Biosafety at the IIC Homewood Flow Cytometry Resource (HFCR)

Cell sorting experiments that include human or non-human primate cells or cell lines must be scheduled for sorting in a biosafety cabinet. The HFCR currently has two cell sorters: 1) the Sony SH800 located in Rm 281 Croft; and 2) the SY3200 located in Room 109 Dunning Hall. We adhere to well established polices in accordance with the *International Society of Analytical Cytology* (Schmid et al. 2007). These policies have been adopted by our Johns Hopkins University, Office of Health, Safety & Environment (HS&E); and are summarized below. Importantly, all unfixed human or non-human primate samples submitted to the HFCR for analysis or sorting must be scheduled in advance identifying the source of the sample as well as all potential biohazards.

Our HFCR request form must be filled out; approved by the HFCR manager Ms Hanhvy Bui <mailto:hanhvy.bui@jhu.edu?subject=HFCR request form> and presented at least two days prior to all sorts. Further detailed biosafety information/guidelines can be found at the International Society for Advancement of Cytometry website: <http://isac-net.org/Resources-for-Cytometrists/Biosafety.aspx>.

Biosafety/Biohazard Protocols:

The Johns Hopkins University, Office of Health, Safety & Environment (HS&E), and the HFCR adhere to the well established guidelines put forth by the International Society of Analytical Cytology (Schmid et al. 2007). The IIC has downloadable packets of our standard operating procedures (SOP) available through HFCR website (http://www.jhu.edu/iic/Flow%20Cytometry.htm). Each instrument has its own custom SOP for users tofamiliarize themselves with prior to training. This is also the case for our flow cytometers and cell sorter. Specifically, as it pertains to the cell sorter, our IIC SOP guidelines include a discussion of 1) biological hazards in sorting unfixed cells; 2) laser hazards; 3) personal protective equipment (guidelines for use/application); 4) biosafety design and operation; 5) sample assessment and safe handling; 6) aerosol containment; 7) waste management.

Each of these sections is briefly summarized below:

1) Biological hazards in sorting unfixed cells: All manipulations of unfixed mammalian samples potentially harbor known/unknown pathogens that may be transmitted through droplets and aerosols that are generated during the cell sorting process. This requires treating of all unfixed mammalian specimens (especially human/primate) as potentially contaminated with HIV, Hepatitis, HSV, etc. and other unknown viruses, bacteria, and pathogens. In such cases, appropriate containment and personal protective equipment, and SOPs must be used/followed for sorting. Per the OSHA Bloodborne Pathogens Standard and Hopkins policy, such samples shall be considered as potentially infectious and include:

- Unfixed mammalian materials, including cultured cells and environmental samples;
- Unfixed cells from primary culture or immortalized cultures deriving from human/primate or of human origins (e.g. HeLa cells, Cos7, Vero, tumor cells lines, etc);
- Unfixed cells from primary and immortalized cultures from animal donors, including nonhuman, primates, other species, and transgenic cells and animals.

It is incumbent upon the PI or other research staff requesting the analysis or cell sorting, to thoroughly divulge any and all potentially infectious or biologically hazardous materials in any specimen submitted. The HFCR questionnaire can be downloaded from the HFCR website and must be filled out in full and approved by Ms. Hanhvy Bui <mailto:hanhvy.bui@jhu.edu>

2) Laser Hazards: Lasers are used in flow cytometers and cell sorters. The instruments have safety interlocks that present a laser hazard if the safety covers are removed or the interlocks are bypassed. All users will be instructed to NOT CIRCUMVENT the interlocks!

3) Personal Protective Equipment (PPE): The operator, and any guest in the sorter room, must wear appropriate PPE. These items are provided by the IIC, and includes, at a minimum, nitrile gloves, a disposable Tyvek gown, and eye protection. With many materials, especially anything mammalian, respiratory protection is required. The IIC provides disposable N-95 and N-100 respirator masks. Additionally, the sorter-operator, when deemed appropriate for certain materials, will use the 3M Air-Mate™ Powered Air Purifying System (PAPR) available in Room 109. The sorter operator will don appropriate PPEs in the anteroom prior to entering the sorter room.

4) Biosafety design and operation: All mammalian materials, including tissues, primary and immortalized cell lines, are defined as Risk Group 2 (RG2) materials for the purpose of laboratory manipulations; and must be handled with Biosafety Level 2 containment and practices. Room 109 Dunning Hall is designed with unidirectional, inward airflow (negative pressure) ventilation at a minimum of Biosafety Level 2 containment. That is, the cell sorter room dedicated to the SY-3200, will be at negative pressure to the anteroom; and the anteroom will be at negative pressure to the hallway.

When the sorter/room 109 is in use, the hallway door will be closed, locked, and the hallway sign, 'Sorter-in use', will be illuminated. When in the sorter room, the sorter-operator will have the sliding door separating the anteroom from sorter-room closed and the 'Sorter-In-Use' light illuminated. When deemed appropriate, the sorter-operator will use the appropriate PPEs provided.

As a strict rule, all samples will be opened, manipulated, and loaded in the Baker biosafety cabinet containing the sorter-head. The sorter-operator will not exit the sorter-room during sorting. The operator will either leave after initial alignment and calibration, and after loading the sample, prior to sorting; or exit after sorting. When exiting the room, the sorter-operator should de-gown; dispose of the gown in the biosafety box; and wash his/her hands. Turn the 'sorter-in-use' light to off; and exit to the anteroom and close the sliding glass door behind you.

When exiting the anteroom to the hallway, the sorter-operator will turn off the 'Sorter-in-use' light, exit the room, and close/lock Room 109 behind you. The sorter room will remain locked when not in use. Please note that food and drinks are prohibited in Room 109; and only mechanical pipetting devices are permitted. Work surfaces in both the sorter room and anteroom must be disinfected with 70% EtOH.

Any spills will be cleaned and decontaminated according to posted, well-established Hopkins' HS&E biosafety protocols.

5) Sample assessment and safe handling: An assessment of each sample must be carried out by the HFCR manager, Ms. Hanhvy Bui, to ascertain the origin of the sample and evaluate the potential presence of pathogens or genetically modified material. This is critical; there will be no exceptions! People presenting material for sorting will have completed the biosafety questionnaire provided and will have received signed approval by HFCR manager (Ms. Hanhvy Bui) prior to the scheduling/start of any new sort. Importantly, the appropriate biosafety containment level must be followed as it pertains to the source and status (e.g. live vs. fixed cells) of the sample. Additionally, posted guidelines on handling and proper disposalof biological materials must be followed in the IIC and HFCR at all times.

6) Aerosol Containment: Only personnel who have been trained by Ms. BUI, the HFCR manager, in appropriate safety protocols will be permitted to operate the flow cytometers and SH-800 (Rm 281 Croft Hall); and only Ms Bui will operate the SY 3200 cell sorter (Rm 109 Dunning Hall.

The efficiency of aerosol control measures on the instruments will be tested periodically. The Biosafety hood must be on and operational throughout the sort. The hood will be tested regularly and records will be maintained to confirm that the hood is functioning normally. Monthly testing, as suggested by the Hopkins' HS&E office, will be carried out and documented in writing. Monthly testing of the flow scattering using the calibrated fluorescent microspheres 'Glo Germ'™ is done to monitor/assay the aerosol containment.

7) Waste Management: Liquid waste resulting from sorting will be collected from the instrument into the provided container that contains fresh concentrated bleach in sufficient quantity to achieve a minimal 10% final concentration. Fluid lines shall be decontaminated routinely with freshly diluted (1% to 10%) bleach solution. After sitting overnight in 10% bleach, waste may be disposed of down the drain.

8) Biosafety training: In accordance with the OSHA BBP Standard, Hopkins' Biosafety policies, and in compliance with the NIH Guidelines for Research Involving recombinant DNA, all personnel working with potentially infectious materials must participate in the campus biosafety training upon initial hire and on an annual basis thereafter for refresher training. In accordance with these guidelines, HFCR supervisor Ms. Hanhvy Bui, IIC Manager Ms. Erin Pryce, and IIC Director McCaffery are available to advise and consult on safe handling of samples prior to, during, and post sorting. No one may work in the IIC until they have first taken and passes the Hopkins' biosafety course.

Biological Spill Response Guide
(Courtesy Northwestern University)

Biological Spills

A biological spill shall be followed by prompt action to contain and clean up the spill. When a spill occurs, warn everyone in the area and call for assistance as needed. The degree of risk involved in the spill depends on the volume of material spilled, the potential concentration of organisms in the material spilled, the hazard of the organisms involved, the route of infection of the organisms, and the diseases caused by the organisms.

Spills of biological agents can contaminate areas and lead to infection of laboratory workers. Prevention of exposure is the primary goal in spill containment and cleanup, exactly as in chemical spills. In evaluating the risks of spill response, generation of aerosols or droplets is a major consideration. If an accident generates droplets or aerosols in the laboratory room atmosphere, especially if the agent involved requires containment at Biosafety Level 2 or higher, **the room shall be evacuated immediately**.

Doors shall be closed and clothing decontaminated. Call Hopkins' HS&E (410.955.9213) to supervise the cleanup. In general, a 30-minute wait is sufficient for the droplets to settle and aerosols to be reduced by air changes. Longer waiting periods may be imposed depending on the situation. Laboratory personnel and/or HS&E must exercise judgment as to the need for outside emergency help in an evacuation.

If a spill of a biological agent requiring containment at Biosafety Level 2 or higher occurs in a public area, evacuation of the area shall be immediate. The principal investigator shall be responsible for designating the extent of evacuation until HS&E or emergency personnel arrive. Prevention of exposure to hazardous aerosols is of primary importance. Anyone cleaning a spill shall wear personal protective equipment (for example, laboratory coat, shoe covers, gloves, and possible respiratory protection) to prevent exposure to organisms. An air-purifying negative-pressure respirator with P-100 filter cartridges is generally adequate protection against inhalation of most biological agents. However, there may be exceptions. Contact HS&E for advice in choosing the correct respiratory protection and for information regarding the requirements that must be met to wear a respirator. An appropriate chemical disinfectant (see attached chart at the end; or use 70% EtOH or 10% Bleach) should be chosen that is effective against the organisms involved in the spill.

Sterilization, Disinfection, and Decontamination.

The Environmental Protection Agency recognizes the following categories of chemical germicides (a germicide is an agent that kills pathogenic organisms). The information in this section is drawn from *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*, Approved Guideline, NCCLS Document M29-A, Vol. 17, No.20 (National Committee for Clinical Laboratory Standards, December, 1997).

Sterilizer or **Sterilant**: An agent intended to destroy all microorganisms and their spores on inanimate surfaces.

Disinfectant: An agent intended to destroy or irreversibly inactivate specific viruses, bacteria, or pathogenic fungi, but not necessarily their spores, on inanimate surfaces. Most disinfectants are not effective sterilizers.

Hospital Disinfectant: An agent shown to be effective against specific organisms such as *Staphylococcus aureus*, *Salmonella choleraesuis*, and *Pseudomonas aeruginosa*. It may also be effective against other organisms and some viruses. The labels of all commercially available hospital disinfectants contain a claim (which must be documented) of effectiveness for specific agents.

Antiseptic: A chemical germicide formulated for use on skin or tissue. Antiseptics should not be used as disinfectants.

Decontamination: A procedure that eliminates or reduces microbial contamination to a safe level with respect to the transmission of infection. Sterilization and disinfection procedures are often used for decontamination.

The OSHA Bloodborne Pathogens Standard requires that all equipment and environmental and working surfaces shall be cleaned and decontaminated after contact with blood or other potentially infectious materials. The standard also requires decontamination of contaminated work surfaces after completion of procedures, immediately or as soon as feasible after any overt contamination of surfaces or any spill of potentially infectious material, and at the end of the work shift if the work surface has become contaminated. All reusable equipment shall be decontaminated immediately or as soon as feasible upon visible contamination.
It should be emphasized that, for any infectious material, adequate precleaning of surfaces is important for any disinfection or sterilization procedure. Ten minutes of exposure to a disinfectant may not be adequate to disinfect objects that have narrow channels or other areas that can harbor microorganisms.

Alcohols, for example, are effective for killing hepatitis B virus (HBV) but are not recommended for this purpose because of their rapid evaporation and the consequent difficulty of maintaining proper contact times.

Chlorine compounds are probably the most widely used disinfectants in the laboratory. You can easily prepare an inexpensive, broad-spectrum disinfectant by diluting common household bleach. Bleach is a 5.25% sodium hypochlorite solution-this is equal to approximately 50,000 ppm of free available chlorine. This level of chlorine can be harmful to skin and eyes. Lower concentrations are effective in disinfection and are less hazardous for the worker. The concentration to be used is based on your assessment of the severity of the contamination or spill of infectious materials.

- For small spills of infectious agents or for contamination on hard, smooth surfaces, a 1:100 dilution of commercial bleach is adequate. This is equivalent to 500 ppm of free chlorine.

- In the case of large or concentrated spills of infectious agents, a higher level of chlorine is needed to be effective in destroying the microorganisms. Use a 1:10 dilution (5,000 ppm of free chlorine) and flood the contaminated area with the solution. Alternatively, you can mix the disinfectant with the spilled material. This higher concentration is more suitable for porous surfaces that may harbor organisms in tiny cracks or pits.

Make the solution fresh each day. Be aware that chlorine compounds may corrode metals, especially aluminum. While a 10% household bleach solution is a commonly used decontaminant concentration, it is probably stronger than necessary for ordinary uses. It can be extremely irritating to personnel. Therefore, the use of higher concentrations of bleach in chemical fume hoods, and the autoclaving of materials that have been treated with bleach, should be reserved for significant contamination.

* Note that bleach will react with water to form hypochlorous acid (HOCl), which will decompose to chlorine (Cl2) and hydrogen chloride (HCl). Special care should be taken when autoclaving hypochlorite solutions because the procedure can generate chlorine gas, which will corrode steel. To avoid evolution of chlorine, the hypochlorite solution should be neutralized with sodium thiosulfate prior to autoclaving.

Formaldehyde is an OSHA-regulated chemical that is a suspect carcinogen, so its use as a disinfectant is not recommended.

Iodophors that are registered with the EPA may be effective hard-surface decontaminants when used per manufacturer's instructions, but iodophors formulated as antiseptics are not suitable for use as disinfectants.

Peracetic (peroxyacetic) acid and hydrogen peroxide mixtures minimize the negative effects of corrosiveness sometimes seen with chlorine compounds and high concentrations of peracetic acid alone. A limited number of trade-name products containing <0.1% peracetic acid and <1.0% hydrogen peroxide and registered with the EPA as sterilants/disinfectants are available. The benefit of these products is their rapid action and broad-spectrum of germicidal activity, in addition to the reduced corrosiveness.

In Section 5.0 of the Laboratory Safety and Chemical Hygiene Plan, the use of any chemicals with the prefix "per" is discouraged for cleaning glassware due to the reactivity of oxidizing materials. Peracetic acid is generally a strong irritant. The low percentage in these products reduces this danger. Nonetheless, these products are intended only for highly concentrated spills of biological materials.

Quaternary ammonium compounds are low-level disinfectants and are not recommended for spills of human blood, blood products, or other potentially infectious materials. are low-level disinfectants and are not recommended for spills of human blood, blood products, or other potentially infectious materials.

Decontamination of Spills.
The following procedure is recommended for decontaminating spills of agents used at BSL-2:
- Wear gloves and a laboratory coat or gown. Heavyweight, puncture-resistant utility gloves, such as those used for housecleaning and dishwashing, are recommended.
- Do not handle sharps with the hands. Clean up broken glass or other sharp objects with sheets of cardboard or other rigid, disposable material. If a broom and dustpan are used, they must be decontaminated later.
- Avoid generating aerosols by sweeping.
- Absorb the spill. Most disinfectants are less effective in the presence of high concentrations of protein, so absorb the bulk of the liquid before applying disinfectants. Use disposable absorbent material such as paper towels. After absorption of the liquid, dispose of all contaminated materials as waste.
- Clean the spill site of all visible spilled material using an aqueous detergent solution (e.g., any household detergent). Absorb the bulk of the liquid to prevent dilution of the disinfectant.
- Disinfect the spill site using an appropriate disinfectant, such as a household bleach solution. Flood the spill site or wipe it down with disposable towels soaked in the disinfectant.
- Absorb the disinfectant or allow it to dry.
- Rinse the spill site with water.
- Dispose of all contaminated materials properly. Place them in a biohazard bag or other leakproof, labeled biohazard container for sterilization.

Spill in a Biological Safety Cabinet

A spill that is confined within a biological safety cabinet generally presents little or no hazard to personnel in the area. However, chemical disinfection procedures are to be initiated at once while the cabinet continues to operate. The disinfectant shall be one that is active against the organisms of potential hazard. Flammable liquids, such as ethanol or isopropanol, shall not be used, even if effective, because of the fire hazard of generating dangerous vapor concentrations within the cabinet that could be ignited by an electrical spark or other source.

Spray or wipe the walls, work surfaces, and equipment with the chosen disinfectant. Allow the disinfectant to remain on the surface for the appropriate contact time (see chart at the end). Minimize the generation of aerosols and use sufficient disinfectant to ensure that drain pans and catch basins below the work surface contain disinfectant. The front exhaust shall also be wiped and the disinfectant drained into a container.

- Maintain cabinet ventilation.
- Warn others in the laboratory.
- Notify the principal investigator.
- Wear protective gloves, a lab coat or gown, and eye protection during the procedure.
- Spray or wipe walls, work surfaces, and equipment with appropriate disinfectant. A disinfectant with detergent has the advantage of detergent activity that will help clean the surfaces by removing both dirt and microorganisms.
- Use sufficient disinfectant to ensure that drain pans and catch basins below the work surface contain the disinfectant. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container.
- Observe the recommended contact time for the disinfectant.
- Dispose of in ORS biowaste programs.

This procedure will not disinfect the filters, fans, air ducts, and other interior parts of the cabinet. If the entire interior of the cabinet needs to be disinfected, contact ORS for direction.

Spill in the Open Laboratory

For a spill in the open laboratory outside a biological safety cabinet, the spill response depends on the size of the spill and hazard of the material. A minimally hazardous material spilled without generating appreciable aerosols can be cleaned with a paper towel soaked in a chemical disinfectant.

A spill of a larger volume of hazardous material with aerosol generation requires evacuating the room, waiting for aerosol reduction, donning personal protective gear (including appropriate respiratory protection), selecting a disinfectant effective against the organisms involved, and cleaning as described above. Following cleanup, response personnel shall wash or shower with a disinfectant soap.

For a small spill of biological material in the open laboratory, take the following action:

- Warn others in the laboratory.
- Notify the principal investigator.
- Wear gloves and protective clothing.
- Decontaminate with an appropriate.
- Dispose of as described above.
- If clothing is known to be contaminated, carefully remove it, folding the contaminated area inward.
- Place the clothing into an autoclavable bag.
- Wash arms, face, and hands.

Spill in a Centrifuge

A biological spill in a centrifuge has the potential for producing large volumes of aerosols. On becoming aware that a spill may have occurred within a centrifuge or other piece of equipment, turn off the equipment, warn others in the area, notify the principal investigator, allow aerosols to settle, and decontaminate following the principles described above.

- Turn off the centrifuge and allow time for the aerosols to settle.
- Warn others in the laboratory.
- Notify the principal investigator.
- Wear gloves and protective clothing.
- Decontaminate with an appropriate disinfectant. Place contaminated equipment in a leakproof bag and move it to a biological safety cabinet, if possible, for decontamination.

Biological Spill on a Person

If a biological material is spilled on a person, emergency response is based on the hazard of the biological agent spilled, the amount of material spilled, and whether significant aerosols were generated. If aerosol formation is believed to have been associated with the spill, a contaminated person shall leave the contaminated area immediately. If possible, (s)he should go to another laboratory area so that hallways and other public areas do not become contaminated.

If a biological material is spilled on a person, emergency response is based on the hazard of the biological agent spilled, the amount of material spilled, and whether significant aerosols were generated. If aerosol formation is believed to have been associated with the spill, a contaminated person shall leave the contaminated area immediately. If possible, (s)he should go to another laboratory area so that hallways and other public areas do not become contaminated.

Disinfectants

Summary of Practical Disinfectants

Disinfectant	Dilution	Contact time (minutes)		Irritant type		
		Lipovirus	Broad-Spectrum	Skin	Eye	Respiratory
Quaternary ammonium cpds. (L)	0.1-2.0%	10	Not effective	Yes	Yes	No
Phenolic cpds. (L)	1.0-5.0%	10	Not effective	Yes	Yes	No
Chlorine cpds. (L)	500ppm*	10	30	Yes	Yes	Yes
Iodophor cpds. (L)	25-1600ppm	10	30	Yes	Yes	No
Ethyl alcohol (L)	70-85%	10	Not effective	No	Yes	No
Isopropyl alcohol (L)	70-85%	10	Not effective	No	Yes	No
Formaldehyde (L)	0.2-8.0%	10	30	Yes	Yes	No
Glutaraldehyde (L)	2%	10	30	Yes	Yes	No
Ethylene oxide (G)	8-23g/ft^3	60	60	Yes	Yes	Yes
Paraformaldehyde (G)	0.3g/ft^3	60	60	Yes	Yes	Yes

L = liquid; G = gas

*Commercially available chlorine bleach is 5.25% chlorine (52,200 ppm). A dilution of 1 to 100 will yield a 525 ppm solution, which is suitable for disinfecting purposes.

Source: *Laboratory Safety Monograph*, U.S. Department of Health, Education, and Welfare, Public Health Servic, and National Institutes of Health, 1979.

Decontaminants and Their Use in Infectious Waste Management

	Ethylene Oxide	Para-form-aldehyde (gas)	Quaternary Ammonium Compounds	Phenolic Compounds	Chlorine Compounds	Iodophor Compounds	Alcohol (ethyl or isopropyl)	Form-aldehyde (liquid)	Glutar-aldehyde
Use Parameters									
Concentration of active ingredient	400-800mg/l	0.3g/ft^3	0.1-2%	0.2-3%	0.01-5%	0.47%	70-85%	4-8%	2%
Temperature, ºC	35-60	>23							
Relative humidity, %	30-60	>60							
Contact time, minutes	105-240	60-180	10-30	10-30	10-30	10-30	10-30	10-30	10-600
Effective Against [a]									
Vegetative bacteria	+	+	+	+	+	+	+	+	+
Bacterial spores	+	+			×			×	+
Lipo viruses	+	+	+	+	+	+	+	+	+
Hydrophilic viruses	+	+		×	+	×	×	+	+
Tubercl bacilli	+	+		+	+	+	+	+	+
HIV	+	+	+	+	+	+	+	+	+
HBV	+	+		×	+	×	×	+	+
Applications [a]									
Contaminated liquid discard				+				×	
Contaminated glassware	×		+	+	+	+		×	+
Contaminated instruments	×			+	+			×	+
Equipment total decontamination	×	+							

[a] + denotes very positive response; ×, a less positive response; and a blank, a negative response or not applicable.

Adapted from *Laboratory Safety, Principles and Practices*, D. Fleming, J. Richardson, J. Tulis, D. Vesley; American Society for Microbiology, 1995: 226-227.

Footers