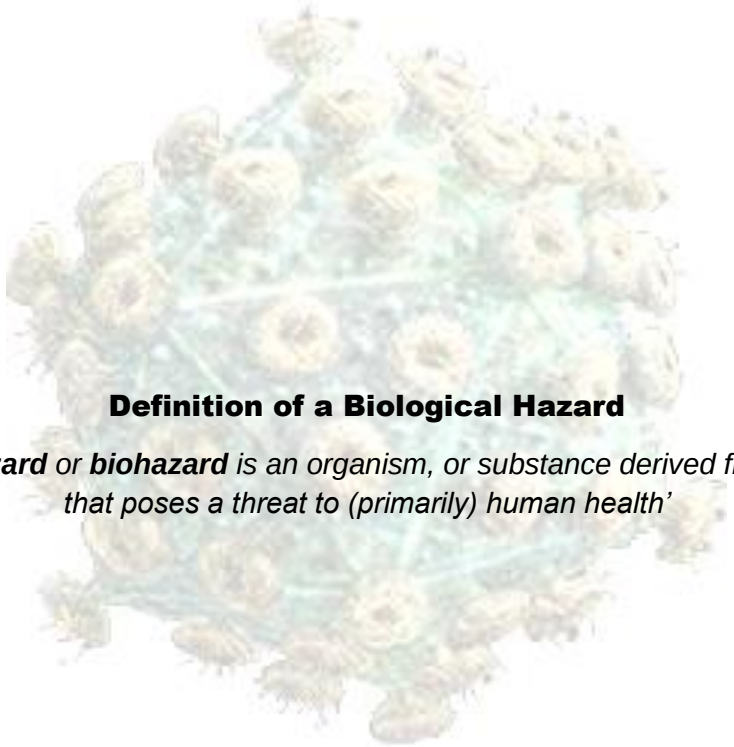
BIOLOGICAL HAZARDS TRAINING



Definition of a Biological Hazard

*'A **biological hazard** or **biohazard** is an organism, or substance derived from an organism, that poses a threat to (primarily) human health'*

Contents

What is a Biological Agent?

A biological agent is defined as a micro-organism, cell culture, or human endoparasite, whether or not genetically modified, which may cause infection, allergy, toxicity or otherwise create a hazard to human health. Due to the often 'invisible' nature of biological agents, staff and students must be very disciplined when working with them, making sure they follow all policies and procedures, risk assessments, safe working practices etc to avoid infection to themselves and others. In addition, it is very important that staff and students ask for help if they are unsure about anything when working with a biological agent.

Types of Biological Agent

Most biological agents are micro-organisms of which there are 5 basic categories: Bacteria; Rickettsiae and Chlamydiae; Viruses; Fungi and Protozoa.

Each of these categories will have thousands of different types of organisms which may have varying affects on both living and dead tissue. For example:

NAME	AFFECT
Saprophytic	Live freely on decaying matter
Parasitic	Live in or on a living host
Commensals	Live in harmony with the host
Symbiotic	Live in harmony for the mutual benefit
Pathogenic	**Produce disease in the host**

Biological agents are also unlike any other hazardous substance as they can change and evolve. For example, bacteria may become resistant to antibiotics. The types of biological agents you will encounter at the University and the types that will be discussed throughout this course will be pathogenic micro-organisms that fall within the bacteria, virus and fungal categories.

What is a bacteria, virus, fungi?

FUNGI

Fungi can be split into three groups: fungus, moulds, yeasts.

Fungus: Are a group of unicellular, multinucleate organisms that are non-photosynthetic (don't contain chlorophyll) and feed on organic matter. They are a simple parasitic life form with many containing minute spores which are like seeds that can carry in air and if settle in a suitable location will grow rapidly. Although most fungus are harmless or beneficial to health, some can cause fatal diseases and illness in humans.

Sickener Fungus

Exposure to fungus at the University could be as a direct result of ingesting mushrooms or for example, as a result of inhaling / ingesting spores when working in wood deterioration resulting in aspergillus fumigates.

Moulds: Grow rapidly in moist conditions and can produce clouds of dusty spores. As spores are usually about 1 micron in size they can be easily inhaled and penetrate to the alveoli region of the lung.

Bread Mould

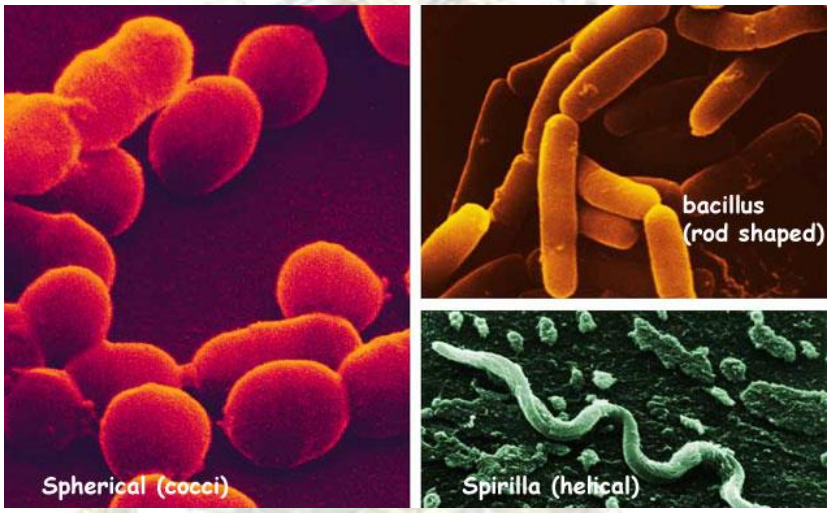
Yeasts: Are a type of fungi which cause infections of the skin or mucous membranes. *Candida Albicans* (thrush) is the most common fungal disease and is often handled during class practicals.

Some fungus, moulds and yeasts produce spores as part of their life cycle. These spores are often very small and can be inhaled or ingested, causing problems in people with respiratory problems such as asthma. They may also cause sensitisation leading to future allergic responses.

BACTERIA

Are a group of single-celled micro-organisms, more commonly known as 'germs', and although most are harmless to humans some known as 'pathogens' have the ability to cause disease in humans. Bacteria are classified into three broad groups dependent on their shape: cocci (spherical); bacilli (rod-shaped); and spirilla (spiral-shaped).

Bacteria are generally in the size range of 1 – 10 microns, although some may be smaller than 1 micron if they are starved. Commonly used pathogenic bacteria at Bangor University include *E-coli*, *Campylobacter* and *Salmonella,* all of which have the potential to cause enteric diseases.



Bacteria

VIRUSES

Are the smallest known types of infectious agents, which under specific conditions can self-replicate although outside living cells they are totally inert. Viruses are about one half to one hundredth the size of the smallest bacteria and have a size measured in nanometers. The sole activity of viruses is to invade the cells of other organisms and when they do so they often cause damage or death to those cells.

It is unsure how many viruses there are, as new types are being discovered all the time, however, there is the common belief there are more types of virus than any type of organism. Many viruses are able to cause disease and although they are generally quite host specific they can 'jump' between species as shown by the recent Swine Flu pandemic.

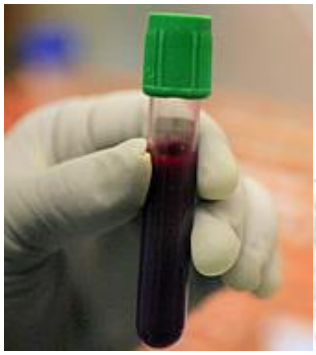
The most likely route of exposure to pathogenic viruses at Bangor University will come from handling human blood and tissue samples.

Sources of Biological Agents

There are a number of different sources of hazardous biological agents that may be encountered by staff and students working and studying at the University. Many of these sources are well understood and their dangers known by most people (for example laboratory cultures of known pathogens). Other less apparent sources may pose an equal or greater risk, especially to the inexperienced since their hazards are less obvious.

The following are common sources of biological agents that you will find at the University.

Human Specimens: Work with human specimens takes place in several Colleges and is a growing area of research, thus increasing the number of people that could potentially be affected. It is important that you treat all clinical specimens as if they contain infectious agents as even with screening it is difficult to determine if the specimen is risk free.



Blood Sample

Sources and types of human derived hazardous agents include:

- Blood borne pathogens eg HIV, hepatitis, malarial parasites.
- Tuberculosis from sputum samples.
- Enteric pathogens in gut tissue or faeces.
- Pyogenic agents eg *Streptococcus* in pus, wounds etc.
- Agents of Transmissible Spongiform Encephalopathy (TSE), especially brain / neural tissue.

Tissue culture of human cells also presents a potential source of hazardous biological agents. The most hazardous types of cell culture are uncharacterized primary cell lines.

Zoonoses: Animals can harbor infections (Zoonoses) that are harmful to humans, and in general, the closer an animal is to humans genetically the greater the risk of such diseases. It is usually wild animals that pose the greatest risk as experimental animals will often have been screened for specific pathogens. It is worth noting that at the University the majority of animals that staff and students will have contact with will not have been screened for Zoonoses.

The following are examples of animal borne diseases:

- Anthrax in cattle, sheep, goats, pigs and horses.

- Leptospirosis in rodents, dogs or cattle.
- Salmonella infection and cryptosporidiosis in vertebrates.
- Ovine chlamydiosis in sheep.
- Psittacosis and Newcastle disease in birds.
- Tuberculosis in cattle.
- Orf in sheep or goats.

Environmental Hazards: Hazardous biological agents are present in a number of environments and may on rare occasions pose a risk to both staff and students. The following are the most common environmental diseases.

- **Legionnaires' Disease:** The causative agent of Legionnaires' disease may be present in plumbing and heating systems and is a particular threat when aerosols are generated since its only route of entry is via the respiratory system.

  The University has a system to manage the Legionnaires' risk but in a laboratory environment there may still be other potential sources eg water baths and emergency showers. As a precaution water baths should be heated to 60^{o}C for one hour every month and emergency showers tested weekly to prevent the build-up of stagnant water.

Aerosols

- **Leptospirosis:** The causative agent of Leptospirosis is present in rat urine and waters contaminated by rat's urine. Staff and students working in areas where rats may be present eg Henfaes Farm, Treborth and certain field stations may be at risk. If you notice rats anywhere on University property you must report it immediately to Estates and Facilities. In addition, if you are working in 'at risk' areas you must practice good hygiene, ensure that all cuts and abrasions are covered and wear gloves.

- **Tetanus and Hepatitis B:** The causative agent for Tetanus is found in soil and sewage, which also potentially contains Hepatitis B. Staff and students working in such areas should ensure they have appropriate vaccinations against these illnesses.

- **Culture of Biological Agents:** At the University bacteria, fungus and viruses are cultured for a variety of teaching and research activities, and although some of these will be harmless, many will have the potential to cause disease.

- **Non Pathogenic Agents:** The only biological agents used that we are sure won't cause disease in healthy humans are those that have been sourced from culture collections and scientific suppliers. These organisms will have been subject to a variety of tests to ensure their identity and lack of hazardous properties. However, such organisms should still be used in accordance with good microbiological practice.

- **Known Pathogenic Agents:** Other agents purchased from culture collections and laboratory suppliers are known pathogens, and the use of such agents is subject to a variety of legislation which is explained in detail later in this document. A specific risk assessment **must be prepared before working** with known pathogenic agents and everyone involved must have proper training and supervision.

Bacteria growing on Blood Agar

- **Environmental Isolates:** Water, sediment and soil samples taken from the environment may contain pathogens which can be concentrated or stimulated to grow during laboratory activities. Pathogens are more likely to be found in environments contaminated with human or animal remains or excreta and include *Clostridia* species in soil and enteric pathogens in polluted water. Unidentified isolates cultured from environmental samples should be treated as if they are pathogenic (Hazard Group 2 – see later) until proven otherwise.

- **Large Scale Culture of Biological Agents:** Fermentation is a method of growing biological agents on a variety of scales. Most fermentation work at the University is small scale but there is an increasing amount of larger scale work which can result in the generation of the following hazards:
    - Production of aerosols.
    - The need to collect and process large samples.
    - Difficulties in ensuring effective disposal, disinfection and cleaning of plant.

100 litre Bio Fermenter

Genetically Modified Organisms (GMO): Genetic modification is defined as altering the genetic material of an organism in a way that does not occur naturally. Whilst there are no confirmed reports of people suffering ill effects from GMOs, the potential for harm to both humans and the environment does exist eg:

- Virulence genes incorporated into an organism making it more pathogenic.
- Enhancement of an organism's ability to induce allergic / autoimmune reactions.
- Cloning of genes that may enhance the risk of cancer.
- Harmful characteristics transferred to organisms in the environment.

Because of the potential for GMOs to cause damage, their creation and use is strictly regulated. Anyone wishing to create or use GMOs at the University must follow strict policies and guidance which can be found on the Health and Safety Services Website - A – Z of General Information.

How does Infection Occur?

Biological agents can enter your body by a number of routes which is why you **must** identify the route of entry in your risk assessment so you can put in place appropriate controls. Infections can occur as a result of direct and indirect contact with a laboratory culture or an infected host's tissue, body fluid, secretions and excretions. For example, the main routes of entry into the body are:

ROUTE OF ENTRY	HOW
Skin contact	Absorption through the skin or via cuts, abrasions etc
Mucous membranes eg eye, nose	Splashes to the face
Ingestion	Eating, drinking, putting make up on, pipetting by mouth **(not allowed!)**
Puncture wounds	Needle stick injuries, broken glass
Inhalation	Airborne contaminants eg dusts, aerosols

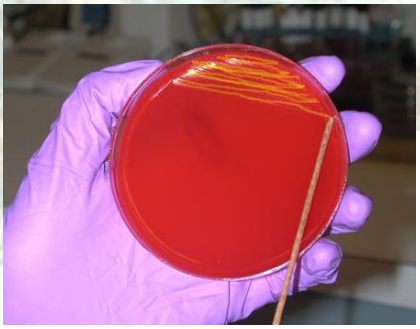
AIRBORNE CONTAMINANTS

Airborne contaminants eg aerosols, spores and dusts leading to inhalation and ingestion are the most hazardous as they are difficult to control / contain and are often very small (less than 5 microns) and can enter the alveoli region of the lung. They also have the ability to travel, potentially infecting large numbers of people over a wide area.

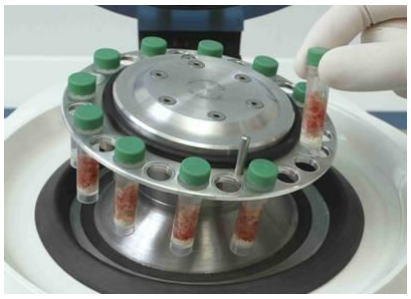
Aerosols can be produced by a number of lab operations and are also easy to create by mistake. If you know there is a risk of airborne infection, you must carry out work in a Safety Cabinet:

- Shaking / pouring cultures.
- Pipetting.
- Making smears.
- Inoculating plates.
- Flaming loops.
- Aerating cultures.
- Homogenisation.
- Ultrasonic disintegration.
- Spills and splashes.

Pipetting. NEVER do by mouth



Inoculating Plates



Homogenisation

Shaking Cultures

The following table shows the probable estimated inhalation dose when manipulating a culture containing 10^9 organisms ml^{-1}. It will give you an idea of how much aerosol can be inhaled during some lab operations.

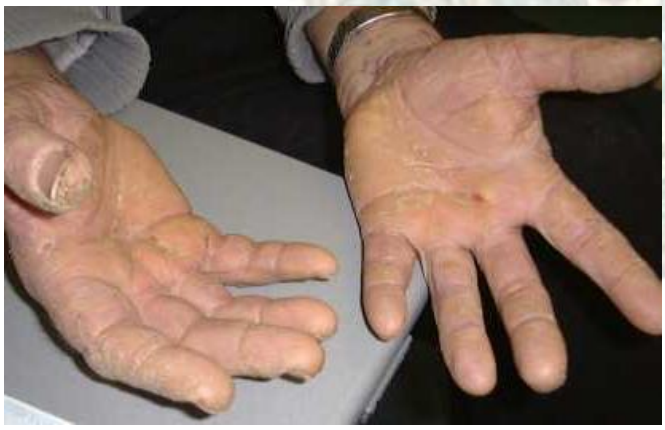
OPERATION	ESTIMATED INHALED DOSE
Flaming Loop	<1
Sonication (without frothing)	10
Sonication (with frothing)	10,000
Homogenisation (delayed opening)	<1
Homogenisation (immediate opening)	10,000
Dropped and broken flash	1,000

However, some airborne organisms do not survive well and may die once the fluid in the droplet has evaporated. The speed of evaporation depends on the droplet size, suspending fluid composition and the temperature and humidity of the environment.

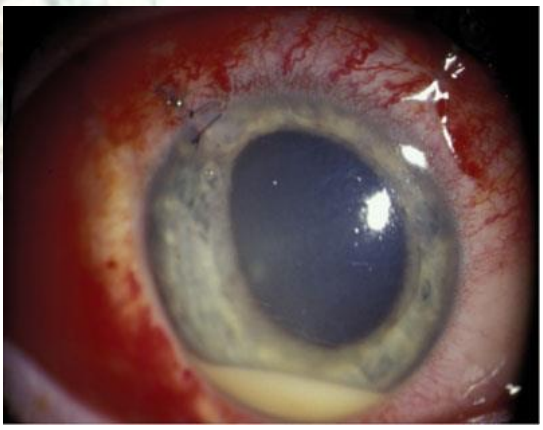
Types of Infections

Micro organisms can produce a wide variety of infections, some of which can be fatal. The seriousness of the infection will not only depend on the strain of the organism but also its' physical condition, the route of entry and the resistance of the host, leading possibly to the following type of infection:

- **Asymptomatic:** There may be no symptoms and the host may be able to pass on infection to others over long periods, possibly a life-time eg HIV.
- **Subclinical:** Symptoms do not need medical attention.
- **Acute:** Symptoms vary over a short period leading to death, asymptomatic transfer or chronic disease.
- **Chronic:** Symptoms change slowly or remain the same over long periods.



Dermatitis



Infection caused by Bacteria

In addition, if not handled correctly biological hazards can also lead to:

HAZARD	AFFECT	POTENTIAL SOURCE (ACDP)
Allergic Sensitisation	Dermatitis, asthma, allergic alveolitis (farmer's lung). Once sensitized exposure to small amounts can trigger an allergic reaction. Serious reactions can be fatal if not treated promptly	*Candida albicans, Penicillium marneffei, Aspergillus fumigates, Coccidioides immitis, Ascaris lumbricoides, Cryptococcus neoformans, Epidermophyton floccosum, Microsporum spp*
Toxin Production	Ingestion of toxins leading to illness, inhalation of microbial products causing toxic reactions in lungs	*Clostridium botulinum and tetani, Corynebacterium diphtheriae, Shligella dysenteriae, Staphylococcus aureus, Vibrio cholerae*
Oncogenicity	Although hard to establish a direct causal relationship with micro-organisms, there are some established associations. Note: Although the risk of occupational cancer from exposure to micro organisms is low **take it into account in risk assessments**	Some Papilloma virus and cervical cancer, Hepatitis B and liver cancer, Human Herpes virus 8 and Kaposi's sarcoma

ACDP - Advisory Committee on Dangerous Pathogens

Identifying Biological Agents

Occupational exposure to biological agents is regulated by the Control of Substances Hazardous to Health Regulations (COSHH). Schedule 3 of COSHH relates purely to biological agents and not only classifies biological agents in accordance with specific criteria but also outlines the controls that must be put in place, in addition to the general requirements of COSHH when working with biological agents, for example, displaying the Bio Hazard Sign, laboratory containment levels, notifications etc.

To identify if a substance is a biological agent, reference should be made to the Advisory Committee on Dangerous Pathogen's **'Approved List of Biological Agents'**. This 'List' classifies biological agents, placing them into one of four Hazard Groups with classification dependent upon the following factors:

- Can the biological agent cause human disease?
- Can the biological agent cause disease amongst employees?
- Can the biological agent cause disease amongst the Community?
- Are effective prophylaxis / treatments available?

Note: Classification does not account for hazards to people with existing disease, lowered immunity / pregnancy or the allergic or toxic hazards of the biological agent and their products. Such issues **must** be considered in the risk assessment.

At the University you will probably only work with **Hazard Group 1** / **2** biological agents. However, **always** speak to your Supervisor if you cannot find a substance on the 'Approved List' as the University may have to provisionally classify the biological agent as follows:

HAZARD GROUP	CAN IT CAUSE HUMAN DISEASE	IS IT A HAZARD TO EMPLOYEES	CAN IT SPREAD TO THE COMMUNITY	TREATABLE
1	Unlikely	No	No	Not applicable
2	Can cause human disease	May be a hazard	Unlikely	Usually effective prophylaxis / treatment
3	Can cause serious human disease	May be a serious hazard	May spread	Usually effective prophylaxis / treatment
4	Causes severe human disease	Is a serious hazard	Likely to spread	Usually no effective prophylaxis / treatment

In addition to COSHH, other legislative requirements that may apply are the Genetically Modified Organisms (Contained Use) Regulations and the Human Tissues Act.

Genetically Modified Organisms (GMOs) (Contained Use) Regulations: Genetic modification is defined as altering the genetic material of an organism in a way that does not occur naturally. The Regulations cover any activity involving GMOs in which measures are taken to limit contact between them and people or the environment and apply to the actual process of genetic modification, and the use, storage, transport and destruction of GMOs.

Human Tissues Act: Human tissue includes any organ, or part of a human body or a substance extracted from, or from a part of, a human body that contains cells. The Act exists to regulate the removal, storage, use and disposal of human bodies, organs and tissue.

Dolly the Sheep - example of GMO

Controlling Risks – legislative requirements / recommended good practice

One of the key controls to minimise the risk from biological agents is to restrict the number of people who could be exposed to the agent. The simplest way to achieve this is to only allow those directly involved with the work into the area where the biological agent is being used. Another method, and in accordance with the COSHH legislative requirements is 'containment' to prevent the spread of the biological agent outside a specified work area.

As the highest Hazard Group you will ever work with at the University is Hazard Group 2, the following containment level 2 requirements as outlined in COSHH must be adhered to with regards to laboratory design / set up:

LABORATORY CONTAINMENT LEVEL 2 – LEGISLATIVE REQUIREMENTS
1. The hazard warning symbol must be displayed 2. Access must be restricted to authorized persons 3. Specified disinfection procedures must be in place 4. Negative air pressure should be maintained if the laboratory is mechanically ventilated 5. Bench surfaces must be impervious to water, easy to clean, resistant to acids, alkalis, solvents and disinfectants 6. Biological agents must be stored safely 7. Procedures must be in place to contain infectious aerosols eg Safety Cabinet

Safety Cabinet protecting Operator from Biological Agent

Biological Hazard Warning Symbol

However, just having a laboratory designed to contain biological agents is only as effective as the people working in it. If staff and students don't also follow procedures to control the risk of infection to themselves and others then an impervious surface or a Safety Cabinet won't control the risk. The Advisory Committee on Dangerous Pathogens (ACDP) has recognized the 'people' element and produced recommendations that not only outline what people working with biological agents should do, but also additional facilities that should be provided to help people control risk.

ADVISORY COMMITTEE ON DANGEROUS PATHOGENS - RECOMMENDATIONS
Personal Protection Equipment (PPE) must be: • Stored in a well defined place • Checked and cleaned at suitable intervals • Repaired / replaced before further use if defective **Contaminated PPE must be:** • Removed before leaving the work area • Kept apart from uncontaminated clothing • Repaired / replaced before further use if defective
Additional Controls: • Laboratory doors must be closed when work is in progress • Lab coats (preferably side fastening) must be worn and stored separately from personal clothing • No eating, drinking, smoking, taking medication, applying make-up, mouth pipetting • Regular decontamination of bench surfaces • Suitable Safety Cabinet to be used for infectious aerosol containment • Hand wash basins near the exit with non hand operated taps • Hands to be decontaminated immediately contamination is suspected • Hands to be decontaminated after handling infectious materials and before leaving the lab • An autoclave should be readily accessible • Material to be moved to the autoclave in robust containers without spillage • Safe collection, storage and disposal of contaminated waste • Contaminated waste to be suitably labeled • Safe storage of material awaiting sterilization • Immediate reporting of accidents and incidents

Controlling Risks at Bangor University

However, although there are legislative requirements and recommendations with regards to what measures should be in place to control the risks from biological agents, what does the University expect?

The University takes its commitment to protect the health and safety of its staff and students very seriously. As such the University has put in place an array of Policies, Procedures and Safe Working Practices which must be followed to prevent accidents and incidents occurring. With regards to work with biological agents, before any work takes place with biological agents the University expects:

1. **All work with Hazard Group 2 biological agents to be notified to the Biological Safety Officer**
2. **Staff and students to be trained in the risks associated with the biological agent**
3. **A specific risk assessment to be undertaken for the work**
4. **Staff and students to follow all Policies, Procedures and Safety Working Practices**

How to notify work with Biological Agents

Always notify the Biological Safety Officer, Dr John Latchford before starting work with a Hazard Group 2 biological agent. Dr Latchford can be contacted on *j.w.latchford@bangor.ac.uk*. In addition, Dr Latchford can provide advice and guidance if needed.

Training and Supervision

This course will provide you with a basic understanding of likely sources of hazardous biological agents, the hazards they pose and the measures that could be taken to control associated risks. You will also have the opportunity to gain some limited practical experience of essential microbiological techniques. Before allowing you to work with biological agents however, your College / Department may provide you with additional training. This training could consist of a local health and safety induction, a laboratory induction as well as what you will learn throughout your research. In addition, you will receive supervision until such time as you are able to work safely on your own.

Risk Assessments

You **must** always **carry** out a **risk assessment** before working with biological agents and the University has a specific Biological Risk Assessment Form which you can use. But don't worry. A risk assessment is simply a careful examination of the hazards that could cause harm to yourself and others, so you can then decide if you can eliminate them or could do more to control them. However, it is **important** your assessment reflects the nature of your work and identifies all foreseeable risks. It is also recommended that you refer to documents such as ACOPs found on the HSE's website www.hse.gov.uk/biosafety/information and seek advice to make sure your risk assessment reflects your work activity.

The following definitions may be useful when carrying out a risk assessment:

- **Harm:** Any physical damage to the body.
- **Hazard:** Anything that may cause harm.
- **Risk:** Is the chance, that somebody could be harmed by the hazard, together with an indication of how serious the harm could be.

But remember, the biological agent itself isn't the only thing you should consider when undertaking your risk assessment. You should also think about:

CONSIDER	THINK ABOUT…
What is the biological hazard?	• Pathogenic, cultures of known origin • Clinical specimens • Environmental isolates • Zoonotic infection • GMO's • Large scale growth • Environmental hazard
What is the Hazard Group?	Refer to the **Approved List of Biological Agents** or ask your Supervisor
What is the route of Entry?	How can the agent enter the body eg skin contact, inhalation
What is the activity?	• The form eg liquid, solid etc • The concentration and amount of organisms to be used • How often will the organisms be used • How will the organisms be cultured / sampled / processed • Is genetic modification involved • Will the organisms be transported • Will glassware / sharps eg needles be required
Will there be by-products?	• Will harmful by products be produced eg aerosols • Will storage of materials be required • Will disposal of materials be required
Who is at risk (individuals and groups)?	• Those directly handling the material • Others working / visiting the laboratory eg cleaners, porters • Vulnerable persons eg new and expectant mothers, allergies etc
Is the working environment suitable?	• Does the laboratory comply with the containment requirements • Is appropriate equipment available eg Safety Cabinet, autoclave
Is the environment at risk?	• Can the agent be released to the environment and survive • Can the agent affect animals, plants • Can environmental harm be remedied
What emergencies could arise?	• Can aerosols / spills be contained

Once you have identified the hazards you can then work out how you can control the risks arising from them. **But** always apply the **Hierarchy of Controls** principle when doing so, which starts by eliminating the hazard completely if possible. The following are examples of practical control methods, some of which are explained further later in the document. But remember, if you are unsure **ask** someone, eg a Supervisor or Lab Technician:

CONTROLS	**EXAMPLE…**
Eliminate the Hazard	• Substitute harmful organisms with less harmful ones • Eliminate / contain the hazardous process
Risk Reduction	• Use attenuated strains of pathogenic organisms • Use screened specimens of human tissues / blood • Reduce viability of pathogens eg by heat treatment of blood • Reduce the number / concentration of organisms used • Reduce the number of people exposed to the hazard
Engineering Controls	• Control access to the laboratory • Use impervious surfaces • Use safety cabinets and autoclaves
Safe Systems of Work	• Check for, and follow Out of Hours procedures • Training / supervision in techniques, equipment is provided • Good personal hygiene / housekeeping • Methods to minimise aerosol production • Maintenance and testing of equipment • Avoidance of the use of needles, glassware etc • Disinfection / decontamination procedures • Safe disposal, storage procedures
Personal Protective Equipment (PPE) – **only use as a last resort or as an additional control**	• PPE appropriate to the hazard • PPE that fits and the wearer is trained how to use • PPE decontaminated / discarded after use
Immunisation / Health Surveillance	The Biological Safety Officer will notify you of such requirements
Emergency Procedures	• Spill control • Evacuation procedures • Decontamination of both personnel and laboratory • Required first aid and medical treatment

Policies, Procedures and Safe Working Practices

In recognition of the risks associated with biological agents, the University has prepared an array of guidance to assist you and which you **must** refer to before working with them. Further information on the available guidance can be found on the Health and Safety Services Website - A – Z of General Information.

In addition, your College will have local rules and safe working practices in place that must be followed eg:

SAFE WORKING PRACTICES - GENERAL
PERSONAL PROTECTION EQUIPMENT (PPE) - Staff and Students must: • **Wear** PPE as required • **Check, clean** and **replace** PPE at suitable intervals • **Remove** PPE before leaving the laboratory **Contaminated PPE must be stored:** • **Apart** from uncontaminated clothing • **Safely** whilst waiting for decontamination / disposal
PERSONAL HYGIENE – Staff and Students must: • **Wear** Howie laboratory coats (fastened) that are left behind in the laboratory • **Never** eat, drink, take medication, apply make up in the laboratory • **Never** pipette by mouth • **Wash hands** in the designated sink before leaving the laboratory • **Report personal contamination immediately**
GOOD HOUSEKEEPING / PRACTICE – Staff and Students must: • **Shut** the laboratory door when working to prevent unauthorized access • **Disinfect** bench surfaces regularly using an appropriate disinfectant • **Use a** Safety Cabinet if there is a risk of aerosol production • **Label** and **store** securely all biological material including waste • **Never** allow waste to accumulate • **Autoclave** equipment before re-use and hazardous biological material before disposal

SAFE WORKING PRACTICES – HANDLING SAMPLES – GOOD ASCEPTIC TECHNIQUE

HANDLING SAMPLES – Staff and Students must:

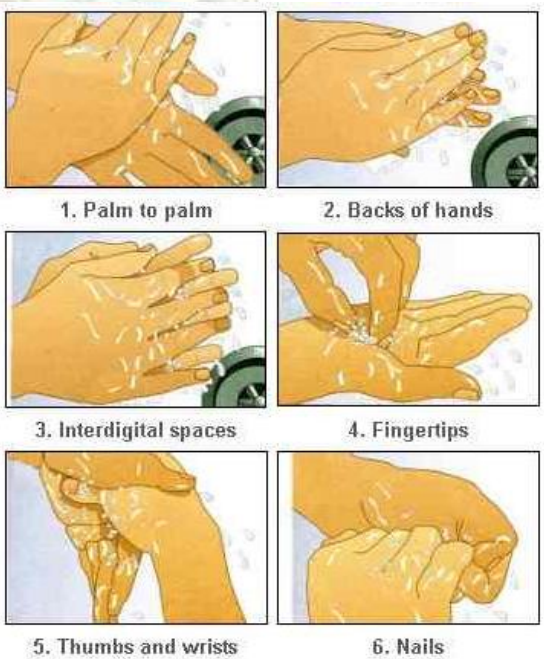
- Keep work areas **uncluttered** and **organised**
- **Disinfect** their bench area before and after work
- **Keep** aerosol production to a minimum eg careful pipetting, or use a Safety Cabinet
- **Keep** draughts to a minimum
- **Open** containers for as short a time as possible
- **Avoid** contact with the tops of tubes / bottles etc
- Use **sterile** disposal containers or flame the tops of glass containers before and after use
- **Carry** out manipulations close to the updraft of a flame
- **Work** methodically eg smooth movements – **never rush**

HANDLING BLOOD - Staff and Students must:

- **Avoid** using sharps to prevent puncture wounds
- **Cover** cuts and abrasions
- **Wear** appropriate PPE to protect eyes, mouth and skin
- **Treat** spills and waste as contaminated
- **Report** needle stick injuries immediately

Howie Lab Coat

Six Stage Hand Washing Technique



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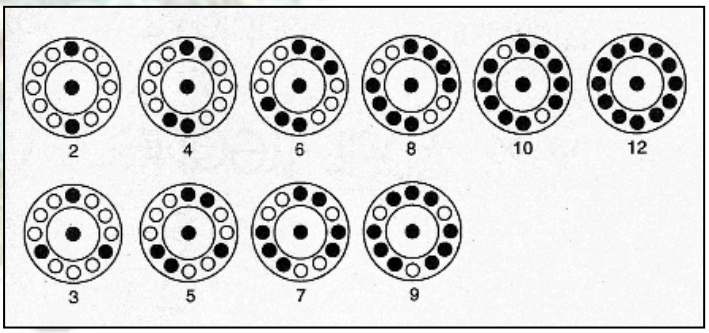
SAFE WORKING PRACTICES – USING GENERAL EQUIPMENT

WHEN HANDLING GENERAL EQUIPMENT – Staff and Students must:

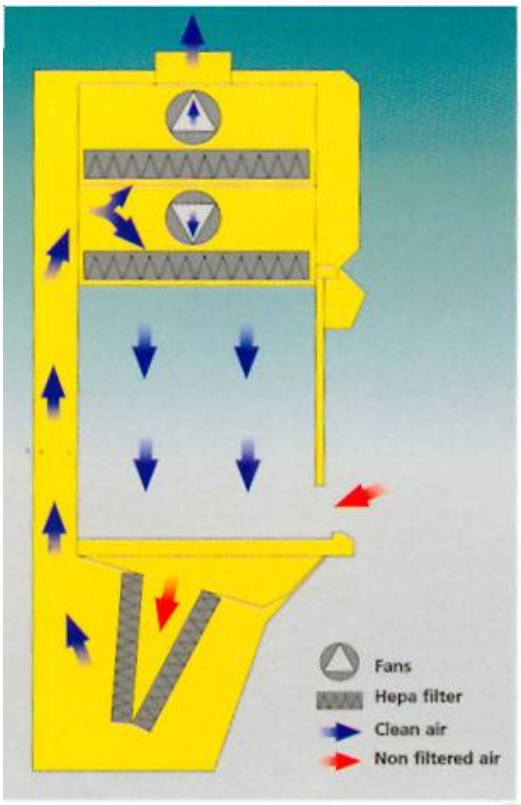
- **Only** use equipment they are trained and authorized to use
- **Use plastic disposable loops** if possible. If using metallic microbiological loops ensure they are closed, less than 5cm long and cooled before touching colonies
- **Check pipette devices** are in good condition and fitted properly. **Never** bubble air through cultures or forcibly expel the last drop
- **Avoid** the use of sharps and glassware
- **Never** stack petri dishes too high or where they could be knocked over
- **Open** ampoules carefully
- **Use** a Safety Cabinet for Hazard Group 2 organisms if there is a risk of exposure via inhalation
- **Use** homogenisers, sonicators in a Safety Cabinet if there is a risk of airborne infection
- **Use** a Safety Cabinet if filtration is to take place under pressure
- **Use** strong sealed tubes and bottles in centrifuges. Centrifuges must be balanced properly and run at the correct speed
- **Secure** flasks in shakers and make sure they are capped / plugged
- **Never** put sealed vessels / metal vessels in the microwave
- **Report defective equipment immediately**

Note: Information Sheets on the safe use of some laboratory equipment eg centrifuges, safety cabinets, microwaves, autoclaves are available on the HSS Website.

Inoculation Loop



Balancing a Centrifuge Properly



Air Flow in a Safety Cabinet

Safety Cabinets: Are used for the containment of infectious materials. At the University Class II Safety Cabinets are generally used which means they are suitable for work with Hazard Group 2 organisms, offering both protection to the operator and the sample. They are particularly useful for handling cell cultures.

Class II Safety Cabinets work by air being drawn through the open front of the cabinet and then drawn downwards through grills in the base of the cabinet. The air passes up the back of the cabinet and is then HEPA filtered. A proportion of the air is then vented (normally to the outside) whilst the rest is re-circulated downwards through the working area providing a curtain of filtered air.

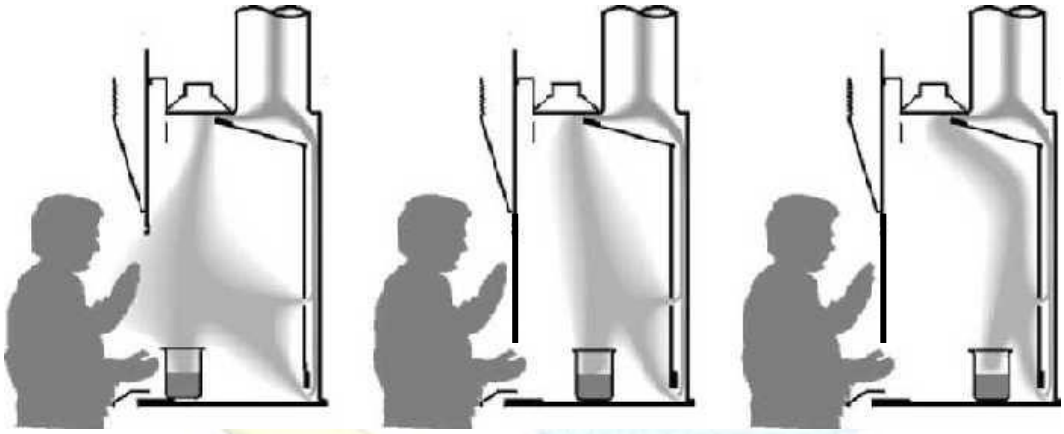
HEPA filters are made of glass fibre paper and should retain at least 99.997% of a challenge dose of particles of a relevant size, and are normally protected by coarse filters which remove large particles. The filters will eventually become clogged and need replacing. At the University their condition is monitored as part of their annual inspection with replacement of filters undertaken as necessary.

SAFE WORKING PRACTICES – USING SAFETY CABINETS

WHEN WORKING WITH SAFETY CABINETS – Staff and Students must:

- **Never** disrupt the airflow eg draughts from people walking past, use of flames in the cabinet, excessive amounts of apparatus or careless work practices
- **Check** the fan is working before use and the airflow is adequate
- **Check** the Inspection label shows it is in test date
- **Keep** apparatus to a minimum
- **Avoid** using gas or use a small flame that does not disrupt the air flow
- **Work carefully.** Avoid creating aerosols and confine operations to the middle and rear of the cabinet
- **Never** use centrifuges inside the cabinets
- **Run** the fan for 10 – 15 minutes after work is finished
- **Clean** surfaces with an appropriate disinfectant
- **Treat** any towels used to wipe / clean the safety cabinet as biological waste and ensure it is autoclaved before disposal
- **Report damage / faults immediately**

To ensure your health and safety it is very important to keep the Safety Cabinet sash as low as possible whilst still being able to work safely. In addition, try to work with the material positioned at the back of the cabinet as both will increase the level of protection the cabinet will afford you.



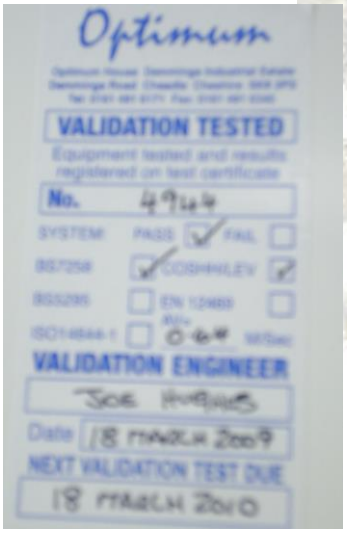
Bad Placement of Materials

Good Placement of Materials

Best Placement of Materials

Other things to look for before working with Safety Cabinets at the University are:

1. Check the Test Label is in date (see below). If it isn't don't use the Safety Cabinet and report it to your Supervisor or Lab Technician immediately.
2. Check the display to ensure the air flow is sufficient.
3. Hold a piece of paper in front of the Safety Cabinet (see below). If the Safety Cabinet is working properly, the paper will drawn into it, however don't let go as the paper could block the vents!



Test Label

Display

Checking the Air Flow

Safe Storage: At the University infectious substances must be stored safely and securely, properly labeled and discarded safely when no longer required. Always:

SAFE WORKING PRACTICES – SAFE STORAGE
Fridges / Deep Freezers: • **Use** leak proof and robust containers • **Never overfill** compartments • **Keep flammable solvents** in explosion proof fridges • **Wear** insulated gloves when handling materials • **Defrost freezers** as necessary • **Secure** fridges etc when not in use
Freeze Drying: • **Assess** the risk of explosion of vessel, contamination of apparatus etc • **Protect** vessels under negative pressure with plastic film, mesh etc • **Screen** apparatus, operators must wear face visors • **Handle** freeze dried powders of hazardous agents in a Safety Cabinet
Ampoules: • **Open** in a Safety Cabinet • **Glass** ampoules can be opened by scoring with a glass file and cracking glass with a red hot glass rod
Liquid Nitrogen Storage: • **Store** in a well ventilated area • **Wear** cryo gloves and face visors • **Store** infectious substances in the vapour phase • **Never** store inhalable infectious agents in liquid nitrogen if there is risk of vials exploding • **After** removal from the bank, cover ampoules with a stout plastic / metal container until gas evolution stops, swab with disinfectant, thaw in tepid water and open carefully • **Always** assume banks and their contents are contaminated

When labeling your samples you must include what the agent is, your name and date so others can instantly identify the risks associated with what is in the container and be able to get in touch with you if they need to.

In addition, you must also make sure biological waste or items awaiting autoclaving are easily identifiable as hazardous biological material.

Correct Labeling of Samples

Transporting Agents: To prevent the spread of infectious agents the University has strict controls in place with regards to their movement. In addition, there are strict legislative requirements which must be met when transporting biological agents externally and failure to comply could lead to legal action being taken against the University. When moving biological agents always:

SAFE WORKING PRACTICES – TRANSPORTING BIOLOGICAL AGENTS
WHEN TRANSPORTING BIOLOGICAL AGENTS INTERNALLY Staff and Students must: • **Be trained** in the safe transportation of biological agents • **Use** robust, leak proof containers • **Label** containers with the biohazard sign, the laboratory of origin and emergency contacts • **Ensure** the external surfaces of containers are free from contamination • **Keep** documentation separate from the containers
WHEN TRANSPORTING BIOLOGICAL AGENTS EXTERNALLY Staff and Students must: • **Contact** the Dangerous Goods Advisor if sending by post or courier • **Be trained** in the safe transportation of biological agents • **Ensure** containers are correctly packaged and labeled • **Agree** the consignment is acceptable with the recipient before sending • **Ensure** documentation is completed correctly • **Keep** documentation separate from the containers
If transporting materials by road / rail, samples must be triple packed which includes: • **Primary** durable, watertight, leak proof container wrapped in absorbent material • **Secondary** durable, watertight, leak proof container (may contain several primary receptacles) • **Outer** protective shipping package • **In addition, contact** the Dangerous Goods Advisor if sending by post or courier

Autoclaving: Another important control to prevent the spread of infectious materials is to ensure equipment which is to be reused and materials waiting for disposal are **sterilised**. The University uses **autoclaves** for this purpose that work by rendering the biological agent non-viable by exposing it to high pressure steam. However, sterilization does depend on the steam penetrating all parts of the load so lids from containers must be loosened and plastic bags undone to allow steam to penetrate.

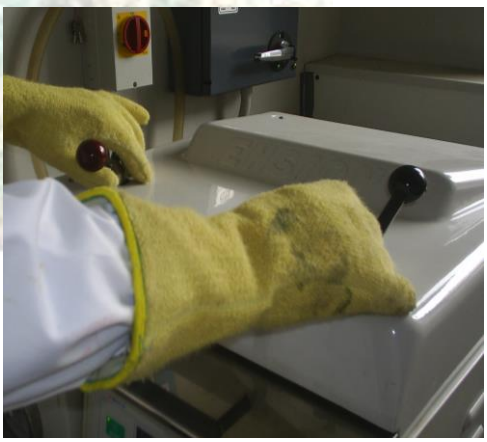
In addition, care must be taken when using autoclaves as they are pressurized, become very hot during the autoclaving process and have the potential to cause serious injuries. Manual handling can also be a problem, especially with top loading autoclaves so always ask for help if you are having difficulty loading / unloading it.

SAFE WORKING PRACTICES – USING AUTOCLAVES

WHEN WORKING WITH AUTOCLAVES – Staff and Students must:

- **Be trained** and authorized before using autoclaves
- **Complete** the autoclave Log Book before using it
- **Always** wear a lab coat and heat resistant gloves – keep sleeves tucked into the gloves
- **Label** all wastes clearly
- **Never** allow waste to build up or autoclave too much in one go
- **Use** appropriate leak proof containers or suitable bags to contain solid wastes
- **Place** petri dishes containing agar is a suitable tray to contain leaks
- **Loosen** tops and **leave** space between items to allow steam to penetrate
- **Select** the right autoclave cycle and ensure the lid is **secure** before starting
- **Check** the chamber pressure is **zero** before opening the door after autoclaving
- **Report damage / faults immediately**

Howie Lab Coat



Heat Resistant Gloves

Disinfection: Is also sometimes used to sterilize materials instead of autoclaving, especially when decontaminating small items of disposable equipment, heat sensitive equipment, surfaces and large items of equipment and spills.

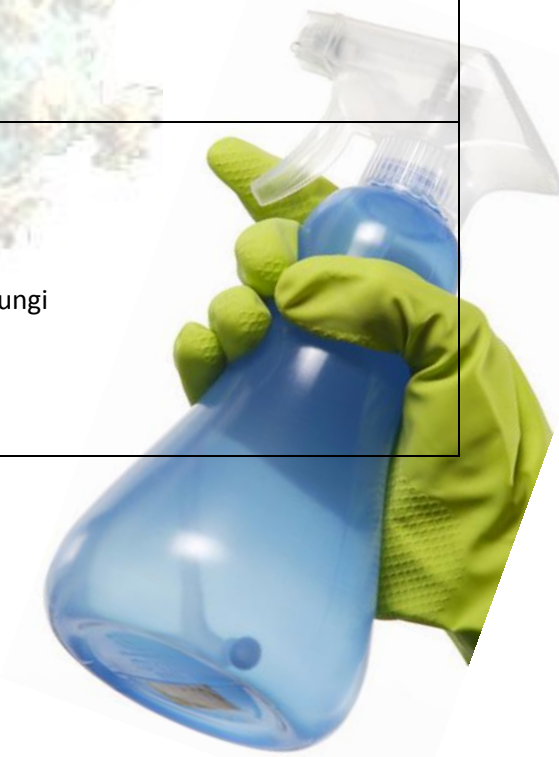
However, because there is no universal disinfectant to deal with all biological agents, disinfection is not as reliable as autoclaving. In addition, they may deteriorate if left to stand for long periods, be inactivated for example by organic matter and most are toxic or irritant creating additional risks when handling them.

Before working with known organisms you must also test the efficacy of the disinfectant against the organism under the conditions of use to ensure it will work. When handling materials which may contain a variety of agents, a disinfectant should also be selected that is likely to be active against all the possible hazards that may be encountered. It is also important that:

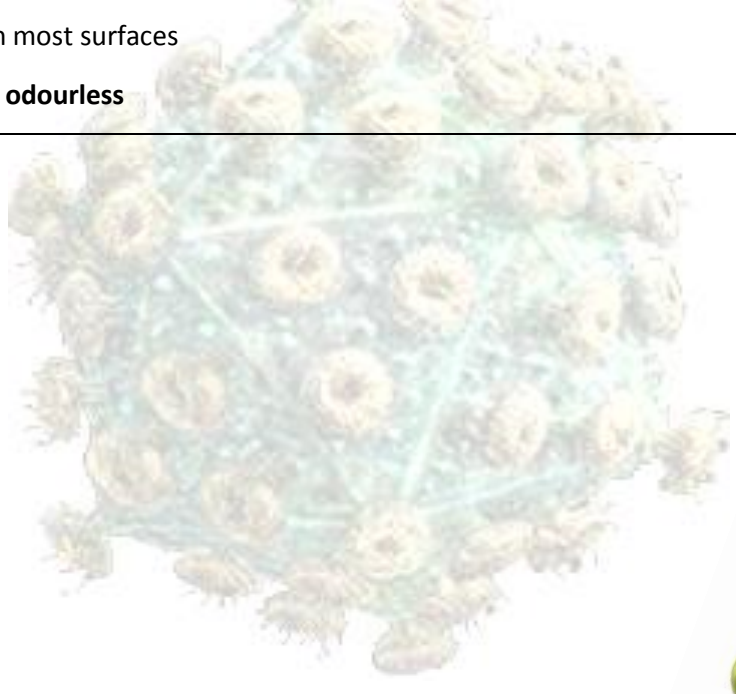
- Disinfectants are diluted accurately and appropriate PPE is worn
- They are used in accordance with suppliers instructions and protocols
- Toxic, irritant, corrosive and allergic hazards are controlled
- Disinfectants that are likely to have become inactive are discarded

Disinfectants commonly used at the University are:

DISINFECTANTS
Hypochlorite Solutions: • **1,000ppm** recommended for surface decontamination, **2,500ppm** for discard containers, **10,000ppm** for spillages • **Active** against bacteria, spores and viruses • **Limited** against fungi and tubercule bacilli • **Compatible** with anionic / non-ionic detergents • **Corrode** metals and damage rubber • **Inactivated** by organic materials and need frequent changing
Clear Soluble Phenolics: • **Active** against vegetative bacteria including tubercule bacilli • **Not active** against spores, many viruses and limited effect on fungi • **Compatible** with anionic / non-ionic detergents • **Inactivated** by rubber and some plastics



DISINFECTANTS
Alcohols: • **Are flammable** • **Normally** used as 60-80% vv solutions in water • **Active** against protozoa, many viruses and vegetative bacteria including tubercule bacilli • **Can be used** as a disinfectant skin rub • **Do not** readily penetrate organic matter
Virkon: • **Claimed** to be active against any type of organism • **Relatively** non-toxic and non-corrosive
Trigene: • **1:100** recommended concentration for all normal laboratory requirements • **Claimed** to be active against spores, bacteria, viruses, fungus, denatures DNA / RNA • **Claimed** to be bio-degradable, non-irritant, non-fuming, non-hazardous, non-corrosive • **Can be used** on most surfaces • **Colourless** and **odourless**



Emergencies: Due to the potential risks associated with biological agents, **emergency procedures** will be put in place in the unlikely event something goes wrong. It is important you **familiarize** yourself with the emergency procedures for the areas you work in, so if for example a spill occurs or you become contaminated you can deal with the situation promptly. In addition, **learn emergency contacts**, first aid arrangements etc. As a general rule:

EMERGENCY PROCEDURES
EVACUATE if there is a risk of **AIRBORNE INFECTION** and **IMMEDIATELY CONTACT** the **BIOLOGICAL SAFETY OFFICER** or **LAB MANAGER**
SMALL SPILLS – Staff and Students must: • **Cover** the spill with disinfectant granules or absorbent paper / cloth soaked in disinfectant • **Allow** the granules / paper to work eg wait 15 minutes • **Collect** the debris eg use stiff card to sweep into a dustpan • **Pick up** glass etc with forceps, swabs or use puncture resistant gloves • **Place** debris, glass etc in a suitable container and label as biological waste • **Disinfect** contaminated surface and any equipment used eg dustpan again
LARGE SPILLS – Staff and Students must: • **Immediately contact** the **Biological Safety Officer** or **Lab Manager** taking care not to spread contamination outside of the laboratory
PERSONAL CONTAMINATION – Staff and Students must: **SKIN CONTAMINATION** • **Remove** contaminated clothing immediately • **Wash** contaminated skin with lots of soap and water • **Immediately contact** the **Biological Safety Officer** or **Lab Manager** **FACE / EYES CONTAMINATED OR CUTS / NEEDLESTICK INJURIES** • **Wash** eyes and mouth with plenty of water • **Call** for first aid attention • **Allow** small puncture wounds to bleed, then wash with plenty of soap and water before dressing • **Wash** minor cuts / lesions with plenty of soap and water before dressing • **Report incidents immediately** to the **Lab Manager** and **Biological Safety Officer** **INGESTION / INHALATION** • **Immediately contact** the **Biological Safety Officer** or **Lab Manager**

Note: All accidents and incidents must be reported on the Accident and Incident Reporting Form to the College Health and Safety Officer. A copy of the Form must also be sent to Health and Safety Services.

A supply of masks to Standard **CE EN149:2001 FFP3** capable of protecting against airborne infectious agents **must** be available in the immediate area outside the Laboratory. A suitable mask is the **3M 1863 Health Care Respirator (P3).** Users must also be trained in the correct fitment and use of the mask.

Health Surveillance

Before starting work with biological agents you must **inform** the Biological Safety Officer who can assess if health surveillance, vaccinations are needed. This is particularly important if you have existing health problems eg dermatitis, allergies or are pregnant. But don't worry, this is not to stop you carrying out work, it is purely to make sure the University has considered the risks fully and done everything it can to ensure your health and safety. Then, if you are asked to attend health surveillance you will be asked to meet the University's Occupational Health Practitioner (in confidence) who may need to undertake some tests eg lung function.

It is also very important you understand the possible risks associated with your work so you can monitor your own health. If at any point you feel your health may be suffering as a result of work, contact the Occupational Health Practitioner or your General Practitioner immediately.

If your situation changes eg you develop an allergy or become pregnant you must let the Biological Safety Officer know immediately.

Further Guidance and Information

Health and Safety Services	**3847** or visit the Website at **hss.bangor.ac.uk**
Biological Safety Officer	j.w.latchford@bangor.ac.uk
Advisory Committee on Dangerous Pathogens	www.dh.gov.uk/ab/ACDP/index.htm
Health and Safety Executive	www.hse.gov.uk