BIOSAFETY APPENDIX E AVOIDING THE PRODUCTION OF BIOLOGICAL AEROSOLS

There are many documented cases of laboratory-acquired infections that resulted from the production and inhalation of infectious aerosols. If adequate precautions are not taken, aerosol production can occur when using laboratory equipment, even when the equipment is used properly and under normal conditions. This SOP covers procedures to be used to avoid the production of bioaerosols.

Centrifuge Operations

When microorganisms or cell cultures are not properly contained within a centrifuge, potentially infectious aerosols can escape during the high-speed spin process. This may occur when spinning uncapped samples, or when there's a leak, a spill, or a tube breaks. Observe the following precautions to minimize the risk of aerosol production while centrifuging:

- Use unbreakable tubes (i.e., not glass).
- Avoid overfilling the tubes. Allow sufficient headroom for sample to rise as it spins (3/4 full maximum but less is better).
- Use only centrifuge tubes with O-ring screw caps to ensure a secure seal.
- Ensure that the centrifuge is properly balanced, with materials of similar densities opposite each other.
- Use outer, sealable safety cups and only load/unload them in a biosafety cabinet.
- Do not open the ultracentrifuge lid during or immediately after a run. Allow the centrifuge to come to a complete stop and wait at least 30 minutes before opening. This allows time for any aerosol to settle if leakage or breakage did occur during the run.
- Never exceed the specified speed limitations of the rotor as listed in the owner's manual.
- Decontaminate the inside and outside of the cups or buckets before and after use, and inspect seals regularly for deterioration. Replace as needed.
- When possible, use the centrifuge in an enclosed, specially ventilated area that discharges air from the space through a HEPA filter.

Mixing Operations

Sonicators, shakers, and homogenizers can all generate an aerosol during operation. Minimize the risk when mixing by observing the following precautions:

- Operate mixing equipment inside a certified biological safety cabinet.
- Use heavy-duty screw caps that include an O-ring.
- Use sealed vessels during mixing. Wait 20 minutes to allow any aerosol that might have developed to settle. Aerosols develop due to the build-up of pressure within the container. NOTE: 20 minutes is acceptable here because these instruments operate at much lower rpm's than ultracentrifuges).
- Open all mixing vessels inside a biosafety cabinet.

- Check the condition of the mixing equipment routinely for wear or deterioration.
- Decontaminate all surfaces of mixing devices before and after use.
- If manual tissue grinders are used, surround the tube with absorbent material.
- **Do not** use household blenders/homogenizers in the lab.

Vacuum and Aspirating Equipment

Minimize the risk of generating aerosols during vacuum and aspiration operations by observing the following precautions:

- Use non-breakable (i.e. not glass) flasks.
- Ensure that vacuum equipment is equipped with a HEPA or similar filter.
- Place a disinfectant in the overflow flask of the aspirating equipment.
- Use containment for the flasks (a grey bin).

Needles and Syringes

Minimize the risk of aerosol production while using needles and syringes by observing the following precautions:

- Perform all operations with needles and syringes inside a biological safety cabinet.
- Discharge air from the syringe before inserting it into a stopper.
- Fill syringes carefully. Avoid frothing or introducing air bubbles.
- Wrap the needle and stopper in a cotton ball or pad moistened with an appropriate disinfectant when removing the needle from the rubber-stoppered bottle.
- Expel excess liquid and air bubbles from the syringe vertically into a cotton ball moistened with an appropriate disinfectant or into a small bottle containing cotton.
- **Do not** use syringes to mix infectious liquids including cell cultures.

Pipettes

Minimize the risk of aerosol production while using pipettes by observing the following precautions:

- Use cotton-plugged pipettes.
- Gently expel the contents of the pipette against the wall of the container and allow it to flow down the side (tip-to-wall technique).
- Do not mix the contents of a container by alternating suction and blowing with a pipette.
- Use TD pipettes instead of TC pipettes. The last drops from a TD pipette do not need to be expelled or blown out to get an accurate measurement.
- Submerge used non-disposable pipettes into a horizontal tray of disinfectant solution inside the BSC immediately after use.

- If you don't generate a lot of pipettes, dispose your decontaminated pipettes to a cardboard box lined with a plastic bag. When the box is full, seal it with packaging tape, label it "Decontaminated Pipettes" and carry out to the dumpster.

  NOTE: If your lab generates a lot of plastic serological pipettes, collect them in a 31 gal red tote and dispose as Regulated Medical Waste on alternate Tuesdays. https://web.uri.edu/ehs/files/2022-BIOWASTE-SCHEDULE.pdf The University's vendor participates in a waste-to-energy program and the pipettes will be incinerated. This is preferable to disposal in landfill. Totes are available in Room 275-A Avedisian or they can be requested in the Supplies section of EHS's new online Biowaste Pickup Request form at https://web.uri.edu/ehs/biohazardous-waste-pickup-request-form/

Transfer Loops

Minimize the risk of aerosol production while using transfer loops by observing the following precautions:

- Substitute an enclosed micro-incinerator for an open flame burner.
- Use disposable inoculating tools. Decontaminate with a suitable disinfectant prior to disposing in a sharps container.