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This Code of Practice and all updates will be issued to each person intending to work in the BSL-3 isolation facility. BSL-3 users must read, understand, accept, and adhere to all the rules herein. A signed form from each authorized user noting receipt, understanding, and acceptance of the Code of Practice will be maintained by Laboratory Manager. Failure to follow the rules of the Code of Practice may result in serious accident. Anyone failing to follow this Code will be ineligible to use the BSL-3 facility further.

1. **Authorized Personnel**

Only authorized users are permitted to work in the BSL-3. In order to become authorized, potential BSL-3 users must:

- Have attended a bloodborne pathogen training session in the past year.
- Be vaccinated against Hepatitis B.
- Receive University of Penn RSO radiation safety training.
- Provide a baseline serum sample.
- Attend a BSL-3 Code of Practice training session with the Safety Officer.
- Attend a hands on training session with the BSL-3 Laboratory Manager.
- Read, understand, accept, and adhere to all rules in this BSL-3 laboratory Code of Practice.

2. **Permitted Work**

The BSL-3 pathogens that may currently be used in the BSL-3 facility are posted at the entrance to the BSL-3 facility. Work that does not require level 3 containment (for example, the manipulation of fixed or non-infectious materials, cultivation of uninfected cell lines, storage of chemicals, and the making of buffers and media) should be conducted elsewhere in external labs whenever possible.

Work involving radioactive isotopes will be permitted within the BSL-3 facility for authorized, licensed users and only in designated areas. A radioactive logbook will be maintained, recording the date, user, isotope, and waste of each experiment. Each radioactive user will be responsible for monitoring and cleaning the designated area and sinks after each use.

Each pathogen to be used in the BSL-3 laboratory must have been pre-approved by the respective Biosafety committee.
3. Access

The laboratory must be kept under restricted access at all times with adequate door labels identifying the area as biohazardous. Negative pressure should be kept at all times with regards to outside environment.

Controlled entry/exit: The external entry door to the BSL-3 facility are controlled by a card-key and biometrix access system. The door lock is deactivated when an authorized user’s identification badge is “swiped” against the door-lock control. An orange light on the door-lock control will appear prompting user to put his index finger against a small screen on the control. Then the light will change to green to indicate that the door may be opened. In the event that two users are entering the BSL-3 facility at the same time, both users are required to swipe their identification badges through the lock control.

The internal entry door to the BSL-3 facility is controlled by negative pressure and will not open until the external door is fully closed. The entryway between the two doors is supplied with personal protective clothing and storage lockers (see Section 4D, “Protective Clothing”). Authorized users may enter the BSL-3 facility on a 24-hour basis.

4. Precautions and Safety Concerns

A. DO NOT eat, drink, mouth-pipette, or smoke in the BSL-3 facility!

B. Sharps and Glass

The major causes of infection of laboratory personnel by human retroviruses are scratches, cuts, and needle-stick injuries. Because of this, the following are not allowed in the BSL-3 facility: hypodermic needles; razors or scalpel blades; glass pipettes; pasteur pipettes; and laboratory glassware. All medium and solutions used inside the facility are to be contained ONLY in plastic bottles. Plastic and/or disposable alternatives must be used for all glassware items which have such suitable alternatives. Any other items which might pose some danger (e.g., damaged apparatus) must be disposed of immediately.

HIV patient samples arrive in glass tubes. Specific handling procedures for these glass tubes are covered in sections 10.D.3 and 11 of this Code of Practice, but a good rule of thumb when handling them is “the less amount of handling, the less chance of accident”.

C. Precautions to prevent genetic recombination.

Each BSL3 research proposal must be reviewed and approved by the BSL3 Facility Director & Institutional Safety Officer prior to initiation. Special consideration should be
given to address the risk and control of genetic recombination that may result from the handling, manipulation or storage of existing viral agents or human materials.

To prevent the chance of genetic recombination experiments using different types of biological agents (such as different viruses) should be conducted in separate Biosafety Cabinets and stored in separate incubators.

D. Personal Protective Equipment

Before entering the BSL-3 facility, users must “gown up” in the entryway. BSL-3 users must wear the provided gloves and back-fastening lab coats at all times while in the facility. Users must also wear conventional shoes, socks, and long trousers or the provided disposable shoe cover and pant alternatives. Disposable sleeves must be used working at the hoods or if working with radioactive materials. Face masks are provided within the entryway and eye shields inside the BSL-3 facility. Other disposable personal protective equipment, such as hairnets, is also provided for users inside the entryway.

The lab coats provided are reusable. There are double-sided lockers within the entry and exit ways of the facility for lab coat storage between uses. The lab coats should be thrown away after 5 uses or if they have become contaminated.

Double gloves are mandatory when working in the BSL-3 facility. The first pair of gloves, ones that should be long enough to tuck gown sleeves under the latex, is provided in the entryway. The second pair of gloves is provided in the entryway and inside the facility and should be changed frequently, particularly when the user changes stations (e.g., when the user is moving from a biosafety cabinet to the microscope or the computer; or when the user is answering the phone). While inside the facility, users should not touch their faces, eyes, hair, or exposed skin.

Minor skin abrasions should be protected by applying bandages under the first pair of gloves. Users should not work in the BSL-3 laboratory if they have skin abrasions, cuts, or conditions that seriously impair the integrity of the skin. BSL-3 users should also not use petroleum jelly or other agents that weaken glove latex.

E. Serological Surveillance

A study concerning the serological monitoring of HIV workers reported by the Division of Safety, National Institutes of Health USA, and published in Morbidity and Mortality Weekly, Vol. 37 No. S-4, pp. 19-22, recommends:

“serum samples should be obtained at least once a year and analyzed for seroconversion. Results should be reported to individual workers in a timely manner. Counseling services should be available for workers who have positive serologic results. Procedures that maintain strict confidentiality should be adopted.”

All personnel working in the BSL-3 facility are required to sign a consent form to have a serum sample drawn prior to working in the BSL-3 facility and then re-drawn every 6 months thereafter. Confidential testing for HIV status and for seroconversion of the drawn samples is at the
discretion of the user, that is, the samples may be immediately tested or they may be stored, untested, after being drawn for testing at a later time, if necessary.

F. Re-evaluation and Safety Meetings

Meetings for the re-evaluation of the Biological Safety Level-3 Code of Practice and to review laboratory procedures will be held once a year. All authorized BSL-3 facility users must attend these meetings in order to maintain their authorization.

5. Equipment and Supplies

The BSL-3 facility is equipped with a multitude of items necessary for autonomous work inside the laboratory. There are many tabletop and micro-centrifuges, biological safety cabinets, tissue culture incubators, microscopes, computers, refrigerators, \(-80^\circ\text{C}\) freezers, and a liquid nitrogen storage tank that can be found in various areas around the BSL-3 laboratory. Some of this equipment is explained in more detail in sections to follow. All common use equipment inside the facility should be treated carefully and used with regard to all other persons authorized to work within the BSL-3 facility.

Supplies for tissue culture and for other required work inside the BSL-3 facility can be found in various designated areas around the laboratory and on the supply carts near each of the biosafety cabinets. General supplies will be kept stocked by the BSL-3 Laboratory Manager. The supply cart near each biosafety cabinet must be stocked with all necessary items by the current user. All personal supplies must be removed from the cart when work in the biosafety cabinet is finished. These supplies should be stored in the users drawer space.

The Laboratory Manager should be notified if stocks within the BSL-3 itself are running low or are out, if a BSL-3 user needs a specific item which they can not find within the facility, or if equipment within the facility needs service or repair.

Each BSL-3 facility user must contact the Laboratory Manager to review equipment and supply charges in order to determine a method of payment or repayment for use of the facility.

6. Biological Safety Cabinets

The BSL-3 facility is equipped with working biological safety cabinets that are rigorously tested. All aseptic procedures, tissue culture work, blood processing, and operations in which infectious liquids or cell cultures are transferred from one container to another is to be done inside one of the biosafety cabinets provided within the BSL-3 facility. In addition, all work that may result in the production of aerosols must be conducted inside a biosafety cabinet as well. This includes filtration of infectious liquids, any work on patient blood sample tubes, or the opening of potentially contaminated containers such as centrifuge rotors and tubes.
Before doing any work in the biosafety cabinets, BSL-3 users should make sure to remove any unnecessary items that may impede the flow of air inside the cabinet. This is facilitated by the supply carts that are provided beside each biosafety cabinet for the stocking of necessary tissue culture supplies and other equipment required for the work.

BSL-3 users should prepare the biosafety cabinet and make sure to have at-hand items for disposal and decontamination of the waste produced (see Section 10 on waste handling and decontamination). Before doing any work in the biosafety cabinets, BSL-3 users must make sure they have sufficient amounts of active Virkon solution and 70% ethanol in squirt bottles. See Section 10A for an explanation of what Virkon is and how to use it effectively). Users should also line the plastic pipette cans found in each of the biosafety cabinets with a new small red plastic bag. Large screw-top plastic 2 liter jars are provided near the sink and should be filled partially with a freshly made active Virkon solution and placed inside the biosafety cabinet. Finally, users should also make sure that the large autoclavable biohazardous waste bucket found near each biosafety cabinet is accessible, is fitted with a large red autoclave bag, and that it’s not overly full. If the bucket is close to full, it should be closed, fitted with it’s lid, and placed in the area designated by the Lab Manager for waste decontamination. A new biohazardous waste bucket should then be fitted with a large red autoclave bag and placed near the biosafety cabinet. Empty biohazardous waste buckets are located beside the autoclave.

At the end of each working session, users must remove all extra materials and all waste from the biosafety cabinet. The internal surfaces of the biosafety cabinet should then be decontaminated and cleaned by first spraying and wiping it with active Virkon solution and then by spraying and wiping it with 70% ethanol.

7. **Centrifuges**

   A. **Non-hermetically sealed centrifugation**

      Any centrifuge operation in the BSL-3 involving non-hermetically sealed containers, carriers, rotors, or tubes must be carried out inside a biosafety cabinet.

   B. **Hermetically sealed centrifugation**

      Centrifuges that have carriers, rotors, or tubes that can be hermetically sealed can be used outside of the biosafety cabinets, provided that all potentially aerosol-producing manipulations are carried out within the biosafety cabinets prior to centrifugation. Tissue culture plate carriers have no hermetically sealable lids. However, plates can be spun in the benchtop centrifuges at low speeds in their dedicated carriers. These plates must first be well sealed with tape and tightly enclosed in a small plastic bag before being placed into the carrier, and that they are not opened at any time outside of the working environment of the biosafety cabinets.

   C. **Spills in a centrifuge**

      If a user suspects that there has been a spillage inside a centrifuge within the BSL-3 facility, the centrifuge should not be opened! The centrifuge should be marked visibly with some sort of sign or some tape placed over the still closed handle that warns other users:
“SUSPECTED CONTAMINATION: DO NOT OPEN!” The incident must be reported to the BSL-3 Laboratory Manager or the Safety Officer immediately. They will do a risk assessment of the situation and decide on a course of action for decontamination.

8. Microscopes, Cameras, and Video Equipment

Users must change their outer gloves before moving to the microscope area from any other work area inside the BSL-3 facility.

In order to be able to remove exposed film from the facility without destroying it by disinfection, users must show extreme caution and be careful that the film does not become contaminated with infectious agents. Users should exercise caution when manipulating infected cell and tissue cultures at the microscope area, and users should address “film work” as a separate from “data collection”. That is to say, users must change gloves between loading/manipulations of the film and any manipulations of infected materials they are working with or photographing.

9. Computer Equipment and Note Taking

Users must change their outer layer of gloves before moving into the computer area from any other work area inside the BSL-3 facility. NO paper may be left in work areas after a user has left the facility. It is recommended that rough notes on paper scraps be discarded into the general waste autoclave buckets before the user leaves the BSL-3 facility.

A networked common-use computer is set up inside the BSL-3 facility in order to allow users external access to any data collected and/or experiments performed inside the BSL-3 facility.

Although permanent notebooks are permitted inside the BSL-3 facility, users are encouraged to use the computer to record experiments and data. Data and notes should be kept inside the individual personal folders for each authorized BSL-3 user and NOT on the desktop of the computer. Unknown-author documents will be periodically removed from the system if not inside a clearly labeled personal folder. All BSL-3 users are responsible for back-up of their own files.

10. Waste Handling, Decontamination/Disinfection, and Disposal

This section provides for the safe storage, decontamination, and disposal of waste from the BSL-3 laboratory. All disinfection and decontamination procedures carried out are designed to eliminate all possibility that users will accidentally contaminate themselves with infectious material while in the facility, or that waste will remain infectious once leaving the BSL-3 facility.

Liquid waste must be immediately decontaminated with disinfectant and contaminated solids must be either rinsed in disinfectant or sealed in spill-proof containers. For the safety of all BSL-3 users, NO contaminated or infectious material should remain exposed to the air or be left in an easily accessible location. The procedures listed below are for decontamination in common situations and are sufficient to inactivate most infectious agents. Other methods of disinfection should be tested under fair conditions first before using in the BSL-3. If a BSL-3
user would like to use alternate methods to decontaminate infectious waste, they must get approval from the BSL-3 Laboratory Manager and the Safety Officer first.

A. The Disinfectant

All or most BSL-3 decontamination procedures involve Clorox or Virkon. A surfactant is present in Virkon to disrupt viral envelopes and cellular membranes and to allow the penetration of its disinfectants (oxidizing and alkylating agents). Virkon is a powerful disinfectant when used at the recommended dilution (1% w/v) and it is known to inactivate most viruses within 5 to 20 minutes of contact. It does not give rise to formaldehyde vapor nor does it corrode stainless steel. Virkon is a red solution when freshly dissolved and active. Since this red coloration fades when the solution is no longer active, solutions of Virkon should not be used unless they are red in color. Virkon must be used for all decontamination purposes except on items that will be placed in an incubator (for these items, exterior swabbing with 70% ethanol is acceptable). BSL-3 users must disinfect any used work surface with Virkon before leaving the facility. Any area of suspected spillage should be sprinkled with Virkon powder, then thoroughly wiped up with paper towels after at least 20 minutes of incubation.

B. Decontamination of Infectious Materials for Removal from the BSL-3 Facility

Any materials that are removed from the BSL-3 Facility must be decontaminated before removal to a room that does not operate under BSL-3 containment procedures. Materials that are decontaminated by heat inactivation (65°C for at least 30 minutes) or fixed by an approved method may be removed from the facility. Infectious material may be removed from the BSL-3 without using decontamination procedures only if it is to be received in a laboratory that is also operating Biosafety Level-3 containment procedures (see Section 14B). If you must remove materials to a room that is not operating under BSL-3 containment procedures contact the BSL-3 Laboratory Manager.

C. General, Uncontaminated Waste

Items such as paper, paper towels, gloves, etc., should be placed directly into the large red-bag-lined autoclavable waste buckets marked with a large biohazard symbol. These are positioned at various places around the BSL-3 facility- they can be found near each biosafety cabinet, in the entryway and the exit. Empty buckets are positioned in the rear of the BSL-3 facility. When a bucket is near full capacity, the current user should twist the red autoclave bag closed, close the lid, and place the entire bucket in the zone set aside for the purpose of disposal by the BSL-3 Laboratory Manager. Users should make sure to replace the bucket with an empty one containing a fresh autoclave red bag. Because these buckets and their contents are handled personally by the Laboratory Manager during autoclaving and disposal, users must make sure that any waste considered potentially “sharp” (such as unprotected pipette tips, broken plastic) not be put into the general waste.

D. Contaminated Waste

D1 Liquid Waste

Liquid waste can be decontaminated by a) pouring it into screw-top 2 liter jars containing an active Virkon solution; b) adding an equal volume of 2% Virkon to the
vessel containing the contaminated liquid; or, c) adding dry Virkon powder to the contaminated liquid to give a final concentration of 1%. After 20 minutes (or preferably overnight), the liquid can then be disposed of down the sink along with plenty of water.

Larger volumes of liquid waste may require autoclaving for total decontamination. The BSL-3 Laboratory Manager or Safety Officer should be notified when large volumes of contaminated liquid needing decontamination is being produced.

**D2 Plastic Ware**

i. *Sealable Items.* (tissue culture flasks, centrifuge tubes, etc.)
   
   Items such as these that have contained infectious material should be emptied of all liquid (see Section 10.D.1), closed tightly, and then placed inside the large red-bag-lined autoclavable biohazardous waste buckets found next to the biosafety cabinet.

ii. *Large Non-sealable Items.* (pipettes, multi well tissue culture plates, etc.)
   
   Pipettes should be rinsed with the active Virkon solution in the screw-top 2 liter bottles before being placed in the red-bag-lined plastic pipette can inside the biosafety cabinet. Multi well plates should be emptied of liquid waste, sprayed with active Virkon solution, closed, and then placed in the red-bag-lined plastic pipette can inside the biosafety cabinet. These bags are then to be sealed at the end of the work session and placed into the large red-bag-lined autoclavable biohazardous waste buckets.

iii. *Small Non-sealable Items.* (pipette tips, cryovials, microfuge tubes)
   
   These are to be placed directly into the active Virkon solution inside the 2 litre bottles. The 2 litre bottles are then sealed by the user at the end of the work session, removed from the biosafety cabinet, and placed in the sink area for at least 20 minutes. The liquid inside the bottles should then be disposed of down the sink with plenty of water and the small items can be tossed into the large red-bag-lined autoclavable biohazardous waste buckets.

**D3 Containers for glass patient blood sample tubes**

Small red sharps containers holding infectious glass vacutainer tubes from HIV patient blood samples (see Section 11) must stay inside the biosafety cabinets so as not to expose any BSL-3 facility user to the potentially infectious material it contains. When they are filled, the current user must close the lid tightly before removing the container from the biosafety cabinet. Then place it into the zone set aside for the purpose of disposal by the BSL-3 Laboratory Manager.
11. HIV Patient Blood Samples

The patient samples are in glass vacutainer tubes and should be handled with the utmost care. A good rule of thumb when handling these tubes is “the less amount of handling, the less chance of accident”. All patient blood sample tubes, due to the vacuum inside, have the potential of creating an aerosol of their contents upon opening. Therefore, vacutainer blood tubes may only be opened in the biosafety cabinet. To open vacutainer tubes first spray a paper towel with Virkon solution and then place the paper towel over the top of the tube. Open the tube slowly with the tube at eye level with hood barrier in between until open. Screw-top plastic 2 litre jars are available at the sink area. These should be partially filled with an active solution of Virkon and placed into the biosafety cabinet. Vacutainer caps and all infectious wastes from the patient samples should be deposited into these Virkon-filled 2 litre jars during processing of the blood. Vacutainer tubes should be gently placed into the red “sharps” containers that are located in each of the biosafety cabinets (see Section 10.D.3). In the case that sample vacutainer tubes arrive broken, the sample tubes are packaged in such a way that any spill or breakage of a vacutainer sample tube will be contained by a larger biohazardous container. This larger vial containing the broken or leaked vacutainer sample tubes should be opened only inside a biosafety cabinet and filled to the point where all it’s original contents are covered by active Virkon solution. After a period of at least 20 minutes, the Virkon solution should be disposed of down the sink (see Section 10.D.1) and all vacutainer sample tubes found inside the larger container should be discarded carefully into the red “sharps” containers (see Section 10.D.3).

12. Radioactive Waste Handling, Decontamination/Disinfection, and Disposal

Work involving radioactive isotopes is permitted in the BSL-3 facility for authorized, licensed users and only in designated areas. All BSL-3 facility users who work with radioactive materials must record all radioactive usage and disposal or decay in logbooks and must adhere to University of Penn Radiation Safety and NRC guidelines. All work surfaces, gloves, and equipment as well as the user must be monitored during and after use of radioactivity. Paper towels, gloves, etc., used during the process must be placed inside a radioactive waste bag for disposal.

Any contamination must be cleaned up with a mixture of 2% Virkon. Large spills must be reported to the BSL-3 Laboratory Manager or the Safety Officer.

A. Liquid Radioactive Waste

If the radioactive liquid waste is contaminated with infectious materials, it must be treated with an equal volume of active 2% Virkon disinfectant for at least 20 minutes (overnight incubation is preferable) before it is disposed of.

Limited amount of liquid non-infectious or decontaminated radioactive waste can be gently poured down the radioactive sink, without splashing and with copious amounts of running water.
Liquid radioactive waste disposed down the sink must be recorded in the sewer disposal logs.

Note that current limits for sewer disposal are $\leq 200\mu\text{Ci of }^3\text{H OR } \leq 50\mu\text{Ci of }^{32}\text{P or }^{125}\text{I}$. The addition of 1% Virkon before disposal helps reduce radioactive contamination to the sink and collection containers.

B. Solid Radioactive Waste

Solid radioactive waste should be placed into a sealable plastic bag, labeled clearly and correctly, and stored in the waste container designated for the particular isotope used. Disposal and decay of solid radioactive waste must be logged in the correct logbook. All solid radioactive waste in the BSL-3 facility is treated as infectious and therefore will undergo a period of decontamination -- this usually consists of an incubation of at least one hour in a 65°C oven - before disposal or decay.

13. Accidents

A. General Guidelines

All dangerous spills, injuries, or other accidents must be immediately reported to the Principal Investigator in charge of the BSL-3 facility, to the BSL-3 Facility Lab Manager, and/or to the Safety Officer.

IF HIV-1 EXPOSURE HAS OCCURRED DIRECT YOURSELF TO THE UNIVERSITY OF PENNSYLVANIA OCCUPATIONAL HEALTH OFFICE:

Hospital of the University of Pennsylvania
Ground Floor
Silverstein Pavilion
3400 Spruce Street, Philadelphia, PA 19104

CALL 215-662-2354 TO GET ADVISE ON COURSE OF ACTION

SITE LOCATED IN #5 in MAP in subsequent page:
Some guidelines for accidents are as follows:

- If any **infected material comes into contact with a BSL-3 user’s outer pair of gloves**, the gloves should be discarded immediately.
- If any **infectious material comes into contact with a BSL-3 user’s exposed skin**, the affected area should be washed immediately and quite thoroughly. The incident must be reported immediately.
- If any **penetrating or “stick” injury** occurs, such as from the breakage of a glass vacutainer blood sample tube, the wound should be encouraged to bleed immediately and the area washed quite thoroughly before bandaging. The incident must be reported immediately.
- As covered in section 7C, if a user suspects that there has been a spill inside a centrifuge, the **centrifuge should not be opened**! It should be marked visibly with some sort of sign or some tape placed over the still **closed** handle that warns other BSL-3 facility users: “SUSPECTED CONTAMINATION: DO NOT OPEN!” The incident must be reported immediately.
- If there has been a **major spill** in the BSL-3 facility which is too widespread for superficial disinfection, the incident must be reported immediately, **all work must stop**, and the laboratory must be sealed shut with warning tape. The Laboratory Manager or Safety Officer will do a risk assessment of the situation and decide on a course of action for decontamination.
• If there has been a minor spill that is both infectious and radioactive, first cover the area with paper towels to prevent spread and then saturate the site with virkon and leave it for at least 20 minutes to inactivate the infectious material. Then use the decontaminating spray or soap remove the radioactive material. The area should be monitored by wipe test after decontamination to insure that all radioactive material has been removed. Laboratory Manager should be informed to help with the clean up.

B. Laboratory Fumigation

If there has been a spill of infectious materials that is so widespread, to the extent that standard clean up can not contain it or decontaminate it, the BSL-3 facility must be entirely fumigated for decontamination. The following procedure will be performed by the BSL-3 Laboratory Manager or the Safety Officer. Materials stored in vials at \(-80^\circ\text{C}\) and over liquid nitrogen should be safe from the effects of this fumigation. However, no similar guarantees can be made about the viability of materials stored in incubators or elsewhere within the BSL-3 facility.

**Formaldehyde Fumigation Procedure:**

Prepare BSL-3: Remove all lab personnel. Close all incubator and refrigerator doors. Set air conditioning to 28\(^\circ\)C (heat room). Turn off biosafety cabinets (timed period, for 13 hours only).

Fumigation: Make formalin mixture in fume hood (75 ml 37\% formaldehyde, 150 ml \(\text{H}_2\text{O}\)). Transport mixture into BSL-3 in a suitable carrier. Add mixture to a metal fumigation beaker on hot plate that has been hooked up to a timer set for two hours and that has been placed in view of the window. Leave BSL-3 immediately after turning on timer to the hotplate. Seal outer door and window filters, put biohazard tape visibly around door, and lock securely.

Incubation: Allow beaker to boil dry and leave room “as is” overnight. The formaldehyde is removed from the facility by the Class II cabinets, which turn on automatically after 13 hours. Once the room is at negative pressure, unseal tape from around the window filters.

After 24 hours, the formaldehyde should have been evacuated. Unseal the door and return air conditioning to normal setting. Make sure formalin has dispersed before allowing any BSL-3 personnel in.

14. **Storage and Transport of Infected Material**

The BSL-3 facility is equipped with 4\(^\circ\)C/-20\(^\circ\)C, -80\(^\circ\)C, and liquid nitrogen storage freezers. Space for individual users is available. All material stored in these freezers should be securely closed and clearly labeled (i.e. name, date, and description of the sample) so the containers...
cannot be opened accidentally. A few other guidelines for storage and transportation of infected materials are as follows.

A. Storage of Infectious Materials

A1 Room Temperature, 4°C, and -20°C

It is inadvisable to store any infectious material under these conditions for long term. Samples stored for longer than three months under these conditions and left untouched will be subject to disposal without notice.

A2 -80°C

Storage racks are provided in the -80°C freezers. BSL-3 users should contact the Laboratory Manager for space. Material should not be stored outside the racks. Any clutter is subject to removal and destruction without notice.

A3 Liquid Nitrogen

When infected cells are to be frozen for storage, they must be suspended in the freezing medium and transferred into freezing vials within a safety cabinet. These vials should then be slowly cooled (at one degree/minute) in the -80°C freezers before they are transported to the liquid nitrogen freezer. Contact the Laboratory Manager for space.

B. Transport of Infectious Materials

All materials that are handled within the BSL-3 facility, with the exception of photographic film (see Section 8), are automatically considered to be infectious unless specifically disinfected by one of the approved methods (see Section 10) and should NOT be removed from the facility. Infectious material may be removed from the BSL-3 without using decontamination procedures only if it is to be received in a laboratory that is also operating Biosafety Level-3 containment procedures. Before transport, storage vials containing contaminated materials need to be securely closed and then surface decontaminated, usually by a swabbing of the outside of the container with 70% ethanol. The vial should then be placed inside a larger container (i.e., a sealable or taped-shut bag or a screw-top vessel) that carries enough absorbent material inside it to soak up any spill that may occur, and securely closed. This larger container should then be placed inside a second container that is to be used as the transportation carrier and which is clearly marked with a biohazard symbol and the words “BIOHAZARDOUS MATERIAL INSIDE”.

The containers used for the transport must be sufficient to prevent accidental contamination and must be approved by the Laboratory Manager and Safety Officer.

15. Preparing Equipment to be Serviced By Outside Personnel

Some equipment in the BSL-3 facility will need to be serviced regularly or might need maintenance. The Laboratory Manager should be notified that equipment within the facility needs service or repair. The equipment will either have to be shipped out to the contractor or be serviced in-house.

A. Shipping Equipment

The equipment to be serviced must be completely disinfected before removal from the facility. This procedure is usually no more extensive than a thorough swabbing of the equipment with 70% ethanol.
B. In-House Service

In such circumstances, the BSL-3 facility must be made safe for the outside personnel before service can be carried out. The Laboratory Manager will be given written notice of the appointed time of service, and will then inform all the BSL-3 facility users of the expected disruption with as much time in advance as possible. One day before the work is due to start, the Laboratory Manager will ensure that the equipment to be serviced and all surfaces with which the service personnel are likely to come in contact with have been thoroughly disinfected. The Laboratory Manager will also bar access to all other areas of the BSL-3 facility. Service personnel will then inform the Laboratory Manager when work has been completed.

In addition, all work by BSL-3 users must stop. Under no circumstances may any manipulations of infectious material be carried out by any BSL-3 user during the period of in-house servicing by outside personnel.

If superficial disinfection procedures are inadequate to protect the service personnel, fumigation of the laboratory will be necessary (see Section 13B). In such cases, all authorized personnel will be contacted before fumigation and given at least one week’s notice, whenever possible.

16. Additional Resources:

Local:  Wistar/CHOP/Upenn Biosafety Office


17. Inactivation of HIV-1 References


iii. Bloomfield SF, Smith-Burchnell CA, Dalgleish AG. Evaluation of


xv. Household bleaches may be too weak. New Scientist 1978:115:25


xxi. Lionel Resnick, MD; Kaith Varen; S. Zaki Salahuddin, MS; Sue Tondreau; Phillip D. Markham, PhD: Stability and Inactivation of HTLV-III/LAV Under Clinical and Laboratory Environments (JAMA 255:1887-1891, 1986)


INTRODUCTION: The recommendations of the expert team are directed to industrial-scale facilities for the production of large quantities of highly concentrated HIV. Their recommendations are similar to and complement those in the preceding "1988 Agent Summary Statement for Human Immunodeficiency Virus," which updates the one published in 1986 (1). Laboratory directors and others responsible for the health and safety of laboratory employees working with HIV and HIV-containing material should carefully consider these relevant recommendations and guidelines in developing an appropriate safety program.

COMMITTEE REPORT

Two workers in different laboratories producing large quantities of highly concentrated HIV have been reported to have laboratory-acquired HIV infections (1). One worker's infection was presumed to be caused by "undetected skin contact with virus culture supernatant" (2). The other worker's infection followed "an injury with a potentially contaminated needle" (2). After the first case was identified, the Director of NIH convened a team of experts to investigate the incidents and to visit seven different laboratories that produced large volumes of HIV. After facilities inspections and separate, confidential interviews with the workers, the team prepared a report of their findings. The conclusions and recommendations from that report follow.

The most probable cause for the first laboratory-acquired infection was inapparent parenteral exposure. Frequent opportunities for unrecognized direct contact with contaminated materials and surfaces were reported to be present. Gloves of questionable integrity, skin cuts and abrasions, and one episode of a dermatitis-like condition represented portals for possible exposure and routes of infection. The inexperience of the first infected worker in microbiologic procedures and Biosafety Level (BSL) 3 practices, coupled with the reliance on obtaining necessary skills through on-the-job training in a setting in which episodes of contamination may have occurred frequently, suggests that the worker might not have possessed an appropriate level of proficiency when the infection may have occurred.

The most probable cause for the second worker's infection was parenteral inoculation. This worker recalled incurring an injury with a blunt cannula approximately 6 months before the first seropositive sample. Incidents of contamination, such as those reported by the first worker, occurred infrequently in the second worker's laboratory.

Aerosol transmission is considered to be the least likely cause of infection in both cases. Operations in which aerosols may have been generated were carried out in biological safety cabinets to reduce the potential for inhalation exposure. Although some aerosols may have been released during the few reported rotor-seal failures involving the continuous-flow zonal centrifuge, the potential for contact exposure was greater. Aerosol transmission was unlikely because: a) in situations in which overt aerosol exposure has occurred in laboratory and production operations involving HIV, no exposed workers have seroconverted; b) no evidence
exists that suggests aerosols may be a natural mode of HIV transmission; c) the probable cause identified above is consistent with documented modes of transmission of bloodborne pathogens in the laboratory.

The occurrence of these two infections emphasizes the finite risk that exists for laboratory workers who handle concentrated preparations of HIV. The conclusions of a National Cancer Institute prospective cohort study (2) indicate that this risk is low and may be similar to the risk for infection of health-care workers who have experienced a needlestick injury.

The occupational risk for infection by parenteral exposure is substantially reduced or eliminated by strict adherence to BSL 2 practices. The recommended use of BSL 3 practices for highly concentrated preparations of HIV is appropriate. The review of these two infected laboratory workers does not suggest the need to alter current CDC/NIH biosafety recommendations for HIV or for patient care (3), research (1), or virus production. There is a need, however, for more proficiency and discipline in laboratory safety practices.

The following recommendations will help assure maintenance of a safe and healthy environment for laboratory and production-facility workers who handle concentrated preparations of HIV:

A. Strictly adhere to standard microbiologic practices and techniques

The most important recommendation is to adhere strictly to standard microbiologic practices and techniques. Persons working with HIV must be aware of potential hazards and must be trained and proficient in practice and techniques necessary for self-protection. Employees must be informed that parenteral exposure is the most serious potential hazard for causing a laboratory-acquired infection. They must be able to recognize how such exposures occur and how they can be prevented. Although on-the-job training is an acceptable approach for learning techniques and practices, it is imperative that proficiency be obtained BEFORE virus is actually handled.

B. Assure that workers are proficient in virus-handling techniques

Selection criteria for employees who will work in production operations or with concentrated preparations of HIV should require experience in the handling of human pathogens or tissue cultures. If an employee has not had such experience, s/he should participate in carefully structured, well-supervised on-the-job training programs.

The director or person in charge of the laboratory or production facility must ensure that personnel are appropriately trained and are proficient in practices and techniques necessary for self-protection. Initial work activities should not include the handling of virus. A progression of work activities should be assigned as techniques are learned and proficiency is developed. Virus should only be introduced into the work activities after the supervisor is confident it can be handled safely.

C. Monitor work practices

Periodically, the biosafety officer or a person with expertise in biosafety should closely observe
practices and techniques used in handling HIV. This can be helpful in identifying activities or behavior that may increase the potential for contact with contaminated material or for inapparent parenteral exposures. If deficiencies are noticed, corrective measures should be specified and implemented.

D. Continuously reinforce safe practices

Practices that reduce the potential for direct contact and inapparent parenteral exposure should be continuously reinforced:

- Gloves should always be worn when concentrated preparations of HIV are handled and when contact with a contaminated surface or material may be unavoidable. If a gloved hand accidentally touches a contaminated surface or material, the glove should be removed immediately and the hands washed.

- Work surfaces should be decontaminated at the end of each day and any time contamination is recognized.

- Workers must develop the habit of keeping hands away from the eyes, nose, and mouth in order to avoid potential exposure of mucous membranes. Wearing filter masks and eye goggles or face shields may assist in accomplishing this objective.

- Needles and sharp implements must not be used when HIV is handled unless no acceptable alternative is available. When possible, unbreakable containers should be substituted for glassware, in order to avoid accidental cuts from broken pieces.

- In the absence of advice and consent of an occupational physician or nurse, no worker should handle any virus-containing material when s/he has cuts or skin abrasions on the hands or wrists.

E. Establish a medical surveillance serology program

Each medical facility should have a medical-surveillance serology program. Serum samples should be obtained at least once a year and analyzed for seroconversion. Results should be reported to individual workers in a timely manner. Counseling services should be available for workers who have positive serologic results. Procedures that maintain strict confidentiality should be adopted.
F. Revalidate integrity of process, transport, and containment equipment

The operational integrity of all equipment used to process, transport, and contain fluids containing HIV should be revalidated at least once a year. The integrity of such equipment should be revalidated after any system failure that releases contaminated fluids into the work environment.

G. Develop production processes that enhance biosafety

Efforts should be made to explore and use production systems and strategies that reduce operational complexity and manual manipulations.

H. Validate efficacy of decontamination methods

Special attention should be given to demonstrating the adequacy of decontamination methods when high organic content, such as cellular debris, is present.

Sponsor and conduct biosafety training initiatives Responsible institutions should orient such programs toward the application of biosafety practices to work involving HIV. Presentation strategies and materials to make the training widely available should be encouraged.

References


C. Recommendations for prevention of HIV transmission in health-care settings. MMWR 1987;36(suppl 2S):3S-18S. *Expert Team: W. Emmett Barkley, PhD, Director, Division of Engineering Services, National Institutes of Health; Robert McKinney, PhD, Director, Division of Safety, National Institutes of Health; John Richardson, DVM, MPH, Biosafety Officer, Emory University; Gerald Schochetman, PhD, Chief, Laboratory Investigations Branch, AIDS Program, Center for Infectious Diseases, Centers for Disease Control; David Henderson, MD, Hospital Epidemiologist, Warren Grant Magnuson Clinical Center, National Institutes of Health.

19. Additional Safety-Related Links:

http://www.biosafety.anl.gov/IBC_BiosafetyLinks.htm#HIV
20. National Clinicians’ Post-Exposure Prophylaxis Hotline

Phone: 1-888-448-4911

Exposure to blood-borne pathogens can present serious risks to health care providers. Prompt post-exposure treatment for HIV and hepatitis B virus can be effective, but because each exposure case is unique, determining who should receive prophylaxis and which drugs are most appropriate is not always easy.

The National Clinicians’ Post-Exposure Prophylaxis Hotline (PEPline) offers treating clinicians up-to-the-minute advice on managing occupational exposures (i.e. needlesticks, splashes, etc.) to HIV, hepatitis and other blood-borne pathogens.

**PEPline clinicians will respond to your call 24 hours a day, 7 days a week.**

Emergency calls made during evening, weekend, and holiday hours are forwarded to on-call clinicians. Non-emergency calls will be returned during business hours. Clinicians will help assess the risk of the exposure, discuss the most recent post-exposure prophylaxis protocols, and review specific treatment and follow-up options. Written materials supporting the telephone discussion are sent by mail or fax whenever needed.