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1.0 PURPOSE

It is pertinent that the tissue/cell culture (which refers to culturing of mammalian cells in a laboratory setting) procedure is performed using aseptic technique. Aseptic technique encompasses environmental control, personal hygiene, equipment, media sterilization, and associated quality control procedures. Contaminations can be avoided by sticking to strict aseptic protocols. Microorganisms are pervasive-bacteria is present on all surfaces, including our skin, fungal hyphae and mold spores are airborne and can enter from air ducts or open doors. In addition, most researchers will use the same microorganisms in experiments in a laboratory setting. Therefore, 'everything' that comes in contact with the cells must be sterile.

Sometimes the cell culture media is supplemented with antibiotics which can aid the aseptic technique. Autoclaving renders pipettes, glassware, and solutions sterile. The nutrient medium cannot be autoclaved and should be sterilized using a filter with a small pore size to filter out bacteria and mycoplasmas (Millipore Sterivex - GS 0.22µm disposable filter units).

Mastering an excellent aseptic technique will save you considerable frustration in the future. Furthermore, the same principles for good aseptic technique also minimize biohazard risk to the researcher when infectious organisms or dangerous chemicals are used.

2.0 PROCEDURE

General Guidelines

- Absolutely no food or drink is allowed in the cell culture room.
- Wash hands before and after handling any cell culture.
- Cell culture rooms are maintained under positive pressure therefore the main door to the anteroom and the inner door to the cell culture suite must be kept closed.

- Lab coats and gloves should be worn when handling any of the biological material in the cell culture facilities. Keep a lab coat dedicated for use in the cell culture room and change it at least every two weeks. Lab coats are cuffed, make sure the gloves go over the cuff.
- The use of Ethanol to disinfect gloves is not recommended due to its tendency to compromise the integrity of the glove material. Should researchers wish to disinfect their gloves, it is recommended that they double glove.
- Gloves must be removed before exiting the cell culture room. If you need to pass between two adjacent rooms, remove one glove to allow you to open the door.
- Keenan Research Centre for Biomedical Sciences (KRCBS) has shared cell culture facilities. Therefore, all users are restricted to working in their assigned biosafety cabinet (BSC) and CO_2 incubator space.
- Users must place their waste in the proper containers (yellow biohazard bag, sharps, regular waste).
- Ensure that the microscope is turned off when not in use.
- It is imperative to discard liquid vacuum waste. This must be done daily in order to prevent fungal growth.
- Be considerate of those who will be using the facility after you. Disinfect all surfaces (including bench tops and microscope) after using the cell culture room.
- Avoid continuous long-term use of antibiotics within cell cultures. The overuse of antibiotics as prophylaxis may lead to cytotoxicity and pose an increased risk of covert mycoplasma contamination within the cell lines.
- If you have encountered a contamination, please inform the Research specialist and the other researchers using the tissue culture room so that appropriate action can be taken.
- Report any equipment problems or incidents of contamination to the Research specialist.

Specific Guidelines

Getting started:

- Check your media for contamination before use in the cells. Warm up the media ahead of time in the bead bath. Use of a bead bath significantly reduces the risk of media contamination.
- Use only sterile media and solutions. When possible, use aliquots instead of the full bottles. If needed, sterilize your reagents, media, or solutions prepared in the laboratory by filtering. Sterilize instruments and equipment by autoclaving.

- Wash your hands, don clean gloves and lab coat.
- Turn on the biosafety cabinet (BSC; see biological safety cabinet guidelines), sterilize BSC work area and flush aspirating tube with 70% ethanol before starting.
- Before placing anything in the BSC ensure that it has been wiped with 70% ethanol (media bottles, tubes stands, pipette boxes).

Working in the BSC using aseptic technique:

- Organize the BSC such that clean items are kept on the left, inoculated cultures/cells are in the middle and dirty collection containers and aspirators are on the right. This reduces cross contamination via airborne aerosols and droplets and helps optimize hand movements.
- Work 6 inches away from the window sash and do not block the airflow by over crowding or blocking the grille.
- Please note that the use of UV light as a germicidal is now strongly discouraged.
- Never uncover a sterile flask, bottle, petri dish, etc., until you are ready to use it. Use both hands to open and close bottles to avoid your fingers touching the inside of the cap. When removing the cap from a bottle, flask, etc., set the cap down on the BSC surface with the interior facing down.
- Avoid pouring sterile liquids from one vessel into another. The drop of liquid that usually remains on the lip of the vessel can easily form a liquid bridge between the nonsterile outside and sterile inside of the vessel. This allows microorganisms from the outside to enter and contaminate the vessel.
- Always use separate media bottles for every cell line. This important step reduces both the possibility of cross contamination with another cell line and limits the spread of contamination if the bottle of medium becomes contaminated.
- Sterile pipettes should never be taken out of the wrapper until they are to be used.
- Never insert a pipette back into a bottle of medium after it has been used to feed a culture. This "double pipetting" saves on pipettes but can easily lead to widespread contamination by other cell lines or mycoplasma.
- Do not draw from different bottles with the same pipette. Use a sterile pipette for each bottle.
- Cultures should be inspected daily for signs of contamination. In addition, testing at regular intervals for mycoplasma should be conducted to ensure the purity and integrity of the culture.

- Promptly discard any contaminated cultures. Retention of these cultures poses a serious threat of cross contamination to other cultures in the laboratory- put contaminated cultures in biohazard bag and autoclave before discarding.
- When aspirating media, use a sterile glass transfer pipette at the end of the aspirating tube.
- Clean a spill containing biological agent immediately by wiping with paper towel sprayed with 70% ethanol. If spilled material goes through the grille, the base plate of the biosafety cabinet should also be cleaned immediately.

Waste disposal:

- All soft edged biological waste should be disposed in the bin with the yellow bag (e.g. tubes, flasks, plates, serological pipettes, gloves).
- All sharps waste must be disposed in the yellow sharps receptacle (e.g. glass Pasteur pipettes, needles, pipette tips).
- Treat aspirated liquid waste with 1:10 bleach before disposing down the drain followed by running copious amounts of water.
    - See Biomedical and Biohazardous Waste Disposal Guidelines.
- If you collect waste (tips or liquid) inside the BSC, place the waste container towards the rear of the cabinet workspace. Dispose the waste in the appropriate bin once you finish your work.
- Everything that is NOT contaminated with biological material will be disposed in the black/grey bin (landfill).

After completion of the work:

- Allow the cabinet to run for 5 minutes with no activity.
- Close or cover open containers before removing them from the cabinet.
- Wipe down the biosafety cabinet with 70% ethanol. Never use bleach as it causes pitting and corrosion to the cabinet.
- Surface-disinfect objects in contact with contaminated material before removal from the cabinet.
- Clean the aspirator line using sterile water, followed by 70% ethanol, until the line becomes clean with no colored medium in it. Never aspirate bleach as it will corrode the components of the aspirator lid.
- Turn off the fluorescent light and cabinet blower by returning sash to designated lower height.
- If the liquid waste has been sitting with 1:10 bleach for 20-30 min empty the Vacusafe container down the drain (with plenty of water), rinse with water.

- If you used the microscope, wipe the stage with ethanol. Check that the microscope and centrifuges are turned off.

Incubators:

- Each lab is assigned an incubator and is responsible for its maintenance.
- Everything stored in the incubator must be labeled with your name, the cell type, and the date.
- Check your cells daily. Look for signs of contamination. These include cloudiness, change of medium color, floating particles, change in the growth rate of cells, cell death, altered cell morphology. Discard old flasks.
- Open the incubator door only briefly to avoid air-borne contaminants getting inside. When finished, check that the door is properly closed. Never leave the door open for an extended period.
- Regularly check water levels in the pan, add autoclaved double distilled water if the water level is low.
- Be careful when moving dishes/flasks to avoid spills. Clean all spills immediately by first wiping down with a clean paper towel, then paper towel with mild detergent followed by wiping down the area with 70% ethanol.
- Incubators must be regularly cleaned. Each user must follow the incubator cleaning processes defined for the incubators. Check with Research specialist for details.

In case of contamination:

- Remove the contaminated cells from the incubator right away. Do not put them in the biosafety cabinet. Treat the affected plates/flasks with 1:10 bleach before discarding. Wait until the color of the medium turns white, then pour the liquid down the sink. Discard of the plastic ware in the yellow biohazard waste bin.
- Until the source of the contamination has been found, consider all your media and cells as contaminated. You may need to discard everything and prepare fresh medium.
- Notify your supervisor, as well as other users who share the cell culture suite about the contamination. Report it to the Research specialist as well.

3.0 DEFINITIONS

Term/Acronym	Definition

BSC	Biosafety cabinet
Vacusafe	Laboratory vacuum pump for safe and efficient collection and containment of biological liquid waste.
Bead Bath	dry, metal beads designed to replace water in non-circulating and non-shaking laboratory water baths

4.0 REFERENCES

Aseptic technique for cell culture- https://pubmed.ncbi.nlm.nih.gov/18228291/

https://www.jove.com/v/5036/an-introduction-to-working-in-the-hood

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01	Director, Research Facilities	October 5, 2022
02	Research Specialist- cell culture	October 5, 2022
03		