

Generic BSL2 Cell Culture SOP

The following SOP has been prepared from OEHS Biosafety Guidelines for performing cell culture experiments and the manipulation of tissues from animals infected with Risk Group 2 Agents.

Training

- All researchers working on the protocol have completed Biosafety, Bloodborne Pathogens (if applicable), and Lab Chemical Safety Training.

Door Signs/Access:

- The entry door to the lab will be posted with a biohazard (BL2) door sign which will remain in place for the duration of the experiments or as long as Risk Group 2 agents or materials are stored in the laboratory.
- The entry door will be posted with a Laboratory Information Card, which will check off BL2 and list the agents requiring BSL2 containment.
- The lab door will remain closed during BL2 experiments.
- The BL2 Work Practices Poster will be placed on a wall in the lab near the designated work bench to remind researchers of the required biosafety procedures.
- Access to the laboratory will be restricted while BL2 experiments are in progress.

Labels:

- The biohazard label will be placed on all equipment storing Risk Group 2 Agents or materials. This includes incubators, freezers, refrigerators and centrifuges.
- The sign should include the word Biohazard, the designation BL2, the identity of the agent, and the name of the primary researcher and contact information.
- Fellow lab members not involved in the experiments shall be informed not to touch experimental materials, only authorized personnel shall handle Risk Group 2 Agents or materials.

Personal Protective Equipment (PPE)

- Researchers may wear disposable personal protective equipment or reusable lab coats for their BSL2 experiments.
- Personal protective equipment worn at BSL2 containment is dedicated to the BSL2 laboratory. BSL2 protective clothing is removed before leaving the lab and stored within the BSL2 lab area, and not worn to non-lab areas or in public spaces, such as hallways outside the laboratory.
- A disposable or reusable lab coat, gloves and face shield can be utilized. Sleeve covers may also be provided to cover the potential gap between gloves and the sleeves of the lab coat.
- Double gloves are recommended for high risk BSL2 experiments (i.e. those involving human pathogens). The outer gloves are removed behind the shield before moving to the incubator or the centrifuge. The inner (or clean) gloves are used to open the incubator or load and operate the centrifuge.
- Disposable protective clothing can be discarded as biological waste at the end of the experiment.

- Reusable lab coats can be inspected and if no overt contamination is observed can be placed on a hook for reuse the next day. Any contamination observed on the lab coat can be disinfected by spraying with 10% bleach or other suitable disinfectant and allowed to air dry, or autoclaved if it will withstand the process. \
- PPE will be removed before leaving the laboratory.
- Researchers will wash their hands with soap and water for 30 seconds following the removal of personal protective equipment.

Engineering Controls

- All work will be performed within a Class II Biological Safety Cabinet according to the Biosafety Cabinet Checklist just below.
- Plastic will be used in place of glass where feasible.
- Vacuum systems will be protected by hydrophobic or HEPA filters.
- The Biosafety Cabinet is disinfected before and after use
- Items will be disinfected with 10% bleach and 70% ethanol for 10 minutes after use for terminal decontamination.
- A sealed rotor or aerosol canister will be used for centrifugation. Rotors or canisters will be loaded and unloaded within the biological safety cabinet.
- Items will be transported according to Yale OEHS policy as noted below:

Biosafety Cabinet Checklist Before Use

- Cabinet on?
- Drain valve closed?
- All waste containers and supplies loaded inside?
- Disinfectant prepared and in cabinet?

Biosafety Cabinet Checklist During Use

- Perform all work inside the cabinet
- Keep grilles clear
- Discard waste inside the cabinet
- Disinfect all items prior to removal
- Remove gloves or disinfect them before removal from the cabinet

Biosafety Cabinet Checklist Work Completion

- Seal all waste containers before removal from the cabinet
- Disinfect all items before removal
- Disinfect all work surfaces upon completion
First the back, sides, inside front view screen;
Then the rear grill, work surface, and front grill

Protocol Safety Centrifuge Procedures

- Transfer Risk Group 2 Agents or materials to centrifuge tubes using filtered plastic pipettes. Do not use glass pipettes or other sharps. Decontaminate the pipettes by

drawing 10% bleach up the pipette and allowing it to bathe down into a beaker. Place the disinfected pipettes into the radioactive sharps container following use.

- Wipe the exterior of the centrifuge tubes down with 10% bleach before transfer to the centrifuge in the equipment alcove. Use a rack to hold the tubes and transport them within the same transport tray used for transfer to the incubator.
- Verify that the O-ring is in place on the centrifuge rotor before use. Do not use the rotor if the O-ring is missing, cracked, peeling or otherwise damaged. Always use sealed tubes for centrifugation.
- Load and seal rotor and place a biohazard sign on the centrifuge.
- Wait at least 2 minutes following the centrifuge run before retrieving tubes for transfer back to the designated work area.
- Spray inside of centrifuge rotor and bowl of centrifuge with 70% ethanol and remove sign from centrifuge.
- Don new 2nd (outer) pair of gloves at work area and resume research experiments.
- Remove disposable personal protective equipment and discard as biological waste.
- Wash hands with soap and water for 30 seconds before leaving the laboratory.

Transport of Biohazards

On Campus Transport (between labs or buildings):

- Must have two leak proof containers, including the following:
 - a sealed primary container
 - a sealed secondary container
 - absorbent (paper towels) between the primary and secondary containers suitable for the volume transported
 - a biohazard sticker on outside of the secondary container with agent name, lab address and phone number
- Utilize plastic containers whenever feasible, avoid glass.
- Sealed plastic (not glass) primary vials can be transported within sealed, labeled plastic bags.
- If glass primary containers must be used, place containers within a sealed rigid plastic container with absorbent and padding to cushion vials during transport.

Emergency Procedures

- Exposures to facial mucous membranes or through the skin from a puncture or contact with broken skin must be flushed immediately and washed for 15 minutes. 15 minute eyewash for facial exposures and 15 minute hand wash with soap and water for punctures and skin exposures.
- All accidents and spills that result in exposure are immediately reported to the Principal Investigator, who is responsible for completing a Department Head's Report of Injury.
- Researchers are knowledgeable in exposure response procedures (refer to the OEHS yellow exposure response card).
- All exposure incidents are reported to Yale Health Services, 55 Lock Street (Urgent Visit phone: 203-432-0123).

- Workers report compromised immunity and skin conditions which may facilitate an exposure to their Principal Investigator and/or the Employee Health Office for evaluation. Researchers may not work with biohazards unless wounds can be adequately covered with a waterproof bandage and double gloves.
- Researchers are familiar with BL2 biohazard spill response procedures. A biohazard spill kit is available within the laboratory.
- In the event a spill (incubator, centrifuge, or dropped flask on floor) resulting in the release of trypanosomes or potential generation of aerosols, researchers must evacuate the spill area and notify the Office of Environmental Health & Safety at 785-3555 (emergency line) or 785-3550 to request assistance with the response to the mixed biohazard and radioactive spill.
- EHS Biosafety Spill Response Guide can be accessed at:
<https://ehs.yale.edu/sites/default/files/files/biosafety-spill-response.pdf>
- EHS Biological Safety FAQs can be accessed at:
<https://ehs.yale.edu/biological-safety-faqs>