

Healthcare and Energy Materials

Laboratory

(E3 05-14, E3 05-15)

Standard Operating Procedures (SOP)

Biological Safety

(08 May 2008)

Prepared by

Teo Wee Eong

Lab Technologist

Email: engtwe@nus.edu.sg

Cheng Ziyuan

Professional Officer

Email: bieczy@nus.edu.sg

Approved and Verified by:

Prof Seeram Ramakrishna

Principal Investigator and Lab Supervisor

Email: seeram@nus.edu.sg

1. Safe handling of mammalian cell cultures

Safe practices

- a) Practice good microbiological practices, especially those that are aimed at avoiding accidental contamination. Be trained in aseptic techniques.
- b) Wear a long-sleeved lab coat, gloves and personal eye protection.
- c) Remove contaminated gloves carefully. Wash hands thoroughly with antiseptic soap after removing gloves.
- d) Keep the work area clean and tidy
- e) Avoid the use of sharps.
- f) Avoid the creation of aerosols through vigorous pipetting.
- g) Never lay the pipette on its side when it is filled with liquid.
- h) Sterile handling in a biosafety cabinet (BSC):

- Swab down work surfaces and materials with 70% ethanol before and after use.
- Plan ahead and take all needed supplies to the BSC before beginning work.
- Delineate areas for clean and dirty materials.
- Avoid talking during culture manipulations as aerosols may be drawn into the work area.
- 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 open.
- Discard unwanted, non-sterile tubes or pipettes immediately into pipette jar. Remove cotton bud using forceps before putting into pipette jar.

i) Cell Lines:

- Use well characterized cell lines where possible.
- Read all information such as Certificate of Analysis provided with the cells and ensure you are aware of the hazards and precautions for handling.
- Treat each new culture that is manipulated for the first time in the laboratory facility as potentially infectious.
- Handle cell cultures from undefined sources as risk group 2 agents. If there is a reasonable likelihood of adventitious agents of higher risk class, the cell line should be handled under appropriate containment level until tests have proven safety.
- NEVER handle or manipulate cells derived from yourself. Autologous cells, if accidentally reintroduced, can evade normal immune responses.
- Quarantine new cell cultures brought to the laboratory until the culture does not show growth of contaminating bacteria, fungi, or mycoplasmas.

- Work with one cell line at a time and disinfect the work surfaces between two handlings involving cell lines.
- Routinely carry out quality control checks of cells to ensure the absence of likely contaminating pathogens.

j) Culture media:

- When preparing media to be used with more than one cell line, aliquot the media into separate containers for each cell line. This will reduce the chances of contamination.
- Restrict use of antibiotics in growth media to reduce the chance of a resistant agent from growing. Rely on good aseptic technique rather than on antibiotics to keep cell lines free of contamination.
- Obtain animal sera from a reputable source as serum can be contaminated with adventitious bovine viruses or prions eg. the agent responsible for bovine spongiform encephalopathy (BSE) or “mad cow disease”.

k) Decontamination:

- Decontaminate all reusable glass and plastic ware immediately after use. Do not allow a lab worker/dishwasher to be exposed to contaminated lab ware.
- Clean up any culture fluid spills immediately with a validated disinfectant.

Working with Human and Other Primate Cells

a) The potential laboratory hazards associated with human cells include:

- bloodborne pathogens e.g. Hepatitis B virus and HIV,
- cells transformed with oncogenic viruses agents, such as SV-40, EBV, or HBV, as well as cells carrying viral genomic material,
- tumorigenic human cells with potential hazards as a result of self-inoculation
- contamination with adventitious bacteria, fungi, mycoplasma, and viruses that cause disease in humans.

b) Handle all human and primate cells using **Biosafety Level 2** practices and containment.

Since most cell lines are not fully characterized, it is wise to regard such cell lines as potentially infectious and handle them at the same biosafety level as a cell line known to carry HIV or hepatitis B virus.

c) Perform all work in a biosafety cabinet when handling infectious materials, or when there is a potential of aerosol production. **Decontaminate all material by autoclaving or disinfection before discarding.**

d) A Hepatitis B immunization is required for all personnel handling human cell lines.

e) All established or permanent cultures of human lymphocytes should be handled on the assumption that they harbor the Epstein-Barr virus.

2. Safe operation of biological safety cabinet

a) Preparation & material placement

- Read the Material Safety Data Sheets (MSDS) for materials being used in a hood. Note any precautions regarding the use of the chemical or microorganisms in the BSC.
- Turn on the fluorescent light and cabinet fan 15 minutes before beginning work.
- If the cabinet is equipped with an alarm, test the alarm and switch it to the “ON” position.
- Confirm inward air flow by holding a tissue at the middle of the edge of the viewing panel and ensuring it is drawn in.
- Decontaminate the cabinet surface and surface-decontaminate all materials to be placed inside the BSC with 70% alcohol or preferably some non-flammable disinfectant.
- Work may be performed on disinfectant-soaked absorbent towels to capture splatters and splashes.
- Bulky items, such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet.
- Aerosol generating equipment (e.g. mixer, centrifuges, etc) should be placed towards the rear of the cabinet.
- Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may impede proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.
- Wear double gloves to minimize skin contact. If the outer gloves are contaminated, discard them and put on a fresh pair. Gloves should be pulled over the wrists of the gown rather than worn inside.
- Generally front opening gowns should be worn for Biosafety level 1 work and back opening gowns for Biosafety level 2 work.

b) Operation

- Active work should flow from clean to contaminated areas across the work surface.
- Arms should be moved in and out slowly, perpendicular to the front opening to minimize disruption of the air curtain and laminar flow. Manipulations of materials within BSCs should be delayed for about 1 min after placing hands and arms inside to allow the cabinet to adjust and to “air sweep” the surface of the hands and arms.
- Work as far to the back (beyond the air split) of the BSC workspace as possible, but within comfortable reach.
- Always use mechanical pipetting aids. No mouth pipetting is allowed.
- Heat sources such as Bunsen burners are strictly prohibited inside the BSCs as they significantly disrupt the laminar flow of air.
- To sterilize bacteriological loops, microburners or electric “furnaces” shall be used.
- Do not work in BSC while a warning light or alarm is signaling.
- The UV lamp should never be on while an operator is working in the cabinet.

c) Spillage

- If there is a spill of biological materials during use, surface decontaminate all objects in the cabinet; disinfect the working area of the cabinet while it is still in operation (do not turn the cabinet off).
- If spillage occurs outside the cabinet, involve lab technologist immediately to activate your spill control/response group.

d) Cleaning and disinfection

- When work is completed, all equipment and supplies from the cabinet should be surface-decontaminated and removed from the cabinet.
- The interior surfaces should also be wiped with an appropriate disinfectant that would kill any microorganisms that could be found in the cabinet. Corrosive chemicals such as sodium hypochlorite should be avoided, but if used, should be followed with a wipe down of sterile water.
- Allow the cabinet to run for 15 minutes.

3. Safe operation of centrifuge

a) Operation

- Lids shall be closed at all times during operation.
- Rotors must be used only with the correct centrifuge.
- Observe maximum speed and sample density ratings designated by the manufacturer for each rotor, and speed reductions required for running high-density solutions, plastic adapters, or stainless steel tubes.
- The user shall not leave the centrifuge until full operating speed is attained and machine appears to be running safely without vibration.
- If vibration occurs the centrifuge should be stopped immediately and load balances checked. Swing-out buckets should be checked for clearance and support.
- Sample loads must be balanced and swinging bucket rotors must not be run with missing buckets. ($\frac{1}{2}$ g at 1 G is approximately equivalent to 250 kg @ 500,000 G's!)
- Plastic centrifuge tubes should be discarded after one cycle of ultracentrifugation. The failure rate for used tubes is a hazard which justifies the use of new tubes for each high G centrifugation.
- Nitrocellulose tubes should be used only when transparent and flexible (fresh). They must never be heated because of explosive possibility.
- Store all fixed angle vertical tube and near-vertical tube rotors upside down, with the lids or plugs removed. Swinging bucket rotors should be stored with the bucket caps removed.
- If vibration occurs, stop the run immediately; wait until the rotor stops, and check the load balances.
- In the event of a power failure, do not try to open the lid to retrieve samples for at least half an hour. Follow the instructions in the manual for recovery of the samples.

b) Spill response

- Turn off centrifuge, notify others in laboratory and evacuate if necessary.
- Post temporary hazard warning sign.
- Notify lab technologist.

4. Safe operation of autoclaves

- ◆ The autoclave chamber is a pressure vessel. Never attempt to open the door while the machine is operating.
- ◆ Wear lab coat, eye protection, heat insulating gloves and covered toe shoes.
- ◆ After the slow exhaust cycle, open autoclave door.

- ◆ Slowly open door slightly. Beware of the rush of steam. If you feel any resistance, do not force open the door.
- ◆ Allow liquids to cool down to ambient conditions before handling them.

5. Biological waste disposal

a) Biowaste disposal:

- All wastes containing biohazardous material should be handled with gloves.
- Free flowing liquid waste eg. Culture of microorganisms, tissue culture wastes, shall not be disposed off with solid waste nor discarded down the drainage system. The waste shall be contained in leak proof, rigid durable containers labeled with the biohazard symbol and the word “biohazard”. Liquid wastes shall be decontaminated by autoclaving or with the use of an appropriate chemical disinfectant in accordance with the manufacturer’s recommendations. The treated waste can be disposed off in the sewer system.
- Sharps shall be placed in appropriate sharps containers that are labeled “biohazard”.
- If the biological waste is contaminated with chemical agents, the waste has to be treated as chemical waste.

b) Sharp-waste disposal

- Sharps, including blood-drawing equipment, needles, syringes, slides, glass pasteur pipettes, capillary tubes, broken glass and scalpel blades, should be disposed into containers labeled as “SHARPS” and biohazard symbol.
- Contaminated needles should not be broken or clipped unless the clipping device can effectively control the aerosol generation. Needles shall not be recapped or separated from syringes prior to disposal.
- Sharps containers shall remain closed at all times except when sharps are being deposited into the container. Sharps containers shall not be placed on the floor in the lab at all times. Sharps containers shall not be filled beyond the recommended fill line.

6. Biological spill response

a) Spills inside a Biological Safety Cabinet

- Leave the cabinet turned on.
- Put on gloves and a lab coat.
- Spray or wipe cabinet walls, work surfaces, and equipment with disinfectant equivalent to a 1:10 bleach solution. If necessary, flood the work surface including drain pans and catch basins below the work surface with disinfectant.
- Wait at least 20 minutes.
- Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
- Autoclave all clean-up materials before disposal in the biohazardous waste container according to the procedure for biohazardous waste disposal.
- Wash hands with appropriate soap/disinfectant and any exposed surfaces thoroughly after the clean-up procedure.

b) Biological spill outside a Biological Safety Cabinet in BSL 2 Lab

- Hold your breath and leave the room immediately.
- Warn others to stay out of the spill area to prevent spread of contamination.
- Post a sign on the door warning others of the spill.
- Remove any contaminated clothing and put it into a biohazard bag for autoclaving.
- Wash hands and exposed skin and inform your PI or supervisor about the spill.
- Put on protective clothing (lab coat, gloves, mask, eye protection, shoe covers) and assemble clean-up materials.
- Wait 30 minutes before re-entering the contaminated area to allow dissipation/settling of aerosols.
- Cover the spill with paper towels and gently apply disinfectant, proceeding from the outer edge of the spill to its center. Refer to MSDS for appropriate type of disinfectant.
- Leave in place for at least 30 minutes. (Refer to MSDS for contact time).
- Collect all treated materials and discard in a biohazard container. Use forceps to pick up any broken glass and place in a sharps container.
- Re-wipe the spill area with disinfectant. Remove gloves and wash hands thoroughly.
- Dispose biohazardous wastes according to procedures.