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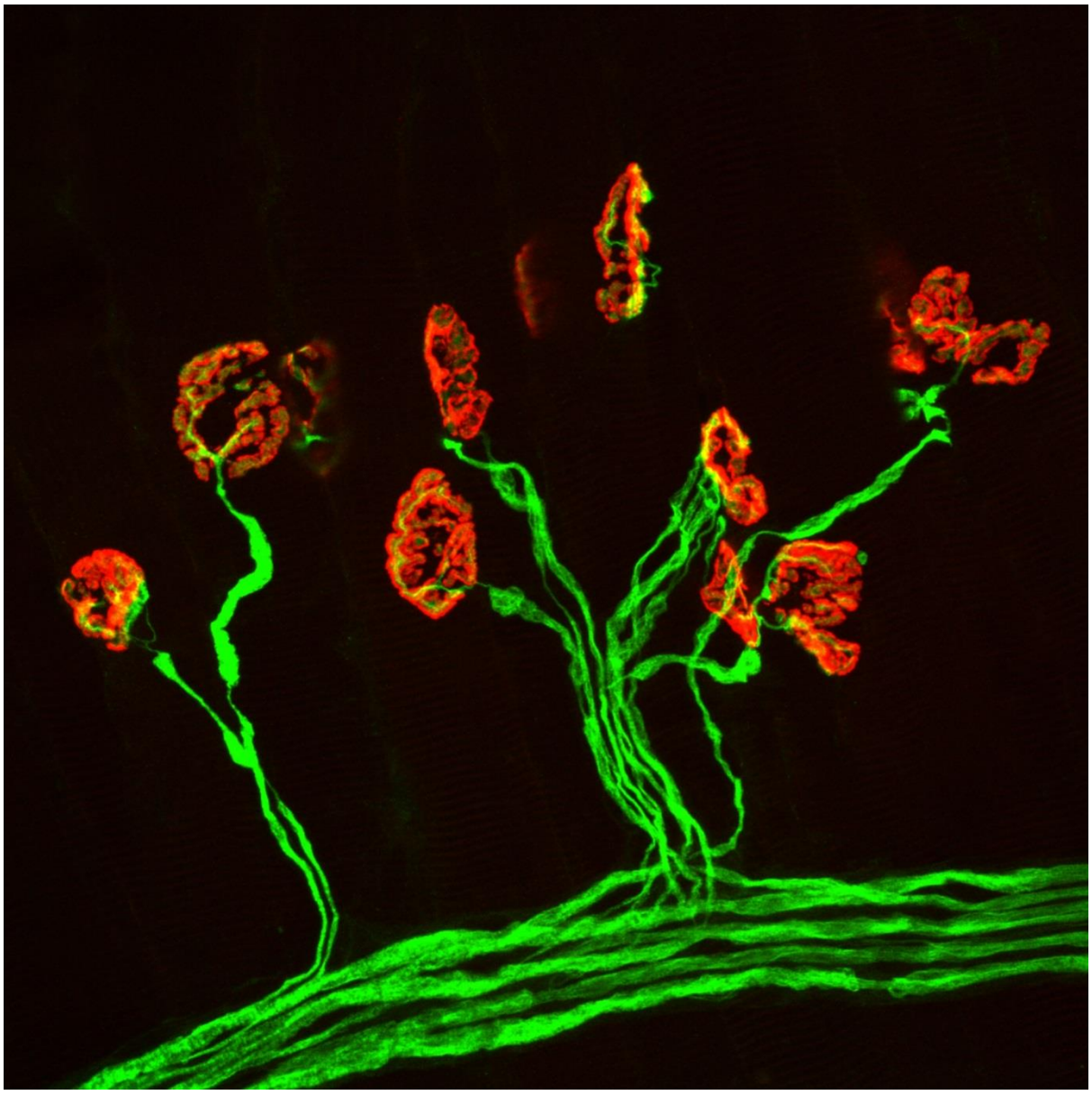


Photo – 2018 Sukrat Arya

NDCN Laboratory Manual

JOHN RADCLIFFE HOSPITAL WEST WING, LEVEL 5 LABORATORIES

Deluxe edition includes NDCN local rules

Introduction

This manual covers many aspects of operations in the research laboratories on Level 5 of the West Wing. It is also your guide to Health and Safety in the laboratory, as well as giving guidance on laboratory housekeeping and good laboratory practice (GLP).

The Health and Safety at Work Act (1974) requires your employer to ensure that you are not harmed by your work and, as such, gives your employer responsibility for your health and safety whilst you are at work. The University of Oxford has delegated this responsibility to your supervisor, this would be your line manager or the Principal Investigator of your group.

However, you are also equally responsible for your health and safety and must follow safety policies and local rules, undertake the training that is provided, use equipment properly and as instructed and wear personal protective equipment when provided. Further details of health and safety responsibilities can be found in *NDCN Laboratory Safety Policy 0011: Health and safety responsibilities of supervisors and staff*.

The University of Oxford has put in place a number of safety policies and these are available on the University Safety Office (USO) web page, where you will also find the termly syllabus of USO training courses. You can book yourself onto training courses using your University Single Sign-On (SSO) credentials, and the courses are free of charge. NDCN also has its own set of more specific policies on the Department intranet (under "Health and Safety" in the "Facilities Management" section).

1. Health and safety in the laboratory

Rules for CL2 laboratories

All of the laboratory spaces on Level 5 of the West Wing are categorised as Containment Level 2 (CL2). This means that the laboratories have been designed for work with biological agents up to hazard group 2.

In a containment level 2 laboratory you must:

- Wear appropriate PPE while working at the bench. Laboratory coats and safety glasses are a minimum.
- Wear sensible footwear that protects the foot and toes from splashes.
- Never eat, drink, chew gum or store foodstuffs in the laboratory.
- Do not apply cosmetics or hand cream in the laboratory.
- Laboratory personnel have specific training in handling pathogenic agents and are directed by scientists with advanced training.
- Access to the laboratory is limited when work is being conducted.
- Extreme precautions are taken with contaminated sharp items.
- Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

In addition we also ask that you:

- Read and understand relevant departmental laboratory safety policies.
- Be familiar with your risk assessments before carrying out a procedure.
- Follow departmental disinfection policy and learn the correct waste streams.
- Clean up when you have finished.
- Respect shared equipment and spaces.
- Switch off equipment after use.

Safety Inspections

The Departmental Safety Officer and representatives from the University Safety Office periodically inspect the laboratories and deviations from health and safety protocols and good laboratory practice are recorded and presented to the Head of Department at the termly Departmental Safety Advisory Committee (DSAC) meetings. Sanctions can be imposed when persistent or serious problems are found.

Personal protective equipment (PPE)

It is the policy of the University that laboratory coats and eye protection (glasses, goggles or visors) MUST be worn whilst working at the bench in all laboratories, this is very important and is not optional. It is only acceptable not to wear PPE if you are passing through circulation space (walkthroughs and corridors) and are not standing at a bench or performing any procedures that require PPE. If you are standing at a bench and are not working yourself, it is still possible for a colleague to spill something, so you should still be wearing appropriate PPE.

Laboratory coats

Laboratory coats can be obtained from the hospital Linen and Uniform Room on Level 0 of the Main Hospital Block. You will need your Hospital ID card and the staff there will provide you with a clean lab coat. After a few weeks, or if your coat is soiled, take it back and put it in the dirty laundry bin, they will then give you a clean one

Safety glasses and prescription safety glasses

These are provided by the Facilities Team or you can purchase your own through your own lab funds. If you are a glasses wearer, the University Safety Office does not consider normal glasses to provide enough protection from splashes. If you are going to be working in the lab for a short period the Facilities Team can provide you with over-spectacles. If you are staying for longer, then you are entitled to a pair of prescription safety glasses, which will be paid for by the Department. Contact the Facilities Team to make arrangements for an eye test and fitting of your prescription safety specs.

Some procedures require additional PPE (such as handling cryogenic substances or using lasers). Guidance on this will be found in the following sections or in the risk assessments for the procedures you will be carrying out.

Biological hazards

In a Containment Level 2 Laboratory it is reasonable to assume that biological materials can represent a significant risk. Because of this it is recommended that every research worker should undertake the USO Biological Safety Training course. You should arrange to do this as soon as possible, details of the course can be found in the training section of the USO website and courses can be booked using your SSO credentials.

Basic guidelines for working with biological material in a CL2 laboratory

More complete guidance can be found in *NDCN Laboratory Safety Policy 0002: Local rules for working with biological materials and genetically modified organisms*.

- Depending on what you are working with, you may need to register with Occupational Health prior to starting work e.g. animal work.
- Wear a laboratory coat, safety glasses and NON-LATEX gloves whilst carrying out the work.
- Dispose of gloves in yellow or orange clinical waste bags ONLY after use (see waste streams).
- Know the disinfection policy.
- Always disinfect work area before and after work.
- Know emergency procedures.
- Ensure you are trained in the techniques you are using and be familiar with the risk assessment.

Chemical hazards

Most chemicals have the potential to cause some harm, therefore in UK law there is a specific and absolute requirement to carry out risk assessments on their use. This comes under the Control of Substances Hazardous to Health regulations. Hence the term COSHH assessments.

It is accepted that many chemicals pose limited risk if handled according to “Good Chemical Practise”. However, if you work within the laboratory, at some point you will almost certainly come into contact with a particularly hazardous chemical (e.g. Ethidium Bromide, Acrylamide, Phenol, Formamide and 2-Mercaproethanol). How these specific chemicals need to be handled is detailed in COSHH assessments, and although the assessment may have already been completed, you must understand the reasoning behind the assessment to ensure the appropriate control measures are followed.

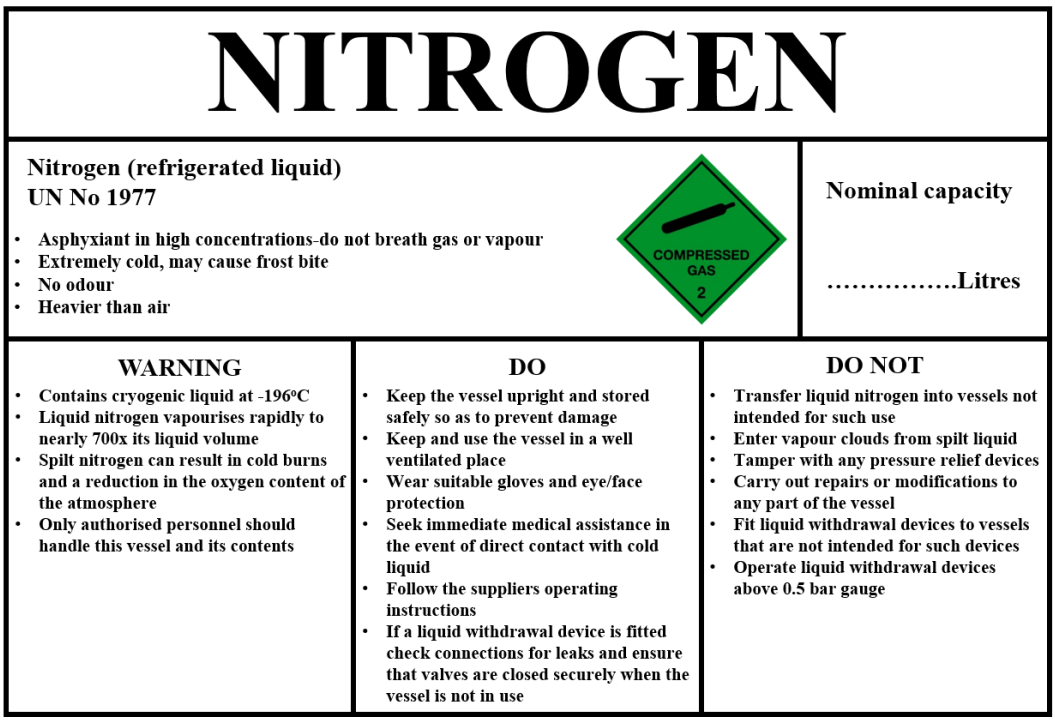
Details on the risks represented by the chemicals used in our laboratories, how to carry out COSHH risk assessments and controls for mitigating chemical hazards can be found in the local rules section on handling chemicals later in this manual.

Cryogenic substances

Liquid nitrogen

Liquid nitrogen is both a cold burn hazard and an asphyxiation hazard. Therefore, before starting work, training must be received on the decanting of liquid nitrogen from the large storage vessels before commencing use of them.

Whilst handling liquid nitrogen you should always wear a lab coat, face mask and suitable cryogenic protective gloves. If you are transporting liquid nitrogen you should never enter enclosed spaces such as lifts. This is because 1L of liquid nitrogen, if spilled, will rapidly evaporate into 1000L of gaseous nitrogen. If this were to happen in a restricted space, with enough liquid nitrogen it will exclude all the breathable air from the immediate environment leading rapidly to unconsciousness and death. If you are a regular user of liquid nitrogen you should sign-up for the cryogenic training course run by the University Safety Office and be familiar with the guidance on the warning panel of any liquid nitrogen container (Illustrated below), also be aware of the low oxygen alarm system.



NITROGEN

Nitrogen (refrigerated liquid)
UN No 1977

- **Asphyxiant in high concentrations-do not breath gas or vapour**
- **Extremely cold, may cause frost bite**
- **No odour**
- **Heavier than air**

COMPRESSED GAS 2

Nominal capacity

…….Litres

WARNING

- **Contains cryogenic liquid at -196ºC**
- **Liquid nitrogen vapourises rapidly to nearly 700x its liquid volume**
- **Spilt nitrogen can result in cold burns and a reduction in the oxygen content of the atmosphere**
- **Only authorised personnel should handle this vessel and its contents**

DO

- **Keep the vessel upright and stored safely so as to prevent damage**
- **Keep and use the vessel in a well ventilated place**
- **Wear suitable gloves and eye/face protection**
- **Seek immediate medical assistance in the event of direct contact with cold liquid**
- **Follow the suppliers operating instructions**
- **If a liquid withdrawal device is fitted check connections for leaks and ensure that valves are closed securely when the vessel is not in use**

DO NOT

- **Transfer liquid nitrogen into vessels not intended for such use**
- **Enter vapour clouds from spilt liquid**
- **Tamper with any pressure relief devices**
- **Carry out repairs or modifications to any part of the vessel**
- **Fit liquid withdrawal devices to vessels that are not intended for such devices**
- **Operate liquid withdrawal devices above 0.5 bar gauge**

The notice found on liquid nitrogen storage vessels carries reminders of hazards with do's and don'ts.

Low oxygen alarms

- Oxygen level panel showing oxygen level from all six monitors.
- When oxygen level falls below 19.0% STOP WORK.
- An Alarm will sound and the amber light will come on.
- This will reset automatically if oxygen level returns to normal.
- When Oxygen level falls below 18% the alarm cannot be reset manually.

O2 Sensor

Low oxygen alarm

Solid carbon dioxide (dry ice)

In its solid form, carbon dioxide is very cold; measuring -78^{O} C. It is normally supplied as pellets or blocks and does not pass through the liquid state as it melts, rather, it sublimes from its solid state into a colourless gas which is odourless at low concentrations but has a characteristic smell at higher concentrations.

- Do not handle with bare hands – use cryogenic gloves.
- Avoid carrying dry ice in the driver's compartment of a lorry or the passenger compartment of a car. If this is not possible, use as little dry ice as possible, ensure that the container is well insulated (though not tightly sealed) and ensure that the compartment is well ventilated (open windows, ensure ventilation system is set to draw fresh air from outside).
- Unload the material as soon as possible at the end of a journey.
- Store dry ice in well ventilated areas away from direct sunlight and sources of heat.
- Use suitable storage containers (there are commercially available insulated containers with vented seals specifically designed for storing dry ice).
- Secure to prevent any unauthorised access.
- Use appropriate warning signage where necessary.
- When opening lids to storage containers, allow a few seconds for gas to dissipate and do not lean in for longer than necessary.
- Do not store or use dry ice in any gas tight container.
- Do not store dry ice in a working refrigerator or freezer – it will sublimate at a faster rate than in an insulated storage container and the extremely cold temperature may cause the thermostat to cut out.
- Dispose of unwanted dry ice by allowing it to evaporate in a well ventilated area – it will sublime leaving no residue.

- Carry out manual handling assessment of bags if necessary.
- Ensure that all users of dry ice are familiar with the hazards and necessary precautions.

More detailed information on the hazards of dry ice and emergency procedures can be found in the *NDCN Laboratory Safety Policy 0004: Local rules on the manual handling and correct use of dry ice*. Also if you are a regular user of dry ice you should attend the cryogenic training course run by the University Safety Office.

Lasers

The Department is legally obliged to keep a register of all lasers, except inherently safe Class 1 lasers (e.g. laser printers, CD players etc.) and laser pointers below Class 3. The inventory must include Class 1 by design products that have embedded Class 3 or 4 lasers whose beams might be exposed during routine service and maintenance.

If you are intending to use high power lasers above (but not including class 2) you should consider the following:

- Before starting work with lasers you must attend the mandatory course and inform the Laser Safety Supervisor.
- All users of Lasers which are above Category 2 must register with the University Safety Office.
- All category 3 and 4 lasers must be accompanied by a written risk assessment, a laser record sheet and local rules.
- The Departmental Safety Officer/Laser Safety Supervisor must be informed of all new equipment containing lasers brought into the department.

Radiation

Although the use of radioactivity in the Medical Sciences Division is lessening all the time, the Department does have some facilities for its use. **It is an absolute requirement that before using radioactive substances you must follow these guidelines**:

- All New radiation workers **must** register with the University Safety Office (University Radiation Protection Supervisor).
- All new workers **must** attend University Radiation Workers Seminar.
- All workers should know the appropriate shielding and monitors used to detect the isotope they are using.
- Be aware of the appropriate methods for recording and disposing of radioactive material.
- **Make sure you are properly trained before attempting to use radiation.**

Compressed gases

Anyone who uses a gas cylinder should be suitably trained and have the necessary skills to carry out their job safely. They should understand the risks associated with the gas cylinder and its contents, and should follow these guidelines:

- Use the correct trolley.
- Get help if required.
- Consider your route.
- Ensure the cylinder is secure during the move and at its final destination.
- Ensure the proper regulator is used for the gas concerned.
- Check the regulator is in date.
- Regulators must be checked annually.

Gas cylinders can be heavy, large and unwieldy, so consideration should be given to all aspects of manual handling involving them. More information can be found in *NDCN Laboratory Safety Policy 0003: Local rules on risk assessment of procedures involving manual handling*. The USO also runs a training course on manual handling.

2. Risk assessment

All laboratory procedures must have a valid, up to date risk assessment

In practical terms a risk assessment is a thorough way of looking at work activities to identify those things, situations, tasks or processes that might cause harm to people. After identifying them assessors need to evaluate the risk and decide what measures are needed to prevent the harm occurring.

In the NDCN the Head of Department has delegated the responsibility for risk assessment to individual supervisors, managers, or persons in control of a particular area of work, or activity, and it is they who must ensure that assessments are done. Those involved in the work should also be consulted since they may have intimate knowledge of the risks involved and may be able to offer practical solutions in controlling them. All procedures in the laboratory must be adequately risk assessed.

NDCN rules for laboratory risk assessments

- Risk assessments should be carried out prior to the start of the work/protocol.
- It is the supervisor's responsibility to make sure that all work performed by their staff has been risk assessed by a competent person.
- Risk assessments should be kept together in an easily accessible place where all members of the group can find them and they should be updated regularly.
- Specific risk assessments need to be assessed by a committee prior to being signed off e.g. genetic modification and radiation.

- Risk assessments for procedures involving hazardous chemicals should include COSHH assessments, see *NDCN Laboratory Safety Policy 0001: Local rules on handling hazardous chemicals* for guidance.
- Pregnant and nursing mothers must perform a pregnancy risk assessment. The Departmental Safety Officer can give advice on this (in confidence if necessary) and information is also available on the Department intranet health and safety web site.

The 5 steps to risk assessment

Step 1 – identify the hazards

The aim here is to identify and record all the possible dangers that could foreseeably cause harm to people in the area where a procedure is being carried out. Hazards may be identified by observation of the laboratory environment, MSDS sheets for the substances involved or safety section of equipment manuals as well as personal experience.

Step 2 - identify the persons or groups who may be harmed and how

The risk assessment should consider everyone who might be affected by the work. This will include the workers themselves, colleagues not directly involved with the work, visitors, external contractors such as cleaners and maintenance engineers. The risks may not be the same for each group and the assessment should consider the different ways that the work might affect them.

Step 3 – evaluate the risks and decide on precautions

Once the hazards have been identified the assessor must decide if they are serious i.e. whether they pose a risk. The most common way of evaluating risk is to rate it as high, medium or low according to the potential outcomes.

(i) Potential severity of the harm (e.g. severe, moderate, insignificant)
(ii) Likelihood that the harm will arise (e.g. very likely, possible, unlikely)
(iii) Numbers of people likely to be affected (e.g. many, some, very few)

Some assessors find it useful to construct a matrix to determine the risk and to award points to the most severe / significant outcomes. This allows the risks to be ranked, remedial action prioritised, and suitable control measures targeted at the most serious problems.

Risk matrix

Risk Matrix		**Likelihood**			
		High	**Medium**	**Low**	**Negligible**
Outcome	**Severe**	High	High	Medium	Near zero
	Moderate	High	Medium	Medium/low	Near zero
	Insignificant	Medium/low	Low	Low	Near zero
	Negligible	Near zero	Near zero	Near zero	Near zero

The assessment should acknowledge any existing measures that control risk. These may have been introduced for other operational reasons but they may, nevertheless, mitigate problems. The assessment should also consider the impact of existing control measures suddenly becoming unavailable.

Hierarchy of controls

(i) **Eliminate** the hazard and remove the risk (e.g. use different equipment, fix faulty machinery, replace worn stair carpet).
(ii) **Substitute** the hazard for something less 'risky' (e.g. use safer materials).
(iii) **Isolate** the hazard from people (redesign equipment e.g. use guards on cutting machines, segregate the work or other engineering controls).
(iv) introduce administrative measures (e.g. **change** the way that the job is done, change practices, introduce protocols, involve workers to ensure they understand what they need to do and provide them with information, instruction and training)
(v) Use **personal protective equipment**.

Step 4 – Record the findings and implement them

The significant findings of the risk assessment should be committed to writing. The aim is not to generate additional paperwork but to ensure that appropriate safety measures, identified during the assessment process, are integrated into existing procedures and routine work patterns.
The assessment must be 'suitable and sufficient'. What this means is that the level of detail in the assessment should be proportionate to the risks identified. Enough will probably have been done when it can be clearly demonstrated that:

(i) Adequate checks were made and that all groups affected by the work or activity have been properly considered.
(ii) Significant risks have been identified and appropriate action taken to eliminate or reduce them.
(iii) Reasonable precautions were put in place.
(iv) Appropriate information, instruction, and training has been given to those directly involved with the work.
(v) Residual risks were low.

Step 5 – Monitoring and review

When control measures have been established and actions (step 4) implemented, the work must be kept under review to ensure they are working properly. If further improvements can be made to reduce the risk further then action should be taken. Risk assessments must be reviewed if information comes to light about the adverse health effects of a particular hazard, so that the control measures can be modified, where necessary. Similarly control measures should be adapted and refined to take advantage of technological advances or improvements.

Pro formas for a number specific types of risk assessment are available for download from the USO website and the Departmental intranet health and safety web site. Available pro formas include:

- COSHH general lab risk assessment
- Animal carer risk assessment

- COSHH biological materials RA
- COSHH DNA RA
- COSHH micro-organisms RA
- GMO class 1 (or initial) RA
- GMO class 2 (and above) RA
- GMO transgenic animal

3. Waste streams

Uncontaminated waste

This waste is treated in the same way as domestic waste and is either put into black bags or is recycled. Laboratory gloves however, even if uncontaminated, must always be disposed of as orange bag waste.

Laboratory waste

If it must be incinerated it will go into a yellow bag (see risk assessment for procedure). If it can be disposed of by other means such as disinfection it can go in an orange bag. Orange bags are the generally preferred disposal route by the OUHT as it is more cost effective and is suitable for most of our clinical laboratory waste.

Large yellow bins are suitable for disposal of contaminated items unsuitable for bags such as disposable pipettes (so called "pointy but not sharp" waste). No sharps, glass or gloves should be put in yellow bins and they should be closed and sealed before disposal. Do not overfill yellow bins.

Incineration only

Disposal by other means (high temperature disinfection)

Suitable for plastic disposable pipettes

Sharps go into a yellow top sharps bin.

- Disposable scalpels and scalpel blades, needles (including syringe barrel).
- **Never** re-sheath needles and scalpel blades.
- Close and seal the bin before disposal.

Autoclave waste goes into clear autoclaveable "biohazard" bag.

- Includes all disposable of genetically modified organisms (GMO) and infectious waste.
- Bags must be closed with cable tie, placed into an autoclave box and taken to the autoclave room on the Level 5.

Chemical waste

Waste chemicals should be assessed for hazard level using COSHH assessments, risk assessments for the procedure they are used in or the material safety data sheets (MSDS available on the supplier's website). If in doubt ask the DSO. If chemicals are mixed assess the most hazardous component.

If the chemical is determined non-hazardous or as low risk and is a small quantity then the chemical can be disposed of down the drain with copious amount of water. This could apply to washing out 2.5 litre Winchester bottles. Once Winchesters are empty and rinsed; cross out the chemical name and any hazard symbols and dispose of next to the wheelie bins in the waste store room on Level 5 (entry code number is 056602).

If a chemical is deemed hazardous it must be disposed of via the University Safety Office. To dispose of chemicals via the University Safety Office you should:

- Complete the form TW 2/10 (disposal of hazardous substances) which is available from the University Safety Office website.
- The information the form will require is, chemical name, quantity, risk number, primary/secondary hazard, whether the substance is organic or inorganic and whether it is in liquid or solid form.
- The completed form (TW 2/10) should be returned to the DSO as an e-mail attachment who will coordinate with disposal with the University Safety Office
- The USO will email the Facilities Management (FM) team the TW number for chemicals to be disposed.
- Once the TW number has been generated by the USO, the Divisional Safety Officer, will contact the FM team to arrange a convenient date and time to collect the chemicals to take to the hazardous waste store (West Wing Level 0 behind the FMRIB building) where he will pack them and arrange for their final disposal by an external waste contractor (Grundon).
- The FM team will email the end user the TW number who should label the chemicals with TW numbers and store them in a container in the laboratory until collection date.

4. Good laboratory practice

Shared equipment

Even if equipment is owned and used by a single research group it is still shared between the people in that group so the same principles apply to equipment that is shared more widely. It is good laboratory practice to keep equipment clean and in good working order, so when you finished using it you must clean it properly and reset it so that it is ready for the next person to use it. Good examples are when using balances, centrifuges, microbiological safety cabinets and fume hoods, cryostats etc.

You should also check that the equipment is within its service schedule and that it is correctly calibrated. If you encounter a problem with shared equipment you should report it to the Facilities Team.

Confocal microscope and bookable equipment

Some equipment may need to be booked before you can use it, for example the confocal microscope. To gain access to the booking system contact the Facilities Manager. Use of the confocal microscope is chargeable (see Section 5).

Shared spaces and general laboratory housekeeping

Many spaces within the laboratories are shared between groups, this includes many of the bays and some the tissue culture rooms. Similarly to equipment, even if they are used by only one group, you must still make sure that you leave the area clean and tidy for the next person. For example, areas where balances are used should be kept clean and any spillages must be cleaned up immediately and in accordance with risk assessment protocols.

You should also ensure that your benches and other areas are kept clean and tidy and that no hazards are left unattended. Benches should be clean and decontaminated in accordance with the associated risk assessment and the disinfection policy if relevant. The floor should be kept free of litter so that the cleaning staff can clean it properly and you must always clean and decontaminate any spills that you have created in accordance with risk assessment protocols.

You should periodically perform a deep clean of your area and arrange with your colleagues to clean communal areas regularly. Do not allow waste chemicals to accumulate and make sure that they are always correctly labelled and stored, including solutions that you have made.

Shared consumables and apparatus

Many consumables and some glassware is ordered communally and the costs are shared between the research groups. There are separate systems for Large Lab 2 and Large Lab 3 with different items being on the shared lists. Speak to the laboratory technician to find out which items are ordered centrally and how to organise restocking. Also contact the Facilities Manager if you would like to discuss having additional items put on the list.

Handling hazardous chemicals

Chemicals should be handled, stored and disposed of in line with the COSHH protocols and risk assessments. Spillages must be cleaned up immediately. Chemicals should always be labelled and the correct GHS hazard symbols should be affixed even to chemical solutions or mixtures that you have made yourself. Hazard symbols stickers can be ordered through the university ordering system or speak to the FM team who have a stock of some labels. Guidance on correct labelling and storage can be found on MSDS information datasheets and also in the *NDCN Laboratory Safety Policy 0001: Local rules on handling hazardous chemicals*. Avoid keeping old chemicals and accumulation of chemical waste.

Safe use of needles and sharps

Exposure to unscreened human blood or tissue carries a potential risk of contracting a blood-borne infection such as Hepatitis B, Hepatitis C, and human immunodeficiency virus (HIV). Although the risk of acquiring infection in this way is low, the universal precautions for people working with human blood or tissue should be scrupulously observed to minimise it. In summary:

- Avoid using sharps if safe alternative devices are available.
- Needles and sharps should be handled with care, and handling kept to a minimum.
- Users of needles or other sharps are responsible for their disposal.
- Never re-sheath, bend or break needles before disposal.
- Dispose of syringes and needles as a unit.
- Never carry used sharps or re-use equipment.
- Sharps disposal containers must be available at the point of use.
- Discard sharps containers when three-quarters full.
- Never clear areas where sharps may be present without hand protection.
- Wear goggles if there is a risk of splashing

Disinfection policy

Disinfection is the reduction of microorganisms to an acceptable level. It is the policy of the Department that all areas likely to become infected with microorganisms must be routinely disinfected. There are many chemicals that are available, but depending upon their particular active agent and the material to disinfect, there may be variable degrees of disinfection. Therefore it is University's policy that all departments should have a clear disinfection policy indicating suitable concentrations, contact times and applications for the typical disinfection requirements of each individual department.

Several disinfectant agents are recommended for particular uses within the NDCN: 1-3% Virkon, Microsol, Chemgene, 70% ethanol, 70% isopropanol, formaldehyde. Further details of disinfection can be found in the *NDCN Laboratory Safety Policy 0005: Disinfection policy*. Methods of disinfection should also be included in the relevant risk assessment for any given procedure. The information in the policy can also be useful when creating risk assessments.

Good tissue culture practice

Bins

Bins should be used accordance with the waste management mentioned above. Yellow plastic bins are for pointy plastic clinical waste e.g. plastic pipettes and pipette tips NOT FOR GLASS, GLOVES OR LIQUIDS. Do not use a bag to line the bin, as this may cause confusion as to the type of use of the bin.

Traps

Should be emptied when reaching half full, washed out and a scoop of Virkon added with a small amount of water.

Stocks

When you are about to take the last items from the tissue culture please restock from tissue culture entrance. This also includes if you are taking one of the last aliquot items from the freezer, it is everyone's responsibility to aliquot more. If you notice stocks are low please inform Khwaja, Sian or Mark.

At the end of the session

- Before and after using a hood it should have all the tips and glass pipette tubs closed and sprayed all over with 70% alcohol and wiped down.
- Aspirator should be checked and if over half full emptied.
- Please always turn microscope's off after use!
- Empty bottles, pipette and tip boxes left on bench nearest door to be taken back to lab for cleaning.
- Pick rubbish off the floor and place in appropriate bin.

If it is the end of the day (5pm onwards) please close the hood if not required by anyone else. IF YOU ARE THE LAST IN TISSUE CULTURE IT IS YOUR RESPONSIBILITY TO CLOSE AND SWITCH OFF ALL HOODS AND ASPIRATORS.

Fluorescent microscope

The microscopes can only be booked 24 hours ahead of time for a maximum of an hour in the morning and an hour in the afternoon, exception being for diagnostic assays, although the diagnostic assayers should consider not booking both scopes at the same time if possible.

Good practice

- If you finish a box of stock items; break it down and place behind bins.
- When a plastic bin is full; close the lid and place near tissue culture exit (NOT IN THE WAY).
- Be tidy; pick stuff up off the floors, throw it in the appropriate bin if rubbish.
- Consider others; you should be leaving the hood how you would expect it to be left for you. Especially if you leave your items in there consider how someone may use it briefly in-between you time in there. Can they use the space? Will it make it easy to accidently contaminate your work?

- The monthly general lab clean is mandatory. All groups and individuals who use the labs are expected to help clean them. A sign-up sheet of jobs goes up the week before so ensure your name is on the list.

Storage of samples in liquid nitrogen

Dos & Don'ts

- When filling Dewars or transferring liquid nitrogen from any other pressurised system,
- When pouring liquid nitrogen from a Dewar, you must wear a suitable Chemical Resistant Face Shield, cryogloves and laboratory coat.
- When handling liquid nitrogen or working on equipment which may be at cryogenic temperatures, you must wear suitable Cryogenic Gloves. The gloves must be loose fitting, so they can be thrown off rapidly if the liquid is spilled into them.
- Open toed shoes must never be worn, particularly when handling liquid nitrogen.
- Use only containers designed for holding Liquid Nitrogen – Do not use domestic thermal flasks.
- When charging a warm container or inserting objects into the liquid, always perform the operation slowly to minimise boiling and splashing.
- Use tongs to withdraw samples immersed in the liquid and handle the tongs and the sample carefully.
- Never store samples in the Liquid Phase when the Vapour Phase will suffice.
- Always bear in mind that Liquid Nitrogen may seep into vials that have been stored in the Liquid Phase. On warming, this liquid nitrogen will turn to large volumes of gas, which can lead to the vials exploding. Therefore when removing vials from the liquid phase, a face shield MUST always be worn and the samples MUST be immediately placed into a secondary container with a closed lid (e.g. sandwich box, larger tube) to allow them to warm up safely.
- Always handle liquid nitrogen in a well-ventilated area. It is worth noting that in an unventilated room, equivalent size to a Cold Room (3m x 3m x 3m), it would only take a spill of 6L of liquid nitrogen to reduce the oxygen content to below the dangerous level of 18%. Therefore always keep the volume of Liquid Nitrogen to a minimum and if you do need to handle large volumes in a poorly ventilated area, then you should always have an Oxygen Meter to hand. Contact H&S Officer for further advice.
- Equally never accompany liquid nitrogen vessels in a lift - always send them up and down on their own.
- Filling of vessels from the supply vent must only be carried out by suitably trained individuals.
- Filling of vessels from the supply vent must never take place outside normal working hours of the Centre.

Emergency Procedures

Oxygen depletion alarms

Oxygen depletion alarm will sound at a warning level when the oxygen level drops below 19% you should leave the area at that point. If the oxygen level drops below 18% then the alarm level will raise and alarms will sound in reception and on Carillion's building management system. The area should be evacuated and not returned to until confirmation the oxygen level has reached a safe level

Eye Contact

In case of freezing or cryogenic burns caused by rapidly evaporating liquid, remove victim from the source of contamination. Open eyelids wide to allow liquid to evaporate. Do not wash the eyes with hot or even tepid water. Contact Medical Assistance. If the victim cannot tolerate light, protect the eyes with a light bandage.

Skin Contact

Contact a First Aider. Remove contaminated clothing, without exposing yourself to the same cryogenic temperature and flush the affected areas with lukewarm water. If severe and particularly if blistering has occurred, Medical Attention must be sort.

Ingestion

Obtain Medical Assistance immediately.

Inhalation

Following a large spillage of liquid nitrogen, evacuate the area and call for help – Contact reception on 234829. If you suspect that someone is suffering from asphyxiation, do not enter the affected area alone – you must call for help. Only if it is absolutely safe to do so i.e. indications are that the Oxygen content is above 18%, then remove the individual to the fresh air and call for a First Aider. If the victim is unconscious, obtain Medical Assistance immediately.

Emergency Procedures for a Major Spillage

If there is a significant release of Liquid Nitrogen, then:

- Evacuate the area and call for help.
- Contact 'reception 01865 234829 (or out of hours tel. 07521161549) at the earliest opportunity.
- Treat any individuals as above for skin, eye, and inhalation and ingestion exposure.
- If there is any uncertainty, then contact BOC (Tel. 0800 222 888).

Microwave ovens

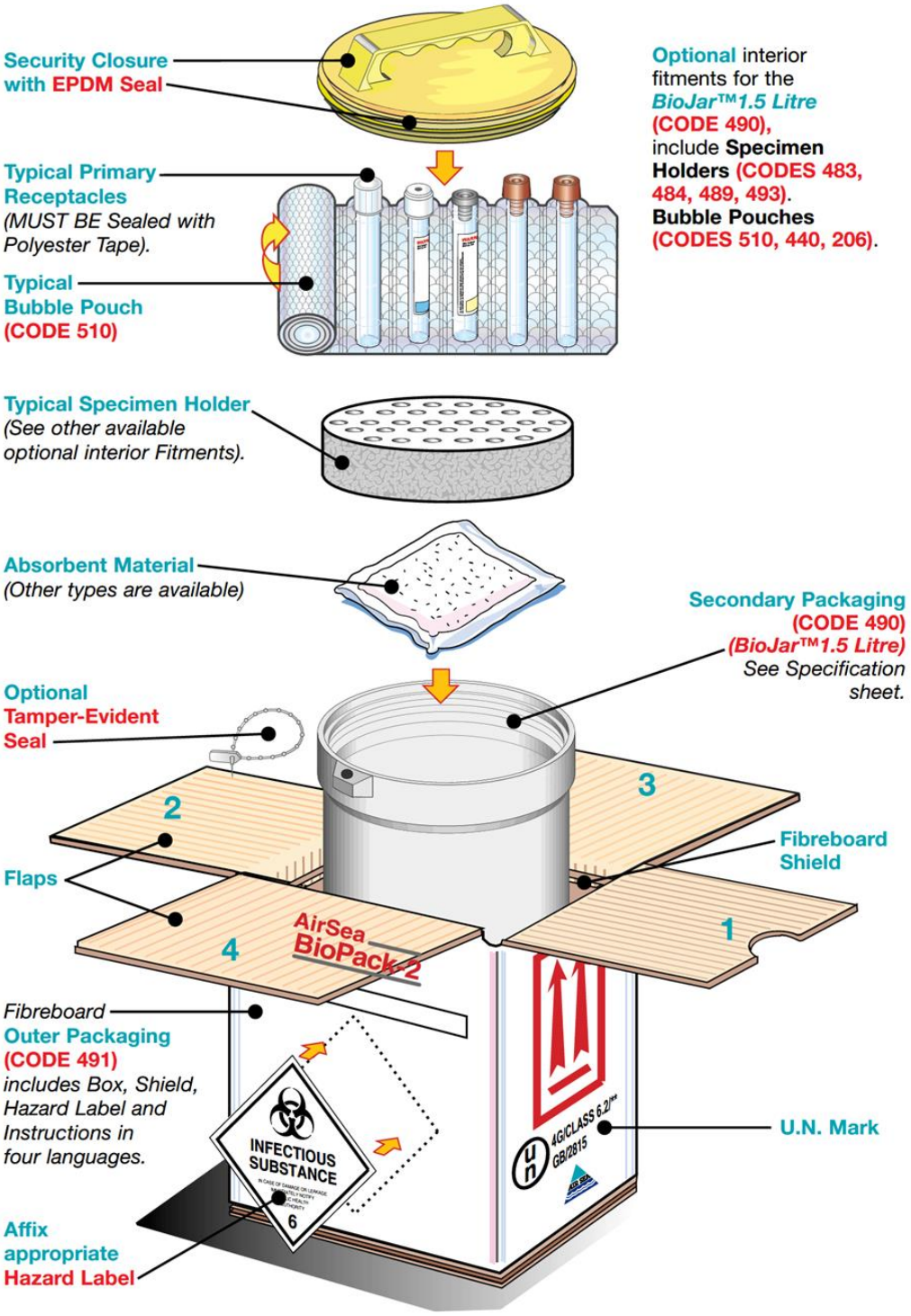
Dos & Don'ts

- Make sure that the Departmental Safety Officer (DSO) is aware that you have or about to obtain a microwave oven, so that regular testing can be carried out.
- Never place metallic objects in a microwave oven as it can lead to electrical arcing and therefore damage to the microwave itself.
- Microwaves are highly energetic and can cause deep tissue wounds to exposed personnel if the safety shielding, such as that around the door seal, has broken down. They should therefore be used in accordance with the manufacturer's instructions and routinely checked for signs of damage to the door, seals, rotating plate or viewing screen. Inform DSO of any problems identified.
- Never seal a bottle – Always completely loosen the cap before heating so as to prevent an explosion. Also, large volumes of solidified agar (> quarter full) should never be warmed in a microwave unless the agar is first chopped up with a disposable pipette or sterile spatula. Microwaves focus the heat at the centre of any substance and can cause explosive vaporisation in solid agar if the vapour cannot escape.
- Solutions, especially agar and agarose, warmed in a microwave can easily become superheated and boil over if moved immediately. Wait for a minute to allow sufficient cooling before carefully removing. Always wear eye protection and heat resistant gloves (Disposable gloves are not sufficient).
- Toxic substances heated within a microwave oven may vaporise causing unnecessary exposure of staff and contamination of equipment, e.g. Ethidium bromide should be added to a gel mix just before the agarose solidifies not before warming in the microwave oven.
- Do not heat flammable liquids or dry off solvents in a microwave oven. Flashpoints are easily exceeded and vapour concentrations can be sufficiently high to cause an explosion or fire.
- Microwave ovens in the laboratory must never be used to heat food.

Transporting biological samples

When transporting or sending biological substances the aim is to contain the item so that it is both preserved for the journey and is isolated from the environment through which it is travelling. Items which are to be posted must be packaged using approved packaging which conforms to national and international standards. Suitable packaging is available from a range of suppliers and must conform to IATA code P620 or P650. Details of this type of packaging and a list of suppliers can be found in NDCN Laboratory Safety Policy 0010.

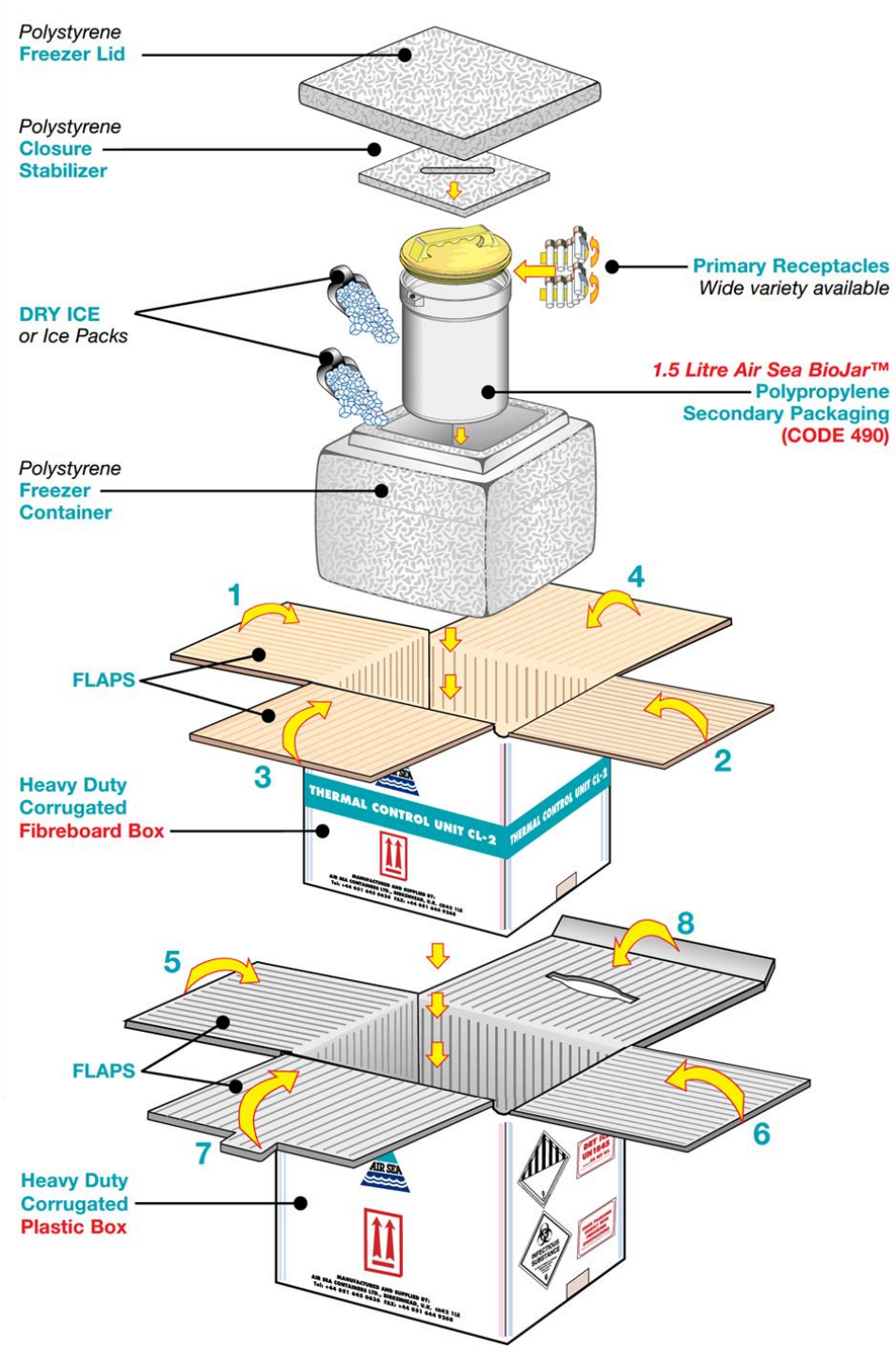
Room temperature packaging system



A triple packing system should be used where the substance is sealed in a primary container such as a vial or capped tube. This then goes into a secondary container which is padded and contains an absorbent material that can soak up any leakage if the primary container breaks. The secondary container is sealed and placed into a tertiary container onto which the appropriate postage and hazard warning labels are affixed.

If the substance is to be cold shipped then the refrigerant (usually dry ice) goes between the tertiary and secondary containers. The tertiary container should be vented if dry ice is used. Dry shippers are an alternative.

Cold temperature packaging system



5. Laboratory procedures

Deliveries

Deliveries arrive at Level 6 reception in the afternoon where they are receipted and assigned to the consignees. The laboratory technician brings laboratory deliveries down to level 5 later in the afternoon and leaves items for individuals in person or near their benches if they are not available. If nobody is available to receive an item; cold items are stored in Cold Room 3, -80C items in the -80C ULT freezer in the procedure room and -20C items are stored in the freezer in the prep room. Absent consignees will be emailed.

Ordering compressed gases

BOC will only accept requisitions from the Facilities Team, so if you require gas you should complete the compressed gas requisition form (available from reception and downloadable from NDCN Facilities Intranet). You will need to provide a valid grant code. There is an ongoing charge for rental also, which will also need an assigned grant code.

Ordering equipment

If you are ordering large or bulky pieces of equipment you must inform the Facilities Team before placing your order so that they can confirm that space is available to locate it. Also, you must give the FM team as much notice as possible when you receive notification of a delivery date.

Servicing equipment

You should tell the FM team if you are expecting a contractor to service a piece of equipment, this is so that a permit to work can be arranged. It is also a requirement that equipment to be serviced must be correctly decontaminated and certified as such. The decontamination certificate is downloadable from the NDCN Facilities Intranet).

Glass washing and sterilisation

Dirty laboratory glassware should be left on the trolleys provided in each lab. The laboratory technician will collect the trolleys when they are full and put the glassware in the glassware washer. When they are clean they will be returned to the appropriate storage areas.

Sterilization runs of glassware and solutions are carried out at 10am and 2pm. Leave the items on the sterilization trolley in the prep room. Items for sterilization should be correctly labelled and bear your name. Use autoclave tape to lightly seal the items, making sure there is adequate venting so that steam can both enter the vessel and escape.

Speak to the laboratory technician for further guidance and special requests.

Bookable resources

Confocal microscope

The confocal microscope in room 66-27 can be booked following appropriate training. There is an hourly charge for using the microscope, which can be booked using Google Calendar. Ask the Facilities Team for more details on training, booking and charges.

Procedure room

The procedure room (66-04B) is a Home Office designated space for performing some types of animal procedures. There is no charge for booking the room, but you will need to arrange swipe card access to use it. The room can be booked using Google Calendar, ask the Facilities Team for more details.

6. Access control

Annual access renewal

Every year you will need to have your access privileges renewed. The date printed on the front of your Hospital ID card is the date that the card was issued. On the anniversary of this date every year, all of your access privileges will expire unless you renew them beforehand. In the few weeks before the expiry date you should visit the Card Issuing Office and ask to have your access rights renewed for another year, you will need to show your Oxford University ID card.

Applying for 'out of hours' access

After you have been here for at least one calendar month you will be eligible to have your access privileges upgraded so that you have "out of hours" access during evenings, weekends and public holidays. To apply you will need to read and understand the Out of Hours/Lone Working Policy and both you and your supervisor will need to sign the Out of Hours Access Declaration document. Once this has been completed, hand the declaration into reception and your access will be upgraded. The Out of Hours pack containing the policy and declaration can be collected from Level 6 Reception or downloaded from the FM Intranet site.

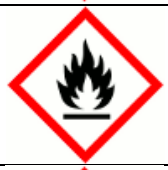
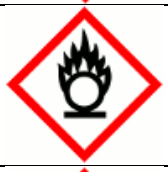
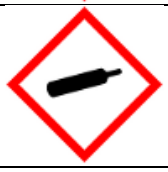
Applying for access to Neuropathology

To apply for access to Neuropathology on Level 1 West Wing you will need to complete Neuropathology Access Declaration document. You will need to obtain the signature of the person in Histopathology that you will be working or collaborating with. Once this has been completed, hand the declaration into reception and your application will be passed on to the Hospital Trust for ratification. The Neuropathology Access pack containing the policy and declaration can be collected from Level 6 Reception or downloaded from the FM Intranet site.

7. Local rules

Handling hazardous chemicals

1) Globally Harmonised System (GHS) Hazard Pictograms

Main hazard	Symbol	Other hazards
Explosive		Explosive; self-reactive; organic peroxide.
Flammable		Flammable; self-reactive; self-heating; pyrophoric; emits flammable gas; organic peroxide.
Oxidising		Oxidising agent.
Corrosive		Corrosive; burns skin; damages eyes; corrosive to metals.
Acute toxicity		Acutely toxic.
Hazardous to the environment		Hazardous to the environment; toxic to aquatic life.
Health hazard /hazardous to the ozone layer		Harmful to health; acutely toxic; irritant to skin, eyes or respiratory tract; skin sensitiser.
Serious health hazard		Carcinogen; mutagen; reproductive toxin; respiratory sensitiser; target organ toxicity; toxic if aspirated.
Gas under pressure		Pressurised gas.

2) Sources Of Additional Information

- COSHH risk assessments
- Material Safety Data Sheets (MSDS)
- Information on containers.
- Internet.

It is your responsibility to ensure you know what controls are needed to ensure no harm will come to yourself or others. You **must not begin** any work until you are absolutely sure of the appropriate precautions to take. If you are unsure, consult your supervisor, group head or the Departmental Safety Officer.

3) Introduction

Most chemicals have the potential to cause some harm, therefore in UK law there is a specific and absolute requirement to carry out 'Risk Assessments' on their use. This comes under the "Control of Substances Hazardous to Health Regulations". Hence the term COSHH assessments.

The following section details when & how these assessments need to be done. It is accepted that many chemicals pose limited risk if handled according to "Good Chemical Practise". However, if you work within the laboratory, at some point you will almost certainly come into contact with a particularly hazardous chemical (e.g. Ethidium Bromide, Acrylamide, Phenol, Formamide, 2-Mercaproethanol). How these specific chemicals need to be handled is detailed in COSHH assessments, and although the assessment may have already been completed, you must understand the reasoning behind the assessment to ensure the appropriate control measures are followed. The next section takes you through the requirements of COSHH and gives you an indication on completing the assessments.

4) COSHH Risk Assessment

Assessments need to be made on those chemicals whose hazards cannot be controlled by good chemical practise (see below) & all biological agents (see NDCN policy on handling biological material).

A formal COSHH assessment is not always required. If the substance has a low hazard rating or the quantity being handled is small or the dilution makes the substance very weak or the exposure is limited, a written assessment is not necessary. To help decide on this status, a useful starting point is to ask whether the hazard rating is such that normal practice and/or good chemical practice is sufficient to control exposure – if the answer is yes, then a formal COSHH assessment is not necessary. However a written assessment will always be required, but certainly not limited to the following instances:

- All microorganisms, classified under the ACDP or DEFRA guidelines as Hazard Group 2 or above.
- Where substances in use have been assigned any of the following risk phrases: H300, H304, H310, H330, H314, H317, H330, H340, H341, H350, H351, H360, H361, H361d, H362, H372.
- Where extreme toxicity is indicated (e.g. LD50 oral, rat < 1 mg/Kg).
- Where special first aid provision is required (e.g. cyanide, hydrofluoric acid, phenol).
- Where significant amounts of substances with a Maximum Exposure Limit (MEL) or Occupational Exposure Standard (OES) are used in unenclosed or partially enclosed circumstances (e.g. use of volatile chemicals on the open bench with no fume cupboard or other local exhaust ventilation).
- Where procedures involve explosive or pyrophoric substances (although not strictly covered by COSHH regulations – the form would provide a convenient format to start the assessment).

5) Documentation & Training

To meet the requirement of the regulations, Group Heads must ensure that individuals within their group carry out appropriate COSHH assessments before they begin work with a new substance. All documented COSHH assessments (and relevant information) must be kept in such a place that all members have access to it. All new staff & visitors to the group should be made aware of the whereabouts of these assessments. They should also be specifically guided as to any action they need to take as and when they commence working with the assessed substances. A copy of the assessments should also be forwarded to the Departmental Safety Officer (DSO), who must ensure that all other visitors, contractors and support staff are made aware of these assessments if they too are to be affected.

As many of the processes carried out within the Centre are the same, there are generic assessments available from the DSO. It is not necessary therefore for all groups to duplicate these assessments, but they must make sure that the assessment is relevant to the work they are carrying out. If for instance a different substance is used or the volumes are significantly different, then they will have to complete a new assessment as before.

6) Individuals Affected

It is also worth noting that when completing the assessment, certain individuals, dependant on the material in question, may also be at higher risk. Anyone who knowingly fits into one of these categories should be made aware of the risk and should be advised to consult the DSO or Occupational Health for further guidance. These people are:

- Anyone who has a compromised or suppressed immunity through existing disease or medication.
- Pregnant (when the foetus may also be at risk) or nursing mothers.
- Anyone who may have a history of asthma (and therefore may be at increased risk from respiratory sensitisers).
- Anyone who has a skin condition such as eczema.

7) Completing The Assessment

Where a Formal COSHH assessment is required, it is recommended that the University COSHH Assessment pro-forma be used (available from the University Safety Office (USO) website). The following information details how to complete this assessment.

8) Guidance On How To Complete The University COSHH Assessment Pro-forma

1. Complete the first sections, detailing the procedure under assessment, who is likely to be affected and therefore the scope of the assessment.

2. In the next section list all the substances to be used in the procedure.

3. For all the substances listed above, identify the hazards associated. Information regarding hazards can be found in the following sources:

    - Substance container label
    - MSDS (contact suppliers directly or search their websites).
    - Previous COSHH assessments
    - Colleagues
    - Technical reference manuals i.e. Merck Index
    - Suppliers catalogues
    - DSO and Divisional Safety Officers (DivSO).

4. By using the following tables, score each of the categories and calculate the associated risk. It is important to take into account quantities and nature of the chemical when assessing a risk.

Table 1. Hazard Calculation

SCORE	HAZARD CATEGORY	QUANTITY	PHYSICAL CHARACTERISTIC
4	Substances classified as Very Toxic, EEC Class 1&2 carcinogens, Respiratory sensitisers, 'Unknowns' suspected of very high toxicity, OEL <0.1ppm, Risk Phrases R26, R2, R28, R39, R42, R45, R46 ACDP Hazard Group 3 & 4 Pathogens (**Must NEVER be handled live within department).**	> 1 Kg > 1 Litre	Substances likely to promote absorption though lung, skin or mucous tissue.
3	Substances classified as Toxic or Corrosive Skin sensitisers, 'Unknowns' not in the very high category, OEL 0.1 to 10 ppm, Risk Phrases R23, R24, R25, R34, R35, R39, R43, R48 ACDP Hazard Group 2 Pathogens	100 g – 1 Kg 100ml – 1 Litre	Gases, Highly volatile liquids, Aerosols, Solutions that promote skin absorption. Infection by inhalation.
2	Substances classified as Harmful or Irritant, EEC Class 3 Carcinogens & Mutagens, OEL 10 to 500 ppm, Risk Phrases R20, R21, R22, R36, R37, R37, R40, R41, R48, ACDP Hazard Group 1 Pathogens	1 – 100 g 1 – 100 ml	Dusty Solids, Lyophilised (easily dispersed) Solids, Volatile Liquids Concentrated Solutions, Low skin absorption. Percutaneous Infection.
1	Substances not identified as Hazardous, OEL > 500 ppm	< 1 g < 1 ml	Dense Solids, Non-volatile liquids, Dilute solutions, No skin absorption Infection by Ingestion

Table 2. Risk calculation

Probability/Risk Maximum Possible Loss	Very Unlikely	Unlikely	Even chance	Probable	Very likely	Certain
No loss	1	2	3	4	5	6
First Aid Required/short rest/recovery	2	4	5	6	7	8
Mild temporary illness	3	5	6	7	8	9
Loss of limb, eye or permanent illness	4	6	7	8	9	10
Fatality	5	7	8	9	10	11

Calculated risk = Hazard score X risk calculation

5. From Table 3 below, note the Risk Rating, and then decide on the appropriate control measures required to control the risk. **Note:** this is **only guidance** and all the factors specified in the table above should be considered before deciding on the appropriate actions. Note also that the first way of limiting risk is to decide if a less hazardous substance can be used. If it can, but you do not use it, then you must record the reasons why this is so.

Table 3. Risk Rating & Control Measures

CALCUL-ATED RISK	RISK RATING	Recommended actions for Substances hazardous by Inhalation	Recommended actions for Substances hazardous to Skin
1 - 10	Low	No special Precautions Required. General Laboratory Rules Apply.	
11-20	Medium	Must handle in Fume Cupboard or MSC (as appropriate) or if risk is such, Wear Appropriate Face Mask	Must Wear Appropriate Gloves and unless risk is such, Safety Glasses should be worn.
21-30	High	Must be handled in a Fume Cupboard/MSC (as appropriate) or special containment Facility	Must Wear Appropriate Gloves & Consider the need for Full Face visor. In some circumstances a special isolation facility may be necessary.
>31	Very High	Contact the H&S Officer before commencing any work.	

6. For those chemicals that have been assessed as Medium or above then record the appropriate control measures in the relevant part of the next section. Consider the following points when recording the control measures:
    - *Engineering controls. Some examples are*:
        - Must be handled in a fume cupboard.
        - Must be handled in a microbiological safety cabinet (MSC, Class I/II/III).
        - Handle in a well-ventilated area etc.
    - *Personal Protective Equipment. Some Examples are*:
        - Wear safety glasses.
        - Wear chemically resistant gloves (Note: You should always specify what type of gloves are recommended e.g. 'wear nitrile gloves', as some chemicals will react with certain rubbers).
        - Wear a face mask (again you may need to specify the type of mask – see DSO for advice).
    - *Management Controls. Some Examples are*:
        - Good microbiological practise.
        - Keep separate from oxidising materials.
        - Store at room temperature & avoid direct sunlight.
        - Never use sharps.
        - Avoid the creation of aerosols.
        - Do not handle out of normal working hours.
        - Inexperienced workers will require supervision.

7. In the final parts of the form, note any emergency procedures that apply. For most substances, this can be recorded as 'normal emergency response applies – contact a first aider'. However, some substances require specific actions above and beyond the normal response and these must be fully documented e.g. Phenol. For your information, the normal emergency response when handling hazardous chemicals is:
    - Contact first aider and if severe, obtain medical attention.
    - If contact with eye, irrigate using one of the eye wash taps provided, for at least 10 minutes.

- If contact with skin, drench the area affected thoroughly with water. If necessary use one of the eye wash taps or an emergency shower to direct the water to the affected area. Remove all contaminated clothing and wash before re-use, also clean up water and dispose as contaminated waste.
- If inhaled, remove from exposure, rest & keep warm.
- If ingested, wash out mouth thoroughly with water. Do not give anything to drink & do not induce vomiting, instead obtain medical attention immediately.

8. Finally complete the sections on waste disposal. Again 'normal waste procedures apply' could be satisfactory, unless there are some specific requirements.

9. One important aspect of the last sections is the 'checks on control measures'. This refers to checks required for any equipment used to limit exposure. Some examples are:
   - Fume Cupboards must be checked annually.
   - Microbiologically Safety Cabinets must be checked annually.
   - Respiratory Protective Equipment (Non-Disposable) i.e. Mechanical Respirators must be checked monthly.

10. Next sign the form as complete and arrange for the Group Head to countersign it. Once completed pass a copy to the DSO and inform all those that will be affected of the assessed requirements.

11. Unfortunately the responsibility does not finish there – ***IN FACT, IT ONLY JUST BEGINS***. According to the COSHH regulations, the control measures need to be monitored & reviewed to ensure that all actions are adequate. At the very minimum, the assessment must be reviewed every year, to ensure that all information is relevant. This will include checking that individuals are following the appropriate control measure and that it is working.

9) Good Laboratory Practice (GLP)

As stated above, the majority of chemicals used within the department do not pose any significant hazard provided they are handled with appropriate caution. The following details the very minimum therefore that everyone must be following to ensure the health of themselves and fellow colleagues.

General Rules

- Appropriate laboratory coats must be worn when working at the bench.
- Eating, drinking, chewing, smoking, mouth pipetting and applying of make-up is forbidden.
- All work should be carried out in a tidy & organised manner. Plan and lay out your work so that everything needed is ready to hand.
- Work must be performed with the minimum of spilling and splashing in order to limit contamination. Suitable dispensing aids must be used and substances handled over spill trays if appropriate.
- Remove all protective clothing before leaving the laboratory completely.
- Wash hands before leaving the laboratory and as often as is necessary. Only use the 'elbow' style taps provided for this purpose.
- Bottles, especially Winchester size, should be transported in appropriate carriers.
- Flames and compressed gas supplies should be shut off when not in use and at the end of the working day.
- NDCNs policy for late or lone working must be adhered to - The Department therefore recommends in general, that staff should not work alone. In particular, working with quick acting, highly toxic or asphyxiating materials is prohibited for persons working alone. Examples include large amounts (>0.5 litre) of: concentrated acids, phenol, ammonia and cryogenic liquids.

Inhalation Controls

- Inhalation of vapours is to be avoided. In general terms, fume hoods must be used for substances that are toxic by inhalation, fuming or have unpleasant smelling chemicals.
- Fume hoods must be left as clear as possible both for the proper functioning of the hood and for the safe handling of materials. All waste must be removed at the end of a period of work.

- Fume Hoods are tested regularly but, if your fume hood is not functioning (e.g. indicator light is not within safe limits), report the fact to the facilities team immediately. Do not use it until it has been passed safe to do so.
- When working in the hood, always keep the sash to the absolute minimum height and generally never above 30cm.
- Recirculating fume hoods provide only limited protection and are not suitable for many operations. A list of approved substances must be drafted in consultation with the DSO and attached to the hood. You must contact the DSO before handling ANY substance not listed in such hoods.
- When not in use, always close the sash to enable the hood to vent correctly.
- Appropriate respirator masks (see DSO for guidance) can be used for handling small quantities of fine powder. However it must be remembered that this only offers protection to the user and that others working in the same area could be affected. Fume hoods should always be used for handling large quantities of fine powder.
- Never handle hazardous substances in poorly ventilated conditions.
- Exposure to gases and vapours should be limited by covering vessels and replacing caps or stoppers promptly.

Skin Contact Controls

- Wear appropriate disposable gloves at all times when handling substances hazardous to skin (or longer sleeved type if the arms are also at risk of contact with the substance).
- Appropriate gloves must always be worn when working with acids, alkalis, and other corrosive liquids and liquefied gases, as a protection against splashing.
- Information as to appropriate gloves is provided within the Material Safety Data Sheet – Further guidance can be given by the DSO or DivSO.
- If the risk of skin contact is significant, it is always advisable to 'double-glove'.
- Gloves should be checked for holes or other signs of damage before use.
- If gloves become heavily contaminated – remove immediately & replace with a fresh pair.
- Never re-use disposable gloves as this is likely to lead to imperfections & limits their protective nature.
- Remove gloves as much as possible, before leaving the laboratory area you are working in. If it is necessary to keep gloves on when travelling between laboratory areas, always take one glove off to open doors and carry items in the other. If is absolutely necessary to keep both gloves on, ensure you use the handles on doors designated for 'Gloves only'.
- Routinely check hands for cuts and abrasions and cover with a waterproof dressing before commencing any work.
- Avoid the use of sharp objects. Dispose of broken glass or sharps via the appropriate route. Never leave sharp objects such as disposable scalpels lying around.
- One very significant risk is in the use of phenol. Phenol not only causes burns on contact with skin but will also permeate the skin and is toxic. Great care must therefore be taken in handling this substance to prevent contact with skin and operators must be suitably trained in the appropriate emergency procedures:

    - Contact first aider & if severe, obtain medical attention.
    - If contact with eye, irrigate using one of the eye wash taps provided for at least 10mins. **DO NOT** use PEG 300 on the eyes. Obtain medical attention.
    - If contact with Skin**,** remove contaminated clothing avoiding contamination of unaffected areas. Wash the affected area with copious amounts of water to remove any excess Phenol that has not been absorbed. Swab the contaminated skin with Phenol Antidote (Polyethylene Glycol 300), but do not scrub as this may lead to spreading the contamination. Obtain Medical Attention for all Phenol burns.
        - If inhaled, remove from exposure, rest & keep warm.
        - If ingested**,** wash out mouth thoroughly with water. Do not give anything to drink & do not induce Vomiting, instead obtain Medical Attention immediately.

Eye Protection

- Suitable eye/face protection may be necessary – these should have been identified within the specific COSHH assessment.
- Safety glasses should always be worn where there is a risk of splashing of substances hazardous to eyes.
- However, suitable goggles or face shield should be worn if the work may generate fumes, mists, dust clouds or heavy splashing.
- Safety glasses (or indeed goggles & face shields) must always be worn when working with acids, alkalis, and other corrosive liquids and liquefied gases, as a protection against splashing.
- Examine all personal protective clothing and equipment before use and replace any that are damaged or likely to be ineffective.

Flammable Materials

- Highly flammable liquids should be stored in sealed containers in fire resisting enclosures.
- Quantities should be kept to a minimum and the total quantity stored should not exceed 50 litres in any one side of a laboratory.
- Never store or hold volumes >500ml on the open bench.
- They must not be stored in refrigerators and freezers which are not deemed 'spark-proof' (units with internal lights, in general, are not). Unsuitable refrigerators and freezers carry standard warning labels – however you should check before use.
- Flammable substances must be kept well away from sources of ignition including naked flames, electric hot plates and non-flameproof electrical equipment.
- Do not overheat substances with low auto-ignition temperatures, or allow their vapours to come into contact with hot surfaces.
- Highly flammable liquids must not be poured down the sink.

Spillage

- Individuals must be aware of the nearest 'emergency response spill kits'.
- Contaminated surfaces and equipment must be cleaned without delay.
- Information relating to hazards of the materials in use & specific spillage procedures should be clearly detailed and accessible in COSHH assessments.
- Any personal contact with a substance should be dealt with immediately by safe and effective decontamination – refer to COSHH assessments.
- All accidents must be reported using the incident report book.

Containers & Storage

- Substances must be put in approved storage enclosures when not in use e.g. flammable material in flammable cupboards; acids/alkalis etc. in Corrosive cupboards.
- All containers must be properly labelled, including any appropriate hazard warning label.
- Chemicals not in use should be returned promptly to their correct storage enclosures.
- Light sensitive substances (e.g. chlorinated solvents) should be stored in amber-coloured bottles away from the light. It is prudent to keep all chemicals out of direct sunlight
- Surplus materials must not be allowed to accumulate in laboratories. Periodically check storage areas & remove duplicated or unused substances.
- Never store hazardous chemicals above the lower shelf on the central shelving.
- Always keep oxidising substances separated from flammable substances & sources of ignition.
- Always check compatibility of chemicals & ensure incompatible substances are stored separately.

10) <u>Chemical Waste Disposal</u>

- Waste chemicals should be assessed for hazard level using COSHH assessments, Risk assessment for the procedure they are used in or the material safety data sheets. If in doubt ask the DSO. If chemicals are mixed assess the most hazardous component.
- If the chemical is determined non-hazardous or as low risk and is a small quantity then the chemical can be disposed of down the drain with copious amount of water. This could apply to washing out 2.5 litre Winchester bottles.*

- If a chemical is deemed hazardous it must be disposed of via the University Safety Office.
- To dispose of chemicals via the University Safety Office first complete the form TW 2/10 (disposal of hazardous substances) which is available from the University Safety Office website.
- The information the form will require is, chemical name, quantity, risk number, primary/secondary hazard, whether the substance is organic or inorganic and whether it is in liquid or solid form.
- The completed form (TW 2/10) should be returned to the DSO as an e-mail attachment who will coordinate with disposal with the University Safety Office
- The USO will email the Deputy Facilities Manager the TW number for chemicals to be disposed.
- Once the TW number has been generated by the USO, the DivSO, will contact the Deputy Facilities Manager to arrange a convenient date and time to collect the chemicals to take to the hazardous waste store (West Wing Level 0 behind the FMRIB building) where he will pack them and arrange for their final disposal by an external waste contractor (Grundon).
- The Deputy Facilities Manager will email the end user the TW number who should label the chemicals with TW numbers and store them in a container in the laboratory until collection date.

* When Winchesters are empty and rinsed cross out the chemical name and any hazard symbols and dispose of next to the wheelie bins in the waste store room on level 5 (code is 05-66-02).

Working with biological materials and genetically modified organisms

1) Introduction

These local rules identifies the measures needed to comply with the University Guidance note S5/09, and subsequently all legal requirements of handling biological material. Therefore all individuals handling biological material within the NDCN must comply by these rules. If for any reason individuals wish to carry out practises outside of these rules, then they should approach the Biological Safety Officer for approval before commencing any new work.

2) Risk Assessments

Micro-organisms (including genetically modified organisms) may be encountered in the laboratory whenever, specific micro-organisms are intentionally cultured for research or diagnostic purposes, where samples or tissues of human or animal origin are handled, where laboratory animals are kept and where hazardous plants or plant materials are grown or used. Therefore in line with the *Control of Substances Hazardous to Health Regulations, 1999* an assessment must be made of the potential risk associated with handling any of these materials.

The basic rules are:

- All material containing or potentially containing (even by contamination), micro-organisms must have a documented COSHH assessment completed before starting any work with that material (Even if the likelihood is that the micro-organism will be assessed as Hazard Group 1 under the ACDP). A generic COSHH assessment can be made that covers a number of similar microorganisms or material, but it must be suitable and sufficient.

- All individuals who may be at risk must have access to this assessment and their attention must be brought to any specific control measures needed. Therefore a copy should be kept by the Group where the material is handled and also a copy must be forwarded to the Biological Safety Officer.

- All laboratories within the NDCN are classed as Containment Level 2. Therefore any organism known to be or assessed as being Hazard Group 1 or 2 can be handled. Equally the use of organisms known to be or assessed as being Hazard Group 3 or 4 are **prohibited**, or at least until appropriate control measures have been taken and approval given by the University Safety Office.

- In the case of Genetic Modification an additional Risk Assessment must be made and approval must be given by the Genetic Modification Safety Committee before work can commence. Information on GM assessments is given later in this policy.

3) Work With Micro-organisms

All new microorganisms brought into the Department must be reported immediately to the Biological Safety Officer, who will maintain a register and inform the University Safety Office as necessary. It is important to use the correct nomenclature and provide as much information regarding the species to prevent any confusion arising.

Most microorganisms have been classified either by the *Advisory Committee on Dangerous Pathogens* (ACDP) for known Human Pathogens or by DEFRA for known Animal Pathogens. The University Biological Safety Advisory Group will assign all microorganisms used within the University Departments to one of the associated hazard groups. Therefore once a new microorganism is brought into the West Wing the BSO will be able to provide you with confirmation as to the hazard group of that organism. Please note that handling some specified Animal Pathogens will require a license before work can commence.

4) Work With Inactivated Micro-organisms

It is acceptable to work with Hazard Group 3 or 4 pathogens within the Department ONLY if they are inactivated before they enter the building. The microorganism must be received with relevant information detailing the exact process of inactivation. If information is received that is contrary to the initial assessment, then the following must be carried out immediately:

- Inform your Group Head and the Biological Safety Officer.
- Wearing the appropriate protective equipment, isolate the sample and any subsequent area that may be affected.
- Mark all possible affected areas with biohazard tape, state 'Do not use or enter' and label with information as to who to contact.
- Wearing the appropriate protective equipment, decontaminate any affected (see disinfection and waste management policies).
- Do not use the material until information is received that states it is safe to handle.
- If it cannot be confirmed as safe to handle, then an alternative arrangement must be made to remove it from the building – BSO can advise where necessary.

Even though a letter of inactivation is received, all samples must be handled as if they are still capable of infecting – this is known as the 'precautionary principle'. Therefore it is advisable to contact the BSO before arranging to bring in samples of this type, so that a thorough COSHH assessment can be completed and appropriate safety precautions identified.

5) Good Microbiological Practice

The fundamental requirement of working with microorganisms is containment, be that within vessels, cabinets or the laboratory. By containing the organism, the risk to individuals is minimised and therefore controlled. These and subsequent other rules outline specific details of how to contain microorganisms. However many of the control measures can be achieved by 'Good Microbiological Practice (GMP)'. GMP are fundamental rules that are adopted by Microbiologists to ensure their samples are not contaminated by unwanted organisms entering their work. Conversely these same practices will prevent organisms escaping their work. These practices must form part of the control measures for all work involving any biological material. Therefore the following rules form part of this GMP and can be referred to when completing the relevant COSHH assessments.

- Ensure you are registered with the University Occupational Health Services for work with Biological Material before commencing any work.
- Side fastening or Back Fastening 'Howie' type Laboratory coats must be worn when working at the bench.
- Disposable gloves (none Latex) may also need to be worn when handling infectious or contaminated material. Specifically gloves should be worn when the likely route of infection is Percutaneous (through skin).
- Never re-use Disposable Gloves as this is likely to lead to infection.
- Remove gloves as much as possible, before leaving the laboratory area you are working in. If it is necessary to keep gloves on when travelling between laboratory areas, always take one glove off to open doors and carry items in the other. .
- Suitable eye/face protection may also be necessary – these should have been identified within by the COSHH assessment.
- Examine all personal protective clothing and equipment before use and replace any that are damaged or likely to be ineffective.
- Remove all protective clothing before leaving the laboratory completely.
- Wash hands before leaving the laboratory and as often as is necessary. Only use the 'elbow' style taps provided for this purpose.
- Routinely check hands for cuts and abrasions and cover with a waterproof dressing before commencing any work. If working with organisms likely to cause percutaneous (through skin) infection, then it is also recommended to wear two sets of gloves over damaged skin.
- Eating, drinking, chewing, smoking, mouth pipetting and applying of make-up is forbidden.
- Keep the laboratory clean and tidy.
- Keep your working area free of clutter: Plan and lay out your work so that everything needed is ready to hand.
- Before starting and once the work has been completed, wash down the bench area with a suitable disinfectant such as 2% Virkon or 80% Ethanol (Further information is given in the departmental disinfectant policy).
- Always clean up spillages immediately using the appropriate disinfectant technique. Therefore always ensure there are freshly made stocks of suitable disinfectant available to hand before starting any work.

- Routinely dispose of all waste generated via the correct Waste Disposal Streams. (Further information is given in the departmental waste policy).
- Never overfill waste containers as this may lead to spills.
- Ensure that appropriate storage, use, disposal and emergency procedures have been established before ordering any hazardous biological agent.
- When carrying out manipulations always use good aseptic technique.
- Use wherever possible techniques that minimise the production of aerosols. For manipulations such as vigorous shaking or mixing and ultrasonic disruption etc., a microbiological safety cabinet or equipment, which is designed to contain, the aerosol should be used. A microbiological safety cabinet should always be used for procedures involving microorganisms that are infectious by inhalation (via the respiratory tract).
- Identify which microbiological safety cabinets are appropriate to your work and learn to use them correctly (Further information on using MSC can be found later in this document.) MSC only protect against airborne hazards – remember that no protection is afforded against skin contamination and so all other techniques must be followed.
- Infectious materials may be centrifuged in the open laboratory if sealed rotors or sealed buckets are used, but they should be opened in a functioning microbiological safety cabinet, particularly if the infection is again by inhalation.
- Never use sharp objects or implements unless there is no alternative, particularly when handling percutaneous infectious material and specifically Blood. If Sharp items are unavoidable make sure that they are disposed of into appropriate puncture-resistant Sharp Containers for safe disposal.
- Routinely check all glassware & equipment for sharp edges. Replace damaged items wherever possible and limit the amount of sharp objects used.
- On a frequent basis, routinely replace the water in, and clean, water baths. This is also essential when a spillage of viable organisms or nutrient media has occurred.
- Do not remove infectious or contaminated material from the laboratory to another area unless it is suitably packaged in a sealed and labelled container.
- Immunisation and regular booster injections should be provided if appropriate as a supplementary safety precaution for those who may be exposed to pathogenic microorganisms.
- Label all storage facilities and equipment used for handling biological material. In particular ensure there is a Biohazard Label affixed, where large volumes of biological material are stored and particularly liable to contain Hazard Group 2 organisms.
- Always transfer Biological Material between laboratories down the rear corridor (Never through the offices). Always ensure it is kept within a sealed container (i.e. sandwich box) that can contain any spillages.
- When sending biological material out of the building, ensure it is packaged and labelled correctly.
- Restrict access to the laboratory areas - Only those people who have good reason for entry should be allowed into the laboratories and even then this should be kept to a minimum.
- Report all accidents and dangerous occurrences at the earliest opportunity, by recording them on an accident report form. It is advisable to inform the Biological Safety Officer or if necessary, University Occupational Health, immediately any puncture wound occurs whilst handling percutaneous infectious material. In any event the wound should be encouraged to bleed and the area washed with soap and water but without scrubbing.

6) Handling And Use Of Genetically Modified Organisms (GMO)

Genetic manipulation relates to any activity involving genetically modified organisms including, but not limited to, culture, storage, transport, destruction or disposal. Under current legislation genetic modification means the altering of the genetic material (DNA or RNA) of an organism using a method that does not occur naturally by mating &/or recombination. Under these regulations *in vitro* fertilisation, natural processes such as conjunction, transduction or transformation and polyploidy induction are not considered to result in genetic modification if they do not involve the use of recombinant nucleic acid molecules or GM organisms.

Any work that does full into this definition of genetic modification must therefore have a separate & specific GM risk assessment carried out before the work can begin. Therefore proposals to carry out such experiments MUST be made to the Genetic Modification Safety Committee for their consideration at the earliest opportunity. Experiments with low risk assessments (e.g. ACGM 1) can be provisionally approved by the Biological Safety

Officer on behalf of the ACGM, however experiments assessed as ACGM 2, 3 or 4 must obtain approval from the Health Safety Executive via the BSO & University Safety Office. This can take up to 45 days to obtain and so once again early notification is important.

Permitted experiments will be subject to the appropriate codes of practice and to the suitable means of containment laid out in the assessment. All individuals working with the assessment must be informed of the required measures and they should also inform the BSO when they begin work.

7) Genetic Modification Risk Assessments

An appropriate risk assessment, approved by Genetic Modification Safety Committee, is required before any work with or storage of genetically modified organisms (GMOs) can commence. These includes both novel modifications of micro-organisms, cell lines, or transgenic animals of any kind within the laboratory or the holding of and work with such GMOs generated elsewhere and imported into the department, laboratory, or biomedical services unit.

NB. Possession of vectors such as phages, plasmids, YACs, BACs etc., will not require an assessment but one must be completed and approved prior to any subsequent transformation or transfection of cells. However, many viral vector systems are themselves considered to be GMOs and must be covered by an assessment before they are obtained or used.

This classification is based on the potential harm it can cause the human population if released.
There are four categories as follows:

- Group 1
  a) Unlikely to cause human disease.
  b) In relation to susceptible animals*, is unlikely to produce disease or is enzootic and does not produce notifiable animal disease.

- Group 2
  a) Can cause human disease and may be a hazard to employees. It is unlikely to spread* to the community and there is usually effective prophylaxis or treatment available.
  b) In relation to susceptible animals*, is exotic, novel* or produces notifiable diseases; and it has both low clinical significance and a low likelihood of spread*.

- Group 3
  a) Can cause severe human disease and may be a serious hazard to employees; it may spread* to the community, but there is usually effective prophylaxis or treatment available.
  b) In relation to susceptible animals* is exotic, novel* or produces notifiable disease; and it is of moderate clinical significance and has a moderate likelihood of spread*.

- Group 4
  a) Causes severe human disease and is a serious hazard to employees; it is likely to spread* to the community and there is usually no effective prophylaxis or treatment available
  b) In relation to susceptible animals* is exotic, novel* or produces notifiable disease; and it has one or both of the following characteristics: the disease has serious clinical significance; has a high likelihood of spread*.

*Susceptible animals are any kind of mammal except man, any kind of four-footed beast which is not a mammal and any species of bird likely to be affected by the biological agent.

*Novel means a new strain of biological agent not previously seen.

*Spread means the passing of the biological agent from one susceptible animal to another and assumes any necessary enzootic vector is present.

8) Work with Blood and Human Tissues

Blood, blood products and other human tissues must not be handled within the NDCN without the permission of the Group Leader and the Biological Safety Officer. A separate biological COSSH assessment must therefore be completed and handed to the BSO at the earliest opportunity.

Blood in particular can arbour pathogens, most notably Hepatitis B (HBV) and Human Immunodeficiency Virus (HIV). Where it is known or strongly suspected that specific Hazard group 3 pathogens are present then the samples must be handled at the corresponding Containment Level. It is often wrongly thought that all blood from such westernised Countries is safe to use. Even in countries such as the UK, there are populations whose blood would be deemed high risk. Therefore before receiving any human tissue or blood material please do consult the BSO for further advice and guidance.

In general where it is permissible to handle blood within the Department, it is not always necessary to confine the work to microbiological safety cabinets, as the likely routes of infection of HBV & HIV is not by inhalation. Therefore provided the work does not generate large droplets or splashes then the samples can be handled in the open laboratory working to the GMP guidelines set out above. However it must never be assumed that the risk is completely negligible, so the following points must also be followed in addition to the ones already described above.

- Gloves must be worn at all times when handling samples and be removed before leaving the laboratory.
- If gloves become damaged or grossly contaminated, then discard immediately, wash hands and replace with new gloves.
- Wear Safety Glasses and a plastic apron, if the work activity is likely to cause splashing.
- Materials must be ONLY be handled in clearly identified and designated work areas.
- Ensure you have plenty of room to work and that you are unlikely to be affected by other individuals
- On completion of the work ensure that all areas and equipment are disinfected and that all waste is disposed of correctly and safely (Further information is given in the West Wing Disinfectant/Waste Policy Document).
- Samples must be centrifuged in sealed buckets.

Appropriate control measures for all other Human tissue in particular Brain tissue, must be made at the time of assessment.

Finally trials involving sampling blood from workers is permissible. However the Blood must NEVER be taken in the laboratory areas. There are rooms available to do this and the BSO must be consulted.

9) Work with Tissue Cultures

Many mammalian cell lines are tumour-derived or have been transformed/immortalised using genes encoding viral proteins. Cell lines, particularly those derived from human sources may therefore contain endogenous viruses such as HIV, and must therefore be handled as potentially infectious human material. Primary cultures from animal or human sources should also be treated as potentially infected.

Therefore all Tissue Culture work can only be carried out within the West Wing with the permission of the Group Leader or Biological Safety Officer. It must always be done within one of the Tissue Culture rooms and unless there is clear reason not to, carried out within a Class II Microbiological Safety Cabinet. Finally all work must be carried out to GMP as set out above.

Two additional points that must be noted are:

- Never carry out transformation of cells from your own blood or other tissue.
- Any incubation of cultures liable to exceed 100 hours must not be undertaken, as the risk of introducing pathogens such as HIV is too great.

10) Work with Naked DNA/Oncogenes

The risks associated when handling naked DNA are generally low since DNA is unlikely to transverse the natural defence barriers of the body. However it can enter the body if the skin is damaged or punctured, so as a very minimum, the points raised in the GMP guidelines appropriate to percutaneous infectious material must be considered i.e. avoid the use of sharps. Therefore naked DNA should never be considered as risk free and as such is subject to the same assessments and precautions as detailed above.

In particular always consider the consequences of expressing that DNA if it was able to obtain access. Does the DNA encode for a potentially hazardous product, such as toxins, allergens, growth factors or does it contain oncogenic sequences (Oncogenic sequences are capable of progressing cells through a stage in the multistage process of cancer). Therefore as above, identify the risk and implement sufficient control measures i.e. denaturing before starting work with any DNA sequence. It must be stressed that this is relevant to the COSHH regulations and therefore must be considered in addition to any GM assessment that may already be completed.

Also consider whether after purification of the DNA there is any chance of contaminants, such as viral particles, being still present. If there is a possibility that they could be, then further steps such as inactivation or secondary purification, should take place to limit the risk.

11) Work with Animals

The BSO must be consulted before any work with animals can be carried out.

12) Microbiological Safety Cabinets (MSCs)

The majority of MSCs are Class II type cabinets (unless stated otherwise). Class II MSC protects both the work and to some extent the user and environment. The work area is bathed with a vertical flow of HEPA-filtered air. Air is also drawn in from the lab and passed downwards through the front grille. The air is then drawn up at the back of the cabinet and through a dust pre-filter. About 20% of this air is then HEPA filtered to the air ventilation system, while the rest is returned as HEPA filtered air to bath the work area once more.

Important rules to note:

- These must never be confused with fume cupboards (see chemical safety) and laminar flow hoods (work protection only).
- All material that contains or potentially contains infectious agents by inhalation must be handled within a MSC.
- Always plan your work so that all materials, including disinfectants, are to hand before starting.
- It is advisable to place materials in the cabinet such that you have dirty materials on one side and clean materials on the other.
- Always limit the amount of material in the cabinet, so that vents are not restricted.
- Always try & deter people from walking behind you when you are working at the cabinet, as this can disrupt the airflow and you may lose some containment.

13) Disinfection and Waste Disposal

There is a specific document that outlines the departmental policy for disinfection & waste disposal.

14) Maintenance and Cleaning

Individuals are responsible for cleaning their work areas and ensuring they are always left clean and safe to use. Groups should routinely review all of their work areas and remove any unwanted biological material and arrange for all surfaces to be cleaned.

Individuals must also ensure that any equipment that becomes contaminated is appropriately disinfected and cleaned immediately. As an absolute requirement all equipment must be disinfected before any maintenance work is carried out. An Equipment Decontamination form must be completed and signed, detailing the

procedure used to disinfect the equipment e.g. 'externally wiped with 1% Virkon'. Only when this is done can any maintenance work commence.

15) Training & Supervision

All individuals likely to handle biological material should read this policy as part of their training. They will also be asked to attend the University's biological training seminars, which are held regularly by the University Safety Office. Individuals will also be given on-the-job training by suitable member(s) of their group. The level of training required must be identified by the Group Head and from this appropriate supervision will be arranged. At the very minimum, Individuals must be taken through all relevant risk assessments and made aware of the safety precautions drawn up.

All individuals are encouraged to review their work and the safety precautions in place. If they identify areas that are not covered or they deem to be unsafe, then they should bring this to the attention of the Group head/BSO immediately.

16) Transport and Postage of Micro-organisms

If necessary, consult Biological Safety Office before sending any transporting or posting any biological material. Please note that University rules restrict any employee from using their own vehicle or hired vehicle for the transport of "Dangerous Goods".

17) Health Registration & Surveillance

All individuals are asked to register with University Occupational Health Service (UOH) before they begin work by completed a "Health Surveillance" form. At this stage they must inform UOH that they are planning to work with biological material and they will then be advised as to the level of surveillance required. If at any point individuals change the degree or nature of biological work, then they should inform UOH immediately & obtain further advice.

18) Accidents

All accidents, incidents and near misses must be reported to the DSO. Details of how to report are given in the NDCN Induction Notes. In particular accidents involving any genetically modified material is reportable to the Health & Safety Executive, therefore prompt reporting is required.

19) Children, Young Persons and Inexperienced Workers

The University has no specific lower age limit for young people being allowed into laboratory areas. However, before young people are allowed in containment laboratories an adequate risk assessment must be in place and the age of the young person will be a significant consideration.

Risk assessment of procedures involving manual handling

1) Manual Handling Risk Assessment

Risk assessments should be carried out by a competent person and so the assessor should be adequately trained to be able to perform the assessment correctly. Manual handling risk assessments should take account of the following factors:

The Task

- How is the load to be manipulated?
- Posture (a very significant factor is to avoid twisting whilst lifting) is stooping involved?
- The distance load is to be moved
- The number of similar tasks to be carried out
- How many people are involved?

The Load

- Heavy
- Bulky or unwieldy
- Unstable
- Sharp or difficult to grasp?

The Environment

- Amount of space around the operation
- Type of floor or work surface
- Lighting etc.

Individual Capability

- Strength of person
- Age and gender
- Existing health problems of the employee

2) Reducing the Risk

The assessment should decide how best to reduce the risk of injury by taking measures which could include:

- Eliminate task.
- Automate task.
- Use mechanical handling aids.
- Share the load.
- Reduce the weight of individual items.
- Make the load easier to manage or grasp etc.
- Improve task layout.
- Use of the body more efficiently.
- Remove any space constraints.
- Improve conditions of floors etc.

3) Training

The University arranges training courses for Departmental Assessors and those involved in regular manual handling activities. Further details can be found on the University Safety Office web site.

- Carry out assessments of the remaining tasks likely to cause injury (the significant parts of the assessments should be in writing).
- Make necessary changes based upon results of assessment.
- Report to the Departmental Safety Committee on progress.

Handling dry ice

1) Properties and Hazards of Dry Ice

In its solid form, carbon dioxide is very cold; measuring -78^{o} C. It is normally supplied as pellets or blocks and does not pass through the liquid state as it melts, rather, it sublimes from its solid state into a colourless gas which is odourless at low concentrations but has a characteristic smell at higher concentrations. It is naturally present as a component of air at a concentration of 0.03% and it is not flammable, in fact it is used in fire extinguishers to supress fire.

There are a few hazards associated with it, chiefly the possibility of cryogenic burns due to skin contact with solid form. Also, because it rapidly sublimates into a gaseous form there is a possibility of asphyxiation in poorly ventilated areas. Because the vapour is heavier than air (relative density to air is 1.52) it may accumulate to high concentrations in confined spaces, particularly at or below ground level. Though CO_2 is a non-toxic gas, it does have an occupational exposure limit assigned to it under the *Control of Substances Hazardous to Health Regulations*.

Other hazards include explosion due to pressure build up if allowed to warm-up in a sealed container and large bags of dry ice present a manual handling challenge due to their weight and low temperature.

2) Exposure Limits

CO_2 Workplace Exposure Limit	**Concentration (ppm)**	**Concentration (%)**
Short Term Exposure Limit (STEL)	15,000	1.5
Long Term Exposure Limit (LTEL)	5,000	0.5

Occupational Exposure Standards are set to help protect the health of workers and represent the concentrations of hazardous substances in the air averaged over a specific time period (time weighted average – TWA). Two time periods are used: long term (8 hours) and short term (15 minutes). The long term limit of 8 hours represents a typical working day, whilst the short term limits are set to help prevent effects which some substances may have following only a few minutes of exposure e.g. eye irritation.

3) Symptoms and Effects

ppm	% Vol	Symptoms and Effects
10,000	1	Slight but un-noticeable increase in breathing rate.
20,000	2	Breathing becomes deeper – rate increases to 50% above normal. Prolonged exposure (several hours) may cause headache and exhaustion.
30,000	3	Breathing becomes laboured. Hearing ability reduced, headache experienced with increase in blood pressure and pulse rate.
40-50,000	4-5	As above. Signs of intoxication after 30 minutes exposure and slight choking sensation.
50-100,000	5-10	Characteristic pungent odour noticeable. Breathing much laboured leading to physical exhaustion. Headache, visual disturbance, ringing in the ears, confusion probably leading to loss of consciousness within minutes.
100,000+	10+	Rapid loss of consciousness with risk of death from respiratory failure.

4) Calculating the potential for CO_2 gas release

It is difficult to evaluate the rate at which the solid form will convert to the gaseous form since this will be dependent on a number of variables such as:

- The form of the dry ice – pellets or flakes, for instance, will sublime at a faster rate than blocks.
- The ambient temperature – sublimation will proceed faster at higher temperatures.
- The degree of insulation provided by the container.

However, the data below can be used to make some approximate estimates as to what concentration of gas will be generated over time in particular circumstances.

- 1kg of dry ice will produce 0.45 m^3 of gas.
- Dry ice to gaseous CO_2 sublimation rate is approximately 1% of total mass per hour in an insulated container.
- Dry ice to gaseous CO_2 sublimation rate is approximately 14% of total mass per hour at room temperature in the open.

5) Control Measures

- Do not handle with bare hands – use cryogenic gloves.
- Avoid carrying dry ice in the driver's compartment of a lorry or the passenger compartment of a car. If this is not possible, use as little dry ice as possible, ensure that the container is well insulated (though not tightly sealed) and ensure that the compartment is well ventilated (open windows, ensure ventilation system is set to draw fresh air from outside).
- Unload the material as soon as possible at the end of a journey.
- Store dry ice in well ventilated areas away from direct sunlight and sources of heat.
- Use suitable storage containers (there are commercially available insulated containers with vented seals specifically designed for storing dry ice).
- Secure to prevent any unauthorised access.
- Use appropriate warning signage where necessary.

- When opening lids to storage containers, allow a few seconds for gas to dissipate and do not lean in for longer than necessary.
- Do not store or use dry ice in any gas tight container.
- Do not store dry ice in a working refrigerator or freezer – it will sublimate at a faster rate than in an insulated storage container and the extremely cold temperature may cause the thermostat to cut out.
- Dispose of unwanted dry ice by allowing it to evaporate in a well ventilated area – it will sublime leaving no residue.
- Carry out manual handling assessment of bags if necessary.
- Ensure that all users of dry ice are familiar with the hazards and necessary precautions.

For transporting samples in dry ice, see also Transporting of Dangerous Goods policy.

6) Emergency Procedures

Temperature related

- For brief, localised contact with cold material - flush the area with tepid water. (Water is used because of its high heat capacity.) Obtain First Aid assistance
- More prolonged contact will require medical treatment. Call a First Aider and ring the emergency number 4444

Vapour related

- Evacuate the area and call for help. On 234829 Reception in working hours and the hospital emergency number 4444 out of hours.
- If you suspect that someone is suffering from asphyxiation, do not enter the affected area alone - call for help on the hospital emergency number 4444. Remove the victim to the fresh air and call for a First Aider. If the victim is unconscious, call Emergency Services 4444 first.

Disinfection

1) Introduction

Disinfection is the reduction of microorganisms to an acceptable level. It is the policy of the Department that all areas likely to become infected with microorganisms must be routinely disinfected. There are many chemicals that are available, but depending upon their particular active agent and the material to disinfect, there may be variable degrees of disinfection. Therefore it is University's policy that all departments should have a clear disinfection policy indicating suitable concentrations, contact times and applications for the typical disinfection requirements of each individual department.

Several disinfectant agents are recommended for particular uses within the NDCN: 1-3% Virkon, Microsol, Chemgene, 70% ethanol, 70% isopropanol, formaldehyde. Should there be a need to use alternative disinfectants, this must be cleared by the Biological Safety Officer (BSO).

2) 1-3% Virkon

Activity

Virkon contains a number of peroxygen compounds that work synergistically to attack key structures within an organism, resulting in destruction of the organism. The components of Virkon have limited health hazards and are compatible with a range of materials. Virkon has also been proven to have a wide range of bactericidal, viricidal and fungicidal activity. Taking into account all these factors therefore, Virkon can be used for the majority of activities where disinfection is required.

Safety Precautions

Although a 1% Working Solution of Virkon has been identified as non-hazardous, care should be taken when handling the powdered form, as it is classified as an irritant.

Preparation of 1% Virkon Working Solution (multiply concentration as required).

Into a suitable container, i.e. bucket or beaker, add the required volume (X ml) of tepid water. Add to this the required amount of Virkon (X ml/100) and stir until the powder has fully dissolved to a clear pink solution. This solution can then be dispensed into smaller containers, such as wash bottles, if required.

The pink colour indicates suitable activity of the disinfectant. Therefore if at any time this pink colour is lost, then the solution should be discarded and a fresh solution made. If the colour is lost immediately on making up the solution, this indicates that the containers are contaminated in some way. Again, the solution should be discarded and all vessels washed out with water and a new solution made.

Disposal requirements

All Virkon solutions can be poured directly down sinks into the drainage system. Contaminated paper, gloves and other waste solid materials, should be collected in autoclave bags and disposed of as biological laboratory waste.

3) Guidelines on the Application of Virkon

Application	Concentration/instructions	Contact time
Hard surfaces, benches, floors etc.		1 hour
Metal parts	A solution containing 1% Virkon	10 mins
Safety cabinets	1% Virkon, followed by 70% alcohol	10mins
Discard jars, plastic tissue culture flasks, glassware	A solution containing 1% Virkon. Ensure all surfaces are in contact with the disinfectant.	1 hour
Supernatants, used tissue culture media, body fluids	For level 1 bacteria in culture broth: - 2% Virkon diluted 1:1 in broth. For level 2 tissue culture medium or other buffered system: - 3% Virkon diluted 2:1 in culture medium.	1 hour
Spillages	Virkon powder directly onto spill, Scrape mixture into yellow bag for incineration. Swab area with 1% solution	Until liquid is absorbed
Skin spillages	A solution containing 1% Virkon, then rinse well with water.	As required
Contaminated clothing	Where autoclaving is not possible/appropriate soak in 1% Virkon (test small area for colour fastness).	1 hour

4) Chemgene HLD4L

Activity

This product contains a number of active ingredients that work synergistically to attack the organism resulting in cell death. It can be supplied at working concentration so is ready-to-use. Therefore this disinfectant again is recommended for a majority of activities, particularly in surface decontamination as it has a wide range of bactericidal, viricidal and fungicidal activity.

Safety Precautions

Chemgene is non-toxic, non-carcinogenic, non-hazardous and non-corrosive. However it can burn skin and eyes especially when undiluted therefore it is recommended to wear lab coat, gloves and eye protection when using the product.

Use of the Solution

Chemgene is supplied in two formats, a concentrate and a 1/20 ready-to-use spray. For the majority of surface disinfection required, the spray should be used. Where cultures are to be disinfected a recommended amount of concentrated Chemgene must be added to produce a final volume of 1:20, the same dilution of 1:20 is used to disinfect blood. For general cleaning a dilution of 1:100 is recommended

Disposal Requirements

Chemgene is biodegradable so all waste solutions can be poured directly down sinks into the drainage system. Contaminated paper, gloves and other waste solid materials should be collected in autoclave bags and disposed of as biological laboratory waste.

5) 70% Ethanol and 70% Isopropanol

Activity

70% ethanol or 70% Isopropanol can only be used to disinfect a physically clean surface as it poorly penetrates organic material. It does provide good bactericidal and fungicidal activity, but is non-sporicidal and is less or in some cases non-effective against viruses. Taking into account all these factors 70% Ethanol or Isopropanol

should only be used where surfaces are relatively clean and where 1% Virkon would not be acceptable i.e. electrical equipment.

Safety Precautions

Both absolute Ethanol & Isopropanol are highly flammable and must be handled within a fume cupboard and away from ignition sources. At 70% these solutions are still deemed flammable, therefore their usage should be minimised and care must be taken at all times to avoid ignition sources. In particular electrical items of equipment should be disconnected from a power source before disinfection can commence.

Preparation of 70% Alcohol Working Solution

The following details the procedure to make up 500mls of 70% Alcohol, which is sufficient to fill an average size wash bottle. Working within a fume cupboard, measure out 350ml of Ethanol or Isopropanol into a measuring cylinder. Make this up to 500ml with water, cover the top of the cylinder with Parafilm, and very carefully invert to mix. Pour contents into a wash bottle for use.

Disposal Requirements

Excess 70% Alcohol can be carefully poured down sinks into the drainage system, and water ran to flush system. Contaminated paper, gloves and other waste solid materials, should be collected in autoclave bags and disposed of as Miscellaneous Laboratory Waste, as described above.

6) Formaldehyde

Applications of formaldehyde

Formaldehyde is extremely hazardous, but does have a wide range of bactericidal, viricidal and fungicidal activity. It is therefore useful as a fumigant for disinfecting microbiological safety cabinets, cryostats and rooms. However Formaldehyde Fumigation must only be carried out by suitably experienced personnel and so the BSO should be consulted for guidance on how this is to be done.

Cold rooms

1) Introduction

These local rules apply to the use of all walk-in cold rooms on the 5th floor of the West Wing (rooms 05-66-31, 05-66-33 and 05-66-36). All Users must ensure they are fully aware of the risks associated when working within the cold rooms & understand the appropriate actions to take before they begin. These guidelines should be read in conjunction with the 'User Guide' for any specialist equipment used.

2) Risk Assessment

The hazards associated with working in the Cold Rooms are summarised below:

- *Temperature*: Cold rooms run at approx. 4°C and as such prolonged exposure to this temperature without appropriate protective equipment could lead to hypothermia. However the expected usage of these rooms is unlikely to cause any problems.
- *Electrical Equipment*: When used in a cold room, condensation may get inside the enclosure of electrical equipment. This could lead to 'short circuiting' across the electrical components, making the equipment inherently unsafe. Provided equipment is stringently monitored and kept to a minimum then this risk should be controlled.
- *Microbial growth*: It is accepted that Mould may grow on surfaces within the cold rooms. If this is not controlled then the risk to the health of the users and also the effect on the users work could be considerable. Therefore stringent monitoring and cleaning, by all staff, is an absolute requirement to keep this risk to an acceptable level.
- *Work at height*: Access to the top shelf is required for storage. The height of this shelf is approx. 190cm. Access to the shelf is via a 'Kick-step'. Provided this shelf is only used to store items less than 5Kgs and do not require regular access, then the associated risk should be minimal.

The most likely incidents to occur are from mould contamination to the room. By following the guidelines set out below, this can be controlled so that the associated risk to the user is minimal.

In exceptional circumstances though, the most serious incident that could occur, would be electrical burns or shocks to the user. However once again as long as the following rules are applied then the likelihood of this happening is low and so the associated risk is minimal.

3) Do's & Don'ts

- Storage of cardboard or polystyrene boxes must be kept to an absolute minimum.
- Where this cannot be avoided then items should be over packed into plastic storage boxes with lids.
- All shelves, boxes and materials used by the group must be labelled with their Group Name or contacts.
- Any spillage must be dealt with immediately & appropriately (See West Wing Waste & Disinfection Policy).
- Health & Safety Officer must routinely arrange for the floor & bench tops to be cleaned & disinfected (at least once every 3 months).
- Groups & Users must routinely clean & disinfect their shelving, boxes & other materials they utilise within the rooms (at least once every 6 months).
- Electrical Equipment used within the room must have been PAT Tested before it is taken into the room.
- Electrical Equipment must be visually checked each time it is used for obvious signs of defects such as loose cable grips, plugs incorrectly fitted, unsafe cable joints, damaged cable, signs of burning/overheating, wrong value fuses (remember Amps=Watts/Volts). If any potential problem is identified then the equipment must be taken out of use immediately and FM team should be contacted for further advice.
- Plugs to electrical equipment must be able to fit into the Damp-proof sockets allowing them to close fully. If this isn't the case then the FM team should be contacted to change the plug before use.
- If electrical equipment is removed from the cold rooms, then it should be allowed to warm up and any condensation should be dried out before using, this can take several hours.
- Where possible, only low voltage equipment should be used within the rooms.

- No item greater than 5Kgs is allowed to be stored on the top shelves.
- Access to the top shelves must be via a Kick-Step.
- Items stored on the top shelves should only require access on a limited basis.
- All hazardous items within the cold room must be stored correctly and appropriately labelled.
- The Departmental Safety Officer will routinely check that the emergency exit handles function correctly in all rooms (at least once every 6 months).

Safe use of sonicators and grinders

1) Introduction

Sonicators are high-frequency sound generators used to disrupt cells or shear nucleic acids and there are two major hazards associated with them: Hearing damage caused by high frequency sound and the generation of aerosols from the sonication process.

2) High Frequency Sounds

Sonicators generate sound waves in the 20,000 Hz range. These sonicator-generated sound waves are outside the normal range of hearing. Often the sound heard while using a sonicator is produced by cavitations of the liquid in the sample container or vibrations from loose equipment. Actions you can take to reduce the hazards include:

- Wear earphone-type sound mufflers to protect your hearing while sonicating.
- If possible, have the sonicator located in a "sound-proof" cabinet while sonicating.
- Do not sonicate in a room containing people not wearing ear protection.
- Shut doors of the room where sonication is taking place.

3) Aerosols Created When blending, Grinding, Sonicating, Lyophilizing

The greatest hazard when using sonicating and other equipment to disrupt cells or shear nucleic acids is the creation of aerosols. These aerosols are generated by cavitations of the sonicator horn in the sample media and mechanical mixing. The following guidelines should be followed.

- Blenders, grinders, sonicators, lyophilizes, etc. should be operated in a biosafety cabinet whenever possible.
- Safety blenders should be used. Safety blenders are designed to prevent leakage from the bottom of the blender jar and to withstand sterilization by autoclaving. They also provide a cooling jacket to avoid biological inactivation.
- Avoiding using a glass blender jar. If a glass jar must be used, it must be covered with a polypropylene jar to contain the glass in case of breakage.
- A towel moistened with disinfectant should be placed over the top of the blender while operating. This practice can be adapted to grinders and sonicators as well.
- Aerosols must be allowed to settle for five minutes before opening the blender jar (or grinder or sonicator container).
- Lyophilizer vacuum pump exhaust should be filtered through HEPA filters or vented into a biosafety cabinet.