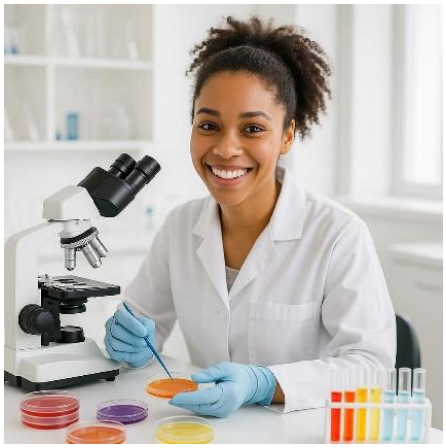
Safety considerations for *Phytotitre* projects

Background to laboratory safety



Being able to work safely in the laboratory is one of the most critical skills you should aim to achieve during your research project. Learning and adhering to laboratory safety protocol is a core element of personal and institutional responsibility. The *Phytotitre* laboratory projects involve the use of diverse chemical and biological agents which could pose significant risks if mishandled. Rigorous adherence to safety protocols helps you to maintain a safe environment not only for yourself and co-workers, but also for visitors and non-scientific staff, such as cleaners and maintenance teams.

Mastering these protocols is a core competency in your development as a scientist, and essential to meet the "Good Laboratory Practice" (GLP) standards required in the global pharmaceutical and biotech industries.

Major types of hazard encountered in the laboratory

Three major categories of hazard are commonly encountered when working in a Life Science laboratory. These are **chemical hazards**, **biological hazards** and **physical hazards**. The following sections list common examples of each of these types of hazard.

Chemical hazards

There are six major forms of chemical hazard that you may come across in the laboratory, as listed below:

1. Corrosives

Corrosives are substances that cause visible destruction or irreversible alterations in living tissue by chemical action at the site of contact. They are particularly damaging to unprotected eyes and skin.

Examples:	Acetic acid, Hydrochloric acid (HCl), and Sodium Hydroxide (NaOH).
Mitigation:	Wear safety goggles when working with corrosive liquids and work near an eyewash station.

2. Irritants and Sensitizers

Irritants can cause temporary inflammation at the site of contact that generally resolves to leave no lasting harm. Sensitizers can cause an allergic reaction after repeated exposure to the same substance, rendering some people sensitive to that substance over the longer term.

Examples:	Sodium Dodecyl Sulfate (SDS) and (in some people) antibiotics like Penicillin.
Mitigation:	Handle fine powders in a fume hood or wear a face mask. Use pre-prepared liquids instead of powders whenever possible.

3. Toxic and Highly Toxic Agents

These chemicals can cause serious biological harm or even death by interfere with metabolic processes.

Examples:	Actinomycin D. This compound, which is used in the anti-inflammatory discovery project, is a potent transcription inhibitor that is very toxic if ingested or inhaled.
Mitigation:	Use a Class II safety cabinet to prevent aerosolization. Double-glove when handling concentrated stocks.

4. Mutagens and Carcinogens

These substances can cause changes to the DNA of cells. Mutagens may lead to genetic mutations, while carcinogens specifically increase risk of developing cancer.

Examples:	Crystal Violet (mutagenic potential) and MTT (a tetrazolium salt). These dyes can interfere with human DNA if absorbed through the skin.
Mitigation:	Use "Benchkote" (plastic-backed absorbent paper) to catch drips. Dispose of all contaminated plastics in the hazardous chemical waste stream.

5. Flammables

Flammables are liquids or gases that can ignite easily at room temperature.

Examples:	Methanol (used for fixing cells) and Ethanol (used for sterilization).
Mitigation:	Keep bottles capped when not in use. Never use a Bunsen burner in the same workspace where alcohols are being used for cell fixation.

6. Penetrants / Carriers

These are chemicals that are not necessarily highly toxic on their own but can transport other hazards into the body.

Examples:	DMSO (Dimethyl Sulfoxide). This is the solvent for your plant extracts. It is capable of crossing the skin barrier, and if it contains a dissolved toxin, it can help transport that toxin into your body through your skin, or even through certain types of gloves.
Mitigation:	Use Nitrile gloves, as DMSO passes through Latex easily.

Quick-Reference Table

Hazard Type	Example	Primary Precaution
Toxic	Actinomycin D	Use in Safety Cabinet; Double-glove.
Mutagen	Crystal Violet / MTT	Prevent skin contact; dispose of via dedicated waste route.
Flammable	Methanol / Ethanol	No open flames; Store in fire-safe cabinet.
Corrosive	Acetic Acid / HCl	Eyewash proximity; Goggles.
Penetrant	DMSO	Nitrile gloves; Wash skin immediately if contact occurs.

GHS pictograms

Containers of hazardous chemicals should be labelled with one or more of 9 **pictograms**, which are part of the Globally Harmonized System (GHS) for chemical labelling. You will need to learn what each of these pictograms means with respect to each type of chemical hazard they represent (please see table below). If no pictogram is shown on the product container, the chemical it contains is typically non-hazardous.

Pictogram	Name	What it Means	Common Lab Examples
	Irritant / Warning	Less severe health effects: skin/eye irritation, dizziness, or allergic skin reactions.	SDS, dilute acids, many standard buffers.
	Serious Health Hazard	Indicates carcinogens, mutagens, reproductive toxins, or respiratory sensitizers.	Crystal Violet, MTT, Formaldehyde.
	Acute Toxicity	Potentially fatal or highly toxic if swallowed or inhaled.	Actinomycin D, Sodium Azide, Cyanides.
	Flammable	Gases, liquids, or solids that ignite easily. Includes self-reactives and pyrophorics.	Ethanol, Methanol, Acetone, Isopropanol.
	Oxidizer	These substances provide oxygen, allowing other materials to burn more fiercely or even explode.	Hydrogen Peroxide, Nitric Acid, Potassium Permanganate.
	Gas Under Pressure	Compressed, liquefied, or dissolved gases. Can explode if heated or if the valve is damaged.	CO_2 cylinders (for incubators), Nitrogen tanks.
	Corrosive	Causes severe skin burns and eye damage.	Sulfuric Acid, Hydrochloric Acid, Glacial Acetic Acid.
	Explosive	Unstable substances that can undergo a sudden, violent chemical reaction.	Picric acid (if dried out), Azides (at high concentrations).
	Environmental Hazard	Toxic to aquatic life with long-lasting effects. Should never be disposed of down the sink.	Crystal Violet, Copper salts, many organic solvents.

Biological hazards

Biological hazards are those living organisms, or products of living organisms, that may cause disease in humans or animals. They are categorized into four different **Hazard Groups** (HG), on the basis of: (i) their ability to cause disease, (ii) the severity of that disease, (iii) the risk of it spreading to the community and (iv) the availability of treatments or vaccines. Laboratories in which such organisms are cultured or studied must meet a minimum level of security to prevent their accidental release, referred to as the **Containment Level**. Examples of micro-organisms in each Hazard Group, and the respective necessary containment level, are listed below.

Hazard Group 1 (Low Risk)

Organisms in Hazard Group 1 (HG1) are very unlikely to cause disease in healthy humans. They generally do not pose a threat to healthy laboratory workers or the public, although they may cause illness in immunocompromised subjects.

Containment:	Containment Level 1 - Standard laboratory practices with no requirement for air handling or use of specialized safety cabinets. Similar to a typical university teaching laboratory.
Examples:	*Micrococcus luteus*, *Escherichia coli* K12 (the lab strains used in your antibiotic projects), and most common baker's yeast (Saccharomyces cerevisiae)

Hazard Group 2 (Moderate Risk)

Organisms in Hazard Group 2 can cause disease in healthy humans and may be a hazard to employees, but are unlikely to spread to the community. Effective prophylaxis (e.g. vaccines) or treatment (e.g. antibiotics) is usually available.

Containment:	Containment Level 2 - Access is restricted, air flow if carefully controlled, and work that may create aerosols must be done in a Class II Safety Cabinet. Commonly seen in university cell culture laboratories.
Examples:	*Staphylococcus aureus*, Hepatitis A, and many common human cell lines (due to their potential to harbour latent viruses)

Hazard Group 3 (High Risk)

Organisms in Hazard Group 3 can cause severe human disease and present a serious hazard to employees. There is a high risk of spreading to the community, but effective prophylaxis (such as vaccination) or treatment (such as antibiotics or anti-sera) is usually available.

Containment:	Containment Level 3 - These are highly specialised laboratories, with strict ventilation (negative pressure) and air filtration procedures applied. There are also strict clothing protocols and an autoclave within the laboratory.
Examples:	Mycobacterium tuberculosis (TB) and HIV

Hazard Group 4 (Extreme Risk)

Organims in Hazard Group 4 can cause severe disease or death in humans and are a serious hazard to employees. They are likely to spread easily to the community if mishandled, and there is usually no effective prophylaxis or treatment available.

Containment:	Containment Level 4 - This is the highest level of containment. These labs are very rare, and only present in heavily secured buildings. Workers must wear positive-pressure “space suits” with a dedicated air supply.
Examples:	Ebola virus, Marburg virus, and Lassa fever

Genetically modified organisms

Some organisms are genetically engineered by scientists to study particular pathways or processes, or to produce useful materials, such as recombinant proteins. Most genetically modified organisms (GMOs) made for these purposes are very safe, but there is always a risk of unintended consequences of genetic alteration of a target organism.

Examples:	*E. coli* BL21 cells or HEK-293 cells expressing a recombinant protein.
Mitigation:	Work with GMOs in a laboratory meeting at least Containment Level 2. All planned work in the use or creation of GMOs must receive approval from an institutional Genetic Modification Safety committee before starting work.

Biological Products & Toxins

Some non-living materials derived from organisms, such as toxins, can be classified as biological hazards.

Examples:	LPS (lipopolysaccharide / endotoxin) is a potent trigger of inflammation. If it enter the bloodstream, it can trigger a massive inflammatory response (fever, chills, or even shock).
Mitigation:	Handle LPS as a toxin. Avoid creating aerosols (don't vortex open tubes) and clean all surfaces after use.

Biological Waste

Anything that has come into contact with any of the agents listed above becomes a biohazard itself, and should be disposed of accordingly via the correct waste stream (discussed below).

Examples:	Used pipette tips, spent growth media, and 96-well culture plates.
Mitigation:	All biological waste must be inactivated before it leaves the laboratory (usually via autoclave or 1% Virkon/Bleach).

Physical hazards

There are eight major forms of physical hazard that you may come across in the laboratory, as listed below:

Sharps

Sharps are any objects that can easily puncture or cut the skin. They increase the risk not only of causing a wound directly, but also accidentally inoculating a chemical or biological hazard into your body.

Examples:	Glass Pasteur pipettes, scalpels, needles, microscope slides, broken glassware.
Mitigation:	Place all used sharps in a specialised sharps bin, which should not be over-filled. Always use a brush and dustpan to pick up broken glass, never your hands. Use plastic alternatives where possible.

Extreme Temperatures

Life Science research frequently requires collection of samples from ultra-cold storage (e.g. -80ºC freezers, -150ºC freezers, -176ºC liquid nitrogen), and the handling of heated samples (e.g. molten agar prior to pouring plates).

Examples:	Contact with cold metal or frozen vials can cause “cold burns” (frostbite) very quickly.
Mitigation:	Use insulated “cryo-gloves” for freezer work and heat-resistant gauntlets for hot liquids. Allow autoclaved media to cool significantly before moving it.

Electrical equipment

Laboratories contain many electrically powered instruments which bring the risk of electrical shock if damaged or mis-used.

Examples:	Plate readers, incubators, shakers and computers.
Mitigation:	Ensure electrical safety checks for laboratory equipment are up to date. Keep liquids away from power strips.

Centrifugation Hazards

Centrifuges accelerate samples to extremely high g-forces - often as high as 13,000 to 15,000 times the force of gravity. If a machine fails at this speed, the result can be a powerful explosion.

Examples:	Mechanical failure due to an unbalanced load can cause the centrifuge to wobble off the bench or, in extreme cases, explode.
Mitigation:	Make sure to balance the masses on both sides of the rotor's central spindle accurately. Always ensure the bucket lids or the rotor lid are securely fastened before starting. Wait for the rotor to stop completely before opening.

Pressurized Gas

Many laboratories contain pressurized gas cylinders, for example, to support the maintenance of a CO_2 atmosphere in mammalian cell incubators.

Examples:	High-pressure cylinders, such as CO_2 cylinders, if knocked over and the valve shears off, can become "missiles" capable of traveling through walls.
Mitigation:	Ensure gas cylinders are always chained or "strapped" to a wall or bench.

Ergonomics, slips and spills

Watch out for trip hazards (don't leave your bag on the lab floor), and slipping from spills. Bear in mind also that repetitive actions can result in Repetitive Strain Injury (RSI).

Examples:	Washing 96-well plates (especially in the L929 assay) often results in water on the floor. Long periods of multichannel pipetting can result in RSI.
Mitigation:	Clean up floor spills immediately. Take micro-breaks every 20 minutes of pipetting to stretch your hands and wrists.

Ionising radiation

Ionising radiation is a specific form of radiation that is capable of detaching electrons from atoms (ionisation). This can cause significant damage to living tissue and DNA. High doses can cause acute radiation sickness, while long-term low-level exposure increases the risk of cancer.

Examples:	Radioactive isotopes such as Tritium (^{3}H), Carbon-14 (^{14}C) and Phosphorus-32 (^{32}P) are commonly used as tracers in life science studies.
Mitigation:	Time, Distance, and Shielding: Minimize time near the source, maximize distance, and use appropriate shielding (e.g., Lead for Gamma rays, or thick Perspex/Plexiglass for high-energy Beta emitters like ^{32}P). Use Geiger counters and personal dosimeter badges to track exposure.

Non-Ionising Radiation

Non-ionising radiation lacks the energy to ionise atoms but can still cause harm through thermal effects or photochemical reactions. UV light can cause eye damage and skin burns. Prolonged exposure increases the risk of skin cancer.

Examples:	Ultraviolet (UV) light (found in transilluminators and Microbiology Safety Cabinets) and Lasers (used in Confocal Microscopy or Flow Cytometry).
Mitigation:	Use wavelength-specific safety glasses when working with UV light or lasers.

Hazards you are likely to encounter during a *Phytotitre* project

The specific chemical, biological, and physical hazards you are likely to encounter during each of the *Phytotitre* laboratory projects are listed in **Appendices 1, 2 & 3**, respectively.

Phytotitre projects are typically NOT associated with the following hazards:

- Use of naked flames
- Use of genetic recombination techniques or genetically modified organisms (GMOs)
- Use of organisms in Hazard Group 3 or Hazard Group 4
- Use of ionising radiation or radiatioactive compounds

Strategies for the mitigation of risk

Once we have acknowledged the types of hazard associated with a new project, we must think about how to minimise the risk of these hazards causing harm to those working on the project and others. These strategies are referred to as risk **mitigations**. The 5 most common forms of risk mitigation in laboratory projects are listed below.

Mitigation 1 - Elimination and Substitution

The most effective mitigation is to remove the hazard entirely or replace it with something less dangerous. For example, if a method suggests the use of hot acid or solvent for a particular step, but alternative substances that are less hazardous can be used to achieve the same results, these should be chosen. Notably, the *Phytotitre* kit has been specifically curated to include extracts from plants with a history of safe human use, eliminating many of the extreme toxicities found in randomly assembled natural product libraries.

Mitigation 2 - Engineering Controls

These are physical changes to the workspace that “engineer” the hazard away from the worker. For example, a Microbiology Safety Cabinet (MSC) provides a physical barrier of filtered airflow to prevent the operator from exposure to biological aerosols. Fume hoods are used when handling toxic powders (such as SDS or actinomycin-D), or volatile solvents like methanol and acetic acid, to prevent inhalation. Safety interlocks on equipment such as centrifuges and UV lights prevent their accidental opening while in operation.

Mitigation 3 - Administrative Control

Standard Operating Procedures (SOPs) are a primary mitigation tool. They are the rules and procedures that govern how work is performed, ensuring that workers follow the same, safety-validated path each time they perform an experiment. Additionally, workers should have appropriate training, and their competency signed off by an experienced supervisor. Further measures include restricting access to higher risk laboratories (such as via card access), introducing specific protocols for lone working and ensuring every secondary container is labeled with the correct GHS hazard symbols.

Mitigation 4 - Implement Safe Working Practices

This refers to the daily habits that prevent cross-contamination and accidental exposure. Workers must become proficient in aseptic technique to prevent the accidental release of microorganisms into the environment. A “One-Glove” rule should be in place for the transport of samples between laboratories to prevent contamination of surfaces in communal spaces, such as door handles. Handwashing should be mandatory every time a worker exits the laboratory, regardless of glove use, and this should be in a dedicated hand-washing sink, not the laboratory sink. Long hair should be tied back before working.

Mitigation 5 - Wear Personal Protective Equipment (PPE)

Personal Protective Equipment (PPE) is considered to be the last line of defense in laboratory safety. It protects the wearer to some extent, but does not remove the hazard itself. It is implemented assuming all other mitigations are in place. The key forms of PPE are as follows:

		
Lab Coats Should be side-fastened and have elasticated cuffs to prevent sleeves from dragging through chemicals or into flames.	**Disposable Gloves** Gloves should be nitrile (not latex) to protect against DMSO, and discarded to the correct waste stream after completing work in the lab.	**Eye Protection** Safety glasses or goggles should be worn whenever working with hazardous liquids (e.g. corrosives, solvents).

<u>At a minimum, the following must be worn at all times when working in the laboratory:</u>

1. Laboratory coat: Buttoned to the top, sleeves rolled down.
2. Gloves: Nitrile, not latex.
3. Safety glasses: Impact-resistant, with side shields.
4. Footwear: Closed-toe shoes only (no sandals).

Completing a risk assessment

The first task to complete when aiming to minimise the risk of harm on a new project is to complete a **Risk Assessment**. You should discuss with your supervisor who will prepare this document, then read and sign the risk assessment associated with your project.

If you are writing the risk assessment yourself, you will need to complete the following tasks:

1) List all of the chemical, biological and physical hazards associated with your project
2) Collect technical information on the types of chemical and biological hazards
3) Prepare a realistic plan for how to protect workers by mitigating the risk

The technical information you require to assess the hazards associated with each chemical is given in the **Safety Data Sheet** (SDS), which is provided by the manufacturer of the chemical. This document lists all of the hazards associated with that substance and gives advice on how to mitigate those risks. You can find the SDS associated with the *Phytotitre* kit on the Downloads page of our website. As part of the risk assessment process, you should collate the safety information from these SDS documents into a separate **Control of Substances Hazardous to Health** (COSHH) document. This document will summarise the risks associated with the substances used in your specific project and how they will be mitigated. Make sure you have read the relevant SDS and COSHH documents for your project before starting.

For the biological hazards section of your risk assessment, you will have to decide what Hazard Group the organism you are choosing to work with belongs to. The bacterial strains we recommend working with are in Hazard Group 1, and the mammalian cell-lines we recommend using are in (at most) Hazard Group 2.

Reminder: Always show your draft risk assessment to your supervisor before submission, as they are the 'Principal Investigator' (PI) responsible for your supervision.

Be aware of and follow your local health and safety rules

In addition to reading the relevant risk assessment, SDS and COSHH documents, there may be other procedures necessary to complete before you are able to work in the laboratory. Please ask your supervisor for advice on what else may be necessary to complete before starting.

Many university laboratories also require students to attend a mandatory safety and general laboratory induction before starting the project. If this is part of the process at your institution, make sure you attend such induction. You should then ask the relevant lab manager, technical staff or day-to-day supervisor to train you in the basic techniques necessary for success on your project.

Disposal of waste

Most laboratory waste **CANNOT** be placed in a normal domestic waste bin. The use of different, hazard-specific waste streams is necessary to protect members of the public from the chemical and biological hazards that may be present in laboratory waste. The major waste streams you may encounter during the *Phytotitre* projects are summarised below.

Infectious Clinical Waste

This is the primary stream for your “wet” laboratory work involving biological agents.

What goes in:	Items contaminated with Hazard Group 1 or 2 organisms, cell culture waste, and gloves used during biological work.
Treatment:	This waste is typically sent for **autoclaving** (steam sterilization) before leaving the premises, then it may go to landfill or incineration.
Bag colour:	Orange

Hazardous Clinical Waste

This stream is for waste that is both infectious and contaminated with hazardous chemicals. Note that most bacterial media and cell culture media are not considered hazardous, so can be dealt with as 'Biological Liquid Waste', as described below.

What goes in:	Items that have been in contact with both biological agents and hazardous laboratory chemicals at the same time.
Treatment:	Sent for incineration (not autoclaved before leaving premises)
Bag colour	Yellow

Sharps Waste

Sharps must never be placed in bags since they may cause injury to those removing the waste. They must go into puncture-resistant plastic bins. As these bins are sent for incinerated, they are often referred to as a "cin bin".

What goes in:	Needles, scalpels, small pieces of broken glass
Treatment:	Sent for incineration
Bin colour	Yellow lid (for chemical contamination), orange lid (for biologicals)

Domestic Waste

This is **ONLY** for standard office-style waste. Anything that has come into contact with laboratory chemicals or biological materials should NEVER enter this stream.

What goes in:	Cardboard packaging from kit deliveries, paper and hand towels from the "clean" handwashing sink.
Treatment:	Sent for landfill or incineration without prior autoclaving
Bag colour	Black (sometimes clear)

Chemical Liquid Waste

Non-hazardous aqueous chemical liquid waste, such as phosphate buffers, saline solutions or other water-based solutions that lack hazardous chemicals or live biological agents, can be poured down the laboratory sink. If the liquid waste is a solvent, or contains any hazardous chemicals, it **CANNOT** be poured down the sink. Check your local rules to establish the correct disposal route for each type of waste liquid you may encounter. However, in UK laboratories, it is most common that such chemical liquid waste is disposed of within large, heavy-duty waste containers, such as Winchester bottles or carboys. There will be one for each of the common forms of liquid chemical waste, for example: (i) general solvents (ii) halogenated waste (iii) crystal violet waste.

What goes in:	Waste solvents, aqueous liquids containing hazardour chemicals.
Treatment:	Collected by a specialist hazardous waste contractor.
Colour	A large bottle, often brown, often kept in or below a fume cupboard.

Biological liquid waste

Any liquid waste that has been exposed to a biological agent, such as a bacterial culture or a mammalian cell culture, must NOT be poured down a sink without first disinfecting the organisms present in the suspension. This can be done either by **chemical disinfection** or by **autoclaving**, as described below.

Chemical disinfection

This is the most common waste stream for liquid biohazards in a university cell-culture lab. Liquid waste is collected in a beaker or bottle containing a concentrated disinfectant - commonly bleach (sodium hypchlorite) or a commercial disinfectant, such as Virkon S or chemgene.

To be effective, the waste must stay in contact with the disinfectant for a specific “kill time” - usually a minimum of 30 minutes to 2 hours, but overnight incubation with disinfectant is most commonly employed. After neutralization in this way, the treated liquid is poured down a dedicated laboratory sink (never the handwashing sink) with copious amounts of tap water.

Autoclaving (Heat Sterilization)

If chemical disinfection is not appropriate (e.g., for large volumes of high-concentration bacterial cultures), heat sterilization is used. In this route, liquids are collected in autoclavable glass bottles with the lids kept loose to prevent the bottle from exploding due to pressure build-up during the heating process. After steam treatment at 121°C and a pressure of 15 psi for at least 15-20 minutes, the liquid can be poured down a dedicated laboratory sink (never the handwashing sink) with copious amounts of tap water. Autoclave tape is commonly attached to the outer surface of the bottle to prove the liquid reached the required temperature to kill all biological agents (the tape will show black markings if the correct temperature was reached).

Mixed Waste

If a liquid is both biologically hazardous AND contains a toxic chemical, you cannot pour it down the sink even after disinfection. Instead, you must inactivate the liquid using either of the previous two methods, then treat the waste as chemical waste by pouring it into the correct chemical waste bottle, as described above. This is the route you would follow for disposal of Crystal Violet waste after staining of cells.

Protecting the safety of others

A key responsibility of every laboratory worker is to protect the safety not only of themselves, but also of other workers in the laboratory, any non-specialists who may visit the laboratory, and the general public outside the laboratory.

This section highlights how responsible laboratory practice extends to protecting the safety of these other groups. You will have to think about these groups when writing your Risk Assessment document.

Safety for other laboratory workers

The first priority with respect to protecting the safety of your co-workers is to follow the local health and safety rules and regulations. Here are some of the most pertinent rules to bear in mind:

Label and store your samples appropriately

You must label your samples with what is contained, particularly with respect to specific hazards (such as micro-organisms or hazardous chemical), your initials and the date. Keep them safely contained in the most appropriate container and location.

Avoid cross-contamination

Never cross-contaminate one chemical with another. Disinfect your working area after completion of a set of experiments. Never touch common surfaces (door handles, elevator buttons, phones) while wearing gloves. If you must transport a plate or tube between labs, use the “one-glove technique”, in which one hand is gloved to hold the sample (transported in a box with a clean non-hazardous outer surface), and the other is bare to open doors.

Safety for non-specialist laboratory visitors

Non-specialists, such as cleaners and maintenance staff, often visit the laboratory out of hours when there are no technical staff present to offer advice on safe working. The following are some key rules to bear in mind for their safety.

Door handles and hand-wash sinks

Cleaners and maintenance staff will often work without wearing gloves. Ensure you never touch door handles, light switches or hand-wash sink taps while wearing gloves. This minimises the risk of contaminating these surfaces with hazardous organisms or chemicals.

Advise guests to follow safety rules

Ensure any guests or visitors you bring into the lab wear a lab coat for the duration of their visit. Enforce the “No Food or Drink” rule strictly, and make sure they wash their hands on leaving.

Safety for those handling waste

Staff removing waste are often not aware of the biological or chemical hazards associated with specific laboratory items. Follow these rules to be considerate of their safety:

Never place hazardous materials in the standard, domestic waste bin - ensure they go only in the correct hazardous waste bin.

Never place sharps, such as needles or broken glass, in a domestic waste bin. Ensure these items go in a specialised sharps bin.

Double bag any biohazard bags if they are heavy or contain a lot of wet material. Leaking “bio-fluid” on the floor is a major slip and infection risk for the facilities team.

Safety for the general public

Laboratory workers must always be mindful of the safety of the general public in their activities. The following are some basic rules to bear in mind:

Follow all biosecurity procedures (e.g. never touching door handle, sink tap etc.) with gloves on to prevent accidental escape of a microbe you are working on from the lab to the external environment. Use the One-Glove rule when transporting samples between labs.

Dispose of waste only via the correct waste stream. Disposal of biological or chemical hazards via the sink or landfill routes can result in serious infection or other health problems for members of the general public.

Safety for workers with specific health circumstances

Your Risk Assessment should give consideration to the fact that some workers may suffer from immunosuppression or other form of specific health circumstance that renders them more susceptible to harm from certain chemicals or biological agents. For example, pregnant women may be more sensitive to certain hazardous chemicals, and recipients of organ transplant may have impaired immunity, even to organisms that are otherwise harmless to most people.

Safety for the environment

Dispose of waste only via the correct waste stream. Disposal of biological or chemical hazards via the sink or landfill routes can result in damage to organisms and ecosystems in the environment.

Leaving the laboratory

Before you leave the lab, perform a quick visual sweep of your area through the eyes of a cleaner:

1. Is the floor clear? (Check for trip hazards)
2. Are all chemical bottles capped tightly? (Prevents escape of toxic fumes).
3. Are there any hidden sharps on the bench? (Protection against puncture wounds).
4. Is your waste in the correct bin? (Prevents accidental contact with hazardous waste).

Emergency Procedures

Another key element of laboratory safety is being aware of what must be done in the case of mishap or emergency. You should take time to learn your own local procedures for dealing with each of the following eventualities.

Dealing with spills on a working surface

In the event of a spill, your priorities are: (1) Safety of personnel, (2) Containment and (3) Decontamination.

Small spills (e.g. inside a Safety Cabinet) can be mopped up with paper towels. Clean the area thoroughly afterwards by spraying on disinfectant solution, 70% ethanol or 70% isopropanol, then wipe and dry the area with paper towels. Dispose of all materials in the biohazard (autoclave) bin.

For large spills (e.g. on the laboratory floor), alert colleagues in the immediate area. Spills of chemicals, such as acids or solvents, can be dealt with by placing an absorbent pad from a chemical spill kit (should be present in your laboratory) over the affected area. Spills of a biological substance, such as a bacterial culture, should be sprayed with disinfectant, allowing 20-30 minutes contact time to neutralise the biological hazard, then soaked up with paper towels. Clean the area thoroughly afterwards by spraying on disinfectant solution, 70% ethanol or 70% isopropanol, then wipe and dry the area with paper towels. Dispose of all materials in the biohazard (autoclave) bin.

Dealing with physical contact with chemicals

If a hazardous chemical, such as crystal violet, splashes on your skin, wash the affected area with copious amounts of lukewarm water and soap for at least 15 minutes.

If a chemical enters the eye, use the Emergency Eyewash Station immediately. Hold eyelids open and flush for a full 15 minutes.

If a chemical contaminates clothing, it should be removed for washing. If a biological agent contaminates clothing, the clothes may need to be autoclaved or treated as hazardous waste.

Reporting of incidents

All spills or accident, regardless of size, should be reported to your University Supervisor and the Technical Lead/Lab Manager. They will advise whether it will be necessary to complete an institutional **incident report form**, which is likely if the spill involved skin contact with hazardous organisms or chemicals.

Hazards associated with *Phytotitre* lab projects

Summary of main hazards by project

The core hazards you will be dealing with which will require risk assessment before starting the different *Phytotitre* laboratory projects are as follows:

	1) Antibiotic drug discovery	2) Anti-cancer drug discovery	3) Enzyme inhibitor disvovery	4) Anti-inflammatory drug discovery	5) Dry data analysis projects
Use of DMSO solvent (a component of the library)	✓	✓	✓	✓	-
Use of other chemicals	✓	✓	✓	✓	-
Culture of a Hazard Group 1 bacterial strain	✓	-	-	-	-
Culture of mammalian cell-lines	-	✓	-	✓	-
Use of electrical equipment	✓	✓	✓	✓	✓
Miscellaneous physical hazards	✓	✓	✓	✓	✓

The specific chemical, biological and physical hazards associated with each type of project are listed in **Appendix 1, 2 and 3**, respectively.

Appendix 1: Chemical hazards associated with *Phytotitre* projects

Chemicals associated with all *Phytotitre* laboratory projects

Chemical	Hazard Category	Specific Risks	Mitigation & Handling
Phytotitre library	Penetrant / Carrier	Contains DMSO as the solvent, which can penetrate the skin and carry dissolved toxins into the bloodstream.	Wear nitrile gloves when handling. If skin contact occurs, wash immediately with soap and water.
DMSO (Dimethyl Sulfoxide)	Penetrant / Carrier	This solvent can penetrate the skin and carry dissolved toxins into the bloodstream.	Wear nitrile gloves when handling. If skin contact occurs, wash immediately with soap and water.
70% Ethanol	Flammable / Irritant	Highly volatile and easily ignited. Causes eye irritation and skin dryness.	Keep away from Bunsen burners or heat sources in the hood. Use in a well-ventilated area/

Additional chemicals associated with the Antibiotic discovery project

Chemical	Hazard Category	Specific Risks	Mitigation & Handling
Luria broth	Non-hazardous	Powder inhalation can irritate lungs.	Wear gloves when handling liquid, and a mask while weighing powder.
Ampicillin (example of a positive control antibiotic)	Sensitizer	Can cause allergic reactions in sensitive individuals via skin contact.	Wear gloves when handling. If you have a known penicillin allergy, notify your supervisor before starting wet-lab work

Additional chemicals associated with the Cancer drug discovery project

Chemical	Hazard Category	Specific Risks	Mitigation & Handling
DMEM or RPMI (cell culture media)	Non-hazardous	None	Wear gloves when handling.
Penicillin/Streptomycin	Sensitizer	Can cause allergic reactions in sensitive individuals via skin contact.	Wear gloves when handling. If you have a known penicillin allergy, notify your supervisor before starting wet-lab work
Crystal Violet	Mutagen / Carcinogen / Environmental Hazard	Causes intense staining of skin and clothing. Suspected of causing genetic	Work on a bench diaper or plastic tray. Wear lab coat and

		defects. Toxic to aquatic life.	gloves. Dispose of as hazardous waste.
Methanol	Flammable / Irritant / Toxic	A fire risk near heat sources.	Keep away from Bunsen burners. Aliquot in a fume hood. Store in a flammable-liquids cabinet.
Acetic acid	Corrosive / Irritant	Pungent vapor causes respiratory distress. Concentrated liquid causes severe skin burns and permanent eye damage.	Handle concentrated stock in a fume hood. Always add acid to water (not water to acid). Keep an eye-wash station nearby.
SDS (Sodium Dodecyl Sulfate)	Irritant / Respiratory Hazard	In powder form, it is a severe inhalation irritant. In solution, it causes skin and eye irritation.	Handle powder in a fume hood. Wear a mask. Use pre-prepared solutions where possible to avoid dust.
MTT (Tetrazolium salt)	Toxic / Mutagen	It is a potential mutagen and can be toxic if absorbed through the skin.	Handle with extreme care. Always wear gloves. Dispose of all MTT-containing media as hazardous chemical waste.

Additional chemicals associated with the Enzyme Inhibitor discovery project

Chemical	Hazard Category	Specific Risks	Mitigation & Handling
Recombinant beta-galactosidase enzyme (1 mg/ml, frozen aliquot)	Sensitizer	Being a protein, it can act as a respiratory sensitizer if aerosolized.	Standard lab PPE (gloves / lab coat).
Ortho-nitrophenyl-β-D-galactopyranoside (ONPG, at least 60 mg, the substrate)	Irritant	May cause mild skin and eye irritation.	Standard lab PPE (gloves / lab coat / goggles).
Galactose (as a positive control for enzyme inhibition)	Non-Hazardous	Very low toxicity, essentially harmless.	Standard laboratory hygiene.
Phosphate buffer (for constitutents see Method sheet 15)	Non-Hazardous	Very low toxicity.	Standard lab PPE (gloves / lab coat / goggles).

Additional chemicals associated with the anti-inflammatory discovery project

Chemical	Hazard Category	Specific Risks	Mitigation & Handling
DMEM or RPMI (cell culture media)	Non-hazardous	None	Wear gloves when handling.

Penicillin/Streptomycin (cell culture supplement)	Sensitizer	Common cell culture antibiotics. Can cause allergic reactions in sensitive individuals via skin contact.	Wear gloves when handling. If you have a known penicillin allergy, notify your supervisor before starting wet-lab work
Actinomycin D	Highly Toxic / Teratogen / Mutagen	Highly Toxic. Can cause genetic defects or harm to an unborn child. Can be fatal if swallowed.	Handle only in the fume hood or safety cabinet. Use pre-diluted stocks wherever possible.
Crystal Violet	Mutagen / Carcinogen / Environmental Hazard	Causes intense staining of skin and clothing. Suspected of causing genetic defects. Toxic to aquatic life.	Work on a bench diaper or plastic tray. Wear lab coat and gloves. Dispose of as hazardous waste.
Methanol	Flammable / Irritant / Toxic	Highly flammable, a fire risk near heat sources.	Keep away from Bunsen burners. Aliquot in a fume hood. Store in a flammable-liquids cabinet.
Acetic acid	Corrosive / Irritant	Pungent vapor causes respiratory distress. Concentrated liquid causes severe skin burns and permanent eye damage.	Handle concentrated stock in a fume hood. Always add acid to water (not water to acid). Keep an eye-wash station nearby.
LPS (Lipopolysaccharide)	Pyrogen / Inflammatory	Can cause a systemic inflammatory response if inhaled or injected.	Avoid aerosolization. Do not vortex open tubes. Wear a mask if handling concentrated powders.
Polymyxin-B	Sensitizer	As an antibiotic, repeated exposure can cause allergic sensitization.	Treat as a bioactive agent. Prevent skin contact.

Appendix 2: Biological hazards associated with *Phytotitre* projects

Use of organisms in Hazard Group 1 will require working in a laboratory meeting at least Containment Level 1. Use of organisms in Hazard Group 2 will require working in a laboratory meeting at least Containment Level 2. Note that most mammalian cell-lines are actually considered to be in Hazard Group 1, but we work with them at Containment Level 2, as if they were in Hazard Group 2. This is a precautionary measure to mitigate the risk from potential adventitious agents (such as latent viruses) that may be present in the culture, even if the cell-line itself is non-pathogenic.

Phytotitre projects

Caithness BIOTECHNOLOGIES

Project	Biological hazard	Hazard Group
Antibiotic discovery	*E. coli* K12, *E. coli* DH5α or *M. luteus*	1
Cancer drug discovery	Mammalian tumour cell-lines (e.g. MCF-7, PC3)	2
Enzyme inhibitor	None	-
Anti-inflammatory	Mammalian tumour cell-lines (e.g. J774, L929)	2
Dry projects	None	-

Appendix 3: Physical hazards associated with *Phytotitre* projects

The *Phytotitre* projects are associated with the following physical hazards. If you are writing a Risk Assessment for your project, you should mention only those that are relevant to your project. You do not need to mention those that are not relevant to your project.

Form of physical hazard	1) Antibiotic drug discovery	2) Anti-cancer drug discovery	3) Enzyme inhibitor drug disvovery	4) Anti-inflammatory drug discovery	5) Dry, data analysis projects
Repetitive strain injury (such as from typing)	✓	✓	✓	✓	✓
Strain from poor ergonomics (e.g. poor seating)	✓	✓	✓	✓	✓
Electric shock from equipment	✓	✓	✓	✓	✓
Tripping on cables / floor items etc.	✓	✓	✓	✓	✓
Slipping on laboratory spills	✓	✓	✓	✓	-
Exposure to naked flames (of other workers)	✓	-	-	-	-
Asphyxiation from CO_2 leakage	-	✓	-	✓	-
Injury from sharp objects	✓	✓	✓	✓	-
Contact with very hot liquids / objects	✓	✓	-	✓	-
Contact with very cold objects	✓	✓	✓	✓	-
Exposure to laser light	-	-	-	-	-
Exposure to ultraviolet (UV) light	-	-	-	-	-
Exposure to radioactive chemicals	-	-	-	-	-

Appendix 4: Summary of waste streams for *Phytotitre* projects

Summary Table for Quick Reference

Item	Contaminant	Correct Waste Stream
Used growth media / liquid bacterial culture	Bacteria / Cells	Liquid biohazard (Disinfectant or Autoclave, then lab sink)
96-well Plate	Bacterial or cell cultures	Infectious clinical waste (orange bag)
96-well Plate	Crystal violet	Hazardous clinical waste (yellow bag)
96-well Plate	MTT / DMSO	Hazardous clinical waste (yellow bag)
Pipette Tip	Actinomycin D	Sharps bin or Hazardous clinical waste (yellow bag)
Paper Towel	Bacterial or cell culture spill	Infectious clinical waste (orange bag)
Paper Towel	DMSO / Extract	Infectious clinical waste (orange bag) or hazardous clinical waste (yellow bag)
Paper Towel	70% Ethanol, no other chemicals or biological contaminants	General waste (black bag)

Disclaimer
These resources are provided for educational purposes only. The user's University Supervisor remains the Principal Investigator and the sole party responsible for the safe conduct, risk assessment, and ethical oversight of all laboratory work. Caithness Biotechnolgies Ltd. accepts no liability for any injury, loss, or damage resulting from the application of the advice or protocols provided herein.