

**Biosafety Concerns for Flow Cytometric HIV Immunophenotyping:
Questions and Answers**

Section Contributors: Ingrid Schmid

Janet Nicholson

Annalisa Kunkl

Ingrid Schmid at schmid@mednet.ucla.edu

Q. Our laboratory has been involved in staining of samples from HIV-infected patients for flow cytometric analysis for many years. With my experience, why would I need to look at safety concerns?

A. It is always important to periodically review safety procedures in the laboratory and increase one's knowledge about the pathogens contained in the samples, because protection of laboratory workers from exposure to pathogens is critical. In addition, information about flow cytometric instrument hazards is not readily available from publications.

Q. I know that HIV is found in peripheral blood; is it also present in other body fluids?

A. Human immunodeficiency viruses, including HIV-1 and HIV-2, have been isolated from various body fluids and secretions, such as semen, cerebrospinal fluid, amniotic fluid, and urine.

Q. What has been the documented mode of transmission for HIV in the laboratory?

A. HIV has been transmitted in the laboratory directly by various routes of infection. The major source for occupationally acquired HIV infection has been through needle sticks; other infection routes are mucous membrane exposure by splashing or spattering of infectious fluids, or contamination of non-intact skin, e.g., through minor cuts, scratches, abrasions, and skin lesions (CDC MMWR, 1987; CDC MMWR, 1988).

Q. How long can HIV survive on inanimate surfaces in the laboratory?

A. Survival time of any virus on environmental surfaces is highly dependent on the concentration of virus in the sample and the environmental conditions in the laboratory, e.g., humidity, temperature. One laboratory study indicated that drying HIV causes a rapid 1-2 log₁₀ reduction in the concentration of infective virus. In this study, potentially infectious HIV could be detected for up to three days after drying. Two other studies which used cell cultures containing high titers of laboratory strains of HIV to investigate virus survival were able to detect potentially infectious virus in dried inocula for up to

seven days (for a review see Sattar, 1991). However, to be a hazard the virus must be re-constituted and inoculated into the worker.

Q. We use bleach and ethanol in our laboratory to clean our work areas. Do these two disinfectants differ in their activity against HIV?

A. HIV can be inactivated by various intermediate-strength disinfectants (Druce, 1995) such as chlorine compounds, ethyl alcohol, and isopropyl alcohol. Alcohols are less effective against HIV than chlorine (Martin, 1985). However, it is important to take into account that diluted bleach (1:100) is not stable and rapidly loses activity against pathogenic microorganisms, particularly when it is exposed to heat and light (NCCLS M29-A, 1997). Furthermore, the purity of the water that is used for the dilution of concentrated bleach influences its germicidal activity.

Q. Is there any difference in the ability of disinfectants to inactivate HIV contained in tissue culture cells or in whole blood?

A. Yes. The type of sample in which HIV is contained alters the contact time of the disinfectant to the virus for inactivation. Exposure time of the disinfectant to HIV needs to be longer for cell-associated than for cell-free virus; inactivation of cell-associated HIV in blood takes the longest. A study by Druce et al. (Druce, 1995) showed that complete inactivation of HIV suspended in whole human blood took 10 minutes of exposure to commercial chlorine compounds, while a 1:100 dilution of a 5.25% hypochlorite solution (concentrated household bleach) with a minimum content of 500 mg/L of free available chlorine could inactivate cell-free HIV within two minutes (NCCLS M29-A, 1997).

Q. How should we clean work areas in our laboratory?

A. Because bleach may lose most of its activity against microorganisms within 24 hours it is important to prepare a fresh 1/10 to 1/100 dilution of 5.25% household bleach for disinfection of work areas every day. Do not scrape off dried blood; always remove it by cleaning it with detergent disinfectant first to remove organic material, because both bleach (Van Bueren, 1995) and alcohols lose germicidal activity in the presence of organic

substances. Furthermore, alcohol is not an ideal disinfectant for work areas because it evaporates rapidly (Van Bueren, 1994). Use of a general hospital disinfectant that also has mycobacteriocidal activity for wiping laboratory surfaces can provide a wide safety margin.

Q. We always turn on the UV lights in the biosafety hood overnight. Does UV exposure have any effect on HIV?

A. Yes, it does. However, similar as with disinfectants, UV-inactivation of HIV is strongly influenced by the biological composition of the medium in which HIV is contained. A published report by Druce et al. (Druce, 1995) demonstrated that cell-free HIV was inactivated in a biological safety cabinet at any distance from the UV light source within 10 minutes; it took 30 minutes to UV-inactivate cell-associated HIV. In contrast, HIV contained in whole blood was not completely inactivated even after a 60 minute exposure to UV light.

Q. We have been getting blood samples for staining delivered to our laboratory, now we will have to pick them up from a blood collection site. How should I transport these samples?

A. You should place blood collection tubes into a second container which is able to contain the specimen in case the primary container breaks; e.g. a plastic bag with a leakproof seal may be used as a container for individual tubes; a rack with tubes may be placed into a plastic carrier with a secure lid. The secondary container should have a biohazard symbol attached. It is not necessary to wear gloves in transition, but they must be put on whenever the primary container is removed from the secondary one in the laboratory. Take care that laboratory requisition slips and any other paperwork that accompanies the collection tubes remain uncontaminated.

Q. I have been asked by my laboratory director to send blood samples to a another laboratory. How should I package these tubes?

A. Samples which contain HIV are required to be shipped as an infectious agent and are classified as an infectious class 6.2 substance under the United Nations (UN) number 2814.

This means that HIV-containing samples can only be transported in packaging that meets UN class 6.2 specifications and associated packaging and labeling instructions. These instructions are available from United States Department of Transportation Hazardous Materials Regulations (DOT, 1998) and from the International Air Transport Association (IATA) Dangerous Goods Regulations.

Q. Is there a procedure I should follow for unpacking when I receive a shipment of blood samples?

A. Carefully inspect the package for leakage and visible damage. Open packages that show signs of leakage or damage in a biological safety cabinet. Before you remove the blood collection tubes from the container inspect them for visible contamination and breakage. If tubes were contaminated, decontaminate them or transfer the contents to a clean sample tube before using them in the work areas for staining. Also, clean any contaminating spills from the secondary container with suitable disinfectants. Store all samples up-right in a suitable rack in low-traffic areas of the laboratory to avoid breakage and accidental spills (NCCLS H18-A, 1990).

Q. We have been staining blood samples in our laboratory for many years, but I would like to know if we need to modify our staining procedure to improve safety aspects. Are there any recent guidelines for sample staining available?

A. Yes. The following procedure has been adapted from the NCCLS document M29-A: Always remove the rubber stopper of evacuated blood collection tubes and caps of other types of tubes in a class I or class II biosafety cabinet using a gauze pad to minimize blood spattering, because of the high probability of splashes and blood spraying. Gauze pads with plastic backing and vacutainer stopper removers are commercially available and can aid in reducing contamination of gloves with blood. If gloves are contaminated during this procedure, they should be changed. Wash your hands immediately after glove removal. After completion of the task, discard all materials used for uncapping tubes into appropriate biohazard containers. Perform aliquoting of blood into sample tubes and any procedure that can produce droplets and aerosols such as mixing and vortexing within a

biosafety cabinet. When samples have not yet been subjected to a fixing reagent, cap all sample tubes before removal from the safety cabinet. For centrifugation of unfixed sample tubes, always cap tubes securely or use safety cups that tightly cover centrifuge carriers.

Q. We routinely fix our samples in 1% formaldehyde solution. Has formaldehyde been shown to inactivate HIV?

A. Yes. Reports in the literature (Martin, 1985; Martin, 1987) have shown that a formaldehyde concentration of 0.5% to 2%, a concentration range commonly used for sample fixation in cytometry, are effective in inactivation of HIV. A thirty minute fixation in a buffered formaldehyde solution before acquisition on the flow cytometer is typical and is considered effective for inactivation of cell-associated HIV contained in samples from HIV-infected patients.

Q. We use 'FACS Lysing Solution' for lysing the red cells in our whole blood samples. Does it have any anti-viral activity against HIV?

A. Yes, it does. Laboratory studies (Nicholson, 1993; Nicholson, 1994) indicated that several commercially available lysing/fixing reagents, including 'FACS lysing solution', 'Coulter Immunolyse', 'Whole blood lyse and fix;' and 'OptiLyse' were able to decrease titers of cell-associated HIV virus by 3-5 log₁₀ when they were used according to the manufacturer's recommendation; this degree of reduction in infective virus concentration is considered effective inactivation.

Q. We use ammonium chloride lysing solution for red cell lysis. Does it have any effect on HIV infectivity?

A. No, ammonium chloride lysing solution has no anti-HIV activity; therefore, when red cells in samples are lysed with ammonium chloride, cells need to be fixed in 0.5% to 2% formaldehyde for inactivation of cell-associated HIV. Always make sure that fixation protocols are followed carefully, i.e., use the fixative at an effective concentration and exposure time, because samples that are considered properly fixed, but in fact are not, may expose laboratory personnel to pathogens contained in the samples.

Q. I have been asked to use a new staining protocol that does not permit sample fixation; should I be concerned about running these samples through the flow cytometer?

A. Samples for flow cytometric analysis should be fixed whenever possible. However, there are flow cytometric applications, e.g., certain methods for measuring apoptosis, measurement of calcium flux, viable cell sorting, that require cells be run through the instruments unfixed. For improved operator safety, newer analytic flow cytometers incorporate biosafety features that reduce the risk of operator exposure to sample hazards; these include enclosed flow cells, automated enclosed sample introduction systems, and droplet containment modules. However, because flow cytometers are generally too large to operate within a biosafety cabinet, unfixed samples have to be handled on the open laboratory bench during these experiments. To compensate for the fact that potentially infectious aerosols are not contained within a safety cabinet, it is advisable for these experiments to combine biosafety level 2 facilities and safety equipment with biosafety level 3 practices (see Table 1).

Q. We have a flow cytometer with an enclosed fluidic system. Are there any safety aspects I need to consider during its operation?

A. If your instrument has a manual sample introduction port, samples need to be mixed individually before they are placed on the cytometer. Because sample mixing is generally done outside of a biosafety cabinet be cautious to prevent the release of aerosols and accidental splashes. If unfixed samples are used, it is recommended that biosafety level 2 safety equipment is combined with biosafety level 3 practices as outlined in Table 1. In this case, mix the capped sample tube gently, then slowly remove the cap with the tube pointing away from you. Place the tube securely onto the sample introduction port to prevent accidental sample spills and splashes. Some sample ports contain a metal sip tube that could pose a risk of damaging gloves when the tube is not inserted diligently. Gloves and protective clothing should be worn during sample loading and during manual acquisition of samples; wearing wrap-around safety glasses will help to protect eyes from accidental sample splashes.

Q. Our flow cytometer has an automated sample loading system. Are there still safety aspects I need to consider for its use?

A. Sample loading systems reduce individual sample handling and perform sample vortexing in an enclosure which prevents operator exposure to aerosols and splashes. Take special care when loading samples onto the instrument tube racks. For unfixed samples, load instrument tube racks inside a biosafety cabinet to contain splashes and aerosols.

Q. In addition to the clinical studies we do in our laboratory, we may need to perform cell sorting of unfixed human cells; eventually we may start to sort samples known to be infected with HIV. Are there any recommendations available for performing these types of experiments?

A. Yes. For details on performing viable cell sorting experiments, refer to the guidelines generated by the Biohazards Working Group of the International Society for Analytical Cytology (ISAC) for sorting of unfixed cells (Schmid, 1997) which include recommendations for sample handling, operator training and protection, laboratory design, instrument setup and maintenance, and testing for instrument aerosol containment. These recommendations also apply whenever unfixed human cells are run through a jet-in-air flow cytometer.

Q. We have a FACSort instrument that we occasionally use for cell sorting. Do I need to be concerned about exposure to aerosols?

A. No. Some enclosed fluid system flow cytometers perform cell sorting using a fluid-switching mechanism. These cytometers, in contrast to jet-in-air cell sorters, do not generate aerosols during cell sorting.

Q. I know how to properly dispose of my blood samples, but is there something I need to consider for the disposal of my stained samples?

A. Stained, unfixed samples are disposed in the same way as primary blood collection tubes according to state and institutional regulations for biohazardous waste (NCCLS GP5-

A, 1993). Stained, formaldehyde-fixed samples are not considered biohazardous waste. Fixed samples should never be autoclaved, because autoclaving formaldehyde-containing samples may release toxic formaldehyde gas and thus lead to exposure of personnel. Fixed samples need to be disposed as chemical waste according to the regulations of your institution for further processing in chemical waste incinerators that are equipped with filters to prevent release of toxic chemicals into the environment.

Q. I have heard that laser beams can do serious damage to the eye. Do I need to be concerned when I operate my flow cytometer?

A. This depends on the type of flow cytometer you run your samples on. Most newer analytic flow cytometers are equipped with low-power lasers which are fully contained and are therefore classified as Class I lasers. Class I lasers do not require special work area safety requirements. However, jet-in-air flow cytometers and cell sorters can contain high-power lasers that may be classified as Class IV lasers, because the laser beam is not fully enclosed during routine laser and instrument alignment procedures. Alignment safety goggles should be worn whenever the laser beams are exposed to prevent eye exposure. In addition, special work area safety regulations need to be followed; these are described in a publication available from the Laser Institute of America (Sliney, 1993).

Q. How do I properly handle the waste fluid from my flow cytometer?

A. Collect the effluent from the instrument in a receptacle containing fresh concentrated household bleach in sufficient quantity to achieve a final concentration of 10% when the container is full. Dispose of the contents of the container according to institutional and local regulations for waste disposal.

Q. Are there any biosafety concerns for performing instrument maintenance and minor repairs?

A. Follow carefully all instructions and maintenance recommendations from the manufacturer of the instrument. All preventive maintenance and repairs should be performed under standard laboratory precautions. Consider all instrument components that

come in contact with sample fluid contaminated and work carefully making sure that gloves are not damaged by instrument parts during service and repairs.

Q. How should I clean fluid lines and disinfect flow cytometer parts?

A. Disinfect flow cytometer fluid lines regularly by running disinfectants through the instrument lines, e.g., a 1:10 dilution of a 5.25% sodium hypochlorite solution (concentrated household bleach) for at least 10 minutes; always follow with distilled water until the disinfectant is rinsed out. As mentioned previously, it is critical that the dilutions of the concentrated sodium hypochlorite solution are made up fresh daily, because diluted bleach may lose most of its anti-microbial effect after 24 hours. For disinfection of flow cytometer parts keep in mind that bleach is corrosive and follow the manufacturer's recommendations for appropriate disinfectants.

Q. We have a new employee who will join our laboratory. Are there any official safety training protocols that I have to follow?

A. Personnel should be trained in laboratory safety procedures before work is started and strict adherence to safety protocols should be emphasized and monitored. Training and monitoring must follow Occupational Health and Safety Administration (OSHA) standards or equivalent local requirements. Cell sorting of known biohazardous samples should be performed by experienced flow cytometry operators, ideally with a minimum of two years experience in cell sorting of non-infectious samples.

Q. Are there any recommendations for the management of accidental laboratory exposure to HIV?

A. At the start of their employment, all laboratory personnel should provide a serum sample for future assay in case of occupational exposure. Educate all personnel in first aid after laboratory accidents and in the standard institutional accident reporting requirements. Establish a written laboratory policy for post-exposure management after occupational exposure to HIV that is routinely up-dated to incorporate the most current CDC guidelines for chemoprophylaxis (NCCLS M29-A, 1997; CDC MMWR, 1996). Collaborate for the

implementation of the recommendations with persons who have expertise in anti-viral therapy and HIV transmission.

Q. Are there any documented transmissions of HIV through the use of a flow cytometer?

A. No. To date there are no reports of laboratory transmissions of HIV due to the use of a flow cytometer. However, it is always important to observe safety precautions to protect instrument operators from exposure to known and unknown pathogens contained in the samples.

References

- Center for Disease Control, Morbid Mortal Wkly Rep 1987;36 (Suppl):2S, 3S-18S.
- Center for Disease Control, Morbid Mortal Wkly Rep 1988;37:377-388.
- Center for Disease Control, Morbid Mortal Wkly Rep 1996; 45:468-472.
- Druce JD, Jardine D, Locarnini SA, Birch CJ. Susceptibility of HIV to inactivation by disinfectants and ultraviolet light. *J Hosp Inf* 1995; 30:167-180.
- Laser Safety Guide 9th edition, Sliney DH (ed.), Laser Institute of America, Orlando, FL, 1993.
- Martin LS, McDougal JC, Loskoski SL. Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy-associated virus. *J Inf Dis* 1985; 152:400-403,.
- Martin LS, Loskoski SL, McDougal JS. Inactivation of human T-lymphotropic virus type III/lymphadenopathy-associated virus by formaldehyde-based reagents. *Applied Environmental Microbiology* 1987; 53:708-709.
- National Committee for Clinical Laboratory Standards 1990. Procedures for the Handling and Processing of Blood Specimens, Vol. 10 No. 12, document H18-A.
- National Committee for Clinical Laboratory Standards 1993. Clinical Laboratory Waste Management, document GP5-A.
- National Committee for Clinical Laboratory Standards 1997: Protection of laboratory workers from infectious disease transmitted by blood, body fluids, and tissue, 2nd ed., document M29-A.
- Nicholson JKA, Browning SW, Orloff SL, McDougal JS. Inactivation of HIV-infected H9 cells in whole blood preparations by lysing/fixing reagents used in flow cytometry. *J Immunol. Methods* 1993; 160:215-218.
- Nicholson JKA, Browning SW. Ability of 'OptiLyse' lysing/fixing reagents to inactivate HIV-infected H9 cells in whole blood. *J Immunol Methods* 1994; 168:283-284.

Sattar SA and Springthorpe VS. Survival and disinfectant inactivation of the human immunodeficiency virus: a critical review. *Rev Inf Dis* 1991; 13:430-447.

Schmid I, Nicholson JKA, Giorgi JV, Janossy, G, Kunkl A, Lopez PA, Perfetto S, Seamer L, Dean PN. Guidelines for sorting of unfixed cells. *Cytometry* 1997; 28:99-117.

United States Federal Code Regulation: Hazardous materials regulation, 1998; 49 CFR Part 171-178.

Van Bueren J, Larkin DP, Simpson RA. Inactivation of human immunodeficiency virus type 1 by alcohols. *J Hospital Infection* 1994; 28:137-148.

Van Bueren J, Simpson RA, Salman H, Farrelly HD, Cookson BD. Inactivation of HIV-1 by chemical disinfectants: sodium hypochlorite. *Epidemiology and Infection* 1995; 115:567-579.

Table 1. Biosafety level 2 containment and level 3 practices*

Biosafety levels	BSL2	BSL2 using BSL3 practices
A. Laboratory facilities		
1. Ventilation	Negative pressure	Negative pressure
2. Posted hazard sign	Required	Required
3. Laboratory separated from the general public	Yes, while experiments are in progress	Yes, while experiments are in progress
B. Containment equipment		
1. Biosafety cabinets or other physical containment system	Required for all aerosol generating processes	Required for all work with infectious agents
2. Biosafety cabinet certification	Annually	Annually
3. Other physical containment	Appropriate physical containment devices , e.g. centrifuge safety cups, are used when procedures with a high potential for creating infectious aerosols are being conducted ^a	Appropriate physical containment devices, e.g. centrifuge safety cups, are used when procedures with a high potential for creating infectious aerosols are being conducted ^b
4. Freezers/refrigerators	Biohazard sign must be posted	Biohazard sign must be posted; all agents are stored separate in closed, labeled, containers
5. HEPA-filtered vacuum lines	Recommended	Recommended
6. Personal protective equipment (i.e. laboratory coats, gloves, etc.)	Required - gloves should be worn when skin contact with infectious material cannot be avoided	Required - combinations of special protective clothing, gloves, respirator masks, wrap-around safety eyeglasses, etc., are used for all activities with infectious materials that pose a threat of aerosol exposure ^c
C. Practices		
1. Public access during experiments	Controlled	Not permitted
2. Decontamination	Daily and upon spills; waste before disposal	Daily, upon finished work with infectious material, and spills; waste before disposal
3. Pipetting	Mechanical devices required	Mechanical devices required
4. Eating, drinking, smoking and application of cosmetics	Not permitted at any time	Not permitted at any time
5. Handwashing facilities	Required	Required
6. Minimization of aerosol production	Recommended	Recommended
7. Autoclave on-site facility	Must be available within the building	Must be available within the building
8. Insect/rodent control program	Required	Required
9. Bench top work	Permitted	Permitted, not recommended
10. Transport of infectious material	Durable leakproof container	Durable leakproof container
D. Training		
1. Technical training	Required	Required
2. Medical surveillance (i.e. baseline serology)	Required when appropriate	Required when appropriate

These procedures include, but are not limited to centrifuging, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures

These procedures include manipulation of cultures and of clinical or environmental material that may be a source of infectious aerosols

Required with aerosol generating equipment, manipulation of high concentrations or large volumes of infectious materials; activity involving all clinical specimens, body fluids and tissues from humans or from infected animals

* This table was adapted from "Working with Biohazardous Materials", Facilities Safety Procedure 360.01, Lawrence Livermore National Laboratory (1992)