ONLY PERSONNEL THAT HAVE BEEN AUTHORIZED BY MMRI MAY ENTER THE SHARED TISSUE CULTURE LABORATORY

1.0 OBJECTIVE

1.1 Guidelines and requirements for use of the Tissue Culture Room

2.0 REAGENTS

2.1 **Sterile PBS**: Do not share bottles or assume that other people will use yours. PBS can be stored at room temperature.

2.2 **Other**: 1X trypsin, 10X trypsin. DO NOT take thawed trypsin from other people. Thaw and use your own.

3.0 INCUBATORS

3.1 **Use**: For the use of an incubator space, contact Dr. Ajith Welihinda or Dr. Jingjing Wang. Space is at a premium, so please be considerate with your cultures. If you will not be routinely maintaining your cultures, THROW THEM AWAY. Leaving flasks indefinitely in the incubator is an unnecessary hazard that threatens the cultures of the people who are actively using the incubator. If you detect contamination, eliminate it **IMMEDIATELY**

3.2 **Maintenance**: If you notice small spills from your flasks or plates, wipe it up immediately with 70% ethanol sprayed onto a paper towel. Do not spray ethanol directly into the incubator, as the fumes will kill the cells. Also wipe down your flask if you will be putting it back into the incubator. Please only use 70% ethanol to clean the incubator. Bleach is damaging to the incubator. There is a tray in the bottom of the incubator that holds water to humidify the incubator. If it runs out of water add 4 liters of autoclaved deionized water. Please keep the tray clean. If the 'add water' alarm starts beeping, this does not refer to the humidifier tray at the bottom of the incubator this refers to the water jacket. Please contact Dr. Ajith Welihinda or Dr. Jingjing Wang when you hear an alarm beeping.

3.3 **Cleaning:** Remove all cells (transfer to one of the other incubators) and turn off CO2. Take out shelves, sides and water reservoir. Wash down everything, including the inside walls with 70% ethanol. Do not use bleach as it can damage the incubator. Let the fumes die down. Autoclave shelves, sides and

water reservoir and put everything back into the incubator. Fill the water reservoir with autoclaved deionized water. Turn the CO2 back on and put the cells back when temperature and CO2 levels are back to normal.

4.0 HOODS

4.1 **Use:** There are three important things to remember when using a tissue culture hood:

1. **NEVER TURN OFF the blower for the hood**. This maintains the sterile field. If the blower has been turned off, wash down hood thoroughly with 70% ethanol. Turn the hood blower on. Wait a minimum of 15 minutes. Wash the hood down again with 70% ethanol. You may now use the hood.
2. **Don't block the airflow**. This is what keeps the workspace sterile. Even though it is convenient, you should never have supplies lying in the airflow tray at the front of the hood.
3. **Keep as few things as possible in the hood** while you are working. This is part of the airflow issue. Objects along the back of the hood block the back air vent. The most sterile spot in the hood is right in the center so keep your flasks well behind the airflow in the front.

4.2 **Maintenance**: Before using the hood, spray it down with 70% ethanol. Always keep the airflow on. Spray down the hood with ethanol when you are finished and turn off the fluorescent light. You can leave the receptacle on but turn off the pipet aid in the hood. Please close the glass shield when finished to reduce noise level in the room.

5.0 VACUUM WASTE

5.1 The vacuum trap is for use with Pasteur pipettes. A canister of sterile pipettes is kept in each hood. Dispose of used pipettes in the sharps container under the hood. Make sure that the glass pipettes are completely within the sharps container and BELOW THE FILL LINE. The disposal company will not pick it up if filled above the line. Also, if tips are sticking out, the next person who tries to dispose of a pipette may stab him/herself. If the container fills to fill line, remove it and replace it. After you are done with the vacuum, run some bleach through the hose. If the media in the receiving flask does not turn clear, indicating that everything has been oxidized, run some more bleach through the hose. Follow with a few squirts of ethanol into the hose to facilitate drying and increasing longevity of the hose. If the flask is almost full, empty it into the sink, flushing with copious water. **Add the equivalent of 10% bleach into**

the empty receiving flask BEFORE starting to fill. DO NOT fill it up so high that liquid flows into the safety filter.

6.0 REFRIGERATORS

6.1 Again, space is at a premium. Throw away unnecessary items. Please store Medium and Tissue Culture related reagents only. Other reagents should not be stored in these refrigerators. Please label and date the reagents.

7.0 GLASSWARE AND TIPS IN HOODS

7.1 **Pasteur pipettes**: Use the sterile canister in the hood. Each group is responsible for their own canisters and sterile pipettes.

7.2 **Plastic ware:**

Tip Boxes: Sterile tip boxes for p20, p200 and p1000 can be kept inside the hood. Please use and keep the boxes sterile inside the hood. When you empty a box, dispose of it properly.

8.0 DISPOSAL

8.1 **Segregation of waste (paper, plastic):** Separate non-contaminated from contaminated waste. Dispose non-contaminated waste into the regular trash and the contaminated waste into the appropriate container (see below)

8.2 **Liquid Waste:** Aspirated media, cell suspensions, used media containing serum and buffers must be treated with a final concentration of 10% bleach for a minimum of 30 minutes. All liquid waste, once treated with 10% bleach may be discarded with running water down the sink. Bleach containing solutions being discarded down the sink CANNOT BE AT CONCENTRATIONS GREATER THAN 10%.

8.3. **Plastic Pipettes:** Remove paper cover and place lengthwise in the large red sharps container.

8.4 **Non-sharps** (**Plastic ware, tubes, plates, paper, gloves):** Place in red bin which must be double-lined with red bags.

8.5 **Sharps (needles, glass pipettes):** Place in the small red sharps container.

9.0 CENTRIFUGE

9.1 **Use**: Always make sure that the buckets are properly balanced. Turn off when done. Please clean the centrifuge if a spill happens.

9.2 **Centrifuge Malfunctions:** Centrifuge malfunctions should be handled in such a way as to avoid personal injury or exposure. In the event of a centrifuge malfunction and/or spill, which may create hazardous aerosols, all personnel should vacate the room for 30 minutes to allow the aerosol to dissipate. Contaminated areas, broken tubes etc. should then be properly decontaminated and cleaned up promptly. The person using the centrifuge, along with the principal investigator and/or laboratory supervisor in charge of the lab, is responsible for insuring that clean up and decontamination is achieved.

10.0 MICROSCOPE

10.1 **Use**: Turning the microscope on and off repeatedly during a short period of time is hard on the bulb. **Therefore, do not turn the scope off until you are completely done**. To increase bulb life, keep the light turned down to its lowest intensity unless you are actively using it. Always clean hemocytometer and coverslip carefully with 70% ethanol after use.

11.0 PRESERVATION AND STORAGE

11.1 Cells are stored in liquid nitrogen. To minimize the effects of freezing, several precautions should be taken. A typical freezing medium is 90% serum, 10% DMSO. In addition, it is best to use healthy cells that are growing in log phase and to replace the medium 24 hours before freezing. Also, the cells are slowly cooled from room temperature to -80°C to allow the water to move out of the cells before it freezes. Please place your cells in a cardboard box at room temperature and place the container in the - 80°C freezer. Once the containers have reached -80°C (about 24 hours or, more conveniently, overnight) transfer the vials and immediately place them in the assigned location/slot in the liquid nitrogen storage tank. **Please see Dr. Ajith Welihinda or Dr. Jingjing Wang for assignment of space in the liquid nitrogen storage tank**.

To maximize recovery of the cells when thawing, warm the cells very quickly by placing the tube directly from the liquid nitrogen container into a 37°C water bath. Once thawed, place the cells immediately into pre-warmed medium, centrifuge to remove DMSO and transfer them into a flask or a plate and place them into a 37^{o}C Incubator.

12.0 SAFETY CONSIDERATIONS

12.1 Assume all cultures are hazardous since they may harbor latent viruses or other organisms that are uncharacterized. The following safety precautions should also be observed:

- pipetting: use pipette aids to prevent ingestion and keep aerosols down to a minimum
- no eating, drinking in the Tissue Culture Rooms
- wash hands after handling cultures and before leaving the lab
- decontaminate work surfaces with disinfectant (before and after)
- use biological safety cabinet when working with hazardous organisms. The cabinet protects the worker by preventing airborne cells and viruses released during experimental activity from escaping the cabinet; there is an air barrier at the front opening and exhaust air is filtered with a HEPA filter make sure cabinet is not overloaded and leave exhaust grills in the front and the back clear (helps to maintain a uniform airflow)
- use aseptic technique
- dispose of all liquid waste after each experiment and treat with bleach.

12.2 **Hepatitis B Vaccination Protection**

12.2.1 **Hepatitis B virus (HBV)** is a pathogenic microorganism that can cause potentially life threatening disease in humans. HBV infection is transmitted through exposure to blood and other potentially infectious materials

12.2.2 **HBV Vaccination**: The OSHA standard requires employers to offer the vaccination series to all workers who have occupational exposure. See attached OSHA Fact Sheet.

13.0 TRAINING REQUIREMENTS

13.1 Initial and annual Bloodborne Pathogen training is required for anyone who works with human blood, human blood components, products made from human blood, human body fluids, unfixed human tissue or organs, human cell lines, animals with human xenografts, bloodborne pathogens, or other potentially infectious materials.

13.2 Required Training is in two parts:

Part 1. **On-line training**:
To register, see Kayla Vuong

Part 2. **Practical on-site training**:

Procedures and policies are covered in the annual Laboratory Safety Refresher training. Initial training is provided by review of this SOP with MMRI staff and tissue culture laboratory orientation.

14.0 RESPONSIBILITES IN THE TISSUE CULTURE ROOM

14.1 **MMRI:** Provides waste containers, red bags, bleach supplies, CO2, and general equipment maintenance.

14.2 **Affiliates:**

1. Change bleach in aspiration traps when required
2. Empty biohazard containers
   - Seal inner and outer bag
   - Move sealed bag of waste to storage freezer in Room 461
   - Double-bag the empty waste container.
3. Seal full sharps containers and move to storage area in Room 461.
4. Affiliates must provide their own hemocytometer.

Questions or concerns?

Please Contact:

Dr. Ajith Welihinda: 408 605-9239 or awelihinda@mmrx.org

Dr. Jingjing Wang: 513 302-6724 or jwang@mmrx.org

OSHA® FactSheet

OSHA's Bloodborne Pathogens Standard

Bloodborne pathogens are infectious microorganisms present in blood that can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV), the virus that causes AIDS. Workers exposed to bloodborne pathogens are at risk for serious or life-threatening illnesses.

Protections Provided by OSHA's Bloodborne Pathogens Standard

All of the requirements of OSHA's Bloodborne Pathogens standard can be found in Title 29 of the Code of Federal Regulations at 29 CFR 1910.1030. The standard's requirements state what employers must do to protect workers who are occupationally exposed to blood or other potentially infectious materials (OPIM), as defined in the standard. That is, the standard protects workers who can reasonably be anticipated to come into contact with blood or OPIM as a result of doing their job duties.

In general, the standard requires employers to:

- **Establish an exposure control plan.** This is a written plan to eliminate or minimize occupational exposures. The employer must prepare an exposure determination that contains a list of job classifications in which all workers have occupational exposure and a list of job classifications in which some workers have occupational exposure, along with a list of the tasks and procedures performed by those workers that result in their exposure.
- **Employers must update the plan annually** to reflect changes in tasks, procedures, and positions that affect occupational exposure, and also technological changes that eliminate or reduce occupational exposure. In addition, employers must annually document in the plan that they have considered and begun using appropriate, commercially-available effective safer medical devices designed to eliminate or minimize occupational exposure. Employers must also document that they have solicited input from frontline workers in identifying, evaluating, and selecting effective engineering and work practice controls.
- **Implement the use of universal precautions** (treating all human blood and OPIM as if known to be infectious for bloodborne pathogens).
- **Identify and use engineering controls.** These are devices that isolate or remove the bloodborne pathogens hazard from the workplace. They include sharps disposal containers, self-sheathing needles, and safer medical devices, such as sharps with engineered sharps-injury protection and needleless systems.
- **Identify and ensure the use of work practice controls**. These are practices that reduce the possibility of exposure by changing the way a task is performed, such as appropriate practices for handling and disposing of contaminated sharps, handling specimens, handling laundry, and cleaning contaminated surfaces and items.
- **Provide personal protective equipment (PPE), such as gloves, gowns, eye protection, and masks.** Employers must clean, repair, and replace this equipment as needed. Provision, maintenance, repair and replacement are at no cost to the worker.
- **Make available hepatitis B vaccinations to all workers with occupational exposure.** This vaccination must be offered after the worker has received the required bloodborne pathogens training and within 10 days of initial assignment to a job with occupational exposure.
- **Make available post-exposure evaluation and follow-up to any occupationally exposed worker who experiences an exposure incident.** An exposure incident is a specific eye, mouth, other mucous membrane, non-intact skin, or parenteral contact with blood or OPIM. This evaluation and follow-up must be at no cost to the worker and includes documenting the route(s) of exposure and the circumstances

under which the exposure incident occurred; identifying and testing the source individual for HBV and HIV infectivity, if the source individual consents or the law does not require consent; collecting and testing the exposed worker's blood, if the worker consents; offering post-exposure prophylaxis; offering counseling; and evaluating reported illnesses. The healthcare professional will provide a limited written opinion to the employer and all diagnoses must remain confidential.

- **Use labels and signs to communicate hazards.** Warning labels must be affixed to containers of regulated waste; containers of contaminated reusable sharps; refrigerators and freezers containing blood or OPIM; other containers used to store, transport, or ship blood or OPIM; contaminated equipment that is being shipped or serviced; and bags or containers of contaminated laundry, except as provided in the standard. Facilities may use red bags or red containers instead of labels. In HIV and HBV research laboratories and production facilities, signs must be posted at all access doors when OPIM or infected animals are present in the work area or containment module.
- **Provide information and training to workers.** Employers must ensure that their workers receive regular training that covers all elements of the standard including, but not limited to: information on bloodborne pathogens and diseases, methods used to control occupational exposure, hepatitis B vaccine, and medical evaluation and post-exposure follow-up procedures. Employers must offer this training on initial assignment, at least annually thereafter, and when new or modified tasks or procedures affect a worker's occupational exposure. Also, HIV and HBV laboratory and production facility workers must receive specialized initial training, in addition to the training provided to all workers with occupational exposure. Workers must have the opportunity to ask the trainer questions. Also, training must be presented at an educational level and in a language that workers understand.
- **Maintain worker medical and training records.** The employer also must maintain a sharps injury log, unless it is exempt under Part 1904 -- Recording and Reporting Occupational Injuries and Illnesses, in Title 29 of the Code of Federal Regulations.

Additional Information

For more information, go to OSHA's Bloodborne Pathogens and Needlestick Prevention Safety and Health Topics web page at: https://www.osha.gov/SLTC/bloodbornepathogens/index.html.

To file a complaint by phone, report an emergency, or get OSHA advice, assistance, or products, contact your nearest OSHA office under the "U.S. Department of Labor" listing in your phone book, or call us toll-free at **(800) 321-OSHA (6742)**.

This is one in a series of informational fact sheets highlighting OSHA programs, policies or standards. It does not impose any new compliance requirements. For a comprehensive list of compliance requirements of OSHA standards or regulations, refer to Title 29 of the Code of Federal Regulations. This information will be made available to sensory-impaired individuals upon request. The voice phone is (202) 693-1999; the teletypewriter (TTY) number is (877) 889-5627.

For assistance, contact us. We can help. It's confidential.

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www.osha.gov 1-800-321-6742

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