

# **BSL-2 SOP**

**(Standard Operating Procedure)**

**University of California Riverside School of Medicine Research Building  
(SOMRB)  
Standard Operating Procedures Manual  
Biosafety Level 2 Laboratory**

**I. Purpose**

The laboratory spaces in the SOMRB are open spaces shared by multiple investigators that are studying different aspects of human disease. This document provides appropriate standard operating procedures to ensure maximal dissemination of information regarding the BSL-2 hazards that can be present at any given moment and appropriate personal protective equipment (PPE) to protect personnel.

This manual applies to all research staff, hosted visitors and guests, volunteers, building staff, and service staff who enter the laboratory. This manual will be reviewed annually by the Principal Investigators (PI) who share the space for changes or corrections to ensure that it is accurate. All PIs will maintain IBC approval for all uses of human or animal pathogens, as well as recombinant DNA materials and activities. All research staff will review annually any Institutional Biosafety Committee (IBC) approved Biological Use Authorizations (BUAs), Exposure Control Plans (ECP) and Standard Operating Procedures held by their laboratory. Appropriate signage will indicate the hazards and appropriate protective personal equipment (PPE) that should be used. Each shared laboratory space will hold a joint meeting semiannually, with a focus on safety to review the list of hazards in use in the common space.

**II. Background**

1) Non hazardous tissues

Tissues harvested from non-infected mice or formalin-fixed tissue (whole, paraffin embedded or on slides with cover) can be manipulated in the open laboratory without additional safeguards.

2) Infected human tissues or cell lines

All human-derived materials (tissues or cell lines) as well as tissues obtained from animals infected with a human pathogen or a pathogen that can pose a hazard to humans must be listed in each investigator's BUA and must be approved by the IBC before any work begins. These materials should be manipulated in a Biological Safety Cabinet (BSC). If tissue/cell lines must be manipulated outside the BSC (for instance during organ 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 rooms in biosafety cabinets (BSC) using dedicated equipment that remains in the tissue culture room. All pathogens are listed in each investigator's BUA and will be

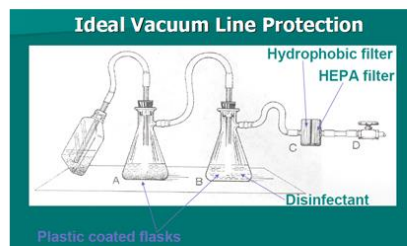
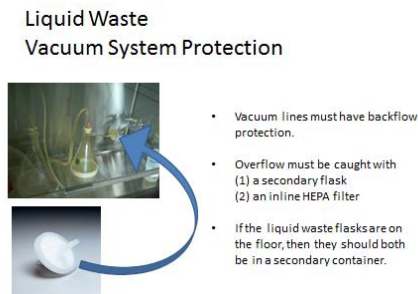
approved by the IBC before any work begins. All human-derived materials (tissues or cell lines) as well as tissues obtained from animals infected with a human pathogen or a pathogen that can pose a hazard to humans must be listed in each investigator's BUA and must be approved by the IBC before any work begins. These materials should be also manipulated in a BSC. General rules for the use of biosafety cabinets (BSC) are listed below, additional rules listed in #7 apply to laboratories using human pathogens only:

3) Vacuum line 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 spill.



Courtesy of Brandy Neilson at the University of Kentucky

1. Turn on the blower in the cabinet at least 10 minutes before starting work and check and magnetic gauge to verify that the biosafety cabinet is working properly. The lab safety officer will schedule BSC certification on an annual basis.
2. Appropriate personnel protective equipment for use in TC 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.
3. No items can be placed on the grills in order to maintain the airflow through the hood. Moreover, the door to the corridor should remain closed in order to minimize disruption to the airflow.
4. After manipulating infectious agents, make sure all containers are tightly closed.
5. All non-sharp materials that were used to manipulate Class Organism (RG) RG-2 pathogens or cell lines/tissues infected with RG-2 pathogens (pipettes, flasks, dishes, etc...) should be decontaminated using an EPA approved disinfectant such as 10% wescodyne/bleach/amphyll after completion of work. After 10 minutes of decontamination, 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 90% full, it is capped and disposed of in biohazard waste. Liquid waste (tissue culture media) should be inactivated by being poured into a container containing undiluted wescodyne/bleach so that the final dilution is 10% wescodyne.

6. After work is completed, clean the inside surfaces of the BSC with an approved disinfectant such as 70% ethanol or 10% wescodyne/bleach/amphyll after completion of work.
7. Allow the blower to run for at least 10 minutes following use.
8. 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 etc...) so it is recommended that these items not be left in the hood while the UV light is on.
9. Dispose of your gloves and wash your hands before exiting the TC room.

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.

#### 4) 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 (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.

#### 5) Chemical hazards

The Division of Biomedical Sciences has a chemical hygiene plan. All laboratory personnel will adhere to this plan when using chemicals.

#### 6) 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.

### **III. Transporting hazardous materials**

There are several instances where biohazardous materials need to be transported between different areas of the SOMRB. For instance, aliquots of pathogens might be transported between the -80 freezers (located in the freezer farm on the second floor) and the tissue culture rooms (third or second floor). Transports might also occur between SOMRB and appropriate areas in Webber or the core facility. All locations to which human/animal pathogens or recombinant materials are brought

must be listed in each investigator's BUA and will be approved by the IBC before any work begins in any location.

For transport of human pathogens, the primary vessel (tissue culture plate/flask/vial) will be placed inside a secondary container, with cover. The outside of the secondary container will be decontaminated before being transported.

#### **IV. Signage and training**

BSL-2 areas are clearly demarcated with appropriate signage provided by EH&S. All personnel in the SOMRB will take the training modules as determined by EH&S. All research staff will also review their laboratory's Exposure Control Plan and IBC approved Standard Operating Procedures prior to working in the laboratory, and annually thereafter.

Access to the BSL-2 laboratories is limited to those who receive appropriate training. All personnel should be able to follow established laboratory safety guidelines and the standard operating procedures. It is the responsibility of each employee to carefully consider every action taken in the BSL-2 lab and its potential impact on possible exposure or contamination, and to follow established Standard Operating Procedures (SOPs) and protocols diligently and without variance.

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.