**Protocol for working with biohazard level-2 samples in the CNF Duffield 201 Lab**

**Tools: Malvern Nano ZS Zetasizer & Malvern NS300 NanoSight**

**Scope:**

The purpose of this document is to outline a procedure to allow the measurement of human derived cell samples on two tools in room 201. Samples that are derived from humans, non-human primates, human cell lines, toxins or pathogens that cause disease in humans require a Biosafety Level-2 (BSL-2) work environment and protocols. The current CNF user population has access to this space and tool set but may not have the appropriate biosafety training to work with the specific hazards the materials present.

The two tools addressed here and approved for this material are only the Malvern Nano ZS Zetasizer and Malvern NS300 NanoSight. Those two tools in addition to several other tools are currently used on a regular basis for non-bio or non-hazardous BSL1 samples.

**Guidelines/Usage Requirements:**

1. All BSL-2 sample work must be approved by the CNF User Program Manager AND the tool manager.
2. BSL-2 work needs to be coordinated with the tool manager(s).
3. The researchers will have completed Cornell EH&S blood-borne pathogen training or equivalent BSL-2 safety training
4. Access to the 201 lab space will be restricted to BSL-2 trained staff and researchers while this work is being performed.
5. Safety glasses, gloves, and an optional lab coat will be used when working with these materials.
6. All sample prep will be performed in the yellow-tape designated areas on the bench and sink next to the tool.

**PPE:**

1. Users should follow the CNF lab dress code and wear a visible wearable badge
2. Safety Glasses, gloves are required when working with BSL2 materials in 201, disposable lab coats are optional

**Procedure:**

1. Schedule tool and room use with Coral and the CNF tool manager. At least 3 days prior, send an email to Xinwei (wu@cnf.cornell.edu) and Beth (rhoades@cnf.cornell.edu) with the date and time that the samples will need to be analyzed. Don’t plan on working until you receive confirmation.
2. Transport samples (50 ml or smaller volumes) in secondary sealed containers. Go directly to the lab. Larger liquid volumes must be transported through Duffield and Philips Halls by CNF staff. Contact them ahead of time.
3. Hang the Biosafety Room Restriction sign on the outside of the lab door.
4. Put on safety glasses and gloves.
5. Don’t start work until other CNF users have finished their work and left the space.
6. Clear the work area that is defined by yellow tape of clutter. This is the area where you are allowed to work with biohazardous samples. Cover any surfaces where you’ll work with white paper mats (in drawer), especially at the tool where samples are loaded.
7. Setup a RED biosafety waste bag in the wire rack holder. Place it on one of the bench mat areas.
8. (OPTIONAL) Don a disposable lab coat.
9. Perform measurements on the tool, stay in lab during all measurements. Do not leave samples unattended in the lab. If you must leave BRIEFLY to go to the restroom, cap all samples.
10. Change gloves often, you can operate the tool keyboard and mouse with gloves on, **NO GLOVES** on the lab phone, door handles or other computers.

**Decontamination and Disposal:**

1. Discard waste in the proper containers.
   1. **Syringes wrappers** in general trash.
   2. **Syringes or Syringe needles** in the Plastic Biohazard Sharps Bin…***Do not place in any other trash container!***
   3. **Micropipette tips** – in the Red Biohazard bag in the wire holder.
   4. **Liquid Samples & waste**- Pour into a capped sample cup with 5ml of 50% bleach:water. Screw on lid and incubate for 10 minutes.
   5. **Empty capped sample cups** – discard into Red Biohazard bag in the wire holder.
   6. **Wipes, paper towels, gloves, bench mat** - in the Red Biohazard bag in the wire holder.
2. Decontaminate the area.
   1. Add 50% bleach:water to the NanoSight glass waste jar. Incubate 10 minutes. Pour the contents down the drain. Rinse the waste jar with 70% ethanol followed by water. Then re-install it on the NanoSight.
   2. Wipe down the pipettes & tip boxes with Conflikt disinfectant-wetted paper towels or pre-saturated disinfectant wipes if available.
   3. Do the same with the exterior of the tool, keyboard, mouse & screen.
   4. Do the same with the lab bench and areas that were exposed to possible sample splashes, spills or contamination.
3. Place all decontamination materials in the Red Biohazard bag in the wire holder.
4. Seal the Red Biohazard bag with a tie or tape. Set it on the right side of the sink.
5. Fill out the surface disinfecting log sheet that is in the tool manual.
6. Remove the room restriction sign from outside of lab door.
7. Log out of CORAL and send an email to Xinwei (wu@cnf.cornell.edu) and Beth (rhoades@cnf.cornell.edu) that you finished, decontaminated and leave any comments.

**Spills:**

1. Cover spills with lab wipes.
2. Saturate the lab wipes with a 10% bleach solution or Conflikt for 5 minutes.
3. Wipe up and discard the wipes in the Red Biohazard bag in the wire holder.
4. Notify CNF Staff of any spills in person or email after hours to safety@cnf.cornell.edu.

**Exposure:**

1. Wash exposed area with soap and water.
2. Perform first aid if needed and seek medical treatment by using the red phones or calling 911 to get Cornell Emergency Responders.
3. Follow up at Gannett Health Center or the Cayuga Medical Center outside normal business hours
4. Notify CNF Staff and CNF Safety of any exposures in person or via safety@cnf.cornell.edu.