



**The University of Sydney**

# **Laboratory Training Document and Safety Manual**

K Block Biochemistry Laboratories  
Room K219 (Main Lab),  
Room K223 (Cell culture Lab),  
**K Block Building**

Multi-User Research Laboratory

Phone: x19753

**Please ensure the workplace is safe for you  
and your colleagues at all times**

Prepared by D Oakes (reviewed by E Hegedus)

Date: September 2006  
Revised: August, 2007; November 2008

**Laboratory Coordinator: Dr Diana Oakes**

**School / Discipline:**

**School of Medical Sciences  
Discipline of Biomedical Sciences**

**Laboratory Coordinator Signature:** \_\_\_\_\_

**Date of Implementation:** September 2007

**Reviewed:** November 2008

**Date of next reviewed:** November 2009

## 1. INTRODUCTION

This laboratory manual has been developed to assist staff and students manage OHS risks in this laboratory.

This manual is provided to document the system(s) used in this Laboratory for the safe management of hazards and risks. This manual is also the basis for training of all laboratory users. This manual will also be used as a training tool for new staff and students.

The Laboratory Safety Manual consists of 9 main sections:

- Introduction
- The Laboratory (p4)
- Administration (p4)
  - Contacts
  - Laboratory Access and Authorisation
  - Laboratory Training
- Risk Management (p5)
  - Risk Assessment - *Assessing Experimental Hazards*
  - Useful Literature and links
- Work Practices (p7)
  - General Laboratory Rules
  - Identified Hazards – Additional Laboratory Work Practices
  - Working with Genetically modified organisms (GMOs)
- Personal Protective Clothing and Equipment (PPCE) (p8)
  - Minimum Protective Clothing and Equipment (PPCE)
    - Laboratory Coats and Gowns
    - Gloves
- Waste Disposal (p9)
  - Chemical Spills
- References (p10)
  
- NEW USER FORM (p11)
- Laboratory Induction Declaration (p12)

The manual also has several appendices containing forms and other information relating to the above sections.

APPENDICES: (pp14-26)

- Appendix 1: Standard Laboratory Procedures for K Block Lab
- Appendix 2: Steps to follow when using the Biohazard Safety (Class 2) Cabinet
- Appendix 3: Good Cell Culture Practice
- Appendix 4: Hazard Identification Form
- Appendix 5: Risk Assessment Form (OHSRM Part B)
- Appendix 6: Standard Operating Procedure - SOP (Sample Form)
- Appendix 7: Laboratory Waste Management Procedures

The system for storing relevant documentation consists of 3 folders:

- MSDS /Hazard Register Folder (YELLOW folder)
- Risk Assessment Register (LIME GREEN folder)
- Copy of this Laboratory Safety Manual (BLACK folder)

These folders are located in Rm K219 on bench immediately to the left of the lab entrance.

This Laboratory Safety Manual will be reviewed at least annually; by the laboratory head or coordinator, and the Discipline Safety Officer (Rebecca Gould), to ensure that it will remain up-to-date.

## 2. THE LABORATORY

The K Block Biochemistry Laboratory consists of a large multi-user laboratory (Rm K219) with a smaller cell culture laboratory (Rm K223) that is accessed from within Rm K219.

- A Fire extinguisher is located in the lab K219 (to the left of door entry).
- The first aid kit is located next to the emergency shower.
- An eyewash sink is located on the emergency shower

## 3. ADMINISTRATION

For all OH&S information, please refer to:

1. The University OH&S webpage: (<http://www.usyd.edu.au/ohs/index.shtml>)
2. Discipline of Biomedical Science website OH&S link: <http://www3.fhs.usyd.edu.au/bio/OHS.html>

**Important: BOOKMARK these sites on your lab computer for ready access.**

### 3.1. Contacts

Further information about the operation of the laboratory can be obtained from the following people:

<b>Name</b>	<b>Role</b>	<b>Extension / Rm</b>
Dr Diana Oakes	<b>Laboratory Coordinator</b>	1-9469, Rm L110a
Dr Fazlul Huq	<b>Supervisor / User</b>	1-9522, Rm LS202B
Dr Helen Ritchie	<b>Supervisor / User</b>	1-9476, Rm L121
Dr Elizabeth Hegedus	<b>Supervisor / User</b>	1-9136, Rm L117
Dr Diana Oakes	<b>Discipline Safety Officer/ OHS Zone 11 committee member</b>	1-9469, Rm L110a
Dianne Eager	<b>K Block First Aid Officer</b>	1-9142, Rm K118
Dr Barrie Egerton	<b>Chair OHS Zone 11 Committee</b>	1-9514, Rm M215
Ray Patten	<b>Chief Building Warden</b>	1-9496, Rm K113

### **EMERGENCY CONTACTS:**

**Campus Security** (24 hours)

**Ext: 3**

**Emergency Services (Police / Fire Brigade / Ambulance)**

**DIAL 0-000**

## 3.2. Laboratory Access and Authorisation

Routine access is only provided to personnel that have:

- been authorised as a laboratory user by completing the NEW USER form (p10) and Laboratory Induction declaration (p11)
- undertaken the relevant Laboratory Training courses (see Section 3.3 below).

The laboratory shall be kept locked at all times when not in use. Only authorised persons will be provided with a key for the laboratory.

- Names of authorised persons will be maintained by the Laboratory Coordinator, and a register of authorised personnel is to be kept in the Risk Assessment Register (LIME GREEN folder) in the Laboratory.

***Working safely is a condition of access to the facility. Repeated failure to observe safe working practices and procedures will result in the withdrawal of access privileges.***

## 3.3. Laboratory Training

1. The University of Learning Solutions (previously Sydney Learning) runs several OH&S courses for both staff and students, you can **enrol on-line** via the following link:

<http://www.usyd.edu.au/learningsolutions/ohs/index.shtml>

**NOTE: Supervisors, please ensure all students complete the following courses:**

- **'Working with Hazardous chemicals'**
- **'Biosafety'**

Records of all Training will be kept in the Risk Assessment Register (LIME GREEN folder) in the Laboratory.

2. A compulsory monthly lab safety meeting of all K Block users (ie lab staff and students) is held each month on Tuesday, 12pm. This meeting is facilitated by Dr Diana Oakes and provides a regular forum in which lab safety issues are discussed. Supervisors also attend this meeting.

## 4. RISK MANAGEMENT

### 4.1. Risk Assessment – *Assessing Experimental Risks*

You or your supervisor will be required to document formal risk assessments prior to any laboratory activity. This involves the assessment and control of risks.

For each project you intend undertaking you must demonstrate that you have identified and are managing the associated hazards and risks by completing a risk assessment of all laboratory activities. A standard operating procedure (SOP) is also required for each experimental process; this gives the step by step details.

To complete the above risk assessment process, please use the following forms:

- 1) Hazards Identification Form (Appendix 4)
- 2) Risk assessment Form (Appendix 5). This form (OHSRM Part B form) is the template provided by the University OHSRM office ([www.usyd.edu.au/ohs](http://www.usyd.edu.au/ohs)).
- 3) The Standard Operating Protocol (SOP). A sample form is provided (Appendix 6).

All of the above forms are also available for download from the Discipline website OH&S link <http://www3.fhs.usyd.edu.au/bio/OHS.html>.

The Discipline Safety Officer must be supplied with the following documentation prior to any work commencing:

- MSDSs of all materials used that are not already in the MSDS / Hazard Register (YELLOW folder). This folder is kept in the laboratory.
- A copy of any licences, certificates, research approvals and any specific conditions stipulated by the licences, certificates, approvals etc

Completed risk assessments must be:

- signed off by your supervisor prior to the work commencing and a copy given to the Discipline Safety Officer, Rebecca Gould.
- A copy of all completed risk assessments and SOPs must be kept in the Risk Assessment Register (GREEN folder) in the lab.

It is important that this risk assessment and SOPs are reviewed whenever:

- The hazards used in the tasks are changed or new hazards are identified.
- An incident resulting in potential exposure occurs.
- New information about the hazard becomes available.
- An annual review is required.

When reviewed, personnel are required to sign the risk assessment. If you have made any changes these must be documented and their impact assessed. Any changes to an existing risk assessment must be authorised by your supervisor.

## 4.2. Useful Literature and Links

The following documents details the relevant safety procedures for this facility, but further reading of general laboratory safety is highly recommended.

1. *Australian Standard AS/NZS 2243.1 Safety in Laboratories (General)*
2. *Australian Standard AS/NZS 2243.2 Safety in Laboratories (General)*
3. *Australian Standard AS/NZS 2243.3 Safety in Laboratories Part 3: Microbiological aspects and containment facilities*
4. *Australian Standard AS 2252.2\_2004 - Biological Cabinet (ClassII) Safety*

A complete copy of the Standards can be accessed via the [University Library database](http://www.library.usyd.edu.au/databases/dbtitles.html) (<http://www.library.usyd.edu.au/databases/dbtitles.html>) under **Standards Australia**.

For all OH&S information or advice, please refer to the University OH&S webpage (<http://www.usyd.edu.au/ohs/index.shtml>) - **Important: BOOKMARK this site on your lab computer**

## 5. WORK PRACTICES

### 5.1. General Laboratory Rules

Safety in the laboratory depends upon you following the prescribed work practices and using the required safety equipment. These work practices are designed to help prevent you from harming yourself, laboratory equipment, your work, other persons in the laboratory and the environment.

Any personnel who have a medical condition that can affect their ability to work safely need to report this to the laboratory manager before commencing any work. All medical information is treated as confidential.

The following work practices apply to all personnel who use or enter this laboratory.

- Only authorised personnel can access the laboratory.
- Use Personal Protective Clothing and Equipment (PPCE) and other protective devices appropriate to the type of work being carried out.
- Ensure that personal clothing is suitable for laboratory conditions.
- Do not wear open-toed shoes in the laboratory.
- Laboratory PPCE (eg lab coats/gowns & gloves) **MUST** be removed before leaving the laboratory.
- Do not bring food or drink for personal consumption into the laboratory or store it in laboratory refrigerators. Eating, drinking, smoking, shaving and the application of cosmetics is prohibited in the laboratory.
- Only self-adhesive labels shall be used.
- Long hair must be tied back.
- Only wear jewellery that either cannot be caught in equipment or contaminated by infectious substances or chemicals, or is protected from these hazards
- Pipetting by mouth is strictly prohibited; always use the pipettors provided.
- The laboratory doors and windows must be kept closed when work is in progress.
- Any hazards, faults, incidents and injuries must be reported to the Discipline Safety Officer immediately.
- Always use safety carriers for transporting chemical containers with a capacity of 2 L or greater.
- Do not carry containers of mutually reactive substances at the same time.
- Wash skin areas which come in contact with chemicals, irrespective of concentration.
- **Always wash your hands when leaving the laboratory.**
- Practise good housekeeping, e.g. immediately cleaning up spills and appropriately dispose of wastes including packaging.
- Dispose of specialised wastes (e.g. broken glassware, chemicals, syringe and needles, biological and radioactive substances) in containers designated for the particular type of waste.

For Further information please refer to Appendix 1 - 'Standard Laboratory Procedures for K Block Lab'.

## 5.2. Identified Hazards - Additional Laboratory Work Practices

**PLEASE NOTE:** Any experimental using human derived tissue are required to be performed in a lab that complies with the standards for a Physical Containment level 2 (PC2) as stated in *Australian Standard AS/NZS 2243.3 Safety in Laboratories Part 3: Microbiological aspects and containment facilities*

The laboratory K119 and K223 complies with the requirements of PC1 and PC2, respectively. **Australian Standard Work Practices** are to be adhered to at all times. These Work Practices are outlined in Sections 4.7 (PC1 Work Practices) and 4.8.6 (PC2 Work Practices) and 4.8.7 (Standard Precautions when handling Human derived tissue, cells or fluids) in *Australian Standard AS/NZS 2243.3 Safety in Laboratories Part 3: Microbiological aspects and containment facilities*.

A complete copy of the Standards can be downloaded via the [University Library database](http://www.library.usyd.edu.au/databases/dbtitles.html) (<http://www.library.usyd.edu.au/databases/dbtitles.html>) under **Standards Australia**.

In addition, read Appendix 3 for some important tips on 'Good Cell Culture Practice' and Appendix 2 for the protocol that must be followed when using the Biological Safety Cabinet (Class 2) located in Rm K223.

## 5.3. Working with Genetically Modified Organisms

All work with biohazards, that include genetically modified organisms (GMOs), must be conducted in accordance with the relevant Australian Standards AS/NZS 2243.3 Safety in Laboratories Part 3: Microbiological aspects and containment facilities AND meet the requirements of the Australian Office of Gene Technology Regulator (OGTR). Please refer to the OGTR for more details: <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/guidelines-1>

Work that use GMOs must be conducted in a facility that has been certified by the Office of Gene Technology Regulator (OGTR) to containment level PC2 or higher, or as specified in a licence granted by the OGTR (see website <http://www.ogtr.gov.au/>). The Regulations require that only exempt dealings with GMOs can be conducted in accordance with the standards for physical containment level 1 (PC1).

Dealings that are required under the Regulations to be conducted in an OGTR-certified containment facility must not commence until OGTR certification of the facility has been confirmed in writing and the appropriate signs provided by the OGTR have been installed. Only dealings for which the project supervisor has written approval may be undertaken.

Further information can be found on the University of Sydney Occupational Health and Safety site: <http://www.usyd.edu.au/ohs/index.shtml> and the OGTR website <http://www.ogtr.gov.au/>

## 6. PERSONAL PROTECTIVE CLOTHING AND EQUIPMENT (PPCE)

Workplace safety can be enhanced and the severity of an injury can be reduced or prevented through the use of PPCE. PPCE is widely recognised as a means of protection for individuals working in an environment where all other methods of hazard control are in place and there is still a risk of injury. You must remember that PPCE is the last barrier or line of defence between you and the hazardous material you are working with.

## 6.1. Minimum PPCE Requirements

The minimum PPCE you are required to wear in this laboratory is:

- Laboratory Coat / Gown
  - White labcoats are worn in Rm K119
  - Blue gowns are worn in Rm K223 (Cell Culture Laboratory)
- Enclosed Shoes

The following additional PPCE is provided and should be used where required eg

- Protective eye glasses (eg face shield or eye glasses)
- Gloves
- Face mask or respirator

The risk assessment and SOPs should indicate any additional PPCE required.

## 6.2. Laboratory Coat or Gowns

Each person carrying out work in this laboratory will be issued with a laboratory coat (a white coat for the main lab Rm K219 and a blue gown for use in the cell culture lab – Rm K223).

**A Labcoat must worn at all times whilst you or anyone else is working in the laboratory.**

- It must be removed and hung on the coat hooks **before** leaving the laboratory.
- A lab-coat washing service is provided by the Discipline. Please leave your named lab-coat in the laundry bag (outside Rm L210).

## 6.3. Gloves

Laboratory gloves are your last barrier or line of protection between you and the hazardous material or process you are working with. It's your responsibility to use the appropriate gloves for the task or hazardous materia. Remember, gloves can get contaminated so care should be taken to ensure that you don't contaminate other areas.

The standard disposable gloves available in this laboratory include:

- Latex disposable examination gloves
- Disposable nitrile gloves
- Disposable vinyl gloves

Some points to remember:

- Gloves must be changed regularly. Ensure that you wash hands between changes.
- Gloves must be changed whenever contaminated.
- Gloves must be removed before leaving the laboratory, before using the computer or before answering the telephone, opening a door.
- Disposable gloves do not provide automatic protection. This is due to the fact that even new gloves may have their integrity compromised. Consider double-gloving for certain tasks.
- Use the right type of glove for the chemical you are working with.

## 7. WASTE DISPOSAL

All laboratory waste must be segregated, stored, and disposed of appropriately. This laboratory segregates it's waste into the following waste streams:

- Non-Hazardous Waste (Domestic)
- Clinical Waste (contaminated with Biological Material)

- Chemically contaminated Waste
- Cytotoxic Waste

For a detailed breakdown of the Laboratory Protocols for Handling these waste streams – refer to Appendix 7 – Laboratory Waste Management Procedures.

## 7.1. Chemical spills

- If chemicals are used, be prepared for spills by doing the following:  
Read the MSDS of all chemicals (MSDS register located in lab) and be aware of the appropriate spill procedure.
- Handle chemicals in containment trays where possible to avoid the possibility of incompatible chemicals mixing.
- Report significant spills and accidents immediately to your immediate Supervisor \_\_\_\_\_ (x\_\_\_\_\_) and Discipline Safety Officer, Dr Diana Oakes (Rm L110a, Extn 1-9469) or Discipline First Aid Officer, Dr Elizabeth Hegedus (Rm L117, Extn 1-9136). Do not proceed without notification.
- For further information on cleaning and decontamination procedures, refer to Section 5 of Australian Standard AS/NZS 2243.3.

## 8. REFERENCES

For all further OH&S information, please refer to the University OH&S webpage (<http://www.usyd.edu.au/ohs/index.shtml>)

### Specific information can be found at the following links:

ChemAlert Database – Material Safety Datasheets (MSDS) and labels available for download from this site

<http://chemicalert.ucc.usyd.edu.au/chemicalert/index/index.do>

Emergency guidelines:

[http://www.usyd.edu.au/ohs/ohs\\_manual/emergency/help.shtml](http://www.usyd.edu.au/ohs/ohs_manual/emergency/help.shtml)

Guidelines on laboratory safety:

[http://www.usyd.edu.au/risk/ohs\\_manual/labsafety.shtml](http://www.usyd.edu.au/risk/ohs_manual/labsafety.shtml)

Hazardous substances:

[http://www.usyd.edu.au/risk/ohs\\_manual/haz-subs/index.shtml](http://www.usyd.edu.au/risk/ohs_manual/haz-subs/index.shtml)

Radiation and laser safety:

[http://www.usyd.edu.au/risk/ohs\\_manual/radiation.shtml](http://www.usyd.edu.au/risk/ohs_manual/radiation.shtml)

Biological safety:

[http://www.usyd.edu.au/risk/ohs\\_manual/bio\\_safety.shtml](http://www.usyd.edu.au/risk/ohs_manual/bio_safety.shtml)

Guidelines for use of genetically modified organisms

<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/guidelines-1>

A complete copy of the Standards can be accessed via the [University Library database](http://www.library.usyd.edu.au/databases/dbtitles.html) (<http://www.library.usyd.edu.au/databases/dbtitles.html>) under Standards Australia.

## K Block Biochemistry Laboratories

### NEW USER FORM

NAME:

PHONE & EMAIL:

LAB:

PROJECT TITLE &  
DETAILS:

EQUIPMENT TO BE USED:

#### CELL CULTURE INFORMATION

Please provide as much  
information as you can  
regarding the cell type  
you are using  
eg. name of organism  
(species, wild type),  
able to replicate etc

#### SPECIAL DECONTAMINATION PROCEDURES

ie. can bleach or  
ethanol be used to  
decontaminate, or is it  
advisable that other  
solutions be used -  
please advise

#### OTHER HAZARDOUS MATERIAL

Indicate any other hazardous material/chemical that will be used and briefly indicate control measures that will be taken (handling, use and disposal etc).

TRAINING Course completion dates (*Biosafety* and *Working with Hazardous Chemicals*):

STUDENT SIGNATURE

RESEARCH SUPERVISOR  
SIGNATURE:

Declares that your student is permitted to independently operate the equipment and will complete risk assessments and SOPs involving hazards named above BEFORE beginning experiments in the laboratory.

## Laboratory Induction Declaration

The following checklist is designed as an aid to inducting new personnel into the safety practices to be followed in the K Block laboratory (Rms K219 and K223).

All laboratory personnel should have access to the Laboratory Safety Manual, and must sign the declaration below that they have read and understood its contents. This must occur before the person commences work in the laboratory.

The Laboratory Induction Checklist must be signed on completion of the induction and a copy should be kept in the Risk Assessment Register (Green folder) in the Laboratory (Rm K119).

Laboratory: K Block (Rm K119 and K223)

Supervisor:

Student/Staff:

Item	Check
Reviewed the contents of the Laboratory Safety Manual	
Laboratory Access Requirements - normal & after hours	
Location of fire fighting & emergency equipment	
Location of first aid kit	
Emergency eye wash and safety shower	
Location of Laboratory Safety Manual and associated folders	
Issue personal protective equipment	
Read and understood the Standard Work Practices for K Block	
Completed Risk Assessments and associated SOPs	
Completed appropriate Safety Training Courses 'Biosafety' 'Working with Hazardous Chemicals'	

I have read and fully understood the Training Document and Safety Manual for the K Block Biochemistry Laboratories.

User's Name: \_\_\_\_\_ Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Supervisor's signature: \_\_\_\_\_

**Please return the completed NEW USER form and Laboratory Induction Declaration to the Discipline Safety Officer, Dr Diana Oakes (Rm L110a, Ext 19469)**



## Appendix 1

### Standard Laboratory Procedures for K Block Lab

LABCOATS SHOULD BE WORN AT ALL TIMES BY PERSONNEL WORKING IN THE LABORATORY.

EATING AND DRINKING ARE PROHIBITED IN THE LABORATORY.

- 1) **Personal Protective Equipment (PPE):** PPE such as gloves, safety glasses and a laboratory coat must be worn whenever biological work is conducted in the laboratory. No sandals or open-toed shoes are allowed in the laboratory. Laboratory coats will be used for general lab work and kept on hooks near Lab entrance.
- 2) **Human Organ and Cell Culture:** All researchers using human organ or cell cultures (primary cultures, cell strains, cell lines), must handle all such cultures in a Biohazard Class II cabinet (see Appendix 2) under PC2 standard lab conditions (Australian standard AS/NZS 2243.3) using appropriate cell culture lab practices (see Good Cell Culture Practice – Appendix 3). Dedicated laboratory coats must be used for cell culture only. When not in use, laboratory coats will be kept on coat hooks near Biohazard cabinet.
- 3) **Blood Borne Pathogens:** All researchers using human or non-human primate blood or blood products, unfixed tissue, body fluids of human or non-human primate origin, must follow standard procedures (see NHMRC publication: '*Infection control in health care settings: Guidelines for the prevention of transmission of infectious diseases*').
- 4) **Decontamination of lab surfaces:** Routinely clean laboratory bench areas before and after use with 70% alcohol. When appropriate, use a 0.5% sodium hypochlorite (using a freshly prepared 1:10 dilution of household bleach) to decontaminate equipment and work surfaces. In locations where bleach would cause corrosion, an disinfectant solution (e.g.,cavicide) must be used to decontaminate.
- 5) **Waste Disposal:** All biological waste must be autoclaved and discarded in clinical waste bags (yellow) or hazardous waste bags (containing EtBr gels). Contact Gautham when yellow clinical waste bag is full (1-9317).
- 6) **Autoclave Safety:** Always wear heat-resistant gloves, safety glasses, and a laboratory coat when opening an autoclave. Be sure to allow the superheated steam to exit before attempting to remove the contents.
- 7) **Handwashing:** Hands must be washed immediately or as soon as feasible after removing gloves or other personal protective clothing.
- 8) **Proper Labeling of equipment and work areas:** Place a color-coded label incorporating the universal biohazard label on the work surface of any potentially contaminated equipment or work surface to warn others of biohazard contamination which may not be easily visible. This includes freezers, refrigerators, and incubators.
- 9) **Clear labeling of solutions:** All bottles of solutions (eg media, buffers etc) must be clearly labeled indicating – contents, name of researcher and date prepared.
- 10) **Storage:** All potentially infectious materials to be stored must be clearly labeled with the universal biohazard symbol. The storage space (e.g., freezer, refrigerator) must also be similarly labeled. In addition, all chemicals stored in the freezer or refrigerator must be clearly labeled and placed in an appropriate container (do not store in cardboard box).
- 11) **Use of Sharps:** Minimize the use and exposure to sharps in the workplace. Keep sharps containers readily available in all locations where sharps waste may be generated. Contact Gautham when sharps container is full (1-9317).
- 12) **Aerosol Generation:** Any procedures that could potentially generate aerosols or other inhalation hazards must be performed in a manner that will minimize airborne pathogen transmission (in Biohazard cabinet in Rm K223 – see Appendix 2 for operating protocol).

- 13) **Mouth Pipetting:** Mouth pipetting may lead to accidental ingestion of biological specimens and is strictly prohibited.
- 14) **All biohazardous spills** must be handled using the following procedure:
  - For spills which researchers are able to clean up safely, a person wearing protective equipment (gloves, goggles, long-sleeved lab coat) must first disinfect the area with a 1:10 dilution of household bleach or disinfectant (e.g., cavicide) before wiping up the spill with disposable paper towels and disposing of all spill materials properly (see 5). Broken glass must be handled only by remote means such as tongs or forceps and disposed of into sharps container.
  - For spills which staff may not be able to clean up safely, the room must be evacuated and personnel must be prevented from entering the area. The Discipline Safety Officer, Dr Diana Oakes (Rm L110a, Extn 1-9469) or Discipline First Aid Officer, Dr Elizabeth Hegedus (Rm L117, Extn 1-9136) must be contacted immediately. Do not proceed without notification.
- 15) **All injuries** and accidental needle-stick injuries, ingestion or inhalations of potentially infectious agents must be reported immediately to the Discipline Safety Officer, Dr Diana Oakes (Rm L110a, Extn 1-9469) or Discipline First Aid Officer, Dr Elizabeth Hegedus (Rm L117, Extn 1-9136).
- 16) **Local Transport of Infectious Materials:** All infectious materials transported to and from the laboratory must be enclosed in a primary container with sealed lid or top, which must then be enclosed in a secondary leak-proof, non-breakable container (e.g., esky) and be appropriately labeled with the biohazard symbol. Any specimens transported to and from off-campus facilities must be escorted by a lab employee.
- 17) **In case of power failure** the following precautions must be taken: immediately discontinue all work until power is restored. If a tissue culture hood is being used, then all open containers must be closed, gas turned off and hood sash closed.
- 18) **In case of fire**, personnel must immediately follow standard emergency procedures (dial 0-000 for fire brigade, then dial 3 for Campus Security).
- 19) **All domestic and international shipments** of biological materials must follow University Policy and all applicable Federal and international regulations. Proper permits/licenses must be obtained before importing or exporting any biological material (see [www.aqis.gov.au](http://www.aqis.gov.au)).

## **Appendix 2**

### **STEPS TO FOLLOW WHEN USING THIS BIOHAZARD SAFETY (CLASS 2) CABINET IN RM K223**

1. Turn on and allow to RUN for 5 mins before use
2. Wash hands thoroughly at sink
3. Put on Blue Lab Coat, followed by gloves (cover coat cuffs with gloves)
4. Wipe down cabinet surface with decontaminant solution (eg CAVICIDE) using paper towel
5. Place experimental material into cabinet (wiping each object with 70% alcohol before putting in cabinet)
6. Wait 2 Minutes
7. Disinfect gloves using 70% alcohol
8. When finished work, place cultures in incubator and remove all material from cabinet
9. Wipe work surface with decontaminant (CAVICIDE)
10. Wait 2 minutes
11. Turn off cabinet
12. Remove gloves and dispose in clinical waste
13. Wash hand thoroughly
14. When leaving room:
  - TURN ON UV light on cabinet. It is set to turn OFF automatically after 20min.
  - TURN OFF ROOM LIGHTS and lock door

## **Appendix 3**

### **GOOD CELL CULTURE PRACTICE**

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#### **Basic Techniques - The Do's and Don'ts of Cell Culture**

Given below are a few of the essential "do's and don'ts" of cell culture. Some of these are mandatory e.g. use of personal protective equipment (PPE). Many of them are common sense and apply to all laboratory areas. However some of them are specific to tissue culture.

#### **The Do's**

1. Use personal protective equipment, (laboratory coat/gown, gloves and eye protection) at all times. In addition, thermally insulated gloves, full-face visor and splash-proof apron should be worn when handling liquid nitrogen.
2. Always use disposable caps to cover hair.
3. Wear dedicated PPE for tissue culture facility and keep separate from PPE worn in the general laboratory environment. The use of different coloured gowns or laboratory coats makes this easier to enforce.
4. Keep all work surfaces free of clutter.
5. Correctly label reagents including flasks, medium and ampules with contents, date of preparation and name of researcher.
6. Only handle one cell line at a time. This common-sense point will reduce the possibility of cross contamination by mislabeling etc. It will also reduce the spread of bacteria and mycoplasma by the generation of aerosols across numerous opened media bottles and flasks in the cabinet.
7. Clean the work surfaces with a suitable disinfectant (e.g. 70% ethanol) between operations and allow a minimum of 15 minutes between handling different cell lines.
8. Wherever possible maintain separate bottles of media for each cell line in cultivation.
9. Examine cultures and media daily for evidence of gross bacterial or fungal contamination. This includes medium that has been purchased commercially.
10. Quality Control all media and reagents prior to use.
11. Keep cardboard packaging to a minimum in all cell culture areas.
12. Ensure that incubators, cabinet, centrifuges and microscopes are cleaned and serviced at regular intervals.
13. Test cells for mycoplasma on a regular basis.

#### **The Don'ts**

1. Do not continuously use antibiotics in culture medium as this will inevitably lead to the appearance of antibiotic resistant strains and may render a cell line useless for commercial purposes.
2. Don't allow waste to accumulate particularly within the microbiological safety cabinet or in the incubators.
3. Don't have too many people in the lab at any one time.
4. Don't handle cells from unauthenticated sources in the main cell culture suite. They should be handled in quarantine until quality control checks are complete.
5. Avoid keeping cell lines continually in culture without returning to frozen stock.
6. Avoid cell culture becoming fully confluent. Always sub-culture at 70-80% confluency or as advised on ATCC's cell culture data sheet.
7. Do not allow media to go out of date. Shelf life is only 6 weeks at +4°C once glutamine and serum is added.

8. Avoid water baths from becoming dirty by using Sigma Clean (Prod. No. S5525).
9. Don't allow essential equipment to become out of calibration. Ensure microbiological safety cabinets are tested regularly.

## **Aseptic Technique and Good Cell Culture Practice**

### **Aim**

To ensure all cell culture procedures are performed to a standard that will prevent contamination from bacteria, fungi and mycoplasma and cross contamination with other cell lines.

### **Materials**

- 10% decontaminant / disinfectant solution e.g. Cavicide
- 70% ethanol in water

### **Equipment**

- Personal protective equipment (gloves, laboratory coat, safety visor)
- Microbiological safety cabinet at appropriate containment level

### **Procedure**

1. Sanitize the cabinet using 70% ethanol before commencing work.
2. Sanitize gloves by washing them in 70% ethanol and allowing to air dry for 30 seconds before commencing work.
3. Put all materials and equipment into the cabinet prior to starting work after sanitizing the exterior surfaces with 70% ethanol.
4. Whilst working do not contaminate gloves by touching anything outside the cabinet (especially face and hair). If gloves become contaminated re-sanitize with 70% ethanol as above before proceeding.
5. Discard gloves after handling contaminated cultures and at the end of all cell culture procedures.
6. Equipment in the cabinet or that which will be taken into the cabinet during cell culture procedures (media bottles, pipette tip boxes, pipette aids) should be wiped with tissue soaked with 70% ethanol prior to use.
7. Movement within and immediately outside the cabinet must not be rapid. Slow movement will allow the air within the cabinet to circulate properly.
8. Speech, sneezing and coughing must be directed away from the cabinet so as not to disrupt the airflow.
9. After completing work disinfect all equipment and material before removing from the cabinet. Spray the work surfaces inside the cabinet with 70% ethanol and wipe dry with tissue. Dispose of tissue by autoclaving.
10. Cell culture discard in cavicide (10%) must be kept in the cabinet for a minimum of two hours (preferably overnight) prior to discarding down the sink with copious amounts of water.
11. Periodically clean the cabinet surfaces with a decontaminant/ disinfectant such as cavicide. or fumigate the cabinet according to the manufacturers instructions.

**Appendix 4 Hazard Identification Form - Identify, prioritise and evaluate the effectiveness of control measures**

Hazard Category (equipment, chemicals, ergonomics)	Hazard Description	Who could be harmed? (staff, visitors, students)	Current Safety controls for performing this task	Risk Rating with current controls			Further work required to reduce or improve level of risk	Completion Date and by whom	Risk Rating after new controls implemented		
				L	S	RC			L	S	RC

**Likelihood Rating**

Low(1) = Unlikely  
 Medium(2)=Possible  
 High(3)=Likely

**Severity Rating**

Low(1)=First Aid Injury  
 Medium(2)=Major injury over 3 days off  
 High(3)=Permanent incapacity or death

**Risk Conclusion(Likelihood+Severity)**

2=Trivial Risk                      5=Substantial Risk  
 3=Acceptable Risk                6=Intolerable Risk  
 4=Moderate Risk

## Appendix 5 Risk Assessment Form

### ohsrm PART B (taken from University OH&S website [www.usyd.edu.au/ohs](http://www.usyd.edu.au/ohs))

Use one PART B form for each hazard or hazardous job identified using the Hazardous Identification Form

#### Assess the risk

Assess the risk for the top priority hazards identified in Hazardous Identification Form ie., begin with those rated 1, then 2 etc.

Ref. #	Description of the hazard or hazardous job	Priority	Identification Date

**What makes it hazardous?** Consult with the workers to find out which factors are relevant:

Tick relevant boxes and record observations or comments.

The physical activity required

*Involves repetitive action, physical exertion, awkward posture etc.*

The work environment

*Lighting, work layout, temperature, egress routes, isolation, traffic etc.*

The nature of the hazard itself

*Hazardous substances, sharps or blades, radiation, potentially violent clients etc.*

The individual(s) involved

*Level of training or experience, physical capacity, health status, age etc.*

Other risk factors or comments

Record the names of those consulted when assessing the risk	Date

#### Control the risk(s)

Control the risks(s) by addressing the risk factors found above.

Consider the **hierarchy of hazard control** and record what controls will be used in the short term and longer term. Record also who is responsible for implementing the control(s) and the due by date(s).

Describe the risk control(s)	Who is responsible for implementation	Due by date

Record the names of those consulted when deciding on risk control measures

PART B completed by: \_\_\_\_\_ Date: \_\_\_\_\_

## Appendix 6

### Standard Operating Procedure SOP (*sample form*)

**Title:** Preparation of fixative solution.

**Prepared by:** John Smith

**Original issue date:** 25/5/07 Fri

**SOP Ref No.:** #00001

**Next Review date:** 25/5/08 Fri

#### Background

The most common usage of fixatives in the laboratory is for the preparation of tissue for histological examination. Fixatives, particularly the formaldehydes, work by cross-linking proteins within the cells of tissues thereby preserving the structural integrity of the cells after the death of the animal. These chemicals can fix the tissues of the user just as readily as experimental tissue, and are therefore highly toxic and carcinogenic with prolonged exposure. The ideal situation is zero exposure to these chemicals.

#### Body parts most at risk

Cornea of the eyes, tissue lining the respiratory tract, skin.

#### Equipment

Scales  
Weighboats (or vessel to contain the fixative)  
Fixative (eg paraformaldehyde)  
0.1M Phosphate buffered saline (PBS)  
2L Beaker  
1L laboratory bottle  
Stirring hotplate  
Magnetic stirrer  
Sodium hydroxide pellets  
Glass funnel  
Retort stand

#### Personal protective equipment required

Safety goggles (or even better a full face shield)  
Gloves  
Lab gown

#### Procedure

- Place 600mL of PBS into the beaker and heat to not more than 60°C
- Add 10 or so pellets of sodium hydroxide (paraformaldehyde requires OH<sup>-</sup> ions in order to depolymerise and dissolve).
- In a fume hood weigh out 40g of paraformaldehyde and add to the beaker.
 

**Risk:** At no time should there be a clear path between the paraformaldehyde solution and the eyes of the user. **ALWAYS** wear safety glasses when using fixatives.

**Procedure (cont.)**

- When dissolved (solution should be completely clear), allow to cool to at least 40°C and filter into laboratory bottle, make up to 1L with PBS.  
**Risk:** Bottles and beakers can get heavy when full. It is sometimes awkward pouring solutions into filtering apparatus when fume hood sash is at appropriate operating height. Raise sash enough to allow comfortable hold on glassware for this step.
- Adjust pH of solution to ~7.2-7.4 with concentrated hydrochloric acid (HCl) solution.  
**Risk:** HCl is a gas thus the concentrated solution used at this step gives off fumes. Fumes will dissolve in the moisture of your respiratory tract if inhaled and form HCl solution, irritating respiratory tract. Perform this step in a fume hood.
- Cool to 4°C before use
- Should make up as close as possible to the day needed. Avoid using paraformaldehyde more than a week old, as fixation efficacy may be reduced.

**Disposal of waste**

Any waste powder should be disposed of in yellow biohazard bags. Take care to contain powder to reduce accidental exposure when handling disposal of bags.

DO NOT pour liquid waste down the sink. Place excess waste into 20L waste container, reuse container until full then dispose as chemical waste.

**References**

1. Sigma (2000) Paraformaldehyde MSDS
2. Leong, A S-Y. Fixation and fixatives. <http://home.primus.com.au/royellis/fix.htm> (accessed 25/5/07 Fri)

## Appendix 7 - Laboratory Waste Management Procedures

Waste Stream	Description	Collection/Treatment Procedures	Disposal Procedure
<b>Non-hazardous waste</b>	<b>Consumable packaging</b> and other materials that have not been contaminated with chemical, biological or radioactive material.	Collect in a general waste bin lined with a black plastic bag.	The general waste bins are provided and emptied by the University Cleaners.  → Landfill
<b>Clinical waste (Biologically contaminated)</b>	<b>Liquid tissue culture waste</b>	<ul style="list-style-type: none"> <li>Decontaminate liquid culture media waste with high level hospital disinfectant eg Cavicide. Mix 1:1 and leave overnight.</li> </ul>	Once decontaminated, dispose of liquid waste down the sink with copious amounts of water.  → Sewer
	<b>Solid waste contaminated with tissue culture, GMOs or associated low hazard chemical residues.</b>  This includes used tissue culture flasks, plastic pipettes, gloves, and paper towel etc.  <i>Note: For tissue culture contaminated with cytotoxic chemicals refer to cytotoxic waste</i>	<ul style="list-style-type: none"> <li>Collect in the provided containers (bin or wire basket) lined with an autoclave bag.</li> <li>Once full, seal the bag and transport to Micro prep room (L204) for autoclaving.</li> </ul> <b>Notes</b> <ul style="list-style-type: none"> <li>Only staff/students trained by Gautham are to operate the autoclave.<sup>1</sup></li> <li>Secondary containment (eg. a locked clinical waste bin) is required for all transport of biologically contaminated waste on campus.</li> </ul>	Once autoclaved, Gautham to place in <b>YELLOW Clinical Waste bin</b> for collection by Stericorp.  Offsite Treatment → Landfill
	<b>Microbiological Plates &amp; Blood Products</b>	<ul style="list-style-type: none"> <li>Leave in the Micro prep room (L204) for disposal</li> <li>For waste contaminated with Human derived tissue, autoclave before placing into Clinical Waste Bin</li> </ul>	Gautham to put in <b>YELLOW Clinical Waste bin</b> for collection by Stericorp  → Offsite treatment → Landfill
	<b>Sharps</b> including hypodermic needles, scalpel blades, pasture pipettes, slides etc., pipette tips contaminated with biological material or chemical residue	<ul style="list-style-type: none"> <li>Collect in an approved <b>YELLOW Sharps container</b> or Qlicksmart scalpel blade removal system.</li> <li>Once full, seal the container and leave in the Micro prep room (L204) for disposal.</li> </ul>	Gautham to place in <b>YELLOW Clinical Waste bin</b> (at top of bin) for collection by Stericorp.  → High Temperature Incineration

	<b>Animal Carcasses</b>	<ul style="list-style-type: none"> <li>Place in prep room freezer L211 or chest freezer in L209.</li> <li>Gautham to organise disposal with clinical waste.</li> </ul> <p><b>Notes</b> DO NOT put in YELLOW Clinical Waste bin</p>	Gautham to arrange for collection by Stericorp.  → Incineration
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Waste Stream	Description	Collection/Treatment Procedures	Disposal Procedure
<b>Chemically Contaminated Waste</b>	<b>Organic Solvents</b> eg, alcohol, hexane, xylene	<ul style="list-style-type: none"> <li>Collect in a labelled hazardous container indicating the type of solvent/s. <sup>2 3</sup> Ensure that incompatible solvents are collected in separate containers.<sup>4</sup></li> <li>Transfer to the Physiology Prep Room (L212) for storage prior to collection.</li> <li>Relevant Laboratory Manager to submit a <i>Request for the Disposal of Hazardous Waste</i> to the OHS &amp; Injury Management Unit (Fax 15868).</li> </ul>	Transfer to the Physiology Prep Room (L212), where they are stored until collection (organised through Matthew Mitchell, OHS & Injury Management Unit).  → Offsite treatment → Reuse as fuel
	<b>Acid waste</b>	<ul style="list-style-type: none"> <li>Collect in a labelled hazardous waste container indicating the type of acid and approximate concentration. Ensure that incompatible waste is collected in separate containers.</li> <li>Transfer to the Physiology Prep Room (L212) for storage prior to collection.</li> <li>Relevant Laboratory Manager to submit a <i>Request for the Disposal of Hazardous Waste</i> to the OHS &amp; Injury Management Unit (Fax 15868).</li> </ul>	Transfer to the Physiology Prep Room (L212), where they are stored until collection (organised through Matthew Mitchell, OHS & Injury Management Unit).  → Offsite Neutralisation → Sewer
	<b>Empty Solvent Bottles</b>	<ul style="list-style-type: none"> <li>Remove lid and dispose of as general waste.</li> <li>Evaporate the bottle dry in the fume cupboard.</li> <li>Remove or deface the label (eg. strike through with permanent pen).</li> </ul>	Place in general waste bins or glass disposal bin in L212, K219 or H207  → Landfill
	<b>Empty 200L &amp; 20L metal drums eg alcohol &amp; methylated spirits</b>	<ul style="list-style-type: none"> <li>Remove bungs, and allow to evaporate dry. All drums must have two holes to ensure drainage or they will not be accepted for recycling by SimsMetal.</li> <li>Leave drums outside, near L006</li> </ul> <p><b>Notes</b> Dianne Borg (Anatomy) to book university ute with Andy Galloway (Phone 19235) &amp; organise drum transport.</p>	Transport to SimsMetal → recycled  <u>SimsMetal</u> Scrap Metal Processing Centre 125 Canterbury Rd, Bankstown Phone 9709 6388

## Laboratory Waste Management Procedures cont....

Waste Stream	Description	Collection/Treatment Procedures	Disposal Procedure
<b>Chemically Contaminated Waste</b>	<b>Low hazard liquid chemical waste</b>	<ul style="list-style-type: none"> <li>• Collect in a labelled hazardous waste container indicating the type of low hazard liquid waste.</li> <li>• Relevant Laboratory Manager to submit a <i>Request for the Disposal of Hazardous Waste</i> to the OHS &amp; Injury Management Unit (Fax 15868).</li> </ul> <p><b>Notes</b> Ensure that all waste is labelled. Any unknown waste is charged a higher disposal fee.</p>	Transfer to the Physiology Prep Room (L212), where they are stored until collection (organised through Matthew Mitchell, OHS & Injury Management Unit).  → Offsite Treatment → Sewer
	<b>Empty Containers of Solid Chemicals</b>	If non-hazardous, remove lid, rinse, deface label and dispose of as general waste.  If hazardous (refer to MSDS), seal the container. <ul style="list-style-type: none"> <li>• Transfer to the Physiology Prep Room (L212) for storage prior to collection.</li> <li>• Relevant Laboratory Manager to submit a <i>Request for the Disposal of Hazardous Waste</i> to the OHS &amp; Injury Management Unit (Fax 15868). Description: Empty Chemical Packaging</li> </ul>	The general waste bins are provided and emptied by the University Cleaners. → Landfill  If hazardous: Transfer to the Physiology Prep Room (L212), where they are stored until collection (organised through Matthew Mitchell, OHS & Injury Management Unit).  → Controlled Landfill
	<b>Batteries</b>	<ul style="list-style-type: none"> <li>• Transfer to the Physiology Prep Room (L212) for storage prior to collection.</li> <li>• Relevant Laboratory Manager to submit a <i>Request for the Disposal of Hazardous Waste</i> to the OHS &amp; Injury Management Unit (Fax 15868) as required.</li> </ul>	Transfer to the Physiology Prep Room (L212), where they are stored until collection (organised through Matthew Mitchell, OHS & Injury Management Unit).  → Offsite treatment → Landfill

## Laboratory Waste Management Procedures cont....

	<b>Sharps</b> used in association with clinical specimens	<ul style="list-style-type: none"> <li>Collect in an approved <b>YELLOW Clinical Waste Sharps container</b>.</li> <li>Once full seal container and transfer to the Micro Lab (L204) for transfer to <b>YELLOW Clinical Waste bin</b> prior to collection.</li> </ul>	Gautham to put in <b>YELLOW Clinical Waste bin</b> for collection by Stericorp.
<b>Cytotoxic Waste</b>	<b>Sharps</b> used in association with cytotoxic drugs (eg. cisplatin)	<ul style="list-style-type: none"> <li>Collect in an approved <b>PURPLE Cytotoxic Sharps container</b>.</li> <li>Once full seal container and transfer to the Micro Lab (L204) for storage prior to collection.</li> </ul>	Gautham to put in <b>PURPLE waste bin</b> for collection by Stericorp. → High Temperature Incineration
	<b>Solid Waste</b> contaminated with cytotoxic drugs or Ethidium Bromide, including contaminated agarose and acrylamide gels and consumables	<ul style="list-style-type: none"> <li>Collect in the <b>PURPLE cytotoxic bucket</b></li> <li>Once full seal bucket and transfer to the Micro Lab (L204) for storage prior to collection.</li> </ul>	Gautham to put in <b>PURPLE waste bin</b> for collection by Stericorp. → High Temperature Incineration
	<b>Cytotoxic Tissue Culture Waste (solid)</b>	<ul style="list-style-type: none"> <li><u>ALL tissue culture waste</u> that is cytotoxic should be collected in an autoclave bag.</li> <li>Transfer to the Micro Lab (L204) for autoclaving prior to placing in <b>PURPLE waste bin</b>. Please ensure that the waste is clearly labelled.</li> </ul>	Gautham to put in <b>PURPLE waste bin</b> for collection by Stericorp. → High Temperature Incineration
	<b>Liquid Ethidium Bromide Waste</b> – buffers contaminated with Ethidium Bromide.	<ul style="list-style-type: none"> <li>Use activated charcoal teabag to deactivate (green bags purchased from Sigma Australia)</li> <li>Leave in contaminated liquid buffer overnight.</li> <li>Transfer charcoal teabag in to laboratory <b>PURPLE cytotoxic bucket</b></li> <li>Dispose of decontaminated liquid waste down sink – flushing with copious amounts of water.</li> </ul> <p><b>Notes</b> See Discipline Safety Officer, Dr Diana Oakes for more information</p>	Once deactivated, dispose of liquid waste down the sink with copious amounts of water. → Sewer