



## Sorting of Unfixed Human Derived Cells

### Biohazard Potential

Unfixed biological specimens can harbor known and unknown pathogens that may be transmitted through droplets and aerosols which are generated during the cell sorting process. Unfixed human materials (blood samples, various body fluids, cultured cells and environmental samples) are *a priori* considered as biohazardous.

Common samples include but are not limited to peripheral blood leukocytes, bone marrow, spleen cells, thymocytes, sperm cells, cells from primary and immortalized cultures from human donors, non-human primates, other species, and transgenic animals. Some clinical applications could include CD34 stem cells, fused dendritic cells + tumor cells, tumor cells which are then subject to gene therapy, separation of alloreactive/non-alloreactive T cells from cell grafts, antigen specific cytotoxic T-cells, specifically CMV or pancreatic islets.

Samples can carry unknown or known human pathogens such as hepatitis viruses (e.g., A, B, C, and D[delta]), human immunodeficiency virus types 1 and 2 (HIV-1 and -2), or cytomegalovirus. Hepatitis B, C, and D viruses and HIV have been classified by the International Association for Research on Cancer as human carcinogens, as have other oncogenic viruses that are encountered in biological and biomedical research (e.g., Epstein Barr virus [EBV], human T-lymphotropic virus types 1 and 2 [HTLV-1 and -2], Kaposi sarcoma herpesvirus/human herpesvirus 8, *Herpesvirus saimiri*).

Although most pathogens encountered when sorting clinical or research samples are transmitted by inoculation (by direct exposure of broken skin or mucous membranes or by ingestion) some may be transmitted by inhalation of organism-containing particles. Therefore, it is important that all laboratory personnel, and in particular the sorter operator, be protected from exposure to aerosols, droplets and accidental splashes. When all relevant biosafety aspects of a flow cytometry facility, according to the biohazard potential of the submitted samples, have been considered flow cytometry experiments can be performed without undue risks to instrument operators and users.

Aerosol containment of the cell sorter may be breached during a sort, for any number of reasons, exposing the operator to potentially biohazardous aerosols. Therefore, to compensate for the fact that aerosols cannot be contained within a biosafety cabinet during cell sorting, it is recommended that BL-2 containment be combined with BL-3 work practices, described in attached appendix, and **for sorting of biohazardous samples, the flow sorter must be equipped with aerosol containment hardware.**

Awareness about the origin of the sample, the potential presence of infectious agents or genetically engineered material is key for protection of the operator and lab. For proper risk assessment, it is critical that relevant biohazard information about the samples be transmitted to personnel before cell sorting experiments. ***A biosafety assessment cell sorting log sheet must be completed.*** This will allow personnel to determine whether the design of the facility and cytometer is appropriate for the planned experiments.

Instrument operators must also possess the necessary skill level and proper training in all safety practices and procedures that apply. Operator training and work experience are particularly critical for viable cell sorting and sorting of unfixed samples known or suspected to contain human pathogens. It is recommended that training should include performing aerosol containment testing<sup>4</sup> of the instrument to be used for biohazardous sorting.



### Recommended Containment Controls

#### Universal Precautions

All laboratory personnel who handle human materials are required to follow procedures as outlined in the Bloodborne pathogen exposure control plan located within the UMDNJ laboratory safety plan. The plan recommends handling all unfixed human specimens as if infected with HIV, Hepatitis B & C. This plan also directs the appropriate safety practices selected by the laboratory director, Hepatitis B vaccinations and post exposure follow-up. Personnel must be trained in the required procedures with strict adherence to the techniques set forth.

#### Environmental Controls

The cell sorter should be located in a separate room where no other laboratory activity is performed, a BL-2 laboratory, with limited access to minimize traffic.

- ♦ **Room air flow** - Proper air flow in the room requires negative pressure and no less than ten changes of air per hour. Air flow should be directed away from the sorter operator.
- ♦ **Personnel access** - Access to the sorting room should be limited in order to allow the operator to concentrate on the sort and to maintain regular air flow and negative air pressure in the room. A sign should be placed on the outside of the door to indicate that a potentially biohazardous sort is in progress. This sign should also contain all necessary information for entering the room safely.

*Ideally*, cell sorting of unfixed samples should be performed in a BSL3-type room which requires a ducted air ventilation system, water resistant interior surfaces (walls, floor, and ceiling), laboratory furniture that can be easily cleaned and decontaminated, sealed windows, and a sink that can be operated without hands.

#### Cell Sorter Operator-Specific Precautions

The protection of operators from infection and biohazard exposure during sorting of unfixed cells is of critical importance. The following recommendations also apply to others who may be present in the room during the sort, e.g., other scientists involved in the experiment. Only experienced and well-trained operators should perform potentially biohazardous cell sorting.

*For sorting of biohazardous samples, the flow sorter must be equipped with an aerosol management system which produces a negative pressure within the sort chamber, where aerosols are forced through a HEPA filter.*

- ♦ **Perform routine aerosol containment testing** by documenting aerosol management hardware is operating, on instrument to be used for biohazardous sorting<sup>4</sup>.
- ♦ **Immunization:** Whenever vaccination against a potential infectious organism that may be present in samples to be sorted becomes available, the sorter operator should consider vaccination.

#### Protective Clothing

- ♦ **Laboratory coats** - The sorter operator should wear a disposable, wrap-around, solid front, long sleeved laboratory coat.
- ♦ **Gloves** - Examination gloves are required whenever manipulating unfixed specimens. For added safety, double gloving may be preferred. When the outer gloves come in contact with potentially biohazardous material, they must be discarded, and new outer gloves are put on over the inner gloves to prevent cross contamination.
- ♦ **Face protection for eyes, nose and mouth** - It is recommended that the sorter operator wear a respiratory protective device, a High Efficiency Particulate Air (HEPA) N-95 (NIOSH certified) air-purifying, particulate respirator, covering nose and mouth, and safety glasses with side shields. For added splash protection, a full face shield can be placed over the respirator and the eye glasses (Contact EOHSS for fit-testing and proper selection of respirator, 5-4058 for Piscataway or New Brunswick and 2-4812 for Newark or Scotch Plains).



### Specimen Handling

It is highly recommended that all specimen processing prior to cell sorting should be performed in a certified Class II biological safety cabinet. Gloves and protective clothing should always be worn by flow cytometer operators. Capped tubes or microtiter plates with sealed covers should be used as sample containers. For specimen centrifugation, use sealed vessels or safety carriers.

- ♦ **Use of “sharps” avoided** - Avoid the use of needles, glass pipets, glass transfer pipets, or glass containers whenever possible for handling or transferring any biological material and use suitable replacements. Dispose of any “sharps” using a leak-proof, puncture-resistant container.
- ♦ **No mouth pipetting** - No mouth pipetting is allowed. Manual pipetting devices must be used.
- ♦ **Work area clean-up** - Discard all contaminated materials, e.g., sample and collection tubes, pipets, pipet tips, gloves, and laboratory coats, using appropriate biohazard containers. Treatment and disposal as regulated medical waste. Either autoclave or decontamination with a 1/10 volume dilution of sodium hypochlorite (household bleach) prior to waste disposal. Wipe off all work surfaces with an appropriate disinfectant solution, taking into account the potential biohazard.
- ♦ **Disinfection of spill** - In general, for small spills on a non-permeable surface, a disinfecting agent, e.g., a 1/10 volume dilution sodium hypochlorite (undiluted household bleach) is applied to a paper towel, placed on the spill, and allowed to make contact for an appropriate time to inactivate any biological organisms. Rapid clean-up of spills should be an established laboratory practice.

### Accidental Exposure

In case of a suspected exposure to a biohazardous agent, laboratory personnel should follow the written protocol on the Emergency Response Guide flip charts posted in the lab. It is recommended that all laboratory personnel including sorter operators provide when they start their employment, a serum sample for storage as a baseline for future assay in the event of accidental exposure. Additional serum samples may be collected periodically, depending on the agents under study in the laboratory.

### References:

1. Biosafety Guidelines for Sorting of Unfixed Cells, Cytometry 28:99–117 (1997)
2. Biosafety Concerns for Shared Flow Cytometry Core Facilities, Cytometry Part A 56A:113–119 (2003)
3. Flow Cytometry : Biosafety recommendations and protective measures, P. Herman, Belgian Biosafety Server
4. Measuring Containment of Viable Infectious Cell Sorting in High-Velocity Cell Sorters, Cytometry Part A 52A:122–130 (2003)

Biohazard Sorting, Schmid, I., Methods in Cell Biology, vol. 75, (2004)

### Contact Information:



# E•O•H•S•S

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## BIOSAFETY ASSESSMENT- CELL SORTING LOG SHEET

**Project Start Date:**

**Biosafety Approval Date:**

1. Project Title and Summary: Provide details about the cells to be sorted:
  
2. Principal Investigator:
  - a. Phone:
  - b. Fax:
  - c. Email:
  
3. Will the samples be fixed before submission to the Flow Cytometry Laboratory? o Yes o No
  - a. If yes, describe the fixation protocol, list concentration and exposure time.
    - i.
  
4. List Type of Sample and Source:
  - a. Eucaryotic cells (circle one):
    - i. Human      Non-human primate      Rodent      Other: \_\_\_\_\_
    - ii. Description : clinical sample      cultured cells      cell lines (ATCC #)
  - b. If it is a clinical sample has it been screened for bloodborne pathogens? o Yes o No
  - c. If yes, for which pathogens? \_\_\_\_\_
  - d. Could the sample contain other known human pathogens?      o Yes o No
  - e. Have the cells been tested for mycoplasma and/or viral infection?      o Yes o No
  - f. Were the cells transformed using any virus such as SV-40, EBV, HTLV-1, or *Herpes saimiri*?      o Yes o No
  - g. Procaryotic cells:
    - i. Bacteria      Fungi      Parasite
    - ii. Description: (ATCC #)
  
5. Does the sample contain any known infectious agent? o Yes o No
  - a. List Agent(s) and provide the Biosafety Level for each agent:
    - i.
    - ii.
    - iii.
  - b. Has the infectious agent been inactivated or rendered noninfectious? o Yes o No
    - i. Describe procedure.
  
6. Are the cells genetically modified ? o Yes o No
  - a. If yes, describe the vector (adenovirus, retrovirus, lentivirus, herpesvirus etc.)
    - i.

I have read the above questions carefully and certify the information provided to be correct.

\_\_\_\_\_  
Signature (Lab Director/ Principal Investigator)

**Risk:** Biosafety level of the sample: **1 - 2 - 3 - 4**

Containment level: **1 - 2 - 3 - 4**



## GUIDELINES FOR SORTING OF UNFIXED CELLS

TABLE 2.  
*Summary of Laboratory Practices, Equipment, and Facilities Associated With Biosafety Levels<sup>a</sup>*

Biosafety levels	BSL1	BSL2	BSL2 using BSL3 practices
A. Hazard levels	Low risk	Low to moderate	Moderate to high
B. Standard microbiological practices			
1. Public access while experiments are in progress	Not recommended	Controlled	Not permitted
2. Decontamination	Daily and upon spills	Daily and upon spills	Daily, upon finished work with infectious material, and spills
3. Infectious waste decontamination	Before disposal	Before disposal	Before disposal <sup>b</sup>
4. Pipetting	Mechanical devices	Mechanical devices	Mechanical devices
5. Eating, drinking, smoking and application of cosmetics	Not permitted at any time	Not permitted at any time	Not permitted at any time
6. Handwashing facilities	Required	Required	Required
7. Minimization of aerosol production	Recommended	Recommended	Recommended
8. Laboratory coats	Recommended (front button coats), not worn outside the laboratory	Required (front button coats), not worn outside the laboratory	Wrap-around disposable clothing required for all workers with potential exposure to infectious agents
C. Special practices			
1. Autoclave on-site facility	Not required	Must be available within the building	Must be available within the building
2. Insect/rodent control program	Required	Required	Required
3. Bench top work	Permitted	Permitted	Permitted in some circumstances
4. Transport of infectious material or waste materials for processing (i.e., decontamination) away from the laboratory	Durable leakproof container	Durable leakproof container	Durable leakproof container
5. Animals not involved with laboratory experiments	Not permitted	Not permitted	Not permitted
D. Containment equipment			
1. Biological safety cabinets or other physical containment system	Recommended for all aerosol generating processes	Recommended for all aerosol generating processes	Required for all work with infectious agents
2. Other physical containment	Recommended that equipment be decontaminated immediately after use	Appropriate physical containment devices are used when procedures with a high potential for creating infectious aerosols are being conducted <sup>c</sup>	Appropriate physical containment devices, such as centrifuge safety cups, sealed centrifuge rotors are used for all activities with infectious materials that pose a threat of aerosol exposure <sup>d</sup>
3. Freezers/refrigerators	Recommended that biohazard sign be posted	Biohazard sign must be posted	All agents must be stored in separate, closed, labeled, containers
4. Biosafety cabinet certification	Annually recommended	Annually	Annually
5. HEPA-filtered vacuum lines	Recommended	Recommended	Recommended
6. Biosafety cabinet decontamination	Recommended after each use	Required after each use	Required after each use
7. Personal protective equipment (i.e., laboratory coats, gloves, etc.)	Laboratory coats recommended; gloves are worn when skin contact with infectious material is unavoidable	Required—gloves should be worn when skin contact with infectious material is unavoidable	Required—appropriate combinations of special protective clothing, masks, gloves, respirators, etc. are used for all activities with infectious materials that pose a threat of aerosol exposure <sup>e</sup>
E. Laboratory facilities			
1. Ventilation	Negative pressure	Negative pressure	Negative pressure
2. Posted hazard sign	Recommended	Required	Required
3. Laboratory separated from the general public	No	Yes, while experiments are in progress	Yes, while experiments are in progress
F. Training			
1. Technical training	Recommended	Required	Required
2. Medical surveillance (i.e., baseline serology)	Recommended	Required when appropriate	Required when appropriate

<sup>a</sup>This table was adapted from "Working with Biohazardous Materials," Facilities Safety Procedure 360.01, Lawrence Livermore National Laboratory (1992).

<sup>b</sup>Infectious waste must be placed in a marked, closed, leak proof container and must be under direct control of the responsible laboratory worker(s) until it is placed in a locked disposal area or autoclaved (waste is not permitted to be left in laboratory overnight).

<sup>c</sup>These procedures include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, and opening containers of infectious materials whose internal pressures may be different from ambient pressures.

<sup>d</sup>These procedures include manipulation of cultures and of clinical or environmental material that may be a source of infectious aerosols.

<sup>e</sup>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.