

BSL2 & BSL2* Biosafety Manual for Employees Working With Human Pathogens

8.17.2022

**Division of Experimental Medicine
Rooms 511, 538, 615, & 623
1001 Potrero Ave.
San Francisco, CA. 94110
Building 3, Floors 5 and 6**

Chief: Joel Ernst, MD 628-206-8101

DEM SAFETY COMMITTEE:

Associate Professor: Timothy Henrich, MD (Co-Chair) 628-206-5518

Division Manager: Justin Vang-Moore 628-206-8106

Laboratory Assistant: Xavier Farias 628-206-8140

Laboratory Managers/Representatives:

Vanessa York	(Co-Chair)	628-206-8096
Florianne Alviar	(CIL)	628-206-5232
Mikias Ilala	(Feeney)	628-206-8366
Charles Le	(Greenhouse)	628-206-6359
Tyler-Marie Deveau	(Henrich)	628-206-4981
Gabriele Beck-Engeser	(Hunt)	628-206-8152
Mary Beth Moreno	(Ernst/Javid)	628-206-8157
Yen (Alice) Wang	(Rutishauser)	628-206-3914
Angel Ruvalcaba	(Suliman)	628-206-3914

Table of Contents

I. Areas

II. Personnel

II-1. Medical Surveillance and On-line Training

II-2. Orientation and Training

II-3. Safety Monitoring and Adherence

III. General Rules and Techniques

III-1. General Rules

III-2. Preparing the Biosafety Cabinet for Work Involving Human Pathogens

III-3. Working with Human Pathogens in the Biosafety Cabinets

III-4. Working with Human Pathogens outside of the Biosafety Cabinet

III-5. Exiting the Biosafety Cabinet

III-6. Leaving the BSL2 Facility

IV. Emergency Response

IV-1. Personal Injury & Exposure

IV-2. Needlestick Hotline

IV-3. Spill Protocol

IV-4. Equipment Failure Protocol

Appendices

Appendix A: Agents

Appendix B: Biosafety Level Work Practice Requirements

Appendix C: Emergency Action Flow Chart

Appendix D: Emergency Spill Procedure

Appendix E: Exposure Incident Checklist

Appendix F: Safe Cryovial Thawing SOP 2017

Appendix G: Herpes B Safety Information

Appendix H: Certification of BSL2/BSL2* Laboratory Training

I. BSL2 Areas (Rooms 511, 538 and 623)

PPE Required: Disposable lab coat, one pair of gloves, and safety glasses worn at all times. No open-toed shoes or uncovered legs are allowed in the facility. Disposable laboratory coats should be changed immediately if one suspects any contamination. Coats should be decontaminated after each use and changed weekly.

Room 538: This room contains the LSRHs. For these machines, you may run both fixed and unfixed samples.

Rooms 511 & 623: Tissue culture rooms.

BSL2* Areas (Room 615)

PPE Required: Disposable gown, two pairs of gloves, and safety glasses. No open-toed shoes or uncovered legs are allowed in the facility. Disposable laboratory gowns should be changed immediately if one suspects any contamination. Gowns should be decontaminated after each use and changed weekly.

All doors to these facilities must remain closed.

II. PERSONNEL**1. Medical Surveillance and Online Training Courses**

The following online safety classes must be completed before beginning work in the BSL2/BSL2*

Biosafety Training
Bloodborne Pathogens Training
Laboratory Safety for Researchers
Safety and Security Awareness for Researchers
Herpes B Training (BSL2* only)

2. Orientation and Training

The principal investigators (PIs) are responsible for ensuring that all proper laboratory practices are followed. New employees must participate in a supervised “on-the-job training” program in the BSL2 facility and prove competency before being allowed to work unsupervised. The employee must establish a level of proficiency in microbiological techniques and practices necessary for the handling of human pathogens and tissue cultures at a BSL2 level before being allowed to work in the BSL2* facility. New employees will work under a “Buddy System” until they both think that the new employee can work safely and efficiently on their own.

The employee’s Lab Manager will provide BSL2/BSL2* Safety Training and Orientation. This will include:

- a tour of both facilities and instruction on safe lab practices and/or techniques
- review of BSL2/BSL2* Safety Manual
- general rules and policies of each facility
- proper usage of the Biological Safety Cabinets (BSC) (see Section III below)
- instruction in proper waste disposal (see Section III below)
- information on Emergency Action Flow Chart (Appendix C).

Under no circumstances should unknown or unauthorized individuals be allowed entry into the BSL2/BSL2* facilities. Authorized employees will be given NON-TRANSFERABLE key access to the BSL2* facility.

3. Safety Monitoring and Adherence

The DEM Safety Committee welcomes all safety questions or comments from every member of DEM.

All personnel should not be offended by safety questions from other individuals, as discussion of such questions is integral to maintaining a safe environment. If you have a question about something you observe, please ask the individual if that practice conforms to safety standards. In addition, all personnel should discuss questions with their Laboratory Manager if they observe behavior that does not appear to conform to the safety standards.

The **SAFETY OF ALL** users requires each individual's commitment to open discussion of safe working practices.

III. GENERAL RULES AND TECHNIQUES

1. General Rules

Communication: All DEM members in the labs will communicate and listen respectfully to each other.

Cleaning/Storage: Each individual is responsible for cleaning up thoroughly after him/herself. Personal items must be labeled and stored in designed drawers, fridge, and freezer spaces. No storage of personal items on the open bench, in the biosafety cabinet hood, or cart.

Sharps: If sharps must be used, the PI, the lab manager, and the safety committee must review the procedure. All work with sharps must be listed on the PI's BUA and approved by the Institutional Biosafety Committee.

No cell phones.

2. Preparing the Biosafety Cabinet (BSC) for Work Involving Human Pathogens

When you first begin using the BSC, switch the blower on. Clean the work surface with 70% EtOH.

The BSC should contain:

1. 20% Bleach (1% sodium hypochlorite) (or 2% triton) waste bucket
2. Vacuum bottles with HEPA filters containing 100% bleach (or triton). **No liquid waste should be aspirated into the secondary vacuum bottle.**
3. Pipette Box
4. A small container lined with double red-bags for the disposal of small, disinfected, dry waste items (BSL2* only).

Next to the BSC, there should be:

1. Large double-bagged biohazard waste step-can for disposal of **dry**, larger disinfected items
2. Garbage can for all non-hazardous items (BSL2 only)

3. Working with Human Pathogens in the BSC

Prior to placement in the BSC, all equipment, reagents and cultures are sprayed or wiped down with 70% EtOH. All biohazardous cell cultures, reagents, and samples should only be opened and physically manipulated within the BSC.

Vortex: All vortexing must occur within the biosafety cabinet. All samples to be vortexed must be in capped tubes

If you detect a spill during vortexing, **IMMEDIATELY STOP WORK**, and clean all work areas.

4. Working with Human Pathogens outside of the BSC

When biohazardous samples are removed from the BSC, they must be capped or covered in order to prevent generation of aerosol vapors. No working with uncovered biohazardous samples outside of the Biosafety Cabinets except for approved procedures such as loading of samples on LSRII.

Centrifugation: When centrifuging tubes, safety cups must be used. Plates must be sealed with disposable plate sealers or lid cover. Do not open centrifuge tubes or plates until the sample is returned to the BSC. Never fill the centrifuge tubes to full capacity.

If you detect a spill during centrifugation, **IMMEDIATELY STOP WORK**, return samples to the biosafety cabinet, and clean all work areas.

5. Exiting BSC

After completion of any work in the BSC, the items in the BSC and the BSC interior should be wiped down with 70% EtOH taking particular care to clean up any spills or splatters.

- Remove all personal and/or extraneous items and store in your personal drawer.
- All liquid waste must be decontaminated for 30 minutes and disposed in the large vacuum bottle or bleach/triton bucket. No liquid waste can go into the red bags.
- Decontaminated pipettes should be drained and then transferred into the pipette boxes.
- The small dry waste bags are placed into the large step can after each use and replaced with new bags. (BSL2* only).
- The larger dry waste bags must be changed after completion of work.

Buckets, vacuum bottles, red bags, and pipette boxes should be changed after each use and brought to the red bins by the autoclave in the BSL2*. A yellow bin is located in the BSL2 for waste collection.

All items leaving the BSL2/BSL2* facility must be sprayed down with ethanol. Samples must be inactivated prior to working in a BSL1 facility. Non-inactivated samples may be brought out of these facilities for shipping or transport to a similar biosafety level but must be packaged with secondary containment.

6. Leaving the Facility

- A. INSIDE BSL2*: Remove outer gloves
 - i. Pinch cuff of outer glove and pull to invert over the fingertips but do not fully remove.
 - ii. Avoid touching outer glove surface by removing 2nd outer glove using inverted 1st glove.
 - iii. Remove 1st glove completely by only touching exposed inner surface.
 - iv. Place gloves in biohazardous waste bin.
- B. EXIT BSL2*
- C. Remove shoe and head covers (if used) and place in biohazardous waste bin.
- D. Remove gown
- E. Remove safety glasses
- F. Decontaminate gown and safety glasses by spraying with 70% EtOH
- G. Place gown and safety glasses in cubby
- H. Remove inner gloves and place in biohazardous waste bin
 - i. Follow procedures described in step #1
- I. Wash hands with soap and water
- J. Exit antechamber

IV. EMERGENCY RESPONSE

If there is a FIRE or Natural Disaster, dial SF POLICE/FIRE: 9-911.

If there is a SFGH security problem, dial SFGH Police: 206-4911

1. Personal Injury and Exposure

First and foremost, attend to yourself: USE COMMON SENSE!

If the injury requires major medical treatment, call 9-911. Get emergency treatment at a hospital. Tell them of the possibility of exposure to a human pathogen. They will contact the Needlestick Hotline.

If the injury/exposure does not require major medical treatment, wash affected area, as recommended for the infectious agent:

HIV-1 and other agents:

PLEASE REVIEW APPENDIX E: "EXPOSURE INCIDENT CHECKLIST"

Instruction Summary: Flush eyes, nose, or mucous membranes with water for at least 10 minutes. Flush abraded or cut skin with soap and water for 10 minutes. If safely possible, retain outer gloves, and contaminated equipment and/or sample(s), in a red biohazard bag, so they can be either tested for the infectious agent or used to review the biosafety practices in place during exposure. If someone else is present in the area, get him or her to assist you.

Herpes B (biohazardous monkey samples): ^[L]_[SEP]

PLEASE REVIEW APPENDIX H: "HERPES B SAFETY INFORMATION" (copies available inside "Herpes B Exposure First Aid Kit" in BSL2* lab, room 615).

Instruction Summary: Immediately proceed to the “Herpes B virus Emergency First Aid Kit” located on the wall by the BSL2* lab telephone. Flush eyes, nose, or mucous membranes with sterile eye solution, then flush with water for at least 15 to 20 minutes. Clean/scrub abraded or cut skin with chlorohexidine-impregnated sponge and water for 15 to 20 minutes. If safely possible, retain outer gloves, and contaminated equipment and/or sample(s), in a red biohazard bag, so they can be either tested for the infectious agent or used to review the biosafety practices in place during exposure. If someone else is present in the area, get him or her to assist you.

2. Needle Stick Hotline: 415-353-7842

The Hotline is a 24/7 pager system: Enter the phone number you are calling from, then the # key. Follow instructions given to you from the Needlestick Hotline call back.

Immediately after seeking medical attention, report exposure to the DEM Division Administrator and to your Principal Investigator (PI). This report will be confidential to the limited number of individuals who need to be notified. All injuries and exposures are required to be reported to UCSF Occupational Health and Safety by the DEM Division Administrator.

3. Spill Protocol (See Appendix D)

BSL2/BSL2* Facility should be evacuated if spill emergency is life threatening.

If a spill is beyond the person’s ability to contain and/or clean:

Call EH&S Emergency Spill Response Team.

Emergency: 415-476-6911

Non-Emergency: 415-476-1414

All lab accidents, injuries or hazardous material exposures must be reported to EH&S **within 24 hours** of the incident. If any employee requires hospitalization, immediately report the incident by phone:

During Business Hours: EH&S [\(415\) 476-1300](tel:4154761300) and Disability Management Services [\(415\) 476-2621](tel:4154762621)

During Off-Hours: UCPD dispatch [415-476-1414](tel:4154761414)

If the spill is small and controllable:

Biohazard Spills Inside the BSC

Immediately disinfect the area. Place dry towels on spill to absorb liquid. Squirt towels with 10% bleach (0.5% sodium hypochlorite), working from the outside in towards the center of spill area.

Wipe or spray all affected areas within the BSC with disinfectant.

Dispose of all contaminated materials in double red bags and place in a red, hard-sided biohazard bin.

Biohazard Spill Outside the BSC

Immediate spill control

Post signs or assign a person to ensure that others will not enter the contaminated area

Decontamination of spill

Immediately disinfect the area. Place dry towels on spill to absorb liquid. Squirt towels with 10% bleach (0.5% sodium hypochlorite), working from the outside in towards the center of spill area.

Wipe or spray all affected areas around spill, including floor, walls, and counters, using paper towels soaked in disinfectant.

Dispose of all contaminated materials in double red bags and place in a red, hard-sided biohazard bin.

4. Equipment Failure Protocol

If equipment or power has a major malfunction and you cannot handle the problem:

Contact SFGH Facilities: (206-8522)

If you can handle the equipment failure:

During the Day: Immediately terminate work, if safely possible.

Shutdown machine, if safely possible.

Remove your samples, if safely possible.

Turn off machine, if safely possible.

Place a sign on equipment describing malfunction, with **Date, Name & Contact Information.**

As soon as safely possible, report equipment malfunction to the Lab Aide and your Lab Manager.

After Hours: Follow all the procedures for equipment malfunction during the day, except you will report equipment malfunction to the Lab Aide and your Lab Manager **as soon as practical.**

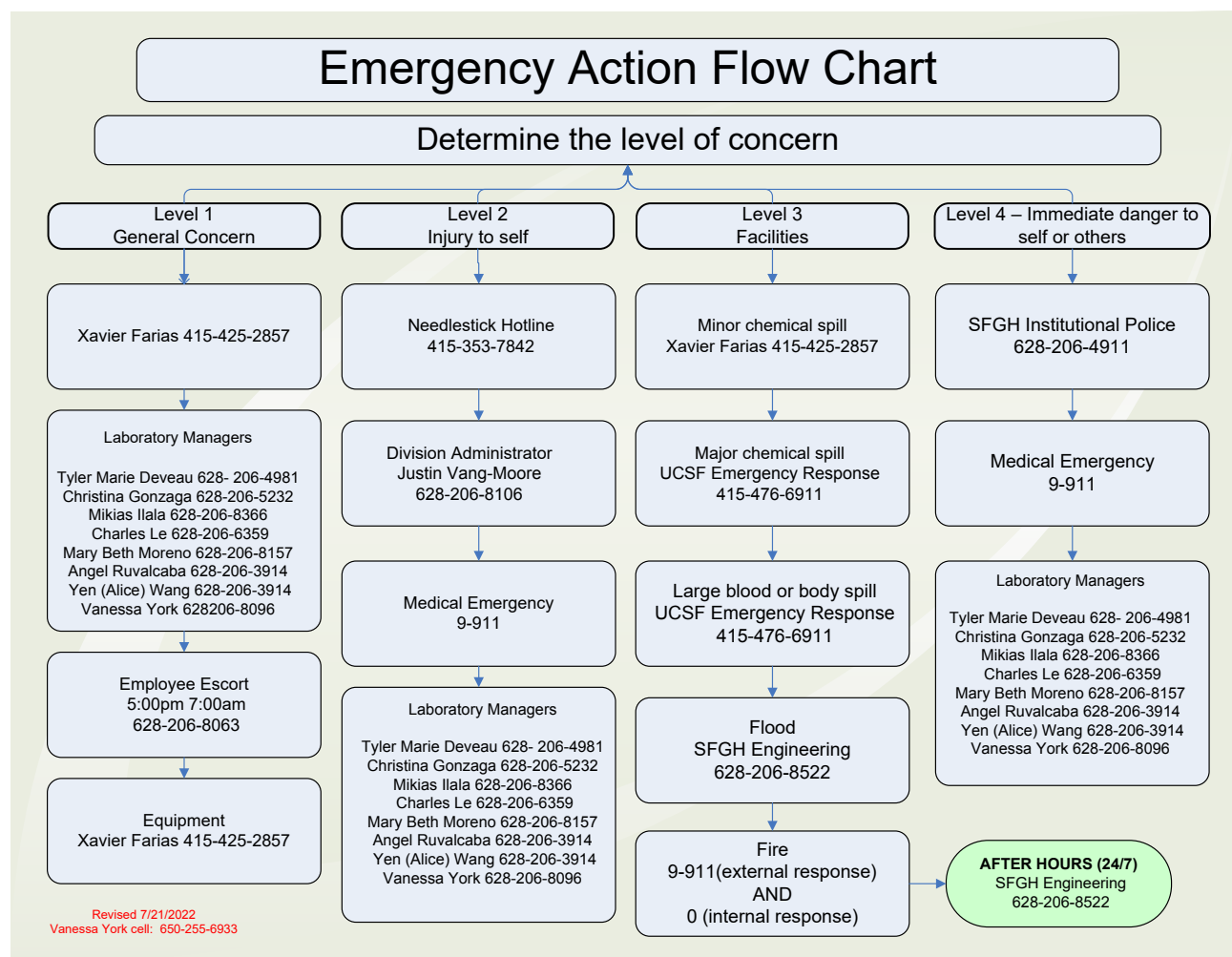
APPENDIX A: LIST OF KNOWN AGENTS IN BSL2/BSL2* LAB:

1. Cytomegalovirus (CMV) (Hunt, Henrich & Rutishauser Labs)
2. Epstein-Barr Virus (EBV) (Ernst, Greenhouse, Henrich, Feeney & Suliman Labs)
3. Hepatitis C (Hunt Lab, Rutishauser Lab)
4. Herpes B (Ernst Lab, Hunt Lab, Rutishauser Lab)
5. Human Herpes Virus 8 (Henrich Lab)
6. Human Immunodeficiency Virus-1 (HIV-1) (Feeney, Hunt, Henrich, & Rutishauser Labs)
7. Human materials infected with SARS CoV-2 (Feeney, Greenhouse, Henrich, Hunt, Rutishauser & Suliman Labs)
8. Inactive Mycobacterium tuberculosis (Ernst & Suliman Labs)
9. Mycobacterium abscessus (Ernst Lab)
10. Mycobacterium bovis BCG vaccine strain (Ernst & Suliman Labs)
11. Mycobacterium smegmatis (Ernst & Suliman Labs)
12. Plasmodium falciparum (Feeney & Greenhouse Labs)
13. Salmonella typhimurium (Ernst Lab)
14. Simian Immunodeficiency Virus (SIV) (Hunt Lab, Rutishauser Lab)
15. Zika Virus (Feeney & Rutishauser Labs)

APPENDIX B**Biosafety Level Work Practice Requirements****DEM UCSF 2022**

Work Practices	BSL-1	BSL-2	BSL-2*
Public access	Not recommended	Restricted	Restricted
Bench-top work	Permitted	Permitted only for low-risk procedures	Not permitted for biohazardous materials
Decontamination	Daily and following any spills	Daily; immediately following work with biohazardous materials and following any spills	Daily; immediately following work with biohazardous materials and following any spills
Eating, drinking, chewing gum, applying lip balm	Permitted only in designated clean areas	Not permitted at any time. Food or drink may not be brought into or through lab	Not permitted at any time. Food or drink may not be brought into or through lab
Lab coats	Recommended	Required	Required (disposable gown)
Personal Protective Equipment	Follow good lab practices	Required: Wear appropriate combinations of special protective clothing for all biohazardous materials.	Required: Wear appropriate combinations of special protective clothing for all biohazardous materials.
Biological Safety Cabinets (BSC)	Not required	Required for all aerosol-generating processes	Required for all aerosol-generating processes. Required for any open sample.
Storage Equipment	Biohazard Signs where required	Biohazard Signs required: all equipment must be labeled with contents	Biohazard Signs required: all equipment must be labeled with contents
Physical containment	Follow good lab practices	Use physical containment devices (centrifuge safety cup, sealed rotor) for all activities using biohazardous materials. Decontaminate equipment immediately after spills.	Use physical containment devices (centrifuge safety cup, sealed rotor) for all activities using biohazardous materials. Decontaminate equipment immediately after spills.
Hand-washing facilities	Required	Required	Required (foot or electronic activation preferable)

APPENDIX C: EMERGENCY ACTION FLOW CHART



Appendix D: Emergency Spill Procedure

UCSF Biological Spill Emergency Procedures will be followed to clean up spills. For all spills the first steps are to remove any contaminated clothing, inform everyone in the laboratory of the spill and for work in the immediate area to stop. If no other person is working in the immediate area, help should be obtained by calling on personnel from other areas before attempting to clean up the spill. For large spills beyond the capacity of laboratory personnel to clean up, the room will be evacuated, signs posted and the UCSF Emergency Response Team will be called. For smaller spills, personnel wearing lab coats, double gloves and face protection will apply paper towels to the spill to absorb the spilled material, carefully (avoiding splashing) apply freshly prepared 10% bleach (0.5% sodium hypochlorite) around the area and then onto the spill. Paper towels will be used to wipe up the spill and all paper towels will be placed in a red biohazard bag. Fresh paper towels will then be placed on the spill area, and a 10% bleach (0.5% sodium hypochlorite) solution applied again and left for 20 minutes. A final wipe-down will be done with clean paper towels soaked in 10% bleach (0.5% sodium hypochlorite) and all contaminated waste disposed of in red biohazardous waste bags. If broken glass is involved, it will be handled with forceps or tongs and disposed of in an approved sharps disposable container before paper towels are applied to the area.

Call EH&S Emergency Spill Response Team.

Emergency: 415-476-6911

Non-Emergency: 415-476-1414

All lab accidents, injuries or hazardous material exposures must be reported to EH&S **within 24 hours** of the incident. If any employee requires hospitalization, immediately report the incident by phone:

During Business Hours: EH&S [\(415\) 476-1300](tel:4154761300) and Disability Management Services [\(415\) 476-2621](tel:4154762621)

During Off-Hours: UCPD dispatch [415-476-1414](tel:4154761414)

Additional Information: Centrifuge Accidents

The potential for multiple infections from a single centrifuge accident is great. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. The opening of a centrifuge must be performed slowly.

Unsealed buckets:

1. If a centrifuge tube breaks while the centrifuge is running, turn off motor. Allow the machine to be at rest for 30 minutes before opening. If breakage is discovered after the machine has stopped, re-close the lid immediately & allow the unit to be at rest for 30 minutes.
2. Unplug centrifuge before initiating clean up.
3. Don strong, thick rubber gloves & other PPE including safety goggles before proceeding.
4. Flood centrifuge bowl with a germicidal disinfectant. Place paper towels soaked in a disinfectant over the entire spill area. Allow 20 minutes contact time.
5. Use mechanical means (such as forceps) to remove broken tubes & glass fragments. Place them in a sharps container for incineration & disposal as infectious waste.
6. Remove buckets, trunnions & rotor then place in disinfectant for 20 minutes or autoclave.
7. Unbroken, capped tubes may be placed in disinfectant & recovered after 20 minutes.

8. Use mechanical means to remove remaining disinfectant soaked materials from centrifuge bowl & discard as infectious waste.
9. Place paper towels soaked in a disinfectant in the centrifuge bowl & allow it to soak overnight, wipe down again with disinfectant, wash with water & dry. Discard disinfectant soaked materials as infectious waste.
10. Remove protective clothing used during cleanup & place in a biohazard bag for autoclaving. Wash hands whenever gloves are removed.

Appendix E: EXPOSURE INCIDENT SOP

The main purpose of this Safety Manual is to prevent laboratory workers from accidental exposures to biohazardous materials. Through the use of (1) PPE [Gloves, Lab Coat, Eyewear Protection] (2) Biosafety Equipment and supplies [BSC, Covered Centrifugation Buckets, etc.] and

(3) explicit Standard Operating Procedure [SOP] instructions for decontaminating and disposing biohazardous waste, lab workers are better able to protect themselves from accidental exposures.

Of course the reality that accidents can occur is very real, despite the large number of precautions implemented and everyone's best efforts. Thus it is important that all BSL2 users understand the step-wise SOP for dealing with an accidental exposure.

EXPOSURE INCIDENT SOP CHECKLIST (Updated 7-2022):

- ☐ 1. Upon an exposure incident, **immediately cease work** and begin to deal with the exposure incident. If other individuals are in the same lab, ask them to help you with clean-up or contacting Needlestick Hotline.
 - *The DEM Emergency Action Flow Chart (**with phone #**) is posted by the telephones in the BSL2* labs and the door of the BSL2 lab.*
 - **If you are having a Medical Emergency, Please call: 9-911.**
 - ☐ 2. **Rinse off the exposed area** with Water/Saline/Soap if required. If available, use any exposure control kit recommended for the particular exposure (i.e. Herpes B exposure kit).
 - ☐ 3. **Call Needlestick Hotline (24/7) at: 415-353-7842.**
 - Describe the exposure incident: Infectious agent(s)/sample; Type of exposure [needlestick or splash]; Body area contacted with infectious material [face, eyes mouth, skin cut].
 - Ask whether you need to be seen by the exposure control medical staff.
 - If you are not seen in-person by the medical staff, ask for information about post-exposure treatments.
 - ☐ 4. If the Needlestick Hotline asks you to report to the clinic, **please report to the clinic ASAP**. Ask other lab members present for assistance if you cannot get to the clinic by yourself. If you need to call a taxi, please use one and ask for a receipt. You can be reimbursed for this expense after treatment.
 - ☐ 5. Contact your PI, the Division Administrator and your Lab Manager when you have time. **Your first priority is treating yourself and receiving proper professional exposure control treatment.** If others are present during the exposure incident, they can contact your PI, Division Administrator and Lab Manager for you.
-

- ☐ 6. After you have received treatment for the exposure incident, please schedule a meeting with the **Division Administrator**. The Division Administrator will ask you and your supervisor to complete (and return) the **Exposure Incident Report Forms**. The Division Administrator will submit the completed forms to UCSF Occupational Health, so your exposure incident can be reviewed. The Biosafety Officer will be informed within 24 hours of RG2 agent exposure as required by IBC for RG2 infectious agents and viral vector exposure.
- ☐ 7. After you have received treatment you should schedule meetings with (1) your **PI**, and (2) with the **Division Chief and your PI**, to discuss the exposure incident, and to review the completed Exposure Incident Report Forms. You may also want to discuss the exposure incident with your **Lab Manager**.
- ☐ 8. Your **PI and Lab Manager** will discuss any additional safety training or SOP changes with you, in order to prevent this type of accidental exposure from re-occurrence.
- ☐ 9. Your Lab Manager will report the exposure incident (without identifying the individual), and the SOP steps taken post-incident, at the next scheduled DEM Safety Committee Meeting.
- ☐ 10. Your PI will write a formal letter to the DEM Safety Committee Meeting discussing the exposure incident and addressing the following points:

1. Describe the exposure incident and the immediate response of the exposed individual. Did the individual seek any treatment? What is the resolution of the exposure incident?

2. Confirm that you have discussed the incident directly with the exposed individual, in order that you have an unfiltered description of the exposure incident. Describe any procedures/practices, or lack of, which may have contributed to the exposure incident.

3. Describe any actions taken by you or your lab, to prevent re-occurrence of this type of exposure incident. This could include additional PPE and/or safety presentations and review of safety SOP literature.

APPENDIX F: Safe Cryovial Thawing SOP 2022

This document is intended to provide a procedure for safely thawing vials that have been stored in a liquid nitrogen freezer. While new liquid nitrogen freezer models employ a jacketed method that prevents vials coming into direct contact with liquid nitrogen, older freezers use a direct immersion method. Often, the storage history of a vial may be unknown, possibly stored immersed in liquid nitrogen. Vials stored immersed in liquid nitrogen may allow infiltration of liquid nitrogen to the vial interior. When the vial is allowed to warm slightly, the liquid nitrogen expands and the vial explodes. This can happen within seconds, even on dry ice. Following safe handling procedures will significantly decrease injury and exposure risk.

1. Store cryovials in a jacketed liquid nitrogen freezer, avoid direct contact with liquid nitrogen.
2. Use cryovials only, with internal threads. External threaded vials allow infiltration of liquid nitrogen to the vial interior.
3. Prior to opening the liquid nitrogen freezer, the operator must wear designated personal protective equipment (PPE) required for the biosafety level of the room. In addition, the operator must be wearing a full laboratory face shield.
4. Prior to removing a vial from the liquid nitrogen freezer, the operator must have a lidded secondary container prepared. The container strength and size must be adequate to contain an exploding vial. Examples:
 - one or two vials can be contained in a capped 50 ml tube
 - multiple vials can be contained in a vial storage box
5. If vials are to be discarded without opening, the procedures above still apply. The unopened vial(s) should be discarded directly into the decontamination bucket in the biosafety cabinet. The bucket should then be capped, and the decontamination protocol for the room followed.
6. If the vial is to be thawed in a water bath, it must be transported from freezer to bath in a secondary container. [The operator must wear a full laboratory face shield while \(a\) transporting and/or holding a vial outside the biosafety cabinet; \(b\) holding a vial during thawing; \(c\) placing or removing the vial from a rack in the water bath.](#) The vial must go directly from the secondary container into the water bath. The water bath must be kept lidded if the operator places the vial in a rack in the water bath.
7. If the entire liquid nitrogen freezer is to be allowed to warm up (for repairs/decommissioning), no loose vials should remain in the freezer. The lid should be left closed so that if vials remain and do explode, they will be physically contained within the freezer chamber. A notice should be posted on the freezer, and users of the room notified.
8. In the event of an exploded vial:

If someone is injured and contaminated, provide first aid assistance if possible and ask an uncontaminated co-worker to call 9-911 for medical assistance. If a person is contaminated but not injured, remove contaminated clothing and help the person use an emergency shower or eyewash as needed.

If a biohazardous spill is in the open laboratory, alert people in the vicinity and evacuate the laboratory immediately. The last person out closes the doors. Do not re-enter for at least 30 minutes so aerosols can be cleared to minimize the risk of inhalation exposure. Proceed as follows:

- 1) If the spill is large or you need help from OEH&S, call the UCSF emergency number: From SFGH: Call 206-8522
- 2) Post signs, and [direct](#) personnel not involved in cleaning up the spill away from the area.
- 3) Remove contaminated sharps from the spill using forceps or tongs, NOT your hands!
- 4) Cover the spill with paper towels or other absorbent material. Take care to avoid making the spilled material splash or spray.
- 5) Pour a freshly prepared 1:10 dilution of household bleach (0.5% sodium hypochlorite) around the edges of the spill and work inward to the center. Allow 20 minutes for the bleach (0.5% sodium hypochlorite) to kill the organisms.
- 6) Use additional paper towels to wipe up the spill, working from the edges into the center.
- 7) After initial cleanup, flood the spill area with bleach (0.5% sodium hypochlorite) and let stand for at least 20 minutes.
- 8) Use paper towels to absorb spill, then wipe-down with clean paper towels soaked with bleach (0.5% sodium hypochlorite).
- 9) Disinfect any equipment, walls, or other areas likely to have been splashed by the spill.
- 10) Notify a Biosafety Officer at 415-514-2824 if the spill is large, involves RG3 materials, recombinant DNA materials, a toxin, or if an injury or inhalation exposure may have occurred

APPENDIX G: Herpes B Safety Information

Herpes B Virus Background

Some DEM labs and individuals are working with non-human primate blood and tissues in their research, including macaque monkeys. No individuals are working directly with live animals, since all non-human primates are housed in facilities at other institutions.

Since non-human primate blood and tissues are processed within the DEM BSL2* lab, there is a risk for accidental exposure to Herpes B virus. Herpes B virus (Macacine herpesvirus 1) is a naturally occurring disease, endemic in over 90% of captive macaque monkeys. Most infected macaque monkeys show no clinical signs of infection, but can shed Herpes B virus into bodily fluids, including blood, saliva, urine/feces and cerebral spinal fluid. Herpes B virus can be present and infectious for several hours on untreated surfaces, equipment and in unfixed dead animal tissue. The transmission of Herpes B to humans is a rare event, but this disease can be fatal. UCSF assumes all macaque monkeys, including blood and tissue samples, to be infected with the Herpes B virus.

Herpes B Virus Infection In Humans

Any mucosal contact with macaque bodily fluids or tissues is considered an exposure to Herpes B virus. Splashes to the eyes, nose, mouth or existing unprotected skin breaks can all lead to viral transmission. The incubation period is the time from exposure to the onset of Herpes B virus symptoms. In humans this time can vary between 5 days to 5 weeks. Symptoms of Herpes B infections can include fever or flu-like illness, conjunctivitis, nausea, abdominal pain or diarrhea, vesicles at the wounds site, dizziness, muscle pain and weakness. Herpes B infection is fatal in up to 85% of cases.

There is no completely effective treatment for Herpes B infection in people. Early diagnosis of Herpes B infection is essential so that antiviral therapy can be initiated immediately. While antivirals may be used for treatment of a potential Herpes B exposure, they may not be effective in preventing development of infection. Preventing exposure to herpes B virus is the best treatment against Herpes B infection.

Prevention of Herpes B Virus Exposure

There are many ways you can reduce your risk of being exposed to Herpes B virus.

1. Complete the annual Herpes B Safety Training (online).
2. Meet with Kathy Nguyen (EH&S) for in-person Herpes B safety training.
3. Review CDC Herpes B virus: First Aid and Treatment PDF (attached below).
4. Always wear your required PPE (Gown, double-gloves, eyewear protection).
5. Consider wearing additional PPE, including disposable face masks or face shield.
6. Change your gloves often.
7. Work in the center of the BSC workspace.
8. Minimize your use of "Sharps" while working with macaque blood/tissues.
9. Properly decontaminate and dispose of all your liquid and dry waste.

10. Spray down and wipe off any items used in the BSC, including hard-sided containers, racks, pipetmen and pipette-gun.
11. Spray down the entire work area after use with 70% EtOH.
12. Wash your hands with soap and water after removal of your PPE.

Post-Exposure First Aid

After exposure immediately go to “Herpes B Exposure Kit” and follow the instructions inside the kit or posted above the kit. **You must begin first aid treatment within 2-3 minutes.** The Herpes B virus can enter your cells within 5 minutes of exposure.

Skin Exposure:

First line treatment of a skin exposure to Herpes B virus is to scrub the wound with a chlorohexidine-impregnated surgeons sponge from the emergency kit. **Scrub/Wash the wound for 15 to 20 minutes.** Wash all the soap out of the wound so that a sample can be cultured from the wound. You can rinse the area with either saline or H₂O. Next, cover the area with a band-aid dressing.

It is essential to scrub for the full 15 minutes. Most detergents and disinfectants actively kill the Herpes B virus. Mechanical scrubbing and flushing are the most effective means of removing the virus before it has an opportunity to enter the host cells.

Eye, Nose or Mouth Exposure:

Immediately following an eye or nose exposure, flush the eyes or nasal passages with sterile eyewash from the exposure kit. Proceed to the eyewash station and **flush eyes or nasal passages for 15 to 20 minutes.**

Immediately following a mouth exposure, proceed to the nearest sink and rinse your mouth with water and spit. Continue to **flush your mouth at the sink or eyewash station for 15 to 20 minutes.**

Post-Exposure SOP

Immediately following your First-Aid treatments:

Call the UCSF Needlestick Hotline: (9)-415-353-7842

Inform the Operator that you have had an exposure to ***Herpes B virus (Macacine herpesvirus 1)***.

The on-call Healthcare Advisor should call you back within 15 minutes. If you do not receive a call-back within 15 mins – CALL BACK. You may also proceed to the UCSF Parnassus Emergency Room.

You will need to be seen in-person at the Emergency Room/Occupational Health Clinic at UCSF Parnassus campus. In most cases you will be given antiviral medication as part of your post-exposure treatments. ***Refer to the “CDC – Herpes B Virus: First Aid and Treatment Document” (see Pages 3-5).***

Please follow the appropriate post-exposure instructions described in the ***Exposure Incident SOP Checklist*** in the DEM BSL2* Safety Manual.

The **Exposure Incident SOP Checklist** is also **posted in the anterooms in the BSL2* lab, Room 615.**

CDC - Herpes B virus: First Aid and Treatment

<http://www.cdc.gov/herpesbvirus/firstaid-treatment.html>

First Aid and Treatment

Although B virus infection in humans is extremely rare, when it does occur, it is often fatal unless treated right away—about 70% of untreated patients die of complications associated with the infection.

Diligence in the recognition of possible exposures, followed by recommended first aid and rapid diagnosis of B virus infection, are the keys to controlling human B virus infection.

First Aid

First aid should begin immediately:

Cleanse the exposed area by thoroughly washing and scrubbing the area or wound with soap, concentrated solution of detergent, povidone-iodine, or chlorhexidine and water, and then irrigate the washed area with running water for 15-20 minutes.

WARNING: a specimen for PCR testing should not be obtained from the wound area prior to washing the site because it could force virus more deeply into the wound, reducing the effectiveness of the cleansing protocol.

After the site is cleansed, a serum specimen should be obtained from the patient to provide a baseline antibody level. See Specimen Collection and B virus Detection ([specimen-collect.html](#)).

Treatment Criteria

The decision about whether to implement antiviral therapy or not should take into account the following criteria [adapted from the Recommendations for Prevention of and Therapy for Exposure to B virus (Cercopithecine Herpesvirus_1 (<http://www.journals.uchicago.edu/doi/full/10.1086/344754>)) , published in Clinical Infectious Diseases in 2002]:

1. Type and physical condition of the implicated animal.

Only monkeys of the macaque family serve as the natural reservoir for B virus infection. No other primates carry any risk of B virus transmission unless they have had the opportunity to become infected by a macaque. Infected macaques will not ordinarily be shedding B virus. Animals with lesions consistent with B virus infection (fluid-filled blisters on the skin) and animals that are immunocompromised or stressed are far likelier to be excreting virus.

2. Thoroughness and timeliness of wound cleansing procedure.

Wounds that have been cleansed within 5 minutes of exposure and that have been cleansed for at least 15 full minutes are less likely to lead to B virus infection. Delay in cleansing or inadequate cleansing of the wound increases the risk of infection.

3. Nature of the wound.

Bites or scratches that penetrate the skin, and particularly deep puncture wounds, are considered higher risk than wounds that are superficial and thus more easily cleansed. Wounds to the head, neck, or torso

provide potentially rapid access to the CNS and thus should be considered higher risk. Prophylaxis is recommended for this type of wound regardless of its severity. Superficial wounds to the extremities are less likely to lead to fatal disease, and antiviral treatment is considered less urgent in such exposures.

4. Exposure to materials that have come into contact with macaques.

Accidental needlesticks with syringes that have come into contact with the CNS, eyelids, or mucosa of macaques are considered to carry a high risk of infection. Punctures from needles exposed to the peripheral blood of macaques are considered relatively low risk. Scratches resulting from contact with possibly contaminated objects, such as animal cages, are considered to carry a lower risk for infection.

It should be stressed, however, that in none of these potential exposures, can the risk of infection be considered zero. As such, the decision to treat with antivirals should be made at the physician's discretion, with liberal consideration of the patient's wishes and concerns.

People with a known risk of exposure should be monitored for symptoms regardless of whether a treatment regimen has or has not been implemented. The animal responsible for the putative exposure should be examined for evidence of disease, and serologic and PCR testing should be done to look for evidence of B virus infection and shedding.

Exposure Scenarios and Treatment Options:

Specific exposure scenarios and the corresponding urgency for post-exposure antiviral treatment, as proposed in the Recommendations for Prevention of and Therapy for Exposure to B virus (Cercopithecine Herpesvirus 1) (<http://www.journals.uchicago.edu/doi/full/10.1086/344754>), are as follows:

Treatment is recommended:

Skin exposure (with loss of skin integrity) or mucosal exposure (with or without injury) to a high-risk source (e.g., a macaque that is ill, immunocompromised, known to be shedding virus, or has lesions compatible with B virus infection).

Inadequately cleansed skin exposure (with loss of skin integrity) or mucosal exposure with or without injury.

Laceration of the head, neck, or torso.

Deep puncture bite.

Needlestick associated with tissue or fluid from the nervous system, lesions suspicious for B virus, eyelids, or mucosa.

Puncture or laceration after exposure to objects (a) contaminated either with fluid from monkey oral or genital lesions or with nervous system tissues or (b) known to contain B virus.

Post-cleansing culture is positive for B virus.

Treatment should be considered

A break in the skin that has been adequately cleaned.

Needlestick involving blood from an ill or immunocompromised macaque.

Puncture or laceration occurring after exposure to (a) objects contaminated with body fluid (other than that from a lesion) or (b) a possibly infected cell culture.

Treatment is not recommended

Skin exposure in which the skin remains intact.

Exposure associated with non-macaque species of non-human primates, unless they were in a situation where they could have been infected by a macaque.

Antiviral Therapy

Recommended dosages for specific antivirals are as follows:

Prophylaxis for exposure to B virus

Valacyclovir—1g by mouth every 8 hours for 14 days, or

Acyclovir—800 mg by mouth 5 times daily for 14 days

Treatment of B virus infection***With no CNS symptoms***

Acyclovir—12.5–15 mg/kg intravenously every 8 hours, or

Ganciclovir—5 mg/kg intravenously every 12 hours

With CNS symptoms

Ganciclovir—5 mg/kg intravenously every 12 hours

It should be noted that while herpesvirus antivirals have been shown to effectively protect rabbits from lethal infectious doses of B virus, no comparable studies of efficacy in humans have been possible.

Herpes B virus Information

On external surfaces, B virus is susceptible to 1% sodium hypochlorite, 70% ethanol, and 2% glutaraldehyde, and formaldehyde. The virus can also be inactivated by heat treatment at 50°–60°C for at least 30 minutes, by lipid solvents, by exposure to acidic pH, and by detergents.

B virus can remain viable in monkey CNS tissue and saliva and in monkey kidney cell cultures. The virus can also survive up to 7 days at 37°C or for weeks at 4°C, and it is stable at !70°C. Although survival studies under conditions of virus desiccation (i.e., dry surfaces) have not been performed, it is presumed that survival times will be comparable to those of other mammalian herpesviruses (with typical survival times of 3–6 hours).

There are no vaccines available for B virus. Experimental vaccines have been evaluated in animal models, but none are being considered for human trial.

Page last reviewed: January 31, 2019

Page last updated: January 31, 2019

Content source: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases

Centers for Disease Control and Prevention 1600 Clifton Rd. Atlanta, GA 30333, USA
800-CDC-INFO (800-232-4636) TTY: (888) 232-6348, New Hours of Operation
8am-8pm ET/Monday-Friday
Closed Holidays -cdcinfo@cdc.gov

APPENDIX H: CERTIFICATION OF BSL2/BSL2* LABORATORY TRAINING**SIGNED Documentation of Training for Access to BSL2/BSL2* Laboratory**

Before entering the BSL2/BSL2* laboratory, you must have:

1. Taken the orientation tour of the BSL2/BSL2* lab with an authorized trainer (e.g., a Lab Manager).
2. Provide documentation of completion of the required UCSF Research Online Training Courses.

Before starting work with *Human Pathogens*, you must have:

1. Read and initialed/signed a copy of the ***“BSL2/BSL2* Biosafety Manual for Employees Working with Human Pathogens.”***
2. Passed the BSL2* Training Quiz administered by the DEM Safety Committee.
3. Completed the training program administered by an authorized BSL2/BSL2* trainer (e.g., a Lab Manager).

This program must include procedures that covered the following topics:

- Adhering to general lab safety procedures, such as gowning, eye protection and wearing double gloves.
- Setting up, cleaning out, and properly using the Biosafety Cabinets
- Manipulating and processing cultures safely, with emphasis on the importance of avoiding aerosol generation during all operations
- Performing the essential procedures required of most protocols, such as centrifugation, plating and incubating
- Decontamination and disposing of waste
- Review “Emergency Action Flow Chart”

By signing this document, you agree to follow all of the practices and Safety Regulations required for working in the BSL2/BSL2* laboratory and that you have met all of the above requirements.

Name (print): _____ **Date:** _____

Signature: _____

Approval: _____ **Date:** _____
BSL2/BSL2* Trainer