1. OBJECTIVE

The purpose of this document is to outline operating procedures for the safe working with mammalian cell culture in order to protect laboratory personnel and students from potential risks of infections and other risks in the NUS Department of Medicine research laboratories.

**The purpose of this SOP template is to outline the general procedures for performing mammalian cell culture. It is the responsibility of the PI/users to customize the information to match his/her specific operations.*

2. SCOPE

This SOP is applicable to all laboratory personnel and students who are conducting mammalian cell culture in the NUS Department of Medicine research laboratories.

3. RESPONSIBILITIES

3.1 Principal Investigators are responsible for making sure that staff are properly trained and equipment and facility are maintained in good working order.

3.2 All staff and students must be aware of the potential risks associated with mammalian cell culture; must obtain the necessary training and work under supervision until proficient in the practices and techniques to work safely.

4. POTENTIAL HAZARDS

4.1 Potential laboratory hazards associated with human samples/cells include bloodborne pathogens e.g. Hepatitis B virus, HIV, etc. and all staff and students should be aware of the risks associated with the biohazards before starting work.

4.2 Ethanol and other disinfectants can be toxic and/or flammable. Identify the risks associated with each reagent before you start work by reading the Safety Data Sheet (SDS).

4.3 Ultra violet lights in biosafety cabinet pose a burn hazard to skin, and blindness hazard to eyes. UV light can cause DNA damage leading to cancer. Ensure UV lights are switched off before starting work.

4.4 The CO_2 gas used in the culture incubator can pose an asphyxiation or toxicity risk if inhaled. Ensure you have also read and understood the risk assessment and standard operating procedure for working with compressed gases.

5. TRAINING REQUIRMENTS

Complete the relevant safety training Chemical Safety, Biosafety for BSL-2 Laboratories and Safe Handling of Human Tissue and Fluids (if the samples are from human) vial IVLE @ https://ivle.nus.edu.sg/.

6. IMMUNIZATION

Immunization for hepatitis B is required for all personnel handling human cell culture.

7. ENGINEERING CONTROL

The biosafety cabinet level 2, BSL2 is the most important equipment to provide containment of infectious splashes or aerosols generated by many microbiological procedures as well as to prevent contamination of the cell culture.

8. PERSONAL PROTECTIVE EQUIPMENT

All personnel are required to wear the following personal protective equipment during the culture work: long sleeved lab coat, latex gloves, safety glasses/goggles and covered shoes.

Hand washing is a critical component of exposure control. Wash your hands after gloves have been removed, before leaving the laboratory and at any time after handling materials known or suspected to be contaminated.

9. SAFETY PRECAUTION

- Do not eat, drink, smoke, handle contact lenses, apply cosmetics, or store food for human consumption in the laboratory. Oral pipetting of any substance is prohibited.

- Read all information provided with the cultured cells and culture methods.
  - Tissue type of origin: Higher risk with haematogenous cells and tissue such as blood and lymphoid tissue.
  - Aware of all the hazards before handling.

- Read the safety data sheet for the materials used in the cell culture. Some of common materials for cell culture might be poisonous or hazardous, such as penicillin-streptomycin, dimethyl sulfoxide, etc.

- Sterile handling in a biosafety cabinet level 2, BSL2.
  - Turn on UV light for at least 10 minutes and then turn on BSL2 cabinet fan before beginning work.
  - Swab down work surfaces and materials with 70% ethanol before and after use.
  - Plan ahead and take all needed supplies to the BSL2 cabinet before beginning work.
  - Delineate areas for clean and dirty materials.

- Avoid pouring actions, which are a potential source of cross-contamination.
- Do not place contaminated tubes, pipettes on work surface.
- Do not leave media bottles or culture vessels opened.
- Work either left to right or vice versa, so that all material goes to one side, once finished.
- Clean up spills immediately and always leave the work place neat and tidy.

- Disinfectants must be available at all times within the areas where the biohazardous material is handled or stored.

- Decontaminate all work surfaces before and after your experiments, and clean up any spill or splash of potentially infectious material immediately with an appropriate disinfectant before discarding into the sink. Clean laboratory equipment routinely, even if it is not contaminated.

- Have a container of an appropriate disinfectant ready for used pipette tips or liquid waste. Containers must be placed inside of the BSL2 cabinet. Decontaminate all reusable glassware and plasticware immediately after use.

- Procedures where aerosols are generated or procedures involving the use of needles or other sharps should be evaluated, Researchers are strongly encouraged to find alternate, less hazardous methods to conduct their research. Discharge pipettes against the wall of containers to avoid splashes. Use of sharps is strongly discouraged.

- Equipment use inclusive of CO_2 incubator, water bath, centrifuge, inverted microscope, etc. needs regular calibration, cleaning and preventive maintenance. The users are adequately trained in use of equipment.

10. PROCEDURE

10.1 Preparation of culture media

- Choice of media depends on the type of cell being cultured
- Commonly used media are RPMI, EMEM, DMEM etc.
- Culture media is supplemented with filter-sterilized antibiotics e.g. penicillin, streptomycin and heat-inactivated fetal bovine serum (FBS) etc.

10.2 Isolation of cells

- Cells are harvested via enzymatic disaggregation by using trypsin, collagenase or some other enzymes.
- To prevent cells from spilling during centrifugation, the tubes containing cell suspension should not be overfilled and are balanced.
- Before centrifugation, load the sample tubes into safety buckets inside the BSL2 and always seal the buckets with safety cups.
- After centrifugation, allow the centrifuge to come to a complete stop.
- Carefully transfer the buckets to the BSL2 and open the safety cups to retrieve the sample tubes.

- Wash the pellet carefully by re-suspending in appropriate media.
- Cells are incubated at 37^{o}C with 5% CO_2.

10.3 Examination of cells

- Cells should be checked microscopically for health of cells, morphology, % confluence, cell debris, contamination, etc. to ensure they are healthy and growing as expected.
- Attached cells should be mainly attached to the bottom of the flask, round and plump or elongated in shape and refracting light around their membrane.

10.4 Changing media

- If cells have been growing well for a few days but are not yet confluent, a media change to replenish fresh nutrients to keep the cells healthy may be required. It is recommended to warm culture media to 37°C. Carefully aspirate spent media from culture vessel and discard. Replace with fresh warmed media and return cells to incubator.

10.5 Sub-culturing or passaging

- Prepare to subculture the cells when the culture has reached around 80% confluence.
- Use appropriate procedures: example by cell scraping or trypsinization

10.6 Freezing of cells

- Check cells for bacterial, yeast, or fungal contamination under a microscope.
- Cells are harvested from tissue culture flask or dish. Cells are pelleted via centrifugation.
- While cells are spinning, calculate total number of cells in flask and determine amount of freeze medium needed. (Cells are usually be re-suspended in freeze medium at 5,000,000 to 20,000,000 cells/mL).
- Prepare freezing medium with appropriate amount of culture media containing 10% FBS and 10% dimethyl sulfoxide (DMSO).
- Label vials with preparation date, cell type and user's name.
- Discard supernatant from centrifuged cells and add freeze medium.
- Quickly aliquot 500 µL to 1 mL of freeze stock per cryogenic vial. Screw each vial closed.
- Put vials into storage box and place box and put entire container into –20°C freezer. After 3 hours, transfer container to -80°C freezer and store overnight.
- Alternatively, cells in the cryotube can be placed in the NALGENE "Mr Frosty" freezing container at -80°C freezer for 2-12 hours.
- Next day, put cells into appropriate rack in liquid N_2 tank.

10.7 Thawing of cells

- Warm culture media to 37°C

- Rapidly remove a vial of frozen cells from liquid N_2 tank and hold in 37°C water bath until sides are thawed but center remains frozen. Spray down vial with 70% ethanol, wipe, and place into BSL2 cabinet.
- Gently transfer partially frozen cells into a new tube containing fresh, pre-warmed culture media.
- Since freeze medium contains DMSO, cells are spun down and re-suspended in fresh culture media supplemented with 5-20% FBS in new tissue culture flask or culture dish.
- Cells are incubated at 37^{o}C with 5% CO_2.

11. WASTE DISPOSAL

11.1 Biohazard Solid Waste

- Solid biohazard waste should be double bagged (yellow bag) in the disposal bin with biohazard GHS label. When the bag is 2/3 full, tie both biohazard waste bags tightly. Label bag with lab location, PI name and contact number.
- The unwanted sharps (including razor blades, syringes, glass pasteur pipettes, needles should not be re-sheathed) should be placed in the sharps bins.
- The biohazard waste will be collected by the licensed service provider.

11.2 Biohazard Liquid Waste

- Culture waste including cell culture media and serum must be treated with appropriate disinfectant - either inactivate for at least 1 hour in freshly prepared Presept solution (5,000 ppm) or appropriate disinfectant prior to disposal to drain with an excess of water.

12. SPILL RESPONSE

If spillage occurs, inform the PI/supervisor/safety lead and spill responders immediately and refer to Spill Clean-Up Procedure: SOP-Medicine-01 Biological Spill Response and/or SOP-Medicine-03 Chemical Spill Disposal.

13. INCIDENT REPORTING

Accidents resulting in injuries must be reported to the PI and/or laboratory safety lead immediately after first aid is applied.

Seek medical attention when necessary at the University Health Centre or proceed to the Accident & Emergency units of National University Hospital after office hours.

All incidents or accidents have to be notified to OSHE within 24 hours via the online NUS Accident and Incident Management System (AIMS) @https://inetapps.nus.edu.sg/osh/portal/eServices/ehs360_aims.html. The AIMS report can be submitted by the injured staff/student, safety leads, his or her supervisor/representative if the staff or student is unfit/unable to do the initial report.

14. REFERENCES

a. NUS Laboratory Biorisk Management Manual (OSHE NUS/OSHE/M/01)
b. NUS Laboratory Chemical Safety Manual (OSHE NUS/OSHE/M/02)
c. SOP-Medicine-01 Biological Spill Response
d. SOP-Medicine-02 Biological Waste Disposal
e. SOP-Medicine-03 Chemical Spill Disposal
f. SOP-Medicine-04 Chemical Waste Disposal