no vaccinations available to guard against fungal infections.

1.2.5 Parasites

Parasites are defined as organisms that infect a 'host' and live on or in that host, often to the detriment of same. Parasites can live inside the body (endoparasites) or on the outside of the body (ectoparasites). Parasites may come from a variety of biological families including nematodes (roundworms); protozoans (single celled organisms e.g. malaria); cestodes (tapeworms); insects (e.g. fleas); etc. Some parasitic infections are easily treatable whilst others can be extremely difficult to treat. In some cases the parasite may have no noticeable negative effect in the body whilst in other cases such infections can be fatal. Suitable disinfectants are available for dealing with parasites in the laboratory or working environment.

1.2.6 Cell Lines

Cell lines / cell cultures fall under the definition of biological agent in the relevant biosafety regulations. The risks from cell lines vary depending on the cells lines in question. Some commercially available human cell lines have been well characterised and are considered to represent a minimal risk to user safety; whilst other cell lines such as cancer cell lines are considered to represent potential risks to human health. Suitable disinfectants are available for dealing with cell lines in the laboratory environment.

Pregnant and breastfeeding persons should not work with any biological agents or in any environment where they may be exposed to same until a person specific pregnancy / breastfeeding risk assessment has been carried out. This can be arranged confidentiality via the SIRC Office (sirc@ucd.ie)

1.3 Routes Of Infection

For biological agents to give rise to a negative effect in a person that person must be 'infected' by that agent. To infect a person a biological agent must either get onto or into a person's body. There are a number routes of infection for a biological agent.

i. *Ingestion Of The Agent*: This may happen through the consumption of contaminated water or food or through the insertion of contaminated fingers, pens, etc. into the mouth.

ii. *Inhalation Of The Agent*: Inhalation of an infectious agent can occur if an infectious aerosol* is present.

iii. *Entry Via Mucosal Membranes*: Some infectious agents can pass through thin body membranes on simple contact and can cause infection e.g. through the eyes, nose, ears or mouth. This route of entry can be further exacerbated if fingers or other items are inserted into the eyes, mouth, nose or ears whilst working with risk material.

iv. *Entry Via Damaged Skin*: Contact between infectious material and broken skin, e.g. skin abrasions, can allow an infectious agent to directly enter the body.

v. *Subcutaneous Entry:* This occurs when infectious agents are physically introduced into the body through the skin, e.g. through a needle stick injury with a contaminated syringe.

vi. *Physical Contamination:* A person may also become contaminated with a biological agent following a simple contact with infectious material. In such cases the agent may cause a disease at the site of contamination or may be spread about the body.

vii. *Transplacental:* The unborn child may become infected by an organism to which the mother has been exposed.

Once in or on a person's body a biological agent may give rise to a recognisable disease. However peoples' susceptibility to a biological agent varies greatly between individuals and depends on a range of factors including age, sex, general state of health, etc.

* An *aerosol* is defined in this instance as a suspension of biological agents in the air.

Studies have shown that bacteria account for the highest number of laboratory derived infections and that the most common routes of exposure to biological agents which lead to infection are:

1. Inhalation
2. Needle stick injury / animal bite
3. Direct contact (skin or mucous membrane)
4. Ingestion

Biological Risks and Laboratory Acquired Infections – A reality that cannot be ignored in health biotechnology. In Bioeng. & Biotechnol. 3(56) 2015. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4412124/

In order to prevent the infection of persons with a biological agent the potential routes of transmission between infectious materials and that person must be identified and eliminated, or the risks minimised to as low a level as is practicable. This is the ultimate aim of any Biosafety Risk Reduction measures implemented within the University.

1.4 Classification Of Biological Agents

Biological agents can be classified into one of four hazard classifications based on their pathogenicity and virulence. The classification of a biological agent has implications for both the design and operation of the laboratory within which it is to be used.

- **Hazard Class I:** a biological agent that is unlikely to cause human disease
- **Hazard Class II:** a biological agent which can cause human disease and might be a hazard to employees, although it is unlikely to spread to the community and in respect of which there is usually effective prophylaxis or treatment available
- **Hazard Class III:** a biological agent which can cause severe human disease and presents a serious hazard to employees and which may present a risk of spreading to the community, though there is usually effective prophylaxis or treatment available
- **Hazard Class IV:** a biological agent which causes severe human disease and is a serious hazard to employees and which may present a high risk of spreading to the community and in respect of which there is usually no effective prophylaxis or treatment available

A comprehensive list of biological agents and their classifications is given in the *Code Of Practice For Biological Agents* as issued by the Irish *Health and Safety Authority.* If the agent in question cannot be found within the listings contained within this document then reference should be made to the *UK Health and Safety Executive's The Approved Lists Of Biological Agents.* If an agent cannot be found in this listing then further enquiries should be made with other researchers to establish the internationally accepted classification for that agent. The classification system for biological agents is based on *World Health Organisation Guidelines* and is standard worldwide. In the eventuality that no classification for a biological agent can be found then the user must assign a classification themselves based on the properties of the agent and similar type agents.

Cell lines by definition will not be listed amongst the organisms listed in the aforementioned documents. Consequently, when utilising cell lines users must assign a classification themselves. Further information in this regard is available in Section 3.10 below.

1.5 Laboratory Containment Requirements

As stated above within UCD two distinct types of potential exposure to biological agents are recognised – the first due to the incidental presence of biological agents in the workplace (including diagnostic laboratories); the second due to the deliberate use of biological agents in the workplace.

In the case of the deliberate usage of biological agents each agent must be classified into one of the four hazard groups outlined above, and must only be used in a laboratory that meets the standards required for such laboratories as laid out in the relevant legislation, i.e. a Class 3 organism can only be deliberately used in a Class 3 compliant lab. An animal carrying or suspected to be carrying a biological agent must be classified in accordance with the hazard class of the agent it is carrying.

Within the relevant Biological Agents Legislation the minimum legally binding containment measures necessary for the safe use of each class of biological agent are laid down. Minimum requirements for health and veterinary care facilities, laboratories, diagnostic laboratories and rooms in which deliberately infected animals or animals suspected of being infected are being kept are mandated (refer to Table 1 below).

In the case of incidental presence of biological agents in a lab, such as when working with human blood samples, the lab should at a minimum meet the standards required by a Class 2 laboratory. This requirement also applies to routine diagnostic work / laboratories, except where there is reason to consider that such containment is not sufficient due to either uncertainty about the characteristics of a potential pathogen or information that suggests that additional containment is required.

The design standards / containment requirements for Class 2, 3 and 4 laboratories are detailed in Table 1 below.

As Class 1 organisms are unlikely to pose a risk to human health there are no formal containment requirements for same and as such the application of good microbiological and hygiene practices is usually sufficient to ensure worker safety – see Section 3.1 below.

Table 1. Extract From 2020 Biological Agents Code of Practice Code of Practice for the Safety, Health and Welfare at Work (Biological Agents) Regulations 2013 and 2020 (S.I. No. 572 of 2013 as amended by S.I. No. 539 of 2020).

Containment Measures At Different Containment Levels For Health And Veterinary Care Facilities, Laboratories, Diagnostic Laboratories And Rooms In Which Deliberately Infected Animals Or Animals Suspected Of Being Infected Are Being Kept.			
Containment Measures	**Containment Level 2**	**Containment Level 3**	**Containment Level 4**
1. The workplace is to be separated from any other activities in the same building	No	Recommended	Yes
2. Input air and extract air to the workplace are to be filtered using HEPA or likewise	No	Yes, on extract air	Yes, on input and extract air
3. Access is to be restricted to nominated workers only	Recommended	Yes	Yes, via airlock
4. The workplace is to be sealable to permit disinfection	No	Recommended	Yes
5. Specified disinfection procedures	Yes	Yes	Yes
6. The workplace is to be maintained at an air pressure negative to atmosphere	No	Recommended	Yes
7. Effective vector control e.g. rodents and insects	Recommended	Yes	Yes
8. Surfaces impervious to water and easy to clean	Yes, for bench	Yes, for bench, floor and other surfaces as determined by risk assessment.	Yes, for bench, walls, floor and ceiling
9. Surfaces resistant to acids, alkalis, solvents, disinfectants	Recommended	Yes	Yes
10. Safe storage of a biological agent	Yes	Yes	Yes, secure storage
11. An observation window, or alternative, is to be present, so that occupants can be seen	Recommended	Recommended	Yes
12. A laboratory is to contain own equipment	No	Recommended	Yes
13. Infected material including any animal is to be handled in a safety cabinet or isolator or other suitable containment	Where appropriate	Yes, where infection is by airborne route	Yes
14. Persons should shower before leaving the contained area	No	Recommended	Recommended
15. Validated inactivation process for the safe disposal of animal carcasses	Recommended	Yes, on or off site	Yes, on site

1.6 Notification of Use Of Biological Agents

There are 2 instances when notification of the deliberate use of a named biological agent is required. Persons involved in incidental exposure to biological agents / non deliberate use do not need to make any notifications.

1.6.1 UCD Internal Notification

Users of named biological entities of Class 2 and above are asked to submit details of their usage via the online form on the SIRC website.

1.6.2 Statutory Notification

All first time users of Class 3 biological agents are required to submit details of their work to the *Health and Safety Authority* (HSA) via the form available at https://www.hsa.ie/eng/Topics/Biological_Agents/. Subsequent use of different Class 3 biological agents by the same user does not need to be notified by the HSA, unless the user has provisionally classified the organism themselves.

Diagnostic laboratories are only required to notify the HSA if they are working with a Class 4 agent or are concentrating or propagating Class 3 organisms.

Users of Class 2 entities do not need to notify the HSA as the University has already registered such usage.

Note that the above requirements are separate but are in addition to the need to notify and obtain a consent from the Environmental Protection Agency for the use of Genetically Modified Organisms / Microorganisms.

1.7 Statutory Requirements

The following requirements are outlined in relevant Biological Safety Legislation and must be implemented when applicable.

1.7.1 Risk Assessment

Risk assessments must be completed for all biological agents in use prior to those agents being used for the first time and for all workplace activities where there is a risk of exposure to biological agents. See Section 2 below for further details.

1.7.2 Classification

Where a named biological agent is deliberately used in the workplace it must be classified into one of four biohazard classifications as outlined in Section 1.4 above.

1.7.3 Containment

In the case of the deliberate usage of biological agents each agent must be classified into one of the four hazard groups outlined above, and must only be used in a laboratory that meets the standards required for such laboratories as laid out in the relevant legislation, i.e. a Class 3 organism can only be deliberately used in a Class 3 compliant lab. SEE Section 1.5 above.

1.7.4 Training And Information

Schools, Units and Research Groups must provide all relevant persons with sufficient and appropriate training and information concerning:

- Any potential risks to health
- The precautions to be taken to prevent exposure
- Any hygiene requirements
- Information on the wearing and use of personal protective equipment
- The steps to be taken by employees in the case of incidents and to prevent incidents

The SIRC Office can assist in providing and developing biosafety training. A minimum training / competency level for all relevant employees must be laid down within the risk assessment.

Table 2 Worker Training Requirements

Containment Level	*Key Competencies*
1 and 2	▪ Awareness of the nature of the biological agents in use and its health effects ▪ Awareness and understanding of all procedures and risk assessments ▪ Technical competency for work in question ▪ Knowledge and understanding of disinfection and hygiene requirements ▪ Knowledge of waste management provisions ▪ Knowledge of the emergency response plan ▪ Safe use of PPE
3 and 4	▪ As for Class 1 and 2 ▪ Safe evacuation, sealing and fumigation procedures

1.7.5 Records

Schools, Units and Research Groups must keep records of all persons exposed to a Group 3 or a Group 4 biological agent (or both), indicating the type of work done by each person, and, whenever possible, the biological agent to which they have been exposed, as well as records of exposures accidents and incidents, as appropriate. These records must be kept for at least 10 years.

1.7.6 Health Surveillance

Where necessary provisions for the Health Surveillance of exposed persons should be made. Health Surveillance is defined as '*the periodic review, for the purpose of protecting health and preventing occupationally related disease, of the health of employees, so that any adverse variations in their health which may be related to working conditions are identified as early as possible'.* Health surveillance may include at least one of the following measures:

- Keeping records of a person's medical and occupational history
- A personalised assessment of the persons state of health
- Where appropriate, biological monitoring as well as detection of early and reversible effects
- Further tests may be decided upon for each person, when such person is the subject of health surveillance, in the light of the most recent knowledge available to occupational medicine

1.7.7 Statutory Notifications

As outlined in Section 1.6 there are requirements to notify the HSA about the use of certain types of biological agents.

1.7.8 Accident Reporting

All accidents and near misses, whether they involve biological material or not, must be reported to the *University SIRC Office* – see https://www.ucd.ie/sirc/healthsafety/accidentreporting/

1.7.9 Pregnancy

No pregnant or breastfeeding person should work with a hazardous biological agent until a *Pregnant Employee Risk Assessment* has been carried out in order to determine if the work being undertaking poses a risk to the employee or their unborn or breastfeeding child's safety. The *University SIRC Office* will arrange the completion of such an assessment – contact sirc@ucd.ie

Part 2

Biological Agents Risk Assessment

2.1 An Introduction To Risk Assessment

A 'hazard' is defined as something with the potential to cause harm (in this case the hazard in question is a biological agent).

'Risk' is defined as the potential of a hazard to cause harm under actual circumstances of use or exposure. In this case this is usually the potential for the biological agent to 'infect' or 'colonise' a person.

The assessment of the risk from any hazard is based on the linkage between the probability of occurrence of an accident / incident involving that hazard, with the severity of injury or material loss resultant from that accident / incident. In terms of biological agents the assessment is based on the potential for exposure to the biological agent and the severity of any resultant disease / infection following exposure (see Section 2.2 below).

'Control measures' are steps that are taken to minimise the risk from a hazard either by minimising the potential for the hazard to cause harm, or if it does cause harm by minimising the severity of the outcome. In the case of biological agents control measures are designed to minimise the risk of exposure / infection, and in the event that an exposure / infection occurs, to minimise the severity of the outcome. For example in the case of working with TB bacterium, one would minimise the risk of exposure by working with the bacteria in a biosafety cabinet located in a Class 3 laboratory; whilst in the event that an exposure occurred one would minimise the risk by ensuring that all persons working with TB were vaccinated against same. In all cases the aim should be to minimise exposure in the first instance.

Residual Risk is the risk that remains once all control measures have been implemented. This residual risk is rated, and this rating dictates if the control measures than have been implemented are adequate (see Section 2.2 below).

A *Risk Assessment* is a written document that identifies a hazard and lists control measures that when implemented are designed to reduce the risk of that hazard causing harm or in the event that harm does occur to minimise the severity of the event. The ultimate aim of a *Biological Agent Risk Assessment* is to assess the risk from the use / presence of the biological agent in the workplace to the health and safety of persons and to identify control measures designed to reduce the risk to as low a level as possible. In

the case of biological agents risk reduction can be achieved through the use of procedural, management and physical controls.

Risk assessments must be completed for all biological agents in use prior to those agents being used for the first time and for all workplace activities where there is a risk of exposure to biological agents. When carrying out the risk assessment in the case of activities involving potential exposure to several types of biological agent then the danger presented by all hazardous biological agents present must be considered.

Risk assessments must be completed by a competent person. That person must have sufficient knowledge and experience to identify and classify the hazards associated with a biological agent and also how to reduce the risks from these hazards.

When completing any Biological Agents Risk Assessment the following should always be borne in mind:

- The use of a less hazardous biological agent whenever possible.
- The keeping as low as possible of the number of employees exposed or likely to be exposed to a biological agent.
- The design of work processes and engineering control measures so as to avoid or minimise the release of a biological agent into the place of work.
- The use of both collective protection measures, and individual protection measures where exposure cannot be avoided by other means.
- The use of hygiene measures compatible with the aim of preventing or reducing the accidental transfer or release of a biological agent from the workplace.
- The use of the biohazard sign and other relevant warning signs.
- The drawing up of plans to deal with accidents involving a biological agent.
- The testing, where it is necessary and technically possible, for the presence, outside the primary physical confinement, of a biological agent used at work.
- The use of means for the safe collection, storage and disposal of waste by employees, including the use of secure and identifiable containers, after suitable treatment where appropriate.
- The making of arrangements for the safe handling and transport of a biological agent within the workplace.

Risk assessments must be reviewed on a regular basis (it is recommended that *Biological Agents Risk Assessments* are reviewed at least annually) and when changes in

work practices necessitate it. Written copies of the most up to date risk assessment must be maintained in the workplace and if deemed necessary extracts should be displayed prominently in or adjacent to the areas to which they refer. All risk assessments should be dated and have a section detailing their review history. The name of the person who wrote or last reviewed the assessment must be clearly indicated also.

Pregnant and breastfeeding persons should not work with any biological agents or in any environment where they may be exposed to same until a person specific pregnancy / breastfeeding risk assessment has been carried out. This can be arranged confidentiality via the SIRC Office (sirc@ucd.ie)

2.2 Risk Assessment Matrix & Residual Risk Rating

As stated above the assessment of risk is based on the linkage of the probability of exposure / infection of workers with the biological agent(s) under review with the severity of injury resultant from that exposure once all identified control measures have been implemented.

The Risk Assessment Matrix below can be used to assess risk.

Table 3. University College Dublin Standard Risk Assessment Matrix

	Severity Of Outcome Of Exposure		
Probability Of Exposure	**Low / Slightly Harmful**	**Medium / Harmful**	**High / Very Harmful**
Low / Unlikely	***trivial risk***	***acceptable risk***	***moderate risk***
Medium / Likely	***acceptable risk***	***moderate risk***	***substantial risk***
High / Very Likely	***moderate risk***	***substantial risk***	***intolerable risk***

Risk assessments are thus graded or rated as 'trivial' risk, 'acceptable' risk, 'moderate' risk, 'substantial' risk or 'intolerable' risk.

Table 4. Severity Of Outcome Of Exposure

Slightly Harmful	***Harmful***	***Very Harmful Outcomes***
Superficial injuries	Lacerations	Fatality
Minor cuts and bruises	Burns	Amputation
Eye irritation	Concussion	Major fracture
Nuisance and irritation	Sprains	Poisoning
Temporary discomfort	Minor fractures	Cancer
	Dermatitis	Life shortening disease
	Asthma	Deafness
		Head injuries
		Eye injuries

Table 5. Risk Ratings

Trivial Risk	No action needed
Acceptable Risk	No additional risk control measures required
Moderate Risk	Implement further risk control measures if possible
Substantial Risk	Further control measures must be implemented. If this is not possible then work must be strictly managed to ensure safety. Consult sirc@ucd.ie for further advice.
Intolerable Risk	Work must be prohibited until further control measures are implemented.

2.3 Completing Biological Agent Risk Assessments

Persons completing biological agents risk assessments in UCD have three choices:

1. Complete the risk assessment using their own format and layout
2. Modification of existing general *UCD Biological Agents Risk Assessments.* The SIRC Office has prepared a number of general risk assessments that can be modified or added to when completing biological risk assessments for lower risk activities. See 2.3.1 below.
3. Use the *UCD Biological Agents Risk Assessment Template*. This is recommended for use when conducting more complex or higher risk biological agents risk assessments, both for incidental exposure and deliberate use of biological agents. See 2.3.2 below.

When carrying out a risk assessment in the case of activities involving potential exposure to several types of biological agents the danger presented by all hazardous biological agents present must be considered.

Risk assessments must be caried out for both incidental exposure and deliberate use of biological agents.

2.3.1 Modifying an existing UCD General Biosafety Risk Assessment

The SIRC Office has prepared a number of general risk assessments that can be modified or added to when completing biological risk assessments for lower risk activities. These can be found on the SIRC Website.

Sample risk assessments include:

- Handling And Use Of Class 2 Biological Agents (General)
- Use And Propagation Of Cell Lines (General)
- Zoonoses (General)
- Use And Propagation Of Cancer Cell Lines (General)

These risk assessments follow a standard layout and include the following sections:

- A note detailing how to use the document
- An introduction to the risk in question
- An initial risk assessment
- A comprehensive list of risk control measures (these can be deleted as necessary if not applicable)

- An assessment of the residual risk when the control measures have been implemented
- A 'Part B' where persons can add specific details of the work / biological agents they are risk assessing

As stated above persons can use these documents as the basis for completing biological agents risk assessments for lower risk activities. These documents are available to download in word format so are editable and can be modified to reflect the specific tasks being undertaken.

For example a person working with a Class 2 bacteria in the lab could download the *Handling And Use Of Class 2 Biological Agents (General) Risk Assessment* and select the relevant risk control measures from the document whilst deleting the non-relevant control measures, and could then complete Part B of the document with the specific details of the task and bacteria under assessment, along with an updated risk rating, thus creating a task specific risk assessment.

2.3.2 Completing a UCD Biological Agents Risk Assessment Template

UCD SIRC Office recommends that the *UCD Biological Agents Risk Assessment Template* be used when completing a biological agent risk assessment for complex or higher risk activities. The template is designed to take the assessor through various key aspects of biosafety on a step by step basis. It can be used for both incidental exposure to biological agents and deliberate use of same.

The template will take users through a series of linked questions highlighting key areas of biosafety. In order to complete the template effectively and fully the user must have an understanding of biosafety and the key areas of biosafety highlighted in Part 3 of this manual.

Once the residual risk rating has been calculated the person completing the risk assessment must confirm that it is acceptable and consequently that the work is safe to proceed. The person completing the risk assessment must also confirm if the activity under review is suitable for lone working.

Part 3

Key Aspects of Biosafety

In addition to the issues highlighted below persons should also refer to the general Risk Assessments available at https://intranet.ucd.ie/sirc/completedriskassessments/index.html

Relevant information can be found in the sections containing Laboratory, Biological, Veterinary, Field Work and Health Sciences risk assessments which will complement the information below.

3.1 Good Microbiological / Hygiene Practices

All persons working in a biological laboratory must adhere to the highest standards of personal hygiene in order to minimise the spread of biological agents within and outside of the laboratory. Persons working with biological materials must:

1. Cleanse or wash their hands after handling material and prior to leaving the laboratory or using the telephone, computer or any other equipment in the laboratory.
2. Not insert their hands or fingers or any pieces of equipment e.g. pens, into their mouth / eye / nose / ears whilst in the laboratory
3. Cover all cuts, abrasion and skin lesions with a waterproof dressing at all times.
4. Persons suffering from any condition that causes them to produce excessive mucosal secretions e.g. colds and flu; or which causes them to sneeze excessively e.g. hay fever; may not be suitable candidates for working with biological agents until their condition has improved.
5. There must be no eating or drinking within the laboratory

At a very minimum the following biosafety precautions should be taken where relevant to avoid exposure to biological agents when working in University laboratories or similar:

1. The avoidance of the use of a harmful biological agent or if the nature of the activity permits its replacement with a less hazardous agent
2. The implementation of good hygiene practices as outlined above
3. The keeping of the number of potentially exposed persons to a minimum
4. The provision of adequate information and training to relevant persons particularly in the recognition of symptoms of infection
5. The provision of vaccinations free of charge if deemed necessary to affected persons
6. The provision and use of suitable personal protective equipment
7. The design of work practices so as to minimise potential for contact with biological agents
8. Ongoing health screening for affected persons if deemed necessary
9. The formulation and implementation of local codes of practice for the safety of personnel where required, especially for the taking, handling and processing of samples of human or animal origin
10. The display of warning notices where necessary
11. The keeping of adequate records of persons potentially exposed to infectious agents where necessary

12. The drawing up of plans to deal with accidents involving a biological agent.
13. The testing, where it is necessary and technically possible, for the presence, outside the primary physical confinement, of a biological agent used at work.
14. The use of means for the safe collection, storage and disposal of waste
15. The making of arrangements for the safe handling and transport of a biological agent within the workplace.
16. The application of *Universal Precautions For The Handling Of Blood And Blood Products* where appropriate

3.2 Universal Precautions For The Handling Of Blood And Blood Products

Universal Precautions are a set of safety guidelines which are based around the *Universal Precautions For The Handling Of Blood And Blood Based Products*. The application of *Universal Precautions* requires that all blood and body fluids be regarded as potentially infectious and appropriate protective action taken. The relevant counter-infection measures outlined in *Universal Precautions* are listed below and should be implemented by all persons working with such materials in all settings:

1. The assumption that all blood products are potentially infectious
2. The implementation of good personal hygiene practices and the avoidance of hand or equipment to mouth/eye/nose/ear contact
3. The use of vaccinations where necessary
4. The wearing of suitable personal protective equipment
5. The prevention of puncture wounds, cuts and abrasions in the presence of blood and body fluids
6. The protection of skin lesions and existing wounds on workers
7. The avoidance of the use of sharps and sharp objects when possible but, where unavoidable, taking particular care in their handling and disposal
8. The development of suitable working area decontamination procedures
9. The elimination or containment of any work practises that could give rise to aerosols or the uncontrolled release of material
10. The development of emergency response plans
11. The disposal of all contaminated waste safely
12. The provision of training and information to all employees engaged in handling potentially infectious material

3.3 Microbiological Safety Cabinets

Microbiological Safety Cabinets (MSC) are a basic tool in laboratories where biological agents are handled. A MSC is a ventilated enclosure intended to offer protection to the user and the environment from aerosols generated when handling biological agents. MSC are not designed to hold radioactive, toxic or corrosive substances. MSC should be used for the handling of all Class 3 and 4 organisms and where there is a risk of generating aerosols when handling Class 2 organisms.

MSC's should be maintained in accordance with the manufacturer's instructions. In particular filters should be changed at the prescribed intervals.

Units must also be located in accordance with manufacturer's advice with the avoidance of disruptive air flows in front of the cabinet key.

All persons working with MSC must receive training in its proper and efficient use.

When using a MSC there must be written decontamination procedures in place which details how to decontaminate the cabinet after each use and also how to decontaminate the cabinet following an unintentional spill of a biological agent. Particular care must be taken to ensure that the disinfectants selected for use in a MSC are compatible with the cabinet components. Generally speaking the use of UV light to disinfect safety cabinets alone is not an effective procedure unless the cabinet is completely empty.

3.4 Selection Of Personal Protective Equipment (PPE)

When working with biological agents the selection and correct use of appropriate PPE is key to controlling the risks presented by biological material. PPE must be stored appropriately, cleaned as required and repaired where defective, or if repair is not possible replaced before further use.

3.4.1 Protective Clothing

All persons working in a biological laboratory must at a minimum wear a closed laboratory coat. Such coats must be removed when leaving the laboratory and must be laundered on a regular basis. Heavily soiled laboratory coats should be removed immediately.

3.4.2 Gloves

Persons involved in the manipulation of biological material must also wear protective gloves. Disposable gloves which provide a mechanical barrier between the users skin and the material being handled are sufficient. Where possible latex gloves should be avoided due to the allergic reactions that such material can generate in wearers. If certain procedures are being undertaken then the use of specialist gloves may be required, e.g. when handling contaminated sharps, cleaning up broken glass, etc. In some cases 'double gloving' may be required.

3.4.3 Eye / Face Protection

Where there is a risk of material splashing or being ejected under pressure safety glasses must be worn by workers. Consideration should be given to the wearing of a full face shield when blood is being handled and there is a risk of splashing.

3.4.4 Respiratory Protection

Under normal conditions respiratory protection should not be required in laboratories handling Class 1 and 2 biological agents, although it may be required in laboratories handling Class 3 and 4 material. If a mask is required to protect the worker from the material being handled then the wider risk control measures have failed. However, respiratory protection may be required during a clean-up following the spillage or unintentional release of biological material. If specialist respiratory protection is required in such instances then appropriate training must be given to the persons responsible for its use.

All required PPE must be clearly indicated in the risk assessment for the biological agent(s) / processes under consideration by that assessment.

3.5 Vaccinations

The use of vaccinations should be considered where persons are handling potential infectious agents and an appropriate vaccine is available. When being offered vaccination persons should be informed of both the benefits and drawbacks of both vaccination and non-vaccination and be allowed to make an informed decision. Vaccinations must be offered free of charge. Note that vaccinations cannot be made mandatory, but certain tasks can be limited to those persons who are vaccinated.

3.6 Emergency Planning / Spillages

All laboratories / facilities where biological agents are handled must have in place an *Emergency Response Plan* which outlines the responses to be taken in the event of an emergency involving the biological agent, such as an unintentional release or spillage of the agent or a worker exposure to the biological agent.

A key part of the *Emergency Response Plan* is the procedure to be followed in the event of a spillage or loss of biological material. Key to the safe management of spillages is the use of appropriate disinfectants, personal protective equipment (PPE) and staff training.

For spillages where aerosols are not likely to be produced persons should don the necessary PPE and treat the affected area with an appropriate dry disinfectant or cover with tissue paper and apply a liquid disinfectant. The treated area should be allowed to remain long enough for the disinfectant to take effect before being cleaned and the waste material being disposed off accordingly. The contact time and the type of disinfectant to be used may vary depending on the biological agent in question and this should be clearly indicated in the *Emergency Response Plan*.

Where a spillage may give rise to aerosols, e.g. during the rupture of a sample tube in a centrifuge, the area must be evacuated and the droplets allowed time to settle. Persons then wearing appropriate PPE may enter the effected area treat the spillage. In some cases extensive decontamination of the working area may be required. If deemed necessary testing for the presence of the biological agent can be done following the completion of the disinfectant procedure. Respiratory protection may be required when dealing with spillages that have generated aerosols. A template spill response poster can be found at https://intranet.ucd.ie/sirc/safetydocumentsandguides/biosafety/

3.7 Safe Use Of Sharps

Sharps pose a particular risk to persons working with biological agents in that in the event of a sharps injury the biological agent may be introduced directly into the victim's body.

3.7.1 Glassware

- Use plastic as an alternative to glass when possible

- Care must be taken when working with glassware. Particular care must be taken when:
  - Inserting pipettes into pipetting aids or Pasteur pipettes into teats
  - Attaching glass to or removing glass from rubber or plastic tubing
  - Removing "frozen" stoppers from glass bottles
  - Breaking glass tubing
  - Washing up glassware
  - Handling broken glassware
- All broken, cracked or chipped glassware must be disposed of immediately
- When handling glass items avoid applying force or excessive pressure in case the item slips or gives way suddenly and breaks. If inserting pipettes into pipetting aids or Pasteur pipettes into teats; attaching glass to rubber or plastic tubing; or removing "frozen" stoppers from glass bottles then the glassware should be held in a cloth to help prevent slipping.
- Carry glassware in suitable trays / cages where necessary.

3.7.2 Blades

- Always handle blades with care.
- Use the appropriate blade for the task. Do not use scalpel blades or razor blades unless absolutely necessary and when in use handle them with care.
- Wherever possible use single unit disposable scalpels rather than changing the blades on a re-useable holder.
- If not being disposed after use blades must always be placed in a safe position and orientation so as to avoid possible accidental injury to others. Do not leave scalpels pointing upwards from beakers or similar or sitting under water in a sink.
- Where a blade is used in a holder particular care must be taken when changing the blade. The blade should not be held in the fingers during the process and the use of excessive force must be avoided. A forceps should be used to hold the blade.

3.7.3 Needles

- Needles should only be used if necessary, and always for the purpose that they were designed. Always consider less hazardous alternatives wherever possible.
- There should be minimal handling of needles in the workplace. Once the seal on the sheath of a needle has been broken carry out any subsequent handling with extreme care and keep handling to a minimum.

- Used needles should be placed directly in a sharps bin at the point of use without either detaching the needle or re-sheathing – there should be no further unnecessary handling of a used unsheathed needle.
- If needles require re-sheathing never hold the sheath with your fingers as if the needle misses the sheath it will puncture a finger, always use a forceps.
- Do not detach the needle from the syringe unless absolutely necessary.
- Unsheathed and used needles must not be left on worktops or mixed with other items. They should be placed into a tray or similar container where they are clearly visible.
- When using needles to inoculate animals the following should be adhered to:
  - Restrain the animal to minimise any unexpected movement in so far as is possible
  - Position hands carefully such that the needle is not pointed either towards your hands or the hands of anyone who may be assisting
  - Ensure you are not likely to be disturbed during the procedure
  - Always wear eye protection.
  - If more than one person is carrying out the procedure establish an agreed technique in advance to ensure the person holding the needle does not inadvertently stick the hand of the person assisting.
- Any needle stick injury caused by an implement contaminated with human, microbial, animal, chemical or radioactive material should be immediately reported to the *SIRC Office* and the victim must seek immediate medical advice.

3.7.4 Disposal

- Place used needles and syringes, or any other types of sharps, directly into a sharps bin for disposal.
- Have a sharps bin available at the point of use to enable immediate disposal.
- Dispose of used sharps only in an appropriate bin. Clearly label sharps bins as to their allowable content.
- Do not overfill sharps bins, fill only as far as the fill line.
- Never place sharps or sharps bins in plastic bags or a domestic refuse bin for disposal.

3.7.5 First Aid

- Cuts caused by sharps should be treated immediately. No attempt should be made to remove broken glass from wounds. Needle stick injuries from contaminated needles should be encouraged to bleed. Wash well under running water and cover with a dry dressing. An attempt should be made to identify any chemical or biological hazard in the needle that may have been injected.
- Apart from very minor injuries, a First Aider should be called.
- If a first aider is not available locally then assistance can be obtained by calling 01 716 7999 / extension 7999.
- In the event of sustaining an accident resulting in a wound:
    - Immediately wash the wound liberally with soap and water but without scrubbing
    - Do not attempt to remove any glass by hand
    - Gently encourage free bleeding of puncture wounds but do not suck the wound
    - Dry the area and apply a waterproof dressing
    - Seek medical advice if the sharp concerned was contaminated with any hazardous materials

There is no evidence available to show that using antiseptics or squeezing a wound will reduce the risk of transmission of a blood borne pathogen. Using a caustic agent such as bleach to wash a wound is not recommended.

3.8 Selection And Use Of Disinfectants

The disinfection process is designed to reduce the number of micro-organisms present to an acceptable level such that the item being disinfected is safe to handle. Disinfection should not be confused with sterilisation, a process that renders an object free from all viable organisms.

There are several types of chemical and physical agents that can be used for disinfection, including chemicals, heat and irradiation. All university laboratories handling micro-organisms should have in place written disinfectant procedures for decontaminating surfaces and equipment and should ensure that working surfaces and equipment are treated on a regular basis as appropriate.

Always consider:

3.8.1 The Target Organism

Disinfectants do not generally kill all the organisms which they come into contact with and do not disinfect against all organisms equally well. A disinfectant which is effective against bacteria may not be as effective against viruses. Some disinfectants are more effective against Gram positive than against Gram negative bacteria. Some disinfectants have a wide spectrum of performance against many organisms. There are many different commercial products available and these will vary in how effective they are against different micro-organisms. Manufacturers of disinfectants should provide advice on the specific antimicrobial activity of their particular products. If the types of micro-organisms in samples or materials handled are unknown then a general purpose disinfectant should be chosen.

3.8.2 Presence Of Other Materials

The presence of other materials in or on the surfaces to be disinfected can have an effect on the activity of the disinfectant. The presence of organic material, other chemical agents including soaps and detergents and the pH and temperature can all reduce the effectiveness of the disinfectant. The concentration of disinfection to be used is likely to vary depending on whether it is used in "dirty" or "clean" conditions.

3.8.3 The Nature Of Surfaces And Equipment To Be Cleaned

Some disinfectants will chemically attack items being disinfected. Stainless steel can be damaged by strong acids and hypochlorite. Plastics may be affected by disinfectants containing organic solvents. Various metals may be attacked by strong acids or alkalis, halogen active substances, or disinfectants containing electrolytes.

3.8.4 Safety Implications Of Disinfectants

Many disinfectants have toxic properties and some are also highly corrosive, causing damage if they come into contact with skin or eyes. Some disinfectants, e.g. glutaraldehyde and hypochlorites, may also have irritant properties and so cause respiratory problems if used in poorly ventilated areas. Some disinfectants may react with other chemicals causing hazardous gases. A *Chemical Agents Risk Assessment* must be completed for all disinfectants in use. Material Safety Data Sheets for all disinfectants in use should be provided by the manufacturer and held within the laboratory.

When selecting a disinfectant both the efficacy of the product and the hazards associated with its use must be taken into account, e.g. given the hazards associated with the use of glutaraldehyde there are unlikely to be any grounds for selecting this agent for routine use within the university.

3.8.5 Working Dilutions

Some disinfectants are provided in concentrated form and have to be diluted in water to the working strength. The manufacturers' instructions should be followed to ensure that the required concentration is achieved. Over dilution will render the disinfectant ineffective. Once made up the disinfecting capacity of diluted products tends to deteriorate rapidly with time, e.g. sodium hypochlorite solutions lose their efficacy rapidly (<24hrs). Manufacturers should recommend how long a made up solution can be stored for and this should be noted in the disinfection policy. Some products contain coloured indicators to show effective disinfecting capacity, e.g. Virkon™. If the disinfectant in use does not contain an indicator then a *use by* or *expiry date* should be clearly marked on the bottle when the solution is made up.

3.8.6. Contact Time

Chemical disinfectants need to be applied to the item they are disinfecting for sufficient time to enable the disinfection to be effective. Always consider manufacturers recommended contact times (in combination with concentrations) for various applications.

Table 6. Some Common Classes Of Disinfectants And Their Activity

Disinfectant Type	***Vegetative bacteria***	***Bacterial spores***	***Fungi***	***Enveloped viruses***	***Non-enveloped viruses***	***Myco-bacteria***	***TSE & Prion Agents***
Phenolic	*Generally Effective*	*Generally Ineffective*	*Generally Effective*	*Generally Effective*	*Depends On The Virus*	*Generally Effective*	*Generally Ineffective*
Hypochlorites	*Generally Effective*	*Generally Effective*	*Limited Activity*	*Generally Effective*	*Generally Effective*	*Limited Activity*	*Generally Effective*
Alcohols	*Generally Effective*	*Generally Ineffective*	*Generally Ineffective*	*Generally Effective*	*Generally Effective*	*Generally Effective*	*Generally Ineffective*
Aldehydes	*Generally Effective*	*Generally Effective*	*Generally Effective*	*Generally Effective*	*Generally Effective*	*Generally Effective*	*Generally Ineffective*
Surface active agents	*Generally Effective*	*Generally Ineffective*	*Limited Activity*	*Depends On The Virus*	*Depends On The Virus*	*Generally Ineffective*	*Generally Ineffective*
Peroxygen compunds	*Generally Effective*	*Generally Effective*	*Generally Effective*	*Generally Effective*	*Generally Effective*	*Generally Effective*	*Generally Ineffective*

3.9 Waste Management

All wastes considered to be contaminated with biological material must be considered as 'hazardous' and must be disposed of in an appropriate manner. The following can be considered to be hazardous / infectious wastes:

- All human tissues, blood and related swabs and wipes from hospitals or laboratories
- Animal carcasses and dressings from veterinary hospitals / practices
- Contaminated needles, glass, instruments, etc.
- Microbiological cultures
- Potentially infected waste from pathology or research labs

Hazardous wastes must be collected and disposed of by licensed waste management companies.

Colour coded bags should be used to separate infectious wastes (yellow bags) from non infectious wastes (black / clear bags). All sharps must go into suitable sharps bins for disposal. Sharps include broken glassware, blades and syringe tips. Sharps must never be placed into normal bins.

The disposal of non infectious wastes which may appear to the untrained / uniformed person to be '*medical wastes*' must be undertaken with great care, e.g. swabs, wipes, syringe bodies, drips, etc. Very often these items are included in the hazardous waste removed from the university in order to avoid any confusion. If these items are to be disposed of as non hazardous wastes then the waste disposal service provider must be made aware of the nature of this waste so that when the material is encountered off site that it is not assumed to be infectious.

3.10 Cell Cultures

Cell cultures can themselves pose a risk to user safety or they may harbour potentially harmful agents. As with all biological agents cell cultures must be assigned a Hazard Classification based on their properties and their ability to infect / harm human health. The four hazard classifications are:

- **Hazard Class I:** a biological agent that is unlikely to cause human disease.
- **Hazard Class II:** a biological agent which can cause human disease and might be a hazard to employees, although it is unlikely to spread to the community and in respect of which there is usually effective prophylaxis or treatment available

- **Hazard Class III:** a biological agent which can cause severe human disease and presents a serious hazard to employees and which may present a risk of spreading to the community, though there is usually effective prophylaxis or treatment available
- **Hazard Class IV:** a biological agent which causes severe human disease and is a serious hazard to employees and which may present a high risk of spreading to the community and in respect of which there is usually no effective prophylaxis or treatment available

The hazard will depend on the type of cells being handled. Well characterised and known cell lines may assigned to Hazard Class 1, although it is worth remembering that even with cell lines such as these there is no absolute guarantee that these cell lines do not carry latent viral agents or viral genome. Unscreened cells or cells with a less well defined history should be handled at the very least at Containment Level 2. As a rule cancer cell lines should be considered as Class 2 agents (unless they are well known and well understood commercially available cell lines). When handling a cell line it is important to be aware of as much of its history as possible and particularly whether or not the cell line has been infected with a viral vector at any stage in its history. Human and primate cell lines must always be considered as having the potential to harbour human viruses. The geographical source of the cell lines should be examined as in some parts of the world Hepatitis B, HIV and similar viral infections are prevalent.

3.10.1 Primary Cell Cultures

When handling primary cell cultures there is an increased risk of spontaneous transformation. This happens relatively frequently with rodent cells and to a lesser extent with human and primate cells. Transformed cells isolated from a worker or a colleague can pose a significant risk to the donor due to the fact that if accidentally reintroduced into the donor body the cells may not be recognised as foreign matter by the body and may in theory form invasive colonies within the body. Therefore the following precautions must always be taken:

- Never culture your own cells and if possible avoid culturing cells from your immediate colleagues
- Never transform your own cells or those of your colleagues
- Whenever possible culture primary cells for short periods only. This reduces the probability of spontaneous transformation.
- If cell lines are unscreened for viral agents then further precautions may be required

Whenever possible only cell strains that have been authenticated and / or have a documented provenance should be used. These are best obtained from a culture collection or from the originator of the cell line. To avoid cross contamination handle only one cell line at a time and ensure appropriate decontamination procedures between the handling of cell lines. The generation of aerosols and the use of sharps should also be minimised.

Table 7 Containment Levels Appropriate For The Handling Of Cell Cultures

Hazard	Cell Type	Containment
Low	Non human, non primate cell lines that have been authenticated and have a low risk of infection with a human pathogen and present no apparent hazard to laboratory workers	Containment Level 1
	Well characterised, screened, and / or authenticated finite cell lines of human or primate origin	
Medium	Cell lines or cell strains that have not been fully characterised	Containment Level 2
	Cancel cell lines	
High	Primary cells from blood, lymphoid cell, neural tissue of human or simian origin.	Containment appropriate to the potential risk
	Primary cell lines cultured for more than 100 hours	
	Cell cultures known, or strongly suspected, to harbour an endogenous pathogen	
	Cell cultures deliberately infected with a human pathogen	

3.11 Working Safely With Animals

Working with animals may expose workers to the risk from allergenic hazards and infectious hazards. Contact sirc@ucd.ie for details of UCD's Lab Animal Allergy Protocol. See the general risk assessment for zoonotic agents at ***https://intranet.ucd.ie/sirc/completedriskassessments/index.html***

3.12 Sources Of Biosafety Information

Websites

- https://www.ebsaweb.eu/ – European Biosafety Association
- Centre For Disease Control, USA.
- Irish Health and Safety Authority
- Public Health Canada - excellent site which include pathogen safety data information for a large range of organisms.
- Health and Safety Executive (UK)
- Belgian Biosafety Server

Publications

- *WHO Biosafety Manual*
- *Biological agents: Managing the risks in laboratories and healthcare premises.* ACDP / HMSO, 2005.

UCD Documents

UCD Use Of Genetically Modified Organisms Manual

UCD Biological Agents Risk Assessments

UCD Biological Agents Risk Assessment Template

UCD Biological Agent Spill / Emergency Response Poster