DEPARTMENT OF MOLECULAR AND CELL BIOLOGY UCT

TISSUE CULTURE BIO-SAFETY MANUAL LEVEL II

Version 2.0

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Contents

1. DEFINITION OF HAZARDOUS AND BIOSAFETY LEVELS AS DEFINED BY THE ADVISORY COMMITTEE ON DANGEROUS PATHOGENS (ACDP)

Biological agents are classified into four Hazard Groups based on the following criteria:-

- Is the agent pathogenic for humans?
- Is it a hazard to employees?
- Is it transmissible to the community?
- Is effective prophylaxis or treatment available?

Anyone intending to work with plant or animal pathogens must first consult the Faculty of Science Biological Safety Committee.

1.1 Definition of Hazard Groups

Hazard Group 1: A biological agent unlikely to cause human disease

Hazard Group 2: A biological agent that 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.

Hazard Group 3: A biological agent that can cause severe human disease and presents a serious hazard to employees; it may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available.

Hazard Group 4: A biological agent that 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.

1.2 Hazards when working with Mammalian cells

The major hazard to laboratory workers working with mammalian cells is exposure to 1) viral agents used to transform cell lines e.g. SV40 and 2) human derived oncogenic cell lines. Many cell lines are not fully screened for presence of infectious agents and should therefore always be treated as a Biosafety Level II HBA as per classification outlined in the Faculty of Science Policy Document on Biological Safety, on the Vula TC site. The most common route of infection is through mechanical injury, ingestion and exposure of mucous membranes of the eyes, nose and mouth to aerosols. Therefore aerolization should be minimized. The use of a Biosafety cabinet and other containment device must be used whenever the creation of an aerosol is possible. Contamination, if it occurs, is usually the result of penetrating injuries caused by sharp objects and from the spilling and splashing of specimen materials. Therefore, the most important elements of Biosafety guidelines are:

To avoid penetrating injuries and to prevent direct contact of skin or mucous membranes with cells and culture medium.
To prevent contamination of an individual or their clothing by wearing a laboratory coat, gloves and good basic hygiene practices, including regular hand washing.
Control of surface contamination by containment and disinfection
Safe disposal of contaminated waste

1.3 Safety Procedures and Containment Levels (Based On: Biosafety in Microbiological and Biomedical Laboratories, Dec 2009 Fifth Edition, CDC and NIH)

There are four Biosafety Levels (BSLs) or containment levels which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed the documented or suspected routes of transmission of the

infectious agents, and the laboratory function or activity. The Biosafety Levels described in this manual are distinct from Hazard Groups, as described in the NIH Guidelines and the World Health Organization Laboratory Biosafety Manual. Hazard Groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The Hazard Group of an agent should be one factor, to be considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.

Biosafety Level 1:
Practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. BSL-1 represents a basic level of containment that relies on standard microbiological practices.

BSL-1 is suitable for use with biological agents in Hazard Group 1.

Biosafety Level 2:
Practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

Biosafety Level 3:
Practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a Biosafety Cabinet.

Biosafety Level 4**:** We do not work with any biological agents or pathogens requiring this level of containment at MCB

2. BIOLOGICAL SAFETY

(The following extract was obtained from the Faculty of Science Policy Document on Biological Safety found on the TC Vula site. Refer to contact details at the end of this document)

Biological safety is the discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials. The principles of biosafety are containment and risk assessment.

2.1 Biological Agents

Biological agents include parasitic protozoa, helminthes, fungi, bacteria and viruses. It is also important to note that handling animals, tissues or secretions e.g. blood and urine derived from them, always carries a risk of infection.

Plant or animal pathogens that are not a hazard to human health, but that pose a hazard to animals or the environment must also be correctly handled and contained

Anyone wishing to work with potentially pathogenic organisms, or samples that may contain them, or who is working with micro-organisms or viruses for the first time, should consult the Faculty of Science Biological Safety Committee. Before starting work with any biological material you must:

- Read this manual and the risk assessment that has already been carried out for your work and ask your supervisor about anything you do not understand or are unsure about OR carry out all necessary risk assessments under the supervision of your PI.
- Risk assessments should be available to all lab members working on the relevant projects and this information should be provided by the PI.
- Receive all training necessary to ensure safe working.

2.2 Containment

The fundamentals of containment include the microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. The use of vaccines may provide an increased level of personal protection. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

2.3 Risk Assessment

- BEFORE commencing a project involving ANY biological material, a Risk Assessment MUST be carried out, submitted and approved by the Faculty of Science Biological Safety Committee.

- Each research project must be assessed with respect to the:
  a) hazard of the biological agents
  b) suitable containment
  c) operational practices
  d) training required

Careful consideration must be given when designing new experiments that result in genetically modified mammalian cells (Refer to Biosafety in Microbiological and Biomedical Laboratories US Department of Health and Human Services, Centers for Disease Control and Prevention fifth Edition; USA department of Labour, Occupational Safety and Health Administration (OSHA).

No work may commence without the approval of the Faculty of Science Biological Safety Committee.

3. GENERAL LABORATORY RULES

- All BSL-1 rules apply so refer to the BSL-1 Health and Safety manual.
- Biosafety manual must be up to date and adopted and all changes must be communicated to all users (see section 6).
- Changes to the safety manual will be adopted after consultation with the users (see section 6).
- All personnel must read, review, sign and follow the instructions on practices and procedures outlined in this manual as well as in, Operations manual and BSL-1 Health and Safety manual, on Vula Tissue culture website.
- Cleaning staff must be informed of the hazards [Refer to section 4.1.2]
- This is a restricted area and therefore all doors must be kept closed
- Lab equipment correctly labeled as biohazardous
- Laminar Flow Hoods are used exclusively for preparation of media.
- No personal belongings allowed in the lab.
- No cell phone conversations in the lab.
- Try to work only during core working hours (8am to 5pm) as far as possible. Do not work alone if working after hours. If this cannot be avoided, a colleague must be informed and be available by telephone in case of an emergency.
- Only one person is allowed to work in a 4ft biosafety cabinet at a time.
- Do your rostered lab duties as required

3.1 Repairs and Maintenance

- Refer to the use of all equipment in the Operations Manual.
- Users need to report faulty equipment. Refer to contact number listed in the table in section at the end of the document. All faulty equipment needs to be decontaminated before repair by users (Refer to Section 7.1).
- Faezah Davids will keep an error log action sheet to record all faults reported
- Faezah Davids will keep regularly updated records of all maintenance, service and validation of equipment.?
- TC will be fumigated annually as arranged by Madhu Chauhan.
- In case Faezah Davids is not available then contact Bronwyn Arendze-Bailey and Tatiana Millard (see contact details at the end of this document)

3.2 Electrical

- Refer to Operations Manual
- Water baths must be topped up to the correct level with water. Only 37 degree baths are allowed to be on all the time. Water levels will be topped up weekly by DAs but users must remain vigilant and top up as necessary.
- No extension cords should be used in this facility.
- Electrical sockets must be kept clear and accessible.

3.3 Gas Cylinders

- Refer to Operations manual
- All gas cylinders must remain secured.
- DA (Keyamdien Diedricks) to monitor the levels of CO_2 gas daily when checking the regulators.
- Users must close Nitrogen cylinder after filtering media and clear tubing of the gas.

3.4 Centrifuge

- Refer to Operations manual as well as the BSL-1 Health and Safety manual for general guidelines on how to use a centrifuge

- Users must ensure that the tubes are balanced and loaded correctly.
- In case of a spill, all users to follow procedure as in 6.4 below.
- Users to grease buckets and their threads regularly after cleaning as part of rostered duties

4. RULES FOR WORKING WITH MAMMALIAN CELL LINES

4.1 Training

Training in laboratory safety is vital and must be continually monitored and strengthened. Poor laboratory practice and human error can negate all safety standards and render equipment hazardous. Continual monitoring of safety practices of users, service staff and DAs by SOs and PIs is essential.

4.1.1 Users

- All users must be familiar with the following MCB documents:
    a) The Operations Manual
    b) BSL-1 Health and Safety Manual
    c) Biosafety Level II Manual

  (All are available on Vula Tissue Culture website)
- New users will be trained by a senior, experienced user with assistance from Faezah Davids or by arrangement with Faezah Davids.
- Final sign off will require observation by Faezah Davids.
- The use of the facility, rostered duties by users and DAs, functioning and maintenance of the equipment will be monitored by random checks by trained, authorized SOs as well as quarterly health and safety checklists by the Health and Safety rep.
- New users will be tested on this document before being signed off to work in the Tissue Culture Facility.
- A quiz will be carried out annually to ensure that all users are up to date with the current safety practices and regulations.
- The above mentioned documents must be reviewed annually to ensure that all users are familiar with the latest version, and the signed declaration form at the back of this document must be signed and submitted annually by the users via Vula as proof of review.

4.1.2 Departmental Assistants (DAs) [Refer to Cleaning Staff Health and Safety Manual]

- Their duties are outlined in detail in the Operations manual
- Trained not to touch any reagents on bench tops or fridges or freezers
- Trained to remove the waste bags by holding at the top of the bag and checking to see that there are no tears in the plastic when removing waste. If there are tears then they will need to double bag.
- Trained to wear gloves and have their own dedicated TC lab coats that are regularly laundered.
- Trained in the dangers of UV and trained to check the notice on the ante room door before entering the laboratory.

4.1.3 Service Staff

- In the case of equipment failure or service, external technical personnel or MCB workshop staff members will be required to gain access to the laboratory
- A designated time slot will be booked when there are no users in the facility
- Neil Bredekamp, who has been informed of the biohazards, will always be present to ensure that the recommended safety procedures are followed by the workers.

4.2 Laboratory Facilities and Practices

All procedures involving cell culture manipulation should be performed in a designated biosafety cabinet (Class II) and preferably sealed centrifuge buckets or rotors.

- Access to the laboratory is to be restricted to authorized persons.
- Work in a laboratory room devoted exclusively to work with mammalian cell lines or insect cells.
- Biosafety cabinets (Class II) should be used. A class II biosafety cabinet (BSC) is a partially open-fronted work chamber that provides protection for personnel and the surrounding laboratory space by means of a barrier air flow at the work opening. The cabinet also provides product and/or experiment

protection against contamination by means of ULPA/HEPA-filtered air flowing in a downwards, uniform, unidirectional manner (Laminar air flow).

- Users must be cognizant of the workings of the BSC in particular the air flow and rate of air flow and positioning of reagents within the hood to maximize safe practices.[Refer to : https://www.youtube.com/watch?v=ZnUW1N-JJz8 which will show you the correct way to work in an ESCO biosafety cabinet.
- These cabinets must be properly installed, routinely checked, validated and serviced annually. Failure to do so may render the cabinet ineffective and dangerous. Sticker with date of validation must be placed on the BSC.
- If repairs require technicians to work in the plenum, then the BSC will need to be decontaminated using Formalin and no access is allowed in that area for 24hrs.
- No flames allowed in the Biosafety cabinets or Laminar Flow Hood.
- While working in the Biosafety cabinets keep the front, side and back grills unobstructed.
- The windows in the laboratory should be sealed closed
- The walls, ceilings and floor should be smooth, easily cleaned, impermeable to liquids and resistant to chemicals
- The bench tops should also be impermeable to liquids and resistant to chemicals
- The laboratory furniture should be sturdy and easily cleaned (no cloth chairs)
- Washbasins should be provided in each laboratory room, preferably near the exit
- An autoclave should be available in the building
- It is recommended that laboratory room doors should be self-closing and have vision panels, and have a “Biohazard” sign posted.
- Floors must be cleared of obstruction
- Haemocytometers must be cleaned and packed away after use
- Long hair must be tied back
- Recommended that UV is switched on after cleaning BSC. All Esco BSC UV will only stay on for 10min before switching off automatically
- When UV light is switched on in the BSC and/or in the Laminar Flow Cabinet a notice must be placed by the user on the ante room door (rm432.b) leading to the laboratory and outside the room door of rm3.16.

4.3 Precautions for Laboratory Workers and Personal Protective Equipment (PPE)

- In addition to the information provided in this manual, the Operations Manual provides additional information on the correct use and maintenance of the equipment and rostered duties of the facility.
- PPE must be stored in the ante room (4^{th} Floor lab) or well defined place (3^{rd} Floor lab), checked and cleaned at suitable intervals
- Closed shoes must be worn in the laboratory.
- Each user has been given a special lab coat and these are laundered regularly with all other lab coats unless the coat has been contaminated during a spill. When being laundered, disposable lab coats can be used.
- Wear gloves while working in the facility. Discard gloves whenever they are thought to have become contaminated or damaged, wash your hands with soap and water and put on new gloves. In the 4^{th} floor lab, users will need to discard compromised gloves, leave facility to wash hands in ante room and put on fresh gloves before returning to the lab.
- Perform all technical procedures in a way that minimizes the creation of aerosols, droplets, splashes or spills.
- Access to the laboratory should be restricted to essential personnel
- The Biosafety Cabinets and Laminar Flow Hood are fitted with UV. Do not work in the room while **UV LIGHT IS ON** as UV **EXPOSURE IS HARMFUL** to skin and eyes

4.3.1 Fourth floor Facility

- Enter via the Ante room and wash hands, don gloves and dedicated lab coat.

- On leaving, remove the gloves and discard in the in biohazardous bin in the lab. Remove the lab coat in the Ante room and wash hands

4.3.2 Third Floor Facility

- On entering, wash hands at the designated sink and don gloves and a tissue culture designated laboratory coat.
- On leaving, discard gloves in biohazardous bin, hang up lab coat and wash hands in the designated sink.

4.4 Transfer of Material to and from the Laboratory

4.4.1 Stored Mammalian cells lines

- Mammalian cell lines are stored in cryovials at -80^{o}C. When cells are transferred from the P2 lab to and from the -80^{o}C freezer they must be transported in a carrier box clearly labelled with a "BIOHAZARDOUS" sticker.

4.4.2 Contaminated medium

- The removal of all contaminated cell cultures must be done immediately on detection without opening the flask. Make sure the flask is tightly closed, spray with ethanol and place in a waste disposal bag tied with masking tape. Place the bag directly into the redlined biological waste bin, ensuring that the flask is in a secure, vertical position. Discard the gloves into the waste bin, wash your hands in the ante room and return newly gloved.
- Bottles of contaminated medium must be transported and discarded outside the Facility in the Wash Room 4.14 where it can be opened to add Biocide and left overnight before discarding it. Please rinse the bottle and autoclave it before returning it to TC, where it can be soaked and washed with the rest of the glassware. You should notify Faezah Davids (alternatively refer to table at the end of this document) and supervisor of any sort of contamination, and alert other users by filling in details on the sheet posted on the Incubator.
- Secure the area should a spill occur in a public space and call supervisor and other users to assist with the cleanup. Follow appropriate procedure as outlined in the section on spills (section 6.3)

5. EMERGENCY PROCEDURES

- In the case of fire, follow the general rules outlined in the signage posted in the corridors of MCB
- You need to know the location of the following:
    a) First Aid Kit
    b) Fire-fighting equipment
    c) Eye-baths
    d) Showers
    e) Current contact details of persons needed in an emergency
- Keep emergency exits clear. On the 3rd floor, use the appropriate door in emergencies
- In the case of an emergency, push the emergency exit red button to unlock all doors in the 4th floor facility which also informs campus security.

5.1 Fire emergency

- In a fire drill/emergency, follow the instructions in the BSL-1ealth and Safety manual and those outlined in this document.
- Refer to contact numbers at the end of this document.
- If you discover a fire, close the door to the fire area before taking further action.
- If the fire detectors/smoke alarm is not activated, push the emergency exit red button which will open the doors, provide a pre-recorded message that will inform you of the correct procedures and contact CPS directly.
- Leave the building by the nearest stairwells.
- DO NOT use the elevators.
- Keep entrance ways, access ways and the roadway clear.
- Await instructions from MCB Fire Marshals.
- DO NOT re-enter the building until the 'all clear' signal is given.

5.2 Biosafety Cabinet Failure

- Close all containers inside BSC
- Spray all surfaces with 70% ethanol
- Close Sash if possible
- Tape an "Out of Order" note on the sash of the BSC (At this point, leave the 4th floor lab)
- Discard gloves in biohazardous bin in lab, hang up lab coat in designated area and wash hands before leaving lab.
- Immediately inform the Supervisor and one of the SOs responsible for equipment listed in the contact numbers listed at the end of this document.

5.3 Medical Emergency

- If encountering a medical emergency, contact CPS at extension 2121, followed by the PI.
- Medical Emergencies include:
  1) Needle-stick or Sharps injuries
  2) Splashes on skin, face, eyes
- Skin-piercing wounds, cuts and skin contamination by spilled or splashed specimen material should be immediately washed with soap, followed by rinsing thoroughly with water. Initial bleeding from such a wound, should be encouraged.
- If not able to call, then push the emergency exit red button in the Ante room which will alert CPS.
- Ensure your own safety and that of the injured person. Secure the accident site and ensure that further injury is prevented.
- DO NOT move the injured person unless there is a high risk of further injury or death.

- Keep calm and do not leave the person unattended.
- Provide first aid only if qualified and wait for a first response team to arrive.
- A written record should be prepared and maintained.
- Refer to contacts in table at the end of this document.

- All medical injuries require the completion of an Injury/Incident Report. This is available from the BSL-1 Health and Safety rep, Madhu Chauhan.

6. SPILLS

Experiments in the BSL-2lab will involve culture medium and buffers either with or without exposure to mammalian cell lines. Protocols to clean up these materials will vary depending on the risk.

6.1 General considerations for any spill include the following:

- Worker is not considered contaminated unless a splash or spillage onto their person occurred
- If required, fetch the spill kit which will contain goggles, protective booties, disposable gown, gloves, tongs, thick plastic bags, dustpan, broom, funnel, muslin cloth and mask.
- Contain the spill with absorbent material
- It is recommended to use tongs as much as possible while cleaning the spill to reduce contact with spill liquids or wet absorbent material
- Pay attention to PPE when crouching or bending over to prevent further contamination
- Recruit a helper for spill cleanup
- Consider your course of action before proceeding
- Stay calm and take your time
- Depending on the severity of the spill and potential exposure as listed below, report to the supervisor and HBA Safety Rep (Refer to the table of contacts).
- If the spill results in a medical emergency, refer to the guidelines under section 5.4.3.

6.1.2 General spill in all areas with uncontaminated culture medium or buffers

- Wipe with paper towel moistened with water and then spray with 70% ethanol
- Discard used paper towel in the red biological waste bin.

6.2. Spill involving cell culture material in the Biosafety Cabinet

- Seal all tubes in the biosafety cabinet and place cells in incubator if necessary after spraying with 70% ethanol
- Place paper towel moistened with water to soak up the culture material on to which you spray 1% Virkon and leave for 20min.
- If glass or fractured hard plastic are present, use tongs or forceps to pick up the sharps and place in the thick plastic waste bag in the biosafety cabinet
- Remove paper towel with tongs and discard directly into the plastic bag used for waste in the biosafety cabinet
- As the paper towel might contain contaminants, allow the biosafety cabinet to purge the air for 3min before commencing your experiments
- If the spill has leaked into the catch basin, then the biosafety cabinet will need to be cleared of all material and the work surface will need to be lifted. Recruit a helper.
- Switch off the BSC fan once cleared and purged and BEFORE layering paper towel over the spill to prevent paper towel being sucked into the plenum.
- Spray all material with 70% ethanol and remove from the biosafety cabinet
- Lift the work surface to gain access to the catch basin and layer paper towel saturated with 1% Virkon over the spilt liquid to soak up the fluid.
- Switch off the BSC to prevent paper towel from being sucked into the plenum of the cabinet.
- Leave saturated paper towel for 20min.
- Collect saturated paper towels and place into a thick plastic waste bag. Wipe the surface with paper towel moistened with water. Spray the surface with 70% ethanol.
- Seal waste bag and place directly into a biohazardous bin for incineration.
- Reseat the work surface.
- Run the BSC for at least 10 minutes after cleaning before resuming work or turning BSC off.
- Once finished working, switch on the UV.

- Spray tongs with 70% ethanol and return to spill kit.
- Inform supervisor and HBA safety rep (Refer to contact list) that the spill has been successfully cleaned

6.3 Spill involving cell culture material outside of the Biosafety Cabinet

- Place the spill kit and assemble the waste bag in an area outside of the spill boundary.
- If the spill is large protective booties are located in the spill kit to prevent contaminating user and thus help contain the spill. After use discard as solid waste.
- Use tongs or forceps to pick up any broken glass and place into the thick plastic waste bag.
- Use tongs to pick up any tubes or debris that were part of the spill and place in waste bag.
- Cover the entire spill area including objects with paper towels and apply 1% Virkon for 20min. For vertical objects which will not hold saturated paper towels in place, mist with disinfectant such that those objects remain wet for the entire contact time. After the minimum contact time, use hands or tongs to pick up soaked paper towels and place into a waste bag.
- If at the time of the spill the lab coat was heavily soiled the lab coat should be collected into a thick plastic waste bag and discarded into the biohazardous waste bin for incineration.
- Place all other waste bags generated during spill cleanup directly into the biohazardous waste bin.
- For a final decontamination step, mop entire area with germicide.
- Discard booties.
- Once clean-up is complete normal work within the room can resume.
- Inform supervisor and HBA safety rep (Refer to list of contacts in the table at the end of this document) that the spill has been successfully cleaned.

6.4 Spill involving cell culture material inside a centrifuge

- A biological spill in a centrifuge has the potential for producing large volumes of aerosols.
- If you suspect a spill then wait 30min for aerosols to settle and fetch the spill kit to don goggles, a second pair of gloves and mask before opening the centrifuge.
- Mark the centrifuge with signage or tape which indicates a spill and DO NOT OPEN.
- During the 30 minute wait, inform other users of the spill and that the centrifuge is out of use.
- Contact supervisor and HBA Safety rep (Refer to list of contacts in the table at the end of this document) that a spill has taken place within the centrifuge.
- After a 30-minute wait period, open and remove any broken items or sharps with tongs or forceps and place them in a thick plastic waste bag
- Using tongs or forceps, remove any broken plastic tubes from the buckets/rotors using extreme caution as there may be sharp edges to a thick plastic waste bag. Seal the bag and place in biohazardous waste bin
- Prepare a 500ml beaker with 0.3% Biocide and place the centrifuge bucket into the biocide.
- Transfer the beaker to the biosafety cabinet and wait 20 min.
- Layer paper towels moistened with water in the centrifuge to absorb the spilled fluid, spray with 1% Virkon and wait 20 min. Discard the used paper towels into a thick plastic waste bag.
- Wipe with paper towel moistened with sterile water to remove all traces of corrosive disinfectant and then spray with 70% ethanol.
- After buckets have been submerged and soaked in biocide for 20min, pour off liquid into sink through a funnel lined with muslin cloth to remove any missed fractured plastic.
- Remove the fractured plastic with tongs and place in thick disposable bag.
- Remove the muslin cloth and discard in the thick disposable bag.
- After disinfection is complete, remove outer gloves and place in the waste bag along with any other waste.
- Tightly close waste bag and spray with 70% ethanol.
- Place waste bag directly into a biohazardous waste for incineration.
- Perform routine cleaning of centrifuge, associated parts and surrounding work area as needed.
- Inform supervisor and HBA Health and Safety rep (Refer to list of contacts in the table at the end of this document) that the spill has been successfully cleaned.

7 DECONTAMINATION, FUMIGATION, DISPOSAL OF NON-HAZARDOUS WASTE AND HANDLING AND DISPOSAL OF HAZARDOUS MATERIAL

7.1 General Decontamination

- Swab down hood, bench tops and microscope stage with 70% alcohol regularly. Clean up at the end of every day and switch off equipment.
- Clean haemocytometers by flooding with 70% Ethanol before a final rinse under running water. Dry well before packing away.
- Users need to decontaminate equipment before service and repairs. Spray with 1% Virkon, wipe with paper towel moistened with water followed by spraying with 70% ethanol.

7.1.1 Biosafety Cabinet Decontamination

- If repairs are required that that needs access to the plenum of the BSC, then the SO will arrange the best time to shut down the lab taking the users experiments into consideration and contact a recommended service provider to carry out the decontamination.
- Once a time as been decided upon by the SO and users, the SO will contact the entire department as that floor will need to be evacuated for a period of 24 hrs.
- The SO will post signs on the door and in the stairwell warning all users of the pending decontamination.
- The procedure will involve mixing formaldehyde and ammonia which are classified as toxic, harmful, corrosive and irritant chemicals that are harmful to the environment. Formaldehyde is also classified as a carcinogen.
- The BSC will be sealed to try and contain all the toxic fumes however it can take up to 48hrs before the smell dissipates.
- The service provider takes full responsibility for clearing all subsequent mess and removing all waste generated during the procedure.

7.2 Annual Fumigation

- MCB will be fumigated every year to remove all insects.
- Madhu Chauhan is in charge of this process as the entire building has to be cleared and will inform the users of the date (usually in January).

7.3 Disposal Methods

7.3.1 Non-Hazardous Waste:

This includes any paper or paper-related products, packaging material etc. that is generated in the lab and that hasn't come into contact with cells or media. Such material is to be discarded in normal see-through plastic bags in a rubbish bin.

7.3.2 Solid Hazardous Waste

All used solid waste, including **gloves, plastic pipettes, pipette tips, glass Pasteur pipettes** and all other waste generated in the BSL-2 hood during routine lab work to be discarded directly into a plastic bag and sealed before discarding the bag into the red biological waste bin outside the hood. There should be no paper or evidence of any waste on the floors. Solid waste should be placed in suitable waste container(s) inside the hood, or in the case of large scale experiments with many flasks/ dishes/plates, stacked neatly at the side. In the case of using any glass Pasteur pipettes, these should be placed in a separate suitable waste container. The underlying principle is to minimize movement of arms into and out of the hood while working, as well as to minimize clutter inside the hood, which itself poses a H&S risk. Avoid placing large amounts of mixed waste together with glass Pasteur pipettes in a single full plastic bag, due to the possibility of the bag being pierced while handling. Waste can be

discarded at the end of the experiment, or if waste generates too much clutter during the experiment, it can be discarded in suitable batches. Bags to be sealed in hood.

7.3.3 Liquid Hazardous Waste

The Gilson Safe Aspiration Station allows you to aspirate directly into a bottle already containing 30g Virkon and 100ml water. The tubing must be flushed with f water until liquid runs clear followed by an ethanol rinse at the end of each session. The final volume of the liquid waste should not exceed 3 L (Refer to Operations manual) and must be emptied weekly by pouring the liquid down the drain after waiting 20 min followed by flushing the sink with a fair amount of water. The bottle may be replaced once it's been washed and contains the correct amount of Virkon and water as stated above.

7.3.4 Sharps

The sharps waste bin is used to discard broken glassware. Follow precautions when handling broken glassware and use the dustpan and hand broom available if necessary.

8 DISINFECTANTS

8.1 Virkon preparation

- Virkon is a balanced, stabilized blend of peroxygen compounds, surfactant, organic acids and an inorganic buffer system.
- Add 10g of Virkon to 1 litre of deionised water
- Label the container with the date the Virkon was prepared
- Virkon older than a week should be discarded down the sink and the sink flushed with cold water
- Contact time of 20min is recommended.

8.2 Biocide preparation

- 0.3g dry powder per 100mls of deionised water
- Contact time of 20min is recommended.

8.3 Aquaclean

Our incubators are water jacketed to ensure even distribution of heat, and humidity is obtained by placing a tray of MilliQ water at the bottom of the incubator. Aquaclean is added to the water in the tray to a final concentration of 0.5% and has to be replenished after one month. Dates of additions of Aqualclean are noted on the cleaning roster posted on the door of the incubator.

8.4 70% Ethanol

- Aqueous alcohol solutions are not appropriate for surface decontamination because of the evaporative nature of the solution.
- 70% ethanol can be used to soak small pieces of surgical instruments and for wipe downs following a disinfectant that might leave a corrosive residue
- 70% ethanol is also suitable for spraying plastic consumables and reagent bottles etc. when loading and unloading a biosafety cabinet and for wipe downs of general work surfaces.

9 COMMUNICATION AND AMENDMENT

- Users, supervisors and relevant SOs will meet annually to amend this document unless unexpected emergencies requires urgent response or changes to the manual
- Any changes/amendments made during the year must be submitted to and approved by the MCB Health and Safety Committee in writing
- All changes must be done in consultation with users via email before submission to the MCB Health and Safety Committee
- Accepted, amended documents will be uploaded to Vula and users will be notified by email
- Users are required to read the document and sign the Declaration available on Vula annually irrespective if changes have been made or not.
- An annual quiz will be carried out to ensure that all users remain familiar with safe practices in the Biosafety level II Mammalian Tissue Culture lab.
- The signed declaration form must be submitted by the users to Vula
- Next date for review is June 2015

10. TABLE OF CONTACT NUMBERS

Name	Extension	Purpose	Email
Upper Campus Lower Campus	Ext 2121 Ext 2222	In the event of injury and fire	
UCT Environmental Safety Officer			
Name	**Extension**	**Purpose**	**Email**
Mr Brett Roden	Ext 3487	Assessing Environmental risks	Brett.Roden@uct.ac.za
UCT Health and Safety Officer			
Name	**Extension**	**Emergency Contact Number**	**Email**
Mr Michael Langley	Ext 3552		Michael.Langley@uct.ac.za
Faculty of Science Biosafety Committee Chair			
Name	**Extension**	**Purpose**	**Email**
Dr Laura Roden	Ext 5322	Submission of Risk Assessment Forms	Laura.Roden@uct.ac.za
First-Aiders			
Name	**Extension**	**Emergency Contact Number**	**Email**
Ms Keren Cooper Mrs Shakiera Sattar Mrs Bronwyn Arendze-Bailey Mrs Pei-Yin Liebrich	Ext 2303 Ext 3264 Ext 1401 Ext 2403	 0723779050 0845818202	Keren.Cooper@uct.ac.za S.Sattar@uct.ac.za Bronwyn.Arendze-Bailey@uct.ac.za Pei-yin.Liebrich@uct.ac.za
Supervisors/PI's			
Name	**Extension**	**Emergency Contact Number**	**Email**
Prof Janet Hapgood Dr Inga Hitzeroth Dr Zenda Woodman	Ext 5977 Ext 5712 Ext 2406	082 8731173 083 5063330 076 1282308	Janet.Hapgood@uct.ac.za Inga.Hitzeroth@uct.ac.za ZL.Woodman@uct.ac.za
MCB Health and Safety Reps			
Name	**Extension**	**Emergency Contact Number**	**Email**
Mrs Faezah Davids (HBA) Ms Madhu Chauhan (H&S)	Ext 4631 Ext 2404	0824675849 0824148539	Faezah.Davids@uct.ac.za Madhu.Chauhan@uct.ac.za
Equipment Failure			
Name	**Extension**	**Emergency Contact Number**	**Email**
Mrs Faezah Davids Mr Neil Bredekamp Mrs Tatiana Millard Mrs Bronwyn Arendze-Bailey Mrs Shakiera Sattar	Ext 4631 Ext 2579 Ext 5831 Ext 1401 Ext 3264	0824675849 0823996790 0823322735 0845818202 0723779050	Faezah.Davids@uct.ac.za Neil.Bredekamp@uct.ac.za Tatiana.Millard@uct.ac.za Bronwyn.Arendze-Bailey@uct.ac.za S.Sattar@uct.ac.za
UCT Occupational Health			
Name	**Purpose**		**Emergency Contact No.**
Sister Sue Key	Emergency physician in case of exposure or symptoms		650 3873
Departmental Assistants			
Name	**Purpose**	**Extension**	**Email**
Ms Mietah Andreas Ms Felicia Stuurman	Supply basic consumables and clean the Facility	Ext 3271 Ext 3271	Mietah.Andreas@uct.ac.za Felicia.Stuurman@uct.ac.za
Mr Keyam Diedericks Mr Charles Hendrickse	Monitoring and maintaining a supply of N2 & CO2 gas	Ext 3271 Ext 5310	Keyamdien.Diedericks@uct.ac.za Charles.Hendrickse@uct.ac.za

11 DECLARATION

I, the undersigned, declare that:

- I have read and understood the content of the Department Of MCB Tissue Culture Bio-Safety Manual Level II And I Agree To Abide By The Rules Of This Facility
- I declare that I have been trained in the operational and safety procedures applicable to work in a BSL-2 laboratory

Trainee Name: _____

Trainee Signature: _____

Date:_____

I, the undersigned trainer declares that _____ _has been trained in BSL-2 laboratory techniques, is proficient in these techniques and allowed to work in the laboratory without on-site supervision.

Access to the Laboratories after hours is allowed.

Yes ☐

No, not until further assessment ☐

Trainer Name: _____

Trainer Signature: _____

Date:_____

Confirmed by: _____

Date:_____

I, the undersigned Supervisor declares that _____ is able to work in the BSL-2 laboratory.

Supervisor Name: _____

Supervisor Signature: _____

Date:_____