Biosafety Concerns for Flow Cytometric HIV Immunophenotyping: Questions and Answers

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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.

biosafety cabinet. When samples have not yet been subjected to a fixing reagent, cap all

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

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

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

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 6 ( ) -4 (p) 6 (o) 8 (-7 (e)4 (e)(o) 8 (o) 4 (s) 4 (p) 6 /F3.0 1 Tf [(a) 6 (f)r) m /ȚTJ ab

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.

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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
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	