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Introduction

Biosafety Level 2 laboratories (BSL-2) typically study different aspects of infectious materials that may cause human disease. This document is intended to provide guidelines for individuals that might enter or work in a BSL-2 lab space. These individuals include research staff, students, visitors, guests, volunteers, building staff, and service staff.

Required Documents for BUA Application Process

A. Principal Investigator must submit a BUA application if the research involves:
   - Infectious organisms (bacteria, viruses, mold, fungi, yeast, parasites, prions, etc.) that may cause disease in healthy humans, or cause significant environmental or agricultural impact
   - Work with human and non-human primate tissues, fluids, cells, or cell culture
   - Recombinant DNA (rDNA)
   - Transgenic plants, insects or animals
   - Human gene therapy
   - Select agents
   - Intentional release of recombinant DNA to the environment
   - Work with animals known to be potential reservoirs of zoonotic diseases

B. Exposure Control Plan/ Aerosol Transmissible Disease Plan (ECP/ATD)

C. Standard Operating Procedures

Training

All laboratory personnel that work in BSL-2 facilities should complete the training modules as determined by EH&S. All of the training can be completed on-line and can be accessed by going to the following link on the EH&S website: http://ehs.ucr.edu/training/courses/

The PI is responsible to ensure that he along with his laboratory personnel complete the required training and retraining. The PI may access the training records of his/her laboratory using the Laboratory Hazard Assessment Tool (LHAT).

All research staff should also review the Exposure Control Plan/Aerosol Transmissible Disease Plan, Standard Operating Procedures, and Chemical Hygiene Plan prior to working in the laboratory. These documents should also be reviewed on an annual basis.

Signage

BSL-2 areas are clearly marked with appropriate signage provided by the Biosafety Officer at Environmental Health and Safety (EH&S), after BUA approval.

Transporting Biohazardous Materials

All locations to which human, animal pathogens or recombinant DNA materials are transported must be listed on each investigator's BUA and be approved by the IBC before any work begins in any location.
Place the primary vessel (tissue culture plate, flask, or vial) inside a secondary container with a cover. The outside of the secondary container should be decontaminated before being transported. All transported biological materials within the campus must be kept in the possession of the transporter at all times.

**Biosafety Tips**

**Working with Tissues, Pathogens and Cell-Lines**

1) **Non-Biohazardous tissues**
   Tissue harvested from non-infected mice or formalin-fixed tissue (whole, paraffin embedded or on slides with cover) can be manipulated in an open laboratory without additional safeguards, unless it is required by the chemical hygiene plan.

2) **Infected human tissues or cell lines**
   All human-derived materials (tissue or cell lines) as well as tissue obtained from animals infected with a human pathogen or a pathogen that can pose a hazard to humans must be manipulated in a Biological Safety Cabinet (BSC). If tissue/cell lines must be manipulated outside the BSC (for instance during organ/tissue harvest and experiments), an exemption will be obtained from the IBC, the area should be clearly demarcated and additional required PPE will be used as determined by the PI.

3) **Handling human pathogens or tissues infected with human pathogens:**
   Experiments that require handling of live pathogens that pose a potential threat to human health will only be carried out in designated tissue culture (TC) laboratories in biosafety cabinets (BSC) using dedicated equipment that remains in the tissue culture room.

4) **Nucleic acids/recombinant DNA**
   Nucleic acids (RNA and DNA) may be isolated and manipulated in the open laboratory space. This includes, but is not limited to, conducting polymerase chain reactions, reverse transcription, cloning and isolating plasmids. All recombinant activities must be listed in each investigator’s BUA and must be approved by the institutional biosafety committee before any work begins.

5) **Chemical hazards**
   Laboratories using chemicals must have a chemical hygiene plan. All laboratory personnel will adhere to this plan when using chemicals.

**Working with Aerosols**

Procedures with a potential for creating infectious aerosols or splashes are only conducted in a biological safety cabinet in a tissue culture room. These may include grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures. These procedures are performed carefully to minimize the creation of splashes or aerosols.
Face protection, such as goggles, face mask, face shield or other splatter guard, can be used if splashes or sprays of infectious or other hazardous materials to the face are anticipated.

**Good Microbiological Work practice**
Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

**Biosafety Cabinets (Types and Use Requirements)**
There are 3 classes of BSCs. All exhaust through HEPA filters before leaving the cabinet
- **Class I**
  - Do not protect the work from contamination
  - Air entering cabinet is not filtered
- **Class II** (4 types – A1, A2, B1, B2)
- **Class III**
  - Totally enclosed, ventilated cabinets
  - Work through portals with attached gloves

**General requirements for the use of biosafety cabinets (BSC)**
1. Turn on the blower in the cabinet at least 10 minutes before starting work and check the gauge to verify that the biosafety cabinet is working properly. The BSC must be certified annually, if being used with GR2 materials.
2. Appropriate personal protective equipment for use in the tissue culture room while manipulating live pathogens that pose a hazard to human health or tissues infected with such pathogens include gloves, lab coat and when appropriate eye protection, or face shield.
3. The BSC sash level should be kept to ensure proper airflow.
4. Don’t place items on the grills of the BSC. Doors to rooms containing the BSC should remain closed in order to minimize disruption to the airflow.
5. After manipulating infectious agents, make sure all containers are tightly closed.
6. All non-sharp materials that were used to manipulate RG-2 pathogens or cell lines/tissues infected with RG-2 pathogens, such as pipettes, flasks, dishes, should be decontaminated using an EPA approved disinfectant such as 10% wescodyne, bleach, or amphyll. After 10-20 minutes of decontamination, non-sharps plasticware can be placed into biohazard bags and autoclaved. Tips are discarded into a container containing 10% wescodyne/bleach/amphyll solution. When the container is 75% full, it is capped and disposed of as biohazard waste. Liquid waste (tissue culture media) should be inactivated by being poured into a container containing undiluted wescodyne or bleach so that the final dilution is 10% wescodyne or 10% bleach.
7. After work is completed, clean the inside surfaces of the BSC with an approved disinfectant such as 70% ethanol or 10% wescodyne, bleach, or amphyll after completion of work.
8. Allow the blower to run for at least 10 minutes following use; this process applies to laboratories using human pathogens.

9. The UV light is turned on between procedures and at the end of the day for at least 15 minutes. UV light can be harmful to plastics, such as gloves, tips so it is recommended that these items not be left in the hood while the UV light is on.

10. Dispose of your gloves and wash your hands before exiting the TC room.

11. Laboratory coat should be removed before you exit the laboratory.

**Vacuum Lines in a Biosafety Cabinet**
Vacuum lines in a biosafety cabinet should have backflow protection. Overflow must be caught with: (1) a secondary flask, or (2) an in-line HEPA filter.
If the liquid flask is placed on the floor it should be in a secondary container.
Flask(s) should be placed in secondary container to contain any accidental spills.

**Centrifuges**
High concentrations (several logs higher than a known or presumed infectious dose) or large volumes of tissue culture supernatants containing infectious agents (>100 mls) may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used. However, these rotors or safety cups should be opened only in a biological safety cabinet.

**References**
- CDC-Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition
- University of California-Personal Protective Equipment (UCOP-PPE) Policy
- UCR-Laboratory Supervisor may request PPE for laboratory employees, by calling EH&S at Ext: 4254 to obtain a PPE voucher. PPE can be picked up at UCR Storehouse by appointment