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Biosafety guidelines for diagnostic and research laboratories working with HIV



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1. Introduction

These biosafety guidelines are provided for the protection of workers in diagnostic and research laboratories handling human immunodeficiency virus (HIV) or materials containing HIV. They also apply to work with agents of other bloodborne diseases, such as hepatitis B virus (HBV). While the guidelines are applicable to both developed and developing countries, special care has been taken to provide advice relevant to laboratory workers in developing countries.

The risk of laboratory-acquired infection with HIV or HBV is primarily from contamination of the hands and mucous membranes of the eyes, nose, and mouth by infectious blood and other body fluids. There is no evidence that HIV or HBV is transmitted by the airborne route.

Current studies indicate that the HIV infection rate for laboratory workers is low. The risk of HIV infection following percutaneous needle-stick exposure to HIV-contaminated blood is estimated to be between 0.13% and 0.5%. In contrast, the risk of HBV infection following similar exposure to that virus is 45 – 120 times as great.

While the level of occupational risk is low, the consequences of infection with HIV are dire and should not be underrated by laboratory personnel. Because there is no vaccine, safe work practices provide the only protection at present against job-related HIV infection. A summary of procedures, persons at risk, and modes of HIV transmission appears in Annex 1.

The role of training in laboratory safety is vital; continual on-the-job training in safety measures is essential for all laboratory and support staff. Poor laboratory practice and human error can negate all safety standards and render good equipment hazardous.

Supervisors should ensure that their staff are safety-conscious and properly trained, and should continually monitor their work practices. It must be emphasized that laboratory safety is the responsibility of all laboratory employees. Individual workers should report unsafe acts or conditions to their supervisors.

The guidelines given here are structured around the following basic protective measures:

- Prevention of puncture wounds, cuts, and abrasions, and protection of existing wounds, skin lesions, the conjunctiva and mucosal surfaces.
- Application of simple protective measures designed to prevent contamination of the person and his or her clothing, and good basic hygiene practices, including regular hand-washing.

Biosafety guidelines for laboratories working with HIV

- Control of surface contamination by containment and disinfection procedures.
- Safe disposal of contaminated waste.

Guidelines related to these basic protective measures are provided at the following levels:

- Standard biosafety guidelines that apply to all procedures and levels of laboratory.
- Additional or supplementary guidelines for:
 - serological laboratories,
 - virus isolation laboratories,
 - research and production laboratories.
- Guidelines for the handling, transfer, and shipment of specimens.
- Guidelines for the collection of blood samples.

A written contingency plan describing emergency procedures to deal with accidents and spills of infectious materials should be prepared and be available to all staff.

As indicated above, these guidelines apply particularly to laboratories working with HIV. More general biosafety guidelines for work with all infectious microorganisms, which are particularly useful for laboratories involved in varied microbiological diagnostic and research activities, are given in another WHO publication.¹

¹ *Laboratory biosafety manual*. Geneva, World Health Organization, 1983.

2. Standard biosafety guidelines

The major hazard to laboratory workers is contamination of the hands and mucous membranes of the eyes, nose, and mouth by infectious blood and other body fluids. Such contamination occurs as a result of penetrating injuries caused by sharp objects, and the spilling and splashing of specimen materials. The guidelines given here outline practices and procedures designed to keep such accidents to a minimum.

Precautions for laboratory workers

1. Wear gloves when handling infectious materials or where there is a possibility of exposure to blood or other body fluids. All laboratories that work with material that is potentially infected with HIV require a generous supply of good-quality gloves (see Annex 2).
2. Discard gloves whenever they are thought to have become contaminated, wash your hands, and put on new gloves.
3. Do not touch your eyes, nose, or other exposed membranes or skin with gloved hands.
4. Do not leave the workplace or walk around the laboratory wearing gloves.
5. Wash your hands with soap and water immediately after any contamination and after work is completed. If gloves are worn, wash your hands with soap and water after removing the gloves (see also Annex 2).
6. Wear a laboratory gown, smock, or uniform when in the laboratory. Wrap-around gowns are preferable. Remove this protective clothing before leaving the laboratory.
7. When work with material that is potentially infected with HIV is in progress, close the laboratory door and restrict access to the laboratory. The door should have a sign: 'Biohazard. No admittance'.
8. Keep the laboratory clean, neat, and free from extraneous materials and equipment.
9. Disinfect work surfaces when procedures are completed and at the end of each working day. An effective all-purpose disinfectant is a hypochlorite solution with a concentration of 0.1% available chlorine (1 g/litre, 1000 ppm) (Annex 3).

10. Whenever possible, avoid using needles and other sharp instruments. Place used needles, syringes, and other sharp instruments and objects in a puncture-resistant container. Do not recap used needles and do not remove needles from syringes.
11. Never pipette by mouth.
12. Perform all technical procedures in a way that minimizes the risk of creating aerosols, droplets, splashes, or spills.
13. Do not eat, drink, smoke, apply cosmetics, or store food or personal items in the laboratory.
14. Make sure that there is an effective insect and rodent control programme. (This is a standard biosafety recommendation.)

Spills and accidents

1. Spills of infected or potentially infected material should first be covered with paper towelling or other absorbent material. A disinfectant should be poured around the spill area and then over the absorbent material and left for 10 minutes. The standard disinfectant recommended for cleaning contaminated surfaces¹ is a hypochlorite solution with a concentration of 0.5% available chlorine (5 g/litre, 5000 ppm). However, for laboratories working with HIV cultures and virus preparations, a higher concentration of available chlorine (1.0%) is recommended. The mixture of disinfectant and spilt material should be cleaned up with absorbent material, which should be placed in the contaminated waste container. The surface should then be wiped again with disinfectant. Gloves should be worn throughout the procedure, and direct contact between gloved hands and the disinfected spilt material should be avoided. Broken glass or plastic should be swept up with a dustpan and brush.
2. Needle-stick or other puncture wounds, cuts, and skin contaminated by spills or splashes of specimen material should be thoroughly washed with soap and water. Bleeding from any wound should be encouraged.
3. All spills, accidents, and overt or potential exposure to infectious material should be reported immediately to the laboratory supervisor. A written record should be kept of all such incidents. Appropriate medical evaluation, surveillance, treatment and, if necessary, counselling should be provided (see also page 6).

¹ *Guidelines on sterilization and disinfection methods effective against human immunodeficiency virus (HIV)*. Second edition. Geneva, World Health Organization, 1989 (WHO AIDS Series No. 2).

Handling and disposal of contaminated material and waste

1. Reusable equipment such as pipette tips, syringes, cannulas, needles, and specimen tubes should be placed in a puncture-resistant metal or plastic container at the work station. Such equipment must be chemically disinfected prior to cleaning and then autoclaved or boiled (Annexes 3 and 4). Gloves must be worn during disinfection and cleaning.
2. Contaminated laboratory gowns, coats, and other protective clothing should be placed in a separate container located within the laboratory. Before reuse, such clothing should be autoclaved or disinfected and washed.
3. Disposable contaminated equipment, e.g., syringes, needles, and other sharp instruments or objects, should be placed in a puncture-resistant metal or plastic container at the work station. This and other contaminated material should preferably be autoclaved, boiled, or chemically disinfected in the work area. Alternatively, it may be transported from the work area in a securely covered leakproof container to a central site on the laboratory premises for immediate autoclaving or incineration. If the containers are to be reused they should be cleaned and disinfected before reuse.
4. Incineration is the method of choice for disposing of contaminated material and waste if the incinerator is located on laboratory premises and under laboratory control. If the material has to be removed from the premises it must be autoclaved or otherwise disinfected. Institutional-type incinerators (not less than 1300°C) should be used; supplementary fuel should always be used to ensure complete combustion. Permission must always be obtained from the appropriate local authorities to operate an incinerator or carry out controlled burning operations. Ashes and debris should be buried in a landfill site.
5. Burial of decontaminated material and waste in a controlled landfill site is the only acceptable option when incineration is impossible or not permitted. Extreme care must be taken to ensure that any materials and waste disposed of in this manner have been sterilized or disinfected and that syringes and needles are destroyed mechanically. The materials should be deposited in trenches, covered with earth, and compacted daily. The controlled fill must be fenced off, and scavenging strictly prohibited.
6. Radioactive material should not be incinerated. It should be disposed of in accordance with national codes and requirements.

Health and medical surveillance of employees

1. Laboratory workers should be given an initial clinical examination, and a baseline serum sample should be obtained and kept frozen for possible future reference. All findings should be kept confidential.
2. If a laboratory worker is exposed to blood, other body fluids, or virus-culture material either parenterally or through mucous membranes, the source material should, if possible, be tested for the presence of virus and/or antibody. If the source material is positive for HIV antibody, virus, or antigen or is not available for examination, the worker should be serologically tested and advised to report and seek medical evaluation of any acute febrile illness that occurs within 12 weeks after the exposure. Such an illness – particularly if characterized by fever, rash, or lymphadenopathy – may indicate HIV infection. During the follow-up that must be instituted the worker should be instructed to take general precautions for preventing HIV transmission, and given appropriate counselling.¹ If seronegative, he or she should be retested 6 weeks after the exposure and periodically thereafter (at 3 and 6 months after exposure).
3. Records should be kept of all illnesses and absences of laboratory workers. The results of the HIV testing of laboratory employees should be kept confidential.

¹ For more details, see: *Guidelines for counselling about HIV infection and disease*. Geneva, World Health Organization, 1990 (WHO AIDS Series No. 8).

3. Supplementary guidelines for serological laboratories

For serological laboratories, the standard biosafety guidelines should be supplemented by the following requirements for the laboratory facilities and equipment.

Laboratory facilities and equipment

1. It is desirable for work with known HIV-contaminated material to be carried out in a separate laboratory or laboratory room devoted exclusively to such work. If this is not possible, a secluded and clearly identified working area should be provided within the laboratory.
2. Biological safety cabinets are not required for the serological testing of potentially HIV-contaminated material. Safety glasses, face shields, or other protective devices should be worn when necessary to protect the eyes and face from splashes and impacting objects.
3. Ample space must be provided for carrying out laboratory procedures safely.
4. The walls, ceiling, and floor should be smooth, easy to clean, impermeable, and resistant to the chemicals and disinfectants normally used in the laboratory. The floors should be non-slip.
5. The bench tops should be impervious and resistant to disinfectants, acids, alkalis, organic solvents, and moderate heat.
6. The laboratory furniture should be sturdy and easy to clean.
7. Washbasins should be provided in each laboratory room, preferably near the exit.
8. Doors to laboratory rooms should be self-closing and have vision panels.
9. There are no specific ventilation requirements. A mechanical ventilation system is not necessary. Windows that open should be fitted with fly screens.
10. An autoclave for the decontamination of infectious laboratory material and waste should be available in the same building as the HIV laboratory.
11. Facilities for storing outer garments and personal items and space for eating, drinking, and smoking should be provided outside the workroom.

4. Supplementary guidelines for virus isolation laboratories

For virus isolation laboratories, the standard biosafety guidelines in section 2 should be supplemented by the following requirements for laboratory facilities and equipment and by additional guidelines on work precautions and the health and medical surveillance of employees, as outlined below. The standard guidelines need to be strengthened in this way because workers are likely to be handling material containing a high concentration of the virus.

Laboratory facilities and equipment

1. The requirements for the laboratory facilities are identical to those for serological laboratories (p. 7). It is most desirable that a laboratory or laboratory room be devoted exclusively to work with HIV-contaminated material. Space may be required for the installation of biological safety cabinets or an exhaust-ventilated cubicle, and for essential equipment such as a refrigerator, a centrifuge, and an incubator.
2. Biological safety cabinets are the equipment of choice for work with infectious microorganisms and procedures that produce aerosols or droplets. However, these cabinets may be rendered ineffective and potentially hazardous to the worker if not properly installed and routinely tested and serviced (Annex 5).
3. Procedures that are normally conducted in a biological safety cabinet may be performed in an exhaust-ventilated cubicle (Annex 5).
4. Sealed centrifuge buckets (safety cups) or rotors should be used to prevent the accidental dispersion of material from the centrifuge. They should be loaded and unloaded in a biological safety cabinet or other physical containment device.

Work precautions

1. Access to the laboratory should be restricted at all times to persons whose presence is required for programme or support purposes.
2. All procedures involving manipulation of infected cell cultures, handling of material containing high concentrations of virus and activities that produce aerosols or droplets should be performed using physical containment equipment, such as a biological safety cabinet or sealed centrifuge buckets or rotors.

3. Workers should put on a clean laboratory coat or gown on entering the laboratory. The coat or gown should be replaced when contaminated or soiled; all laboratory gowns and coats should be taken off and left in the laboratory when the worker goes out of the laboratory for any reason.

Health and medical surveillance of employees

Medical examination of all laboratory workers is necessary. A baseline serum sample should be obtained and stored frozen for future reference (see p. 6).

5. Supplementary guidelines for research and production laboratories

For laboratories working with or producing less than 10 litres of virus suspension at any given time, the standard biosafety guidelines in section 2 should be supplemented by the following requirements for laboratory facilities and equipment, and by additional guidelines on work precautions, spills, and accidents, and the health and medical surveillance of employees as outlined below. The standard guidelines need to be strengthened in this way because the work involves procedures that result in materials containing a high concentration of virus and the manipulation of concentrated virus preparations.

Laboratory facilities and equipment

1. Entrance to the laboratory from the access corridors should be through two sets of doors, in the form of a double-doored vestibule, a double-doored clothes-changing room, an airlock, or other similar physical arrangement.
2. The interior surfaces of the room (floor, walls, ceiling) should be water-repellent and easy to clean. Openings into these surfaces, such as for service pipes and ducts and electrical conduits, should be sealed to facilitate decontamination of the area.
3. A washbasin operated by foot, knee, or elbow, or automatically should be provided near the exit door.
4. The access doors should be self-closing.
5. The windows in the laboratory should be kept closed and sealed.
6. A ducted exhaust-ventilation system should be provided, creating a directional air flow that draws air into the laboratory through the entrance and maintains the laboratory under negative pressure relative to the outside. The exhaust air should not be recirculated, but discharged outside through a dedicated sealed exhaust system away from occupied areas and air intakes. The exhaust air can be discharged directly outside without passing through a high-efficiency particulate air (HEPA) filter or otherwise being treated, unless it is discharged through the building ventilation system. If there is a mechanical air supply system, it must be interlocked with the mechanical exhaust system to prevent pressurization of the laboratory in the event of failure of the exhaust fan.

7. The HEPA-filtered exhaust air from biological safety cabinets should be discharged directly outside or through the building's exhaust air system. The exhaust air may be recirculated within the laboratory if the cabinet is regularly tested and has been certified as safe within the past 12 months. If exhaust air is discharged through the building's exhaust system, the exhaust system of the cabinet must be connected in such a manner as to avoid interfering with the air balance in the cabinet or the building's exhaust system, e.g., through a thimble-unit connection.
8. An autoclave for the decontamination of laboratory wastes should be available in the laboratory or within the same building.
9. Sealed centrifuge buckets (safety cups) or rotors should be used. They should be loaded and opened in a biological safety cabinet.

Work precautions

1. Access to the laboratory should be restricted at all times to persons whose presence is required for programme or support purposes.
2. All procedures involving manipulation of infected cell cultures, handling of material containing high concentrations of virus, and activities that produce aerosols or droplets should be performed in biological safety cabinets (Annex 5).

Aerosol accidents

In the event of an accident causing the release of an aerosol, e.g., breakage of a centrifuge or homogenizer, all workers in the laboratory should hold their breath and leave the room immediately, close the door, and report the incident to the supervisor. The room exhaust air system and biological safety cabinet(s) should be left on to ventilate the laboratory. Workers wearing appropriate protective clothing may re-enter the laboratory after 30 minutes to disinfect the equipment affected and the room.

Health and medical surveillance of employees

1. Medical examination of all workers in the laboratory is required. A baseline serum sample should be taken and stored frozen for future reference (see p. 6).

6. Guidelines for the handling, transfer, and shipment of specimens

The handling, transfer, and shipment of improperly packed specimens carry a risk of infection to all people directly engaged in, or in contact with, any part of the process. Improper handling within the laboratory endangers not only the laboratory workers immediately concerned but also the administrative, secretarial, and other support personnel. The transfer of specimens between laboratories and institutions increases the risk to the public and to personnel of the transport and postal services.

Transport of specimens – general guidelines

1. Specimen containers should be of leakproof break-resistant plastic or glass. Screwcaps are preferable for closing containers.
2. After the container is closed and sealed, it should be wiped with a disinfectant – a hypochlorite solution at a concentration of 0.1% available chlorine (1 g/litre, 1000 ppm) – and then dried.
3. When a specimen is received and before the container is opened, it should be wiped with a disinfectant as above.
4. Within the health care facility and laboratory, specimen containers should be placed in racks to maintain them in an upright position. The racks should then be transferred or transported in leakproof plastic or metal containers that will contain accidental leakages or spillages.
5. Specimen containers in racks being transported from field collection sites or between laboratories in laboratory-controlled vehicles should be in leakproof plastic or metal boxes with secure, tight-fitting covers.
6. Transport and storage in liquid nitrogen tanks with long holding times may be necessary in some areas without a reliable electricity supply.

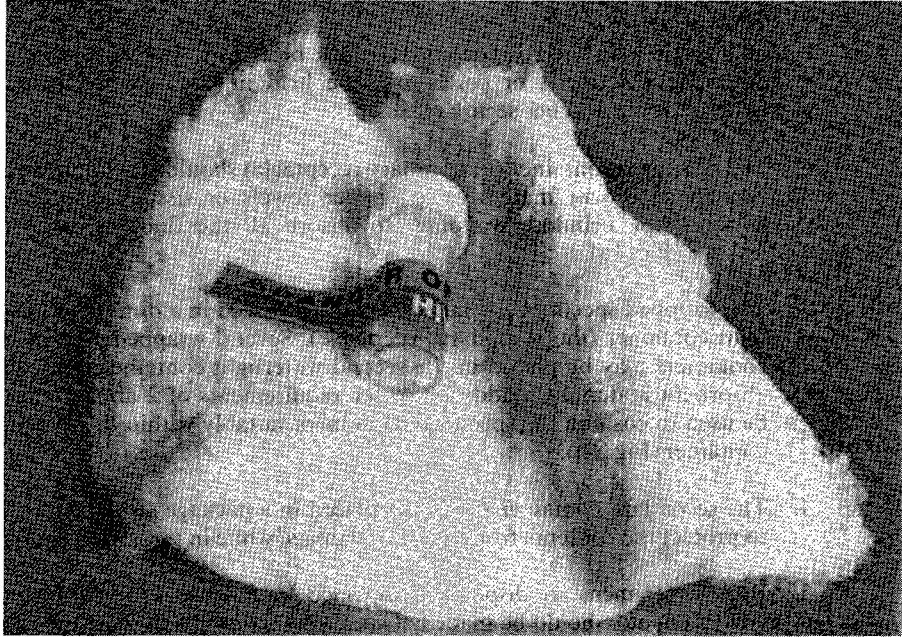
Transport of specimens by public conveyance

The United Nations Committee of Experts on the Transport of Dangerous Goods, the International Air Transport Association (IATA), the Universal Postal Union (UPU), the International Civil Aviation Organization

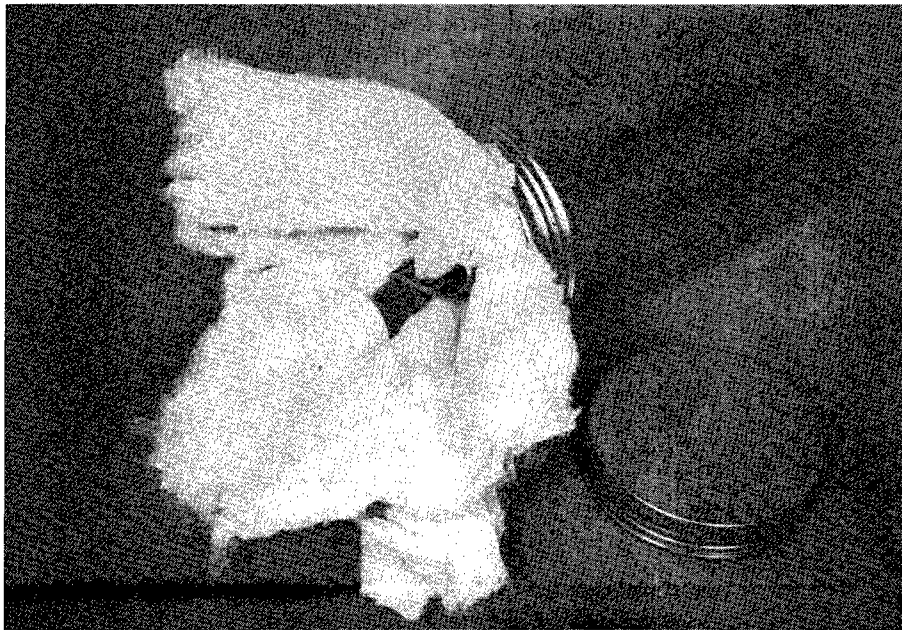
(ICAO), and the World Health Organization (WHO) have developed regulations for the shipment of specimens by mail, air freight, and other common carriers. These may be summarized as follows:

1. The specimen should be placed in a watertight receptacle of good-quality glass or plastic. The closure must be tight to prevent leaking. Screwcaps, stoppers, or corks must be held in position with wire, adhesive tape, or other secure means.
2. The specimen container (the primary receptacle) should be wrapped in enough absorbent material (e.g., paper towels or tissue, absorbent cotton wool, cellulose wadding) to absorb all the fluid in case of leakage (Fig. 1a).
3. The wrapped specimen container should be placed in a durable watertight container (the secondary receptacle). Several wrapped specimen containers may be placed in the second watertight container. Enough absorbent material (in addition to the requirements of 2 above) must be used to cushion the specimen containers suitably within the second container (Fig. 1b).
4. The secondary container should be placed in a package strong enough to protect the contents from physical damage while in transit (Fig. 1c).
5. Before specimens are dispatched, advance arrangements for shipment, collection, etc. should be made between the sender, the carrier, and the receiving laboratory.
6. Specimen data forms, letters, and other information that identify or describe the specimen should be taped to the outside of the secondary watertight container (Fig. 1d).
7. National and international shipping and transport regulations must be observed.

Fig. 1. Recommended containers for packaging of potentially HIV-infected specimens for shipment by mail, air freight, or other common carriers.



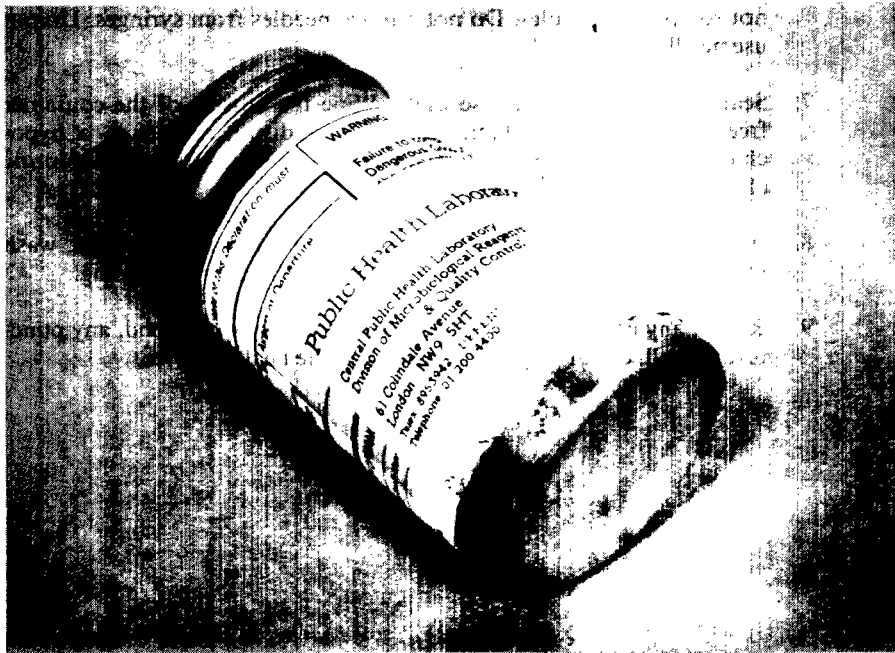
(a) Specimen container wrapped in absorbent material.



(b) Wrapped specimen container placed in durable watertight container.



(c) Secondary container placed in package of sufficient strength.



(d) Specimen forms etc. taped to the secondary container.

7. Guidelines for collection of blood samples

The major hazards to people taking blood specimens are blood contamination of the hands while drawing blood, and penetrating injuries caused by needles and other sharp instruments or objects. The following guidelines describe practices and procedures designed to minimize such accidents.

1. Inspect your hands for cuts, scratches, or other breaks in the skin. If the skin is broken, wear gloves. If blood gets on the gloves, they should be discarded.
2. Take care to avoid contaminating your hands while taking blood.
3. Wash your hands with soap and water immediately after any blood contamination and after work is completed.
4. If you wear gloves, wash your hands with soap and water after removing the gloves.
5. Wear a laboratory gown.
6. Place used needles and syringes in a puncture-resistant container. Do not recap used needles. Do not remove needles from syringes. Do not use needle clippers.
7. Seal specimen containers securely. Wipe the outside of the container free of any blood contamination with a disinfectant, e.g., a hypochlorite solution with a concentration of 0.1% available chlorine (1 g/litre, 1000 ppm) (Annex 3).
8. In the event of a needle-stick or other skin puncture or wound, wash the wound thoroughly with soap and water. Encourage bleeding.
9. Report any contamination of the hands or body with blood, any puncture wound, or cut to the supervisor and the health service.

Selected further reading

Advisory Committee on Dangerous Pathogens. *LAV/HTLV-III – the causative agent of AIDS and related conditions*. London, Her Majesty's Stationery Office, 1986.

AIDS guidelines for microbiology laboratory. Ottawa, Laboratory Center for Disease Control, 1986.

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Collins, C. H. *Laboratory-acquired infections: history, incidence, causes and prevention*, second edition. London, Butterworths, 1988.

Guidelines on sterilization and disinfection methods effective against human immunodeficiency virus (HIV), second edition. Geneva, World Health Organization, 1989 (WHO AIDS Series, No. 2).

Laboratory biosafety manual. Geneva, World Health Organization, 1983.

Working safely with HIV in the research laboratory. Bethesda, MD, National Institutes of Health, 1988.

Procedures that carry a risk of potential HIV exposure

Procedure	Person at risk	Mode of transmission
Collection of blood sample	Patient	<ul style="list-style-type: none"> – Contaminated needle – Contaminated hands or gloves of health worker
	Health worker	<ul style="list-style-type: none"> – Skin puncture by needle or broken specimen container – Contamination of hands by blood
Transfer of specimens (within laboratory)	Laboratory personnel Transport worker	<ul style="list-style-type: none"> – Contaminated exterior of container – Broken container – Spill or splash of specimen
HIV serology and virology	Laboratory personnel	<ul style="list-style-type: none"> – Skin puncture or contamination of skin or mucous membrane – Contaminated exterior of specimen container – Contaminated work surface – Spill or splash of specimen – Broken specimen container – Perforated gloves
Cleaning and maintenance	Laboratory personnel Support staff	<ul style="list-style-type: none"> – Skin puncture or skin contamination – Spills or splashes – Contaminated work surface
Waste disposal	Laboratory personnel Support staff Transport worker Public	<ul style="list-style-type: none"> – Contact with contaminated waste – Puncture wounds and cuts
Shipment of specimens (to other centres)	Transport worker Postal worker Public	<ul style="list-style-type: none"> – Broken or leaking specimen containers and packaging

Selection and care of gloves

The wearing of gloves is generally recommended for laboratory workers who handle blood specimens or other fluids that may be infected with HIV. Gloves reduce the risk of contamination of the hands with blood, but will not prevent the occurrence of penetrating injuries or cuts caused by needles, other sharp instruments, or broken glass or plastic. It is important to remember that gloves are meant to supplement, not replace, good infection control practices, including proper hand-washing.

The following general precautions should be taken regarding the use of gloves in the laboratory:

1. Wear gloves for all manipulations of potentially infectious materials. Laboratories that work with HIV require a generous supply of good-quality gloves, and adequate provision for them must be made in the budget.
2. Discard gloves whenever it is thought that they have become contaminated; wash your hands, and put on new gloves.
3. Do not touch your eyes, nose, or other exposed membranes or skin with gloved hands.
4. Do not leave the workplace or walk around the laboratory wearing gloves.
5. Wash your hands after removing gloves.

Selection of gloves

Non-sterile examination gloves of vinyl or latex are satisfactory for laboratory use. They should be used once only. General-purpose plastic utility gloves, usually known as rubber or household gloves, are satisfactory for use in cleaning instruments, decontamination procedures, and other activities where manual dexterity is not required. Such gloves may be reused.

Care of gloves

1. Surgical and examination gloves are intended for single use only. Nevertheless, in some situations these gloves will have to be reused. Gloves may be reprocessed by the following method:
 - (a) Rinse your gloved hands thoroughly in a hypochlorite solution (0.1% available chlorine) (Annex 3).
 - (b) Rinse your gloved hands in clear water to remove the disinfectant (disinfectants may cause the gloves to deteriorate).
 - (c) Wash your gloved hands with soap and water and rinse thoroughly (detergents may cause “wicking”, i.e., enhanced penetration of liquids through undetected holes in the gloves).
 - (d) Remove the gloves and hang them up by the cuffs to dry.
 - (e) Wash your hands.
 - (f) Test the gloves for holes before reuse by filling each glove with 325 ml \pm 25 ml of water at room temperature, twisting the cuff through 360°, and placing the gloves in a rack for two minutes; look and feel for leaks. If possible, dust the gloves with French chalk or talcum powder before reuse.

2. General-purpose utility gloves may be reused but should be discarded if they are peeling, cracked, or discoloured or have punctures, tears, or other evidence of deterioration. Utility gloves may be reprocessed by the following methods.
 - (a) Rinse your gloved hands thoroughly in a hypochlorite solution (0.1% available chlorine) (Annex 3).
 - (b) Rinse your gloved hands in clear water to remove the disinfectant.
 - (c) Wash your gloved hands with soap and water and rinse thoroughly.
 - (d) Remove the gloves and hang them up by the cuffs to dry.
 - (e) Wash your hands.
 - (f) Test the gloves for holes as described in 1(f) above.

Sterilization and disinfection¹

Sterilization procedures and disinfectants commonly used in laboratories and health care facilities will inactivate HIV at concentrations lower than those commonly used in general practice. Heat is the most effective method for inactivating HIV, and thus autoclaving or boiling is the method of choice, particularly for medical instruments and reusable laboratory equipment. Chemical disinfectants are less reliable, but are extremely useful in general laboratory decontamination.

It is imperative that all reusable instruments and equipment be disinfected and cleaned thoroughly before being reprocessed.

Steam sterilization

Steam sterilization (autoclaving) is the method of choice for reusable equipment, including needles and syringes. Autoclaves and pressure cookers should be kept in operation for at least 20 minutes after a temperature of 121°C (250°F) is reached; this is equivalent to a pressure of 1 atmosphere (101 kPa, 15 lb/in²) above atmospheric pressure. The autoclave should not be overloaded.

An inexpensive substitute for an autoclave is the modified pressure cooker developed by WHO and UNICEF.²

Sterilization by dry heat

Sterilization by dry heat is appropriate for instruments and equipment that can withstand a temperature of 170°C (340°F). Plastic equipment may not withstand this temperature. An ordinary household oven is satisfactory for dry-heat sterilization. Sterilization is accomplished at 170°C (340°F) for a minimum of 2 hours.

¹ Based on *Guidelines on sterilization and disinfection methods effective against human immunodeficiency virus (HIV)*, 2nd ed. Geneva, World Health Organization, 1989 (WHO AIDS Series 2).

² For more information contact: Expanded Programme on Immunization (EPI), World Health Organization, 1211 Geneva 27, Switzerland; or UNIPAC (UNICEF Procurement and Assembly Centre), Freeport, DK 2100, Copenhagen, Denmark.

Boiling

When an autoclave is not available, the simplest and most reliable method of inactivating most pathogenic microbes, including HIV, is by boiling. A high level of disinfection of instruments and equipment is achieved by continuous boiling for 20 – 30 minutes.

Chemical disinfection

Many disinfectants recommended for use in health care facilities have been found to inactivate HIV in laboratory testing. In practice, however, chemical disinfectants may not be reliable, because they may be inactivated by blood or other organic matter present. Furthermore, they must be prepared carefully. They may also rapidly lose their strength, especially when stored in a warm place.

Chemical disinfection must not be used for needles and syringes. Chemical disinfection for other skin-cutting and invasive instruments should be employed only as the last resort, if neither sterilization nor high-level disinfection by boiling is possible, and then only if the appropriate concentration and activity of the chemical can be ensured and if the instruments have been thoroughly cleaned to remove gross contamination prior to soaking (immersion) in the chemical disinfectant. Removal of gross contamination is necessary because disinfectants will not penetrate into some organic matter, such as clotted or dried blood. Instruments other than skin-piercing instruments should be roughly towelled dry before immersion, since repeated immersion of wet instruments may dilute these solutions beyond their range of effectiveness.

1. Sodium hypochlorite

Sodium hypochlorite solutions (liquid bleach, eau de Javel, etc.) are excellent universal disinfectants. A general all-purpose laboratory disinfectant solution for wiping work benches and specimen containers, cleaning gloves, etc., is one with a concentration of 0.1% available chlorine (1 g/litre, 1000 ppm). A stronger solution for laboratory use when disinfecting heavily soiled equipment, blood spills, etc., is one with a concentration of 1.0% available chlorine (10 g/litre, 10 000 ppm).

Required strength	Dilution of sodium hypochlorite solutions (parts of stock solution: parts of water)		
	5% stock solution	10% stock solution	15% stock solution
0.1% (1 g/l, 1000 ppm)	1:50	1:100	1:150
1.0% (10 g/l, 10 000 ppm)	1:5	1:10	1:15

Care is required in the preparation of the sodium hypochlorite solution because the amount of available chlorine in stock solutions varies with the country of manufacture. In some countries the concentration of sodium hypochlorite solution is expressed in chlorometric degrees ($^{\circ}$ chlorom.); 1° chlorom. is approximately equivalent to 0.3% available chlorine.

- Household liquid bleach generally contains 5% available chlorine.
- Eau de Javel (15° chlorom.) contains approximately 5% available chlorine.
- Eau de Javel (48° chlorom.) contains approximately 15% available chlorine.
- Chlorinated lime contains approximately 35% available chlorine.

Sodium hypochlorite solutions gradually lose their strength, requiring daily preparation of fresh solutions for use. The effectiveness of hypochlorite solutions is neutralized by blood, serum, and other proteinacious material. Regular replacement is required.

2. Calcium hypochlorite

Calcium hypochlorite is available in powder, granule, or tablet form. It decomposes at a slower rate than sodium hypochlorite. Calcium hypochlorite is normally formulated with 70% available chlorine. At this strength a 0.1% available chlorine solution is obtained by dissolving 1.4 g of dry chemical in 1 litre of water. A 1.0% solution is obtained by dissolving 14 g of dry chemical in 1 litre of water.

3. Sodium dichloroisocyanurate (NaDCC)

NaDCC is normally formulated with 60% available chlorine in tablet form. At this strength a solution containing 0.1% available chlorine is obtained by dissolving 1.7 g of stock in 1 litre of water. A 1.0% solution is obtained by dissolving 17 g of stock in 1 litre of water.

Tablets are also formulated with 1.5 g of available chlorine per tablet. 1 tablet dissolved in 1 litre of water provides a 0.1% solution for use.

NaDCC is more stable than sodium and calcium hypochlorite.

4. Chloramine

Chloramine is available in powder or tablet form. It is normally formulated with 25% available chlorine. Because it releases chlorine at a slower rate than other chlorine compounds, higher concentrations are required for effective disinfection.

A solution of 20 g of stock in 1 litre of water is recommended as an all-purpose disinfectant: a dilution of 40 g of stock in 1 litre of water is recommended for disinfecting heavily soiled equipment, blood spills, etc.

Chloramine is more stable than sodium and calcium hypochlorite.

5. Ethanol (ethyl alcohol) and 2-propanol (isopropyl alcohol)

Ethanol and 2-propanol are effective disinfectants, particularly for cleaning surfaces such as the exterior of specimen containers and bench tops. For maximum effectiveness, they should be used in a concentration of 70% (70% alcohol, 30% water).

6. Polyiodone iodine (PVI)

Iodophore disinfectant activity is similar to that of hypochlorite solutions. Iodophores are more stable and less corrosive but are expensive (however, they should not be used on aluminium or copper).

7. Formalin

Formalin is an excellent disinfectant, but its uses are limited because the solution and the vapour released are toxic and very irritant. Formalin contains 35–40% formaldehyde, 10% methanol, and water. It should be diluted 1:10 (to give a solution containing 3.5–4% formaldehyde) for disinfection. Rinse the equipment before reuse.

8. Glutaral (glutaraldehyde)

Glutaral is a high-level disinfectant, often used for the disinfection of non-disposable heat-sensitive equipment and instruments. It is usually available as a 2% aqueous solution. To activate, add the powder or liquid supplied with the solution.

Immersion in the activated solution destroys vegetative bacteria, fungi, and viruses in less than 30 minutes. Ten hours' immersion is required for the destruction of spores. Treated equipment must be thoroughly rinsed.

Activated solutions should not be kept more than 2 weeks, or according to the manufacturer's instructions.

Processing reusable needles and syringes

Disposable single-use syringes and needles are generally preferred for all patient care and laboratory procedures. Needle-locking syringes or one-piece needle-syringe units (disposable or reusable) should be used to aspirate fluids, so that the fluid collected can be discharged safely through the needle if desired. However, in some situations, syringes and needles suitable for reuse and sterilization may need to be used for economic and practical reasons. In such situations, it is imperative that needles and syringes are decontaminated before being reprocessed and reused.

Reusable syringes and needles can be processed by the following method. Note that gloves must be worn, and extreme care must be exercised to prevent needle-stick injuries and/or cuts.

- Leave the needle attached to the syringe.
- Aspirate hypochlorite solution, or another suitable disinfectant, containing 0.1% available chlorine, into the syringe (Annex 3).
- Immerse the syringe and the attached needle in disinfectant solution, horizontally in a flat tray.
- Leave them immersed in the disinfectant solution for 20 minutes.
- Discharge the disinfectant solution from the syringe and needle.
- Rinse the syringe and needle with water, filling and emptying several times.
- Examine needles and syringes for needle barbs, integrity of syringe seal (rubber ring), the fit of the needle hub to the syringe, whether syringe markings are readable, etc.
- Sterilize the syringe and needle by autoclaving (in a steam sterilizer) or disinfect by boiling in water for 20 minutes prior to reuse (Annex 3).

Biological safety cabinets and other containment equipment

There is no evidence that HIV or other retroviruses are transmitted by inhalation. Nor is there any evidence that inhalation of aerosols plays any part in hepatitis B infections. It has also been demonstrated that it is exceedingly difficult to make aerosols from blood because of the size and viscosity of the components. Protection from aerosols by the use of biological safety cabinets or other containment devices is therefore not strictly necessary in laboratories engaged in serology and blood screening. However, biological safety cabinets or other containment devices are indicated for use in procedures where specimens may contain other pathogens requiring such containment, for virus isolation, and where HIV is being concentrated or purified in quantities greater than those required for diagnostic procedures, e.g., in the production and manipulation of concentrated HIV.

Biological safety cabinets (class I or II, see below) or other appropriate devices for personal protection or physical containment (e.g., special protective clothing, masks, gloves, goggles, respirators, centrifuge safety cups, sealed centrifuge rotors) are recommended for use in the following circumstances:

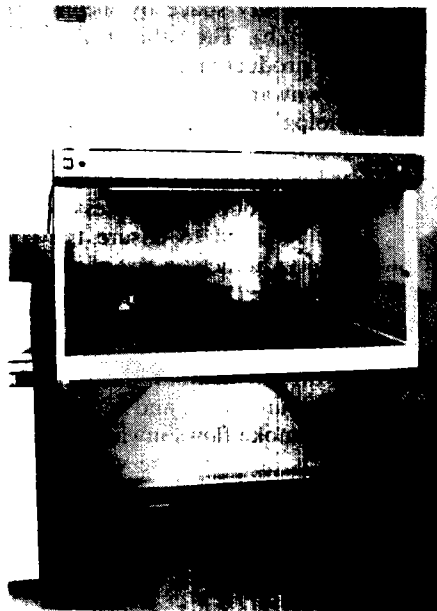
1. When procedures with a high potential for creating infectious aerosols are carried out. These include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption and opening of containers of infectious materials whose internal pressure may be different from atmospheric pressure.
2. When high concentrations or large volumes of infectious agents are used. If materials are centrifuged in the open laboratory, sealed heads or centrifuge safety cups should be used, and the heads or safety cups should be loaded and unloaded only in a biological safety cabinet.

A class I biological safety cabinet is an open-fronted work chamber which is exhaust-ventilated to provide protection for personnel and the surrounding laboratory space by means of an inward air flow away from the operator. The exhaust air is filtered through a high-efficiency particulate air (HEPA) filter before being discharged from the cabinet. A class I biological safety cabinet is recommended for all laboratories that do not have the expertise and equipment required for the routine testing of air filters, cabinet tightness, and balanced air flow. The cabinet is not designed to provide protection for material from airborne contamination (Fig. 2a).

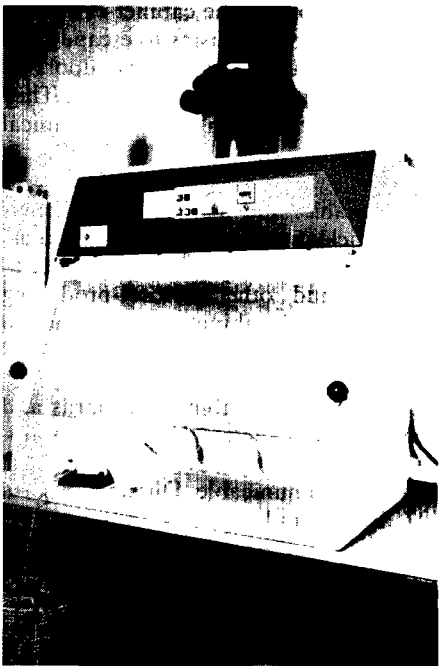
Fig. 2. Biological safety cabinets.



(a) Biological safety cabinet class I.



(b) Biological safety cabinet class II.



(c) Testing the inward airflow by the movement of tissue paper.

A class II biological safety cabinet is a partially open-fronted work chamber which provides protection for personnel and the surrounding laboratory space by means of a "barrier air flow" at the work opening (Fig. 2b). The cabinet also provides protection against contamination of the product and/or experiment by means of HEPA-filtered air flowing in a downward, uniform, unidirectional manner (laminar air flow). Class II biological safety cabinets require routine testing and servicing by trained technicians to maintain the barrier air flow and to ensure the integrity of the pressurized air flow chambers and the efficiency of the air filters. They should not be used unless skilled routine servicing is available. Several tests are required to ensure the containment efficiency of class II biological safety cabinets; a test of inward air velocity is not sufficient.

A rough test to determine if a class I biological safety cabinet provides worker protection involves the use of a chemical "smoke". A cotton swab is dipped in titanium tetrachloride, which produces a white "smoke" on exposure to air, and is passed around the perimeter of the work opening. If the smoke flows inwards, the cabinet is functioning properly. If it flows or drifts outward, the worker is not protected. Sticks or tubes of chemical smoke are commercially available. Note that titanium tetrachloride is toxic and must be used with care. An alternative method is to suspend ribbons of tissue paper around the work face (Fig. 2c) and observe whether they are sucked into the cabinet.

Care must be taken in locating the biological safety cabinet within the laboratory. Air currents across the working front of the cabinet can interfere with the protective air flow, and allow microorganisms to escape from the cabinet. The cabinets should therefore not be located near doors or windows and should be away from traffic patterns within the room. They should not be placed near the supply or exhaust grilles of mechanical ventilation systems.

If a biological safety cabinet is not available, and aerosol protection is required, a negatively ventilated work cubicle can be constructed. Workers in the cubicle should wear a HEPA-filter mask and goggles in addition to normal protective clothing. Centrifuges and other aerosol-producing devices can be placed in negatively ventilated cabinets or isolators to provide the necessary protection.

Additional information regarding biological safety cabinet standards and testing can be found in *Guidelines for biological safety cabinets*, an unpublished document of the World Health Organization (CDS/SMM/81.21), available on request from Division of Communicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland.