Standard Safety Practices for Sorting of Unfixed Cells

In 1994 the International Society of Analytical Cytology (ISAC) recognized the need to formulate safety guidelines for sorting and analysis of unfixed cells to provide laboratories with recommendations for practices to reduce the potential for biohazard exposure of instrument operators. After extensive discussion and review, guidelines were officially adopted and published (Schmid et al., 1997). Many changes have occurred since then, in the field of cytometry, in safety practices, and in regulatory requirements:

1. Advances in cell-sorter technology have made high-speed cell-sorting more prevalent and changed the biohazard potential of cell-sorting experiments.
2. New and less expensive options for personal protection of operators have become available.
3. Instrument manufacturers have responded to the need for improved operator protection and have introduced instrumentation containing novel safety features.
4. Newly designed safety attachments for cell sorters have become commercially available.
5. Simpler, bead-based techniques for measuring the efficiency of aerosol containment during cell sorting have been developed.
6. With the availability of compact, easier-to-operate sorters many more laboratories have incorporated cell sorting into their repertoire, but often do not have dedicated operators to perform cell-sorting experiments.
7. Advances in cell biology have increased the need for live infectious cell sorting for cell culture and experiments involving molecular genetics.

Hence, the previously published guidelines are outdated and the generation of a new standard has become a pressing issue for the flow cytometry community worldwide, particularly as more and more laboratories conduct cell-sorting experiments involving samples with variable and sometimes complex levels of biohazard potential, such as genetically engineered cell preparations.

This unit provides written standard practices for handling and sorting of potentially biohazardous specimens, and includes methods to assess the risk of exposure of laboratory personnel to biological and/or toxic aerosols that may be produced by deflected-droplet fluorescence-activated cell sorters. The possibility exists that operators of cell sorters could become infected with biological agents contained in the specimens they are sorting (Harding and Liberman, 1995; Sewell, 1995; Collins and Kennedy, 1999; Vecchio et al., 2003). The standard practices outlined here aid in preventing exposures of laboratory personnel to infectious agents from sorting of unfixed cells. Recommendations focus on cell sorting of live, unfixed samples. However, it is important to note that functional measurements on cells (e.g., evaluation of calcium flux or membrane potential, certain apoptosis assays, cytokine assays, or live DNA or RNA staining) preclude cell fixation, and when performed on jet-in-air flow cytometers, can also expose operators or bystanders to potentially hazardous aerosols and sample splashes. Therefore, the safety practices outlined here apply whenever unfixed samples are run through a jet-in-air flow cytometer or a cell sorter that combines a flow cell with jet-in-air sorting.

Biohazard potential of unfixed cells

Typical biological specimens that are subjected to cell sorting include, but are not limited to, peripheral blood leukocytes, bone marrow, splenocytes, thymocytes, sperm cells, and cells from primary and immortalized cultures from humans, nonhuman primates, other species, and transgenic animals. These samples can harbor known and unknown infectious agents such as hepatitis viruses (A, B, C, D [delta]), human immunodeficiency viruses (HIV-1, -2), or cytomegalovirus. Hepatitis B, C, D, and HIV viruses have been classified as carcinogenic for humans by the International Agency for Research on Cancer, as have other viruses which are encountered in biological specimens, e.g., Epstein Barr virus, human T-lymphotropic viruses, Kaposi sarcoma herpesvirus/human herpesvirus 8, *Herpesvirus saimiri*, and simian virus 40 (International Agency for Research on Cancer, 1994, 1996, 1998; Ferber, 2002). Samples may also consist of genetically engineered cells that contain

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genomic sequences of potentially infectious organisms or sequences of unknown function which could exhibit toxic or oncogenic effects. Occupational transmissions across species to humans of retroviral agents such as simian type D retrovirus are of particular concern as these animal viruses could be introduced into the human population by this route (Lerche et al., 2001). Most known pathogens encountered when sorting clinical or research samples are transmitted by the percutaneous route, by direct exposure of broken skin or mucous membranes, or by ingestion. Some may be transmitted by inhalation of organism-containing droplets (Table 3.6.1) that are generated through either laboratory manipulations (Hambleton and Dedonato, 1992) or the sorting process (Merrill, 1981). Although HIV viruses and hepatitis viruses are transmitted primarily through the percutaneous route, infection through aerosolization of virus particles has been documented for hepatitis B (Almeida et al., 1971). However, transmission of the HIV virus through ingestion of HIV-infected breast milk has been described (Ruprecht et al., 1999). Therefore, HIV can potentially infect an individual via the oral mucosal route. Samples not only may contain pathogens, but may also be labeled with toxic and/or carcinogenic dyes which create additional health risks for sorter operators (Rachet et al., 2000; Wennborg et al., 2001; *UNIT 3.4*).

Biological particles 0.1 μm to 60 μm in size (e.g., aerosols) have been found to be important in the spread of infectious diseases (Andersen, 1958; Ijaz et al., 1987; Sattar and Ijaz, 1987; Schoenbaum et al., 1990; Musher, 2003). Submicrometer particles formed through dehydration of small droplets (droplet nuclei) can contain inorganic material, organic material, or infectious agents and may stay suspended in air for prolonged periods of time. During inhalation, larger particles are deposited mainly into the nasal passages, 3- to 7-μm particles into the tracheal area and pharynx, and ≤3-μm particles into the lungs of the exposed individual (Andersen, 1958). Droplets that fall out of suspension in air will land on surfaces, and pathogens they may contain can then be transmitted by exposure to broken skin or mucous membranes, or by ingestion. Consequently, protection of all laboratory workers from exposure is critical, in particular during high-risk procedures such as droplet-based cell sorting using instruments with high system pressures.

Creation of droplets and aerosols during cell sorting

Jet-in-air technology utilized for cell sorting involves a liquid stream carrying the cells through a nozzle vibrating at high frequency. At a given distance from the nozzle orifice the stream is broken into individual droplets. These droplets are then passed between high-voltage plates. Droplets containing cells of interest with parameters preselected by the operator are electrostatically charged and deflected into sort sample receptacles. Overall droplet size depends on the instrument operating pressure and the size of the nozzle orifice and its vibration frequency. High-speed cell sorters utilize higher system pressures and sort frequencies (Ibrahim and van den Engh, 2003) and thus produce more smaller droplets compared to older instruments designed for low-speed separations (Leary, 2005). All sorters also generate microdroplets, i.e., satellite droplets, 3 to 7 μm. Owing to the high fluid pressure produced in high-speed cell sorters large amounts of secondary aerosols of various and undefined droplet sizes can occur during instrument failures, for instance, when a partial clog in the nozzle causes a deflection in the fluid stream that is hitting a hard surface, e.g., the waste catcher. Droplets larger than 80 μm constitute the majority of droplets generated during sorting and settle quickly out of the atmosphere; smaller droplets, however, may be aerosolized, particularly when they are elevated by air currents. Because of the potential health risk to sorter operators and the environment if aerosols escape into the room, aerosol containment of a sorter, whether free standing or enclosed in a biological safety cabinet, must be verified using appropriate testing methods.

Risk Assessment and Level of Containment

Considering the potential for exposure to aerosols during cell sorting, it is incumbent on the investigator who wishes to have such live, unfixed cells sorted or analyzed to determine the appropriate biosafety level (BSL), and in conjunction with the flow cytometry laboratory director or manager and the sorter operator, review all the appropriate safety procedures for the particular pathogen for risk assessment. Such a review must also take into account the National Institutes of Health (NIH) guidelines for research involving recombinant DNA molecules (National Institutes of

Table 3.6.1 Infectious Agents Associated with Laboratory-Acquired Infections Resulting From Manipulation of Biological Samples[a]

Agent	Source of infection	Species	Route of infection	Biosafety level (practices, safety equipment, and facilities)
Hepatitis B, C, D virus	Blood, cerebrospinal fluid, urine, tissues	Human, naturally or experimentally infected primates	Inoculation, exposure of mucosal membranes to aerosols, broken skin	BSL-2; BSL-3 in case of aerosol production, large quantities, or high concentrations
Herpes virus simiae (B virus)	Primary culture (Rh monkey kidney cells)	Macaque or human	Inoculation, possibly aerosol inhalation (minimal)	BSL-3; BSL-4 for large quantities or high concentrations
Herpes Simplex 1,2 varicella virus	Ubiquitous	Opportunistic pathogen in immunocompromised host	Direct contact or aerosol inhalation	BSL-2
Cytomegalovirus Epstein-Barr virus (EBV)	Blood, tissues, EBV transformed cell lines	Human	Inoculation, exposure of mucosal membranes to aerosols, broken skin	BSL-2
Herpes 6,7 virus	Blood, bronchoalveolar lavage	Human	Risk not known	BSL-2
Influenza virus	Bronchoalveolar lavage, respiratory tissues	Human, naturally or experimentally infected animals	Aerosol inhalation	BSL-2
Lymphocytic chorio-meningitis virus	Blood, cell cultures, nasopharynx secretions, bronchoalveolar lavage, tissues	Nude mice, SCID mice, naturally infected macaques or marmosets, possibly man	Inoculation, exposure of mucosal membranes to aerosols, broken skin, well documented transmission by aerosol inhalation	BSL-2; BSL-3 in case of aerosol production, large quantities, or high concentrations
Poliovirus	Fluids, tissues, respiratory secretions	Naturally or experimentally infected nonhuman primates, transgenic mice	Ingestion, parenteral inoculation	BSL-2 practices by vaccinated personnel. WHO guidelines for establishing BSL-2/polio and BSL-3/polio laboratories after wild polio has disappeared. When oral vaccination is not administered, BSL-4 for work with wild polio.

continued

Table 3.6.1 Infectious Agents Associated with Laboratory-Acquired Infections Resulting From Manipulation of Biological Samples[a], *continued*

Agent	Source of infection	Species	Route of infection	Biosafety level (practices, safety equipment, and facilities)
Pox viruses Genetically engineered recombinant vaccinia virus	Lesion fluid, tissues, respiratory secretions, bronchoalveolar lavage	Infected volunteers or animals	Inoculation, exposure of mucosal membranes to aerosols, broken skin	BSL-2 practices by vaccinated personnel
Human immunodeficiency virus (HIV-1, 2) Retroviral vectors containing full length infectious genomes	Blood, body fluids, tissues	Human	Inoculation, exposure of mucosal membranes to aerosols (containing concentrated virus), broken skin	BSL-2; BSL-3 in case of aerosol production, large quantities, or high concentrations
Simian immunodeficiency virus (SIV)	Blood, body fluids, tissues	Macaque	Inoculation	BSL-2; BSL-3 in case of aerosol production, large quantities, or high concentrations
Vesicular stomatitis virus	Blood, body fluids, tissues	Infected animals, humans	Exposure to infectious droplets or aerosols, direct skin and mucosal membrane exposure	BSL-3; BSL-2 for laboratory-adapted strains with demonstrated low virulence
HTLV-1, 2 virus	Blood, body fluids, tissues	Human	Inoculation, exposure of mucosal membranes to aerosols (containing concentrated virus), broken skin	BSL-2; BSL-3 in case of aerosol production, large quantities, or high concentrations
Coxiella burnetii	Blood, urine, tissues	Infected animals, humans	Inoculation, exposure to infectious aerosols	BSL-2; BSL-3 for cell culture and manipulation of tissues
Rickettsia prowazekii	Infected tissues	Naturally infected nonhuman primates	Inoculation, aerosol inhalation	BSL-2; BSL-3 for tissue cultures of infected cells
Brucella	Blood, cerebrospinal fluid, tissues	Humans, Experimentally infected animals, sheep	Inoculation, direct skin contact	BSL-2; BSL-3 for tissue cultures of infected cells
Bacillus anthracis	Blood, cerebrospinal fluid, pleural fluid	Naturally and experimentally infected animals	Exposure of intact and broken skin, inoculation	BSL-2; BSL-3 in case of aerosol production, large quantities, or high concentrations

continued

Table 3.6.1 Infectious Agents Associated with Laboratory-Acquired Infections Resulting From Manipulation of Biological Samples[a], *continued*

Agent	Source of infection	Species	Route of infection	Biosafety level (practices, safety equipment, and facilities)
Chlamydia psittaci	Blood, tissues	Birds, humans	Exposure to infectious aerosols and droplets	BSL-2; BSL-3 in case of aerosol production, large quantities, or high concentrations
Leptospira interrogans	Blood, tissues	Infected animals, humans	Inoculation, skin and mucous membrane contact	BSL-2
Listeria monocytogenes	Blood, cerebrospinal fluid	Naturally or experimentally infected animals	Ingestion, eye and skin exposure	BSL-2
Mycobacterium atypicum	Bronchoalveolar lavage, lesion tissues	Humans	Inoculation, direct skin contact, aerosol inhalation	BSL-2
Mycobacterium tuberculosis	Gastric lavage, cerebrospinal fluid, pleural fluid, urine	Humans, naturally infected primates	Aerosol inhalation	BSL-3
Neisseria gonorrhoeae	Synovial fluid, urine, cerebrospinal fluid	Humans	Inoculation, direct skin contact	BSL-2
Neisseria meningitidis	Pharyngeal exudates, bronchoalveolar lavage, cerebrospinal fluid, blood	Humans	Inoculation, direct skin contact, aerosol inhalation	BSL-2; BSL-3 in case of aerosol production or high concentrations
Salmonella Salmonella typhi	Blood	Humans	Inoculation, direct skin contact	BSL-2; BSL-3 for large quantities
Treponema pallidum	Lesion fluid	Humans with primary and secondary syphilis	Inoculation, direct skin contact, aerosol inhalation	BSL-2
Toxoplasma Trypanosoma Leishmania Plasmodium	Blood	Humans or experimentally infected animals	Inoculation, aerosol inhalation	BSL-2
Blastomyces Coccidioides Histoplasma	Tissues	Infected animals	Inoculation, aerosol inhalation	BSL-2; BSL-3 for cultures containing *Coccidioides*, *Histoplasma*

[a]This table was adapted from US HHS Publication: Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999.

Health, 2002). An extensive list of biological agents and their recommended BSL assignment has been published by the CDC (Centers for Disease Control and Prevention, 1999).

Risk assessment is based on relative pathogenicity of the infectious or suspected infectious agent for healthy human adults. The investigator must make an initial assessment based on the risk group (RG) of an agent, which is classified into one of four risk groups. These classifications can be found in the NIH Guidelines, on the American Biological Safety Association Web site (*www.absa.org*) under Resources, or may need to be determined. To determine the appropriate containment level, the initial risk assessment should be followed by a thorough consideration of the agent itself and how it will be manipulated. Factors to consider include virulence, pathogenicity, infectious

dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene-product effects. The containment level may be raised or lowered from the initial risk group classification as a result. Some resources to use in conducting a risk assessment are the MSDSs at Health Canada (*http://www.phac-aspc.gc.ca/msds-ftss/index.html*), the American Public Health Association (APHA) Control of Communicable Diseases Manual, recent scientific articles and textbooks describing pathogenic agents or recombinant vector systems, and experts in the field (in the case of novel agents).

Clinical samples may contain unknown pathogens; in these cases, in the absence of hard data, a cautious approach and adoption of a higher biosafety level is advisable. Risk assessment is likely to be most difficult for samples containing recombinant DNA molecules. In recent years evolving technologies have led to the generation of modified viruses, bacteria, yeast, and other microorganisms. Common recombinant viruses include adenoviruses, alphaviruses, retroviruses, vaccinia, and herpesviruses designed to express heterologous gene products. Selecting the appropriate biosafety level for such work begins by establishing the classification of the nonmodified virus and then proceeds to an evaluation for a possible increase in hazard potential associated with a given genetic alteration. Of particular concern are modifications that result in expression of a toxin or a known oncogene, or of sequences that alter the host range or cell tropism or that allow the virus to integrate into the host genome. If needed, advice from a virologist should be sought to determine the proper BSL for planned flow cytometric experiments.

"Containment" refers to safe methods for managing infectious agents in the laboratory based on the type of organism and the type of risk due to the nature of the procedure. The four biosafety levels (BSL-1, BSL-2, BSL-3, and BSL-4) consist of a combination of laboratory facilities, laboratory practices and techniques, and safety equipment as outlined in Biosafety in Microbiological and Biomedical Laboratories (Centers for Disease Control and Prevention, 1999), which is also available on line at *www.cdc.gov/od/ohs*. The purpose of containment is to reduce or eliminate exposure of laboratory personnel as well as the environment to hazardous agents contained in samples to be processed. Risk assessment of the experiments to be performed on the equipment available will define the appropriate combination of the different safety elements.

BSL-1 containment is used for work with agents not known to cause disease in humans. BSL-2 practices, safety equipment, and facilities are used with pathogens that are known to cause disease in humans but that can be easily contained and are not usually transmitted by aerosols. BSL-3 containment is applicable to biological agents that cause serious and potentially lethal disease as a result of exposure by inhalation. The ultimate level of containment, BSL-4, is reserved for work with exotic and highly dangerous organisms transmitted by aerosols and for which no vaccination or therapies exist. These agents pose a high individual risk of life-threatening disease; therefore, viable sorts should never be done when working with these pathogens. Refer to Table 3.6.1 for the recommended biosafety containment levels for selected agents and to Table 3.6.2 for the corresponding work practices.

At BSL-2 containment all laboratory manipulations that can generate infectious or potentially hazardous aerosols (pipetting, vortexing, and the like) must be performed in a Class I or Class II biosafety cabinet, while at BSL-3 containment all manipulations involving infectious or potentially hazardous materials must be conducted inside a biosafety cabinet. Many cell sorters do not fit within a biosafety cabinet and specimens must be handled on the open bench during cell sorting. Furthermore, for any number of reasons, aerosol containment of a sorter may be breached during the cell sorting procedure and expose the operator to potentially biohazardous aerosols.

When sorting any infectious or hazardous material, even if it is classified as BSL-2, it is critical to understand that **droplet-based sorting procedures are considered BSL-3 practices.** It is therefore recommended that viable, unfixed samples that are potentially infectious be sorted at a minimum on a sorter which has been tested for aerosol containment (described below) located in a BSL-2 facility (modified as described in Environmental Controls) using practices and containment equipment recommended for BSL-3 by the CDC (Centers for Disease Control and Prevention, 1999). However, because of the increased hazard of a sudden quick release of large amounts of fluid or aerosols into the environment, it is highly recommended that high-speed sorting be performed in a BSL-3 laboratory facility under complete BSL-3 containment.

Table 3.6.2 Summary of Laboratory Practices Associated with Biosafety Levels[a]

Biosafety Levels	BSL-1	BSL-2	BSL-3
A. Hazard levels	Low risk	Low to moderate	Moderate to high
B. Standard microbiological practices			
1. Public access while experiments are in progress	Limited	Controlled	Not permitted
2. Handwashing facilities	Required	Required	Required
3. Eating, drinking, smoking, application of cosmetics, storing food for human consumption	Not permitted at any time	Not permitted at any time	Not permitted at any time
4. Pipetting	Mechanical devices	Mechanical devices	Mechanical devices
5. Safe handling of sharps	Required	Required	Required
6. Minimization of aerosol production	Recommended	Recommended	Required
7. Decontamination of work surfaces	Daily and upon spills	Daily and upon spills	Daily, upon finishing work with infectious material, and upon spills
8. Infectious waste decontamination	Before disposal, placed in durable leak-proof container for transport	Before disposal, placed in durable leak-proof container for transport	Before disposal, placed in durable leak-proof container for transport
9. Biohazard sign	Posted at entrance	Posted at entrance	Posted at entrance
10. Insect/rodent control program	Required	Required	Required
C. Special practices			
1. Immunization	Not required	Recommended	Recommended; depending on the specific biohazard vaccination may be required
2. Medical surveillance (e.g., baseline serology, periodic testing)	Not required	Required when appropriate	Required when appropriate
3. Specific biosafety manual and training in policies and procedures appropriate for hazard potential	Not required	Required with annual updates	Required with annual updates
4. Bench-top work	Permitted	Permitted	Permitted in some circumstances, but appropriate combinations of personal protective equipment, e.g., face-shields, respirators, must be used

continued

Table 3.6.2 Summary of Laboratory Practices Associated with Biosafety Levels[a], *continued*

Biosafety Levels	BSL-1	BSL-2	BSL-3
D. Safety equipment			
1. Laboratory coats, gowns, uniforms	Recommended, not worn outside the laboratory	Required, not worn outside the laboratory	Solid-front, wrap-around disposable clothing required for all workers with potential exposure to infectious agents
2. Gloves	Recommended, worn when skin contact with infectious material is unavoidable	Required when skin contact with infectious material is unavoidable; double gloving may be appropriate	Required when skin contact with infectious material is unavoidable; double gloving may be appropriate
3. Biological safety cabinets	Not required	Required for all aerosol-generating processes	Required for all work with infectious agents
4. Other physical containment	Recommended that equipment be decontaminated immediately after use	Appropriate physical containment devices are used when procedures with high potential for creating infectious aerosols are being conducted[b]	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 aerosols exposure[c]
5. Freezers/refrigerators	Recommended that biohazard sign be posted	Biohazard sign must be posted	Biohazard sign must be posted; all agents must be stored in separate, closed, labeled containers
E. Laboratory facilities			
1. Ventilation	No special requirements	Negative pressure	Ducted exhaust air ventilation system
2. Laboratory separated from the general public	Not required	Yes, while experiments are in progress	Required
3. Lockable doors	Not required	Required	Passageway with two self-closing doors
4. Sink	Recommended	Required	Required
5 Laboratory surfaces easy to clean, impervious to water, resistant to chemicals used for disinfection	Work surfaces	Work surfaces	Work surfaces, floors, walls and ceilings
6. Autoclave inside facility	Not required	Not required	Required
7. HEPA-filtered vacuum lines	Not required	Recommended	Required

[a]This table was adapted from US HHS publication "Biosafety in Microbiological and Biomedical Laboratories," 1999.
[b]These procedures include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, and droplet-based sorting.
[c]These procedures include manipulation of cultures and of clinical or environmental material that may be a source of infectious aerosols.

Alternatively, secondary containment systems should be considered for prevention of aerosol escape. For instance, newly developed cell sorters, e.g., BD FACSAria (BD Biosciences) or JSAN (Bay Bioscience Co., Ltd, Japan), are small enough to be completely enclosed in a biocontainment biological safety enclosure (The Baker Co., Sanford, ME), and more recently compact high-speed sorters that can be integrated into a biosafety cabinet, such as the inFlux (Cytopeia, Seattle, WA) and the Reflection (iCyt Visionary Bioscience, Champaign, IL), have become commercially available. These secondary containment systems permit sorting of materials classified as BSL-2 using BSL-2 practices. The effectiveness of aerosol containment should be verified through rigorous testing before the sorting of any potentially infectious samples. Monitoring devices that indicate proper operation during sorting are necessary.

Standard Precautions and Other Regulatory Requirements

In the United States of America all laboratory personnel who handle human cells and other potentially infectious materials, such as specimens from experimentally infected animals, are required to follow universal precautions and procedures as outlined in the Occupational Safety and Health Administration (OSHA) document Occupational Exposure to Bloodborne Pathogens (United States Federal Code Regulation, 1991) and put forth in additional specific local and institutional safety regulation documents. Laboratories also must comply with federal code regulations for possession, use, and transfer of select agents and toxins (United States Federal Code Regulation, 2002). All recombinant DNA experiments have to be performed in compliance with the specific NIH guidelines (National Institutes of Health, 2002) and have to be approved by institutional biosafety committees (IBCs). All institutions receiving grant or contract awards from the NIH are expected to follow the current health and safety guidelines published at *http://grants1.nih.gov/grants/policy*.

Other countries have developed their own stringent regulatory standards and/or have adopted aspects of regulations for work with biological agents as mandated in the US. International biosafety regulations, guidelines, and information sources are available online through the European Biosafety Association (*http://www.ebsa.be/working_grouos_international/biosafety_compendium.htm*). Furthermore, guidelines for specimen handling based on US regulations that are focused on clinical settings are published by the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards, 2005). Relevant details for the preparation of infectious samples containing HIV for flow cytometry, such as shipping and receiving of specimens, local transport, staining, and disposal, have previously been described (Schmid et al., 1999).

Each institution should establish a biosafety committee for review of potentially hazardous laboratory protocols. A thorough review of the protocols and procedures for sorting of unfixed human cells will include, but not be limited to, the procedures used to establish containment of the cell sorter and an evaluation of the containment. Ad hoc review committees could be established for each application. These may consist of the investigator requesting the sort, the sorter operator, a representative of the biosafety office of the institution, and a scientist not involved with the protocol under consideration.

Appropriate safety practices are the responsibility of the laboratory director. Each laboratory needs to develop or adapt a biosafety operations manual that specifies practices designed to minimize risks and takes into account the biohazard potential of the specimens being processed (Schmid et al., 2003). Personnel must be trained in the required procedures, and strict adherence to the techniques set forth is essential.

Handling of all unfixed human specimens and primary cell cultures as if infectious is mandated by OSHA. This practice also applies to established cell lines that are in vitro, or animal-passaged human explanted tissues transformed by spontaneous mutation or a natural or laboratory infection with an immortalization agent, e.g., Epstein Barr virus. In fact, cell lines from the American Type Culture Collection (ATCC) and other sources bear warnings that they may contain bloodborne pathogens, and ATCC recommends they be accorded the same biosafety level as the ones known to be infected with HIV. Likewise, specimens from nonhuman primates and animal tissues, explants, or cell cultures known to be deliberately infected with human pathogens are subject to safety procedures as outlined in the Bloodborne Pathogen Standard.

Only rigorously characterized human cell lines that stringent techniques, such as PCR, sensitive antigen detection, stimulation and

co-culture assays, enzyme analysis, or other suitable method, have proved to be completely devoid of bloodborne pathogens could be excluded. However, as most laboratories are not able to provide reliable confirmation that the samples are pathogen free before they are subjected to cell sorting, following biosafety precautions outlined in this unit is strongly advised.

If samples are fixed, appropriate methods must be selected to reliably inactivate potentially biohazardous agents. Concerns exist about the effectiveness of standard fixation methods to reduce the level of infectivity in samples containing high titers of known viruses or unknown infectious agents resilient to inactivation (Aloisio and Nicholson, 1990; Ericson et al., 1994). Fixation procedures must be performed carefully; otherwise, samples that are considered inactivated, but in fact are not, can pose a serious health risk to laboratory personnel.

Cell Sorter Operator–Specific Precautions

The protection of operators from infection and biohazard exposure during sorting of unfixed cells is of paramount importance (Merrill, 1981; Giorgi, 1994; Ferbas et al., 1995; Schmid et al., 1997; Schmid, 2000; Oberyszyn and Robertson, 2001; Perfetto et al., 2003, 2004; Schmid et al., 2004). The following recommendations also apply to others who may be present in the room during the sort, e.g., scientists involved in the experiment.

Immunization

Whenever a vaccine against a potential infectious organism that may be present in samples to be sorted becomes available, the sorter operator should consider vaccination. Vaccination against hepatitis B virus is highly recommended.

Personal protective equipment

BSL-3 practices, as outlined by the CDC (Centers for Disease Control and Prevention, 1999), require personal protective equipment. The sorter operator should wear a disposable, wrap-around, solid-front, long-sleeved laboratory coat made of fluid-resistant material. Examination gloves are required at all times and in some cases double-gloving should be considered. When an outer glove is contaminated by contact with potentially biohazardous material, it must be decontaminated with an appropriate disinfectant before being discarded. Then a new outer glove is put on over the inner one to prevent cross contamination. Gloves are subject to fatigue caused by laboratory manipulations and should be changed often, or immediately upon being torn. It is recommended that the operator wear a respiratory protective device appropriate for aerosol protection, e.g., N95 NIOSH-approved particulate respirators covering nose and mouth, and safety glasses with side shields. For added splash protection, a full face shield may be placed over the respirator and glasses. Recently, for an added margin of safety a complete system (DePuy Chesapeake Surgical, Ltd., Sterling, VA) consisting of a body suit, a helmet, and a battery-powered respiratory system with electrostatic filter medium has become commercially available (Perfetto et al., 2003). Protective clothing and equipment are never worn outside the work area.

Specimen handling

All specimen processing prior to cell sorting should be performed in biological safety cabinets. Capped tubes or microtiter plates with sealed covers should be used as sample containers. For local transport, place primary collection tubes or sample tubes in a secondary container, such as a plastic carrier with a secure lid that is able to contain the specimen in case of breakage of the primary container. For specimen centrifugation, use sealed vessels or safety carriers.

Avoid the use of needles, glass pipets, glass transfer pipets, or glass containers or tubes for handling or transferring any biological material whenever possible and use suitable replacements. Dispose of any contaminated sharps using a leak-proof, puncture-resistant container as specified by local biosafety regulations.

No pipetting by mouth is allowed. Manual pipetting devices must be used and must be equipped with filters to prevent infectious liquid from contaminating the pipetting device.

Sort samples need to be prepared as single-cell suspensions because aggregated cells can partially or completely clog sort nozzles and stop the sort. Any interruption of a potentially biohazardous sort increases the risk of operator exposure to pathogens contained in the sort sample owing to the increased probability for splashes and escape of sort aerosols during the manipulations required to continue sorting. To reduce the formation of cell aggregates during sample preparation samples should be centrifuged 5 to 10 min at $\sim 300 \times g$ to pellet cells. Higher centrifugation speeds can damage cells and compact them so densely that they are

difficult to break apart. Frozen cell samples that are thawed for sorting frequently contain dead cells that may release DNA into the medium. DNA binds to the surface of live cells, and after centrifugation these samples form solid aggregates that lead to nozzle clogging problems and excessive aerosol formation. In these situations, add 20 μg/ml RNase-free DNase and let samples sit 10 min at 37°C. Select an optimal solution for sample resuspension to maintain cell viability. Highly concentrated cell suspensions have an increased tendency to clump; therefore, dilute them to the lowest possible density for the sort speed used. Sort samples are often chilled to preserve cellular structures and prevent capping of antibodies bound to cell surface receptors. However, the cold can aggravate clumping, so keeping sort samples at an intermediate temperature, such as 15°C, may be preferable over sorting at 4°C. Immediately prior to sorting, all samples should be filtered through filter meshes with the appropriate pore size for the nozzle tip used on the sorter.

Work areas must be cleaned routinely. Discard all contaminated materials, e.g., sample and collection tubes, pipets, pipet tips, gloves, and laboratory coats, using appropriate biohazard containers. Follow the established institutional procedures for storage and disposal of biomedical/hazardous waste. Generally, this involves either autoclaving or decontamination with a 1/10 (v/v) dilution of 0.71 M sodium hypochlorite (straight household bleach) prior to waste disposal. Wipe off all work surfaces with an appropriate disinfectant solution, taking into account the potential biohazard (Table 3.6.3). Summary information on the survival and disinfectant inactivation of HIV has been published (Martin et al., 1985; Sattar and Springthorpe, 1991; Druce et al., 1995; Van Bueren et al., 1995), and is reviewed in Schmid et al. (1999).

After any spill of biological material, the protection of personnel is the first priority. Rapid clean up of spills should be an established laboratory practice. In general, for small spills on a nonpermeable surface, a disinfecting agent such as diluted 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 (Table 3.6.3). For the handling of larger spills or spills on a nonsmooth or permeable surface, local institutional biosafety offices should be contacted.

Accidental exposure

It is recommended that when all laboratory personnel begin employment they provide 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. Each laboratory should develop or adapt a written protocol to be followed in case of a suspected exposure to a biohazardous agent. Current guidelines for post-exposure management of healthcare workers should be used and medical evaluation, surveillance, and treatment records must be maintained (Wang et al., 2000; Mikulich and Schriger, 2002; Schriger and Mikulich, 2002). Guidelines for post-exposure chemoprophylaxis against HIV infection are available from the CDC and should always follow the latest recommendations available on line at *www.cdc.gov/mmwr*.

Environmental Controls

Cell sorting of unfixed samples, especially when free-standing sorters with high operating pressures are employed, should be performed in a BSL-3 laboratory facility because containment of the sorter could be breached any time during a sort for a variety of reasons and release potentially hazardous materials into the vicinity of the instrument. A BSL-3 room is accessible only through a passageway with self-closing, lockable doors and requires a ducted HEPA-filtered air ventilation system, water-resistant interior surfaces (e.g., walls, floor, ceiling) and laboratory furniture that can be easily cleaned and decontaminated, sealed windows, and a sink that can be operated without hands (Table 3.6.2). However, sort facilities in existing institutions may not have all the environmental safety features recommended for BSL-3. Sorting of unfixed cells can still be achieved in a BSL-2 laboratory facility provided that the institution can modify the sorter room taking into consideration the following requirements:

1. The air venting system discharges air towards the outside away from occupied areas or is HEPA filtered.

2. The cell sorter is located in a separate, lockable room where no other laboratory activity is performed.

3. Airflow in the room is balanced to create no less than ten changes of air per hour. It is recommended that negative airflow be measured by a visual monitoring device located at the door.

Table 3.6.3 Summary of Practical Applications of Chemical Disinfectants for Decontamination[a]

	Quaternary ammonium compounds	Phenolic compounds	Chlorine compounds	Hydrogen peroxide	Iodophor	Alcohols		Aldehydes	
						Ethyl	Isopropyl	Formaldehyde (Formalin)	Glutaraldehyde
Practical requirements									
Use dilution	0.1%-2%	1%-5%	1/10-1/100 dilution of 0.71 M sodium hypochlorite, ~50-500 ppm[b]	3%-6%	0.0075% -10%, ~25-1600 ppm[b]	70%-85%	70%-85%	0.2%-8%	2%
Contact time for lipovirus	10 min	10 min	10 min	10 min	10 min	10 min	10 min	10 min	10 min
Contact time for broad spectrum	Not effective	Not effective	30 min	30 min	30 min	Not effective	Not effective	30 min	30 min
Inactivation profile									
Vegetative bacteria	✓	✓	✓	✓	✓	✓	✓	✓	✓
Lipoviruses	✓	✓	✓	✓	✓	✓	✓	✓	✓
Nonlipid viruses		—[c]	✓	✓	✓	—[c]	—[c]	✓	✓
Bacterial spores			✓	✓	✓			✓	✓
Fungi	—[d]	—[d]	✓	✓	✓	✓	✓	✓	✓
Protozoal parasites			✓					✓	✓

continued

Table 3.6.3 Summary of Practical Applications of Chemical Disinfectants for Decontamination[a], *continued*

	Quaternary ammonium compounds	Phenolic compounds	Chlorine compounds	Hydrogen peroxide	Iodophor	Alcohols		Aldehydes	
						Ethyl	Isopropyl	Formaldehyde (Formalin)	Glutaraldehyde
Characteristics									
Stability[e]	✓	✓		✓	✓	✓	✓	✓	✓
Corrosive		✓	✓	✓	✓				
Flammable						✓	✓		
Residue		✓	✓		✓			✓	✓
Organic material Inactivated[f]	✓		✓	✓	✓				
Potential application									
Surfaces[g]	✓[h]	✓	✓	✓	✓	✓	✓	✓	✓[h]
Instrument surfaces and parts			✓	✓		✓	✓		
Flow cytometer fluid lines			✓	✓		✓			

[a]This table was adapted from Lawrence Livermore National Laboratory (1995) and from *http://www.ianrpubs.unl.edu/epublic/pages/publicationD.jsp?publicationId=431*.

[b]Available halogen; iodophors may require prolonged contact time for inactivation of bacterial spores, tubercle bacilli, and certain fungi.

[c]Variable results depending on the virus; activity drops sharply when alcohols are diluted below 50%.

[d]Weak activity only.

[e]Shelf life >1 week when protected from light and air.

[f]Prior to decontamination cleaning with lipophilic detergent/disinfectant necessary.

[g]Work surfaces, decontamination of fixed or portable equipment surfaces.

[h]Usually compatible with optics, but consider interference from residues and effects on associated materials such as mounting adhesives.

4. Access to the sorting room is restricted 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, if needed.

5. All other safety recommendations for BSL-3 practices and personal protective equipment are followed rigorously.

Ultraviolet (UV) room light may be used to sterilize the room after each sort at the end of the day after all personnel have left, although its effectiveness against different pathogens may vary and areas where UV rays are blocked will remain unexposed. Therefore, routine cleaning with disinfecting agents is highly recommended.

Instrument Considerations

Proper operation of the cell sorter

Follow all the manufacturer's recommendations for instrument operation and maintenance carefully. Perform high-speed sorting of unfixed cells only on instruments that were designed for increased sorting rates or have been properly modified by the manufacturer. Never increase the system pressure on a cell sorter designed for low-pressure sorting, because the fluidic lines, valves, and fittings cannot reliably withstand the increased pressure.

Set up a rigorous sorter preventive maintenance schedule, either as part of a service contract offered by the instrument manufacturer or as quality control performed by laboratory personnel. Routinely perform leak checks on the fluid lines of the cell sorter. To do this, gain access to the fluidic lines. Carefully check for wet areas, indicating leaks in the tubing. Inspect tubing for cracks and signs of stress, particularly around the fittings and where tubing passes through valves. Also inspect sheath lines and waste lines. Replace any leaking tubing.

Verify the proper operation of the sort mechanism and the stability of the sort streams and droplet break-off each time immediately before attempting to sort a potentially biohazardous specimen. If the streams and the droplet break-off do not remain stable during the sort setup, correct the problem before attempting a potentially biohazardous sort.

After each sort, the instrument should be decontaminated with a disinfecting agent, taking into account the biohazard under study. Sort collection-tube holders are heavily exposed to sample droplets and must be carefully decontaminated before handling. Appropriate disinfectants to be used for decontamination of equipment or work surfaces exposed to blood or other potentially infectious materials include diluted bleach, Environmental Protection Agency (EPA)-registered tuberculocides, EPA-registered sterilants, or products registered to be effective against HIV or HBV as listed online at *http://www.epa.gov/oppad001/chemregindex.htm*. Common laboratory disinfectants (Harding and Liberman, 1995; Rutala, 1996) applicable for instrument decontamination and their properties are listed in Table 3.6.3. Alcohols are not classified as high-level disinfectants, because they cannot inactivate bacterial spores and penetrate protein-rich materials, and isopropanol is not able to kill hydrophilic viruses. Before designing a cell sorter–specific decontamination protocol, check with the instrument manufacturer that all components exposed to the disinfectant can tolerate exposure. Wipe off all surfaces inside the sort chamber, the sample introduction port, and the holder. Run the disinfectant through the instrument for the appropriate exposure time. Always follow with distilled water to completely remove the disinfectant, because some disinfecting agents are corrosive and residual disinfectant solution can affect the viability of subsequent samples that are run through the sorter. Make sure that the water used for washing out the disinfectant is sterile and does not introduce new contaminants into the instruments.

Sample introduction system

Cell sorters pressurize the sample tube once it is inserted onto the sample introduction port. While newer-generation instruments are equipped with completely enclosed sample introduction chambers for operator safety, some older sorters have an open port, requiring careful operator handling. Each time a sample tube is put on the instrument, check the tube seal and its secure fit onto the sample introduction port. A faulty seal can allow the sample tube to be blown off on pressurization and to splash sample onto the operator. Make sure that the tube material provides sufficient strength to tolerate high instrument pressure. When the tube is removed, the sample line back drips, creating a potential biohazard through spraying of droplets. Permit the back drip to go into a tube until the sample is flushed out of the introduction line to avoid splashing of sample droplets. Alternatively, soft absorbent

pads soaked in disinfectant can be placed in the catch tray to prevent droplet splattering. The catch tray or trough should be decontaminated regularly. Droplet containment features eliminate the back drip from the sample introduction line for prevention of droplet splashes. Alternatively, installation of a plastic shield around the sample introduction port can block droplet spraying from the sample back drip.

Nozzle tip

Since a clogged sort nozzle is one of the major reasons for increased aerosol production on cell sorters, samples should be prepared properly to minimize the formation of cell clumps. Any clumped cells present in the sample to be sorted must be removed. Options include filtration through nylon mesh filters, e.g., different pore-size meshes from Small Parts Inc. (Hialeah, FL), tubes with cell strainer caps (Becton Dickinson, Falcon), or individual cell strainers (Becton Dickinson, Falcon). Filter samples immediately before starting the sort to give cells less time to re-aggregate. For large cell numbers, distribute cell aliquots into separate tubes and filter each sample individually before placing it onto the sorter. If feasible, put an in-line filter, either bought from a commercial supplier or made in the laboratory by heating the end of a clipped-off pipet tip and fusing it with nylon mesh, on the uptake port to prevent cell clumps from reaching the sort nozzle. Select a sort tip with the appropriate nozzle size for the cell size to be sorted.

Smaller nozzle sizes provide optimal signal resolution and easy sort setup. However, to avoid clogged nozzles, it is recommended that the nozzle orifice be at least four times bigger than the cell diameter (Stovel, 1977), ideally six times larger or even more. Sort nozzles should be cleaned frequently by sonication, or other methods recommended by the manufacturer, between sorts to prevent buildup of cellular debris. Inspection under a microscope can help to determine if the nozzle is clear. However, cells can still aggregate inside a clean sort nozzle at the intersecting surface of the sample injection fluid with the sheath fluid, even during sorting of properly prepared samples. Accumulated cells at this intersection eventually break free and may partially clog the nozzle tip. When this happens, stop sample flow, turn off the high voltage, and put the fluidic control into the off position. Modern sorters have safety devices that will stop the sorting process as soon as a clog develops and cover the collection vessels. In any case, however, the sort chamber door must remain closed until aerosol has been cleared from the chamber. Visual verification of the actual time needed for aerosol clearance should be performed with bottled smoke (Lab Safety Supply Inc., Janesville, WI, or equivalent). Remove the sample to be sorted first. Then, only after aerosols have cleared, open the sort collection chamber door and take out the collection vials. Cap all tubes. Clear the clog as appropriate. Before the sort is continued, make sure the stream emitted through the tip is straight and steady, and the droplet break-off and the side streams are stable.

Aerosol control measures

A standard safety feature of cell sorters includes an interlocking sort-chamber door and a sample collection chamber designed to contain aerosols. These barriers are not always completely sealed, and detection of air leaks should be performed with bottled smoke. Sealing of any such openings can aid in achieving aerosol containment. All modern sorters are equipped with a receptacle that is connected with a waste evacuation system for collection of the undeflected center stream in order to reduce aerosol production during sorting. Auxiliary vacuum pumps designed to remove aerosols from the sort chamber are now available as optional attachments for sorters and are highly recommended. For custom installation of a generic vacuum pump, care needs to be taken that no air turbulences are created that will affect the stability of the sort streams. The vacuum lines for removal of airborne particles must be connected to a cartridge-type HEPA filter and to a filter flask containing a disinfectant, usually undiluted household bleach. For each sorting experiment the flask must be emptied and replenished with fresh, concentrated bleach.

Recently, a removable containment hood that is vented by a high-efficiency particulate air filtration (HEPA) filter/fan unit and covers the sort area and the sample introduction port (Cytek Development, Fremont, CA) has become commercially available to improve containment on FACStar, FACSVantage, and FACSDiVa (BD Biosciences) cell sorters. Dako Colorado, Inc. (Fort Collins, CO) provides a Class I biosafety cabinet attachment for their MoFlo high-speed cell sorter. An efficient containment system on the sorter will be able to remove aerosols as long as the sort chamber door remains closed. Because of the potential hazard to operators if aerosol droplets escape, the efficiency of aerosol containment on the cell sorter must be verified in regular sorting mode and in instrument failure

mode before a potentially biohazardous sort is attempted. If aerosol containment is incomplete, the safety features of the cell sorter must be modified such that no escape of aerosol can be detected. Sorters can be placed inside a biological safety cabinet. Older cell sorters with water-cooled lasers are generally too large to fit, although successful adaptation of such a system to accommodate a biosafety cabinet has been described (Lennartz et al., 2005). Recently, some instruments have become small enough to be completely enclosed in a walk-in clean air and biocontainment biological safety enclosure (BioPROtect II, The Baker Co., Sanford, ME), and novel compact sorters, e.g., inFlux (Cytopeia) and Reflection (iCyt), can be accommodated in biological safety cabinets. The efficiency of aerosol containment of these cabinets must be verified using appropriate testing methods as described below.

Stream view cameras

Sort-stream viewing cameras are standard on newer sorters. They prevent the sorter operator from coming close to the area of the instrument that poses the greatest potential biohazard. Viewing systems that illuminate the center stream and the deflected streams near the sort collection vials are recommended as they allow the operator to monitor increased aerosol production due to shifting stream positions and fanning.

Limitations and Alternate Technologies

Complete BSL-3 containment is required when agents known to be transmitted by the inhalation route are sorted since the greatest potential of exposure or infection from these agents is from aerosolization (e.g., *M. tuberculosis*) and they are known to cause substantial morbidity and mortality. The need to perform such viable sorting experiments should be reviewed on a case-by-case basis with the biosafety office and infectious disease professionals of the institution. Cell fixation and alternate cell separation technologies (e.g., manual or automated magnetic bead separation or cell adherence to coated flasks) that can be readily performed in biological safety cabinets should be considered. Future novel high-speed cell-sorting technologies that do not generate aerosols, e.g., ultra-rapid fluid-switching technology, could offer an alternative to droplet-based sorting (Leary, 2005).

Samples labeled with radionuclides pose major problems, as most sorting laboratories do not have authorization for using sources of ionizing radiation. In addition, the stringent requirements for handling such materials, including monitoring their use and disposal, and the issues concerning instrument contamination and the generation of radioactive aerosols during sorting make flow cytometry experiments that involve cells labeled with radionuclide tracers not generally feasible.

Training and Experience

Only experienced flow cytometry operators should perform potentially biohazardous sorts. The time required to obtain cell-sorting proficiency on a given sorter varies, but training periods of 6 months are common. Some novel sorters do not require the complex alignment procedures required by older instruments, and laboratories do not always feel a need for a dedicated instrument operator. It is important, however, that any operator who performs potentially biohazardous sorting be trained carefully in proper instrument operation and all relevant safety procedures, including aerosol containment testing on free-standing sorters, as well as those enclosed in biological safety cabinets. The operator should have previous laboratory experience and a minimum of two years of experience in flow cytometry. Ideally, this should include training in performing sorting on deflected-droplet cell sorters using non-infectious, fixed material of the same type that will contain the known biohazard, e.g., peripheral blood mononuclear cell preparations.

Previous working experience with potentially biohazardous specimens is strongly recommended. Knowledge of the characteristics of common bloodborne pathogens and of the specific infectious organism present in the specimens to be sorted will help the sorter operator to formulate safe working practices (Evans et al., 1990).

Assessment of Aerosol Containment

The classic method for assessment of aerosol containment on deflected-droplet cell sorters using aerosolized bacteriophage and a detection system of bacterial lawns has been described in several publications (Merrill, 1981; Giorgi, 1994; Schmid et al., 1997; *UNIT 3.3*). The T4 bacteriophage method for assessment of containment can also be combined with active air sampling for testing room air (Ferbas et al., 1995; Schmid et al., 1997, 2004; *UNIT 3.3*). Tagging aerosol droplets with bacteriophages is an established technique that, provided the titer of the bacteriophage is sufficiently high, insures that all droplets generated during the test sort

contain T4. Because it has been established that a single phage is sufficient to generate one plaque (Merrill, 1981), the assay provides high sensitivity. Furthermore, the readout of containment results by counting plaques is straightforward. However, the method requires intermediate knowledge of microbiological techniques, depends on the performance of biological materials, and takes overnight to produce results even when all the materials have been preprepared.

Recently, a novel assay for measuring the efficiency of aerosol containment has been described (Oberyszyn and Robertson, 2001; *UNIT 3.5*). The method uses a suspension of highly fluorescent melamine copolymer resin particles (Glo Germ, Moab, UT) with an approximate size range from 1 to 10 μm, simulating a biological sample during the test sort. Aerosol containment is measured by placing microscope slides around the instrument where aerosols are produced and examining the slides under a fluorescent microscope for the presence of Glo Germ particles. Assay sensitivity and reproducibility have been increased through the use of a viable microbial particle sampler (Perfetto et al., 2003, 2004). This device draws room air onto a microscope slide and concentrates the collected Glo Germ particles onto the areas on the slide located directly underneath the intake ports. This technique can be performed immediately before starting a potentially biohazardous sort, but a fluorescent microscope has to be readily accessible. Glo Germ particles are highly fluorescent and therefore easily detected. However, careful handling of the microscope slides and the air sampler is important to avoid false positives, and diligent scanning of the entire slide is needed to reliably detect the escape of a single particle.

Before sorting any potentially biohazardous specimens on a given instrument, it is imperative to validate that aerosols are contained during the regular sorting process and during instrument failure modes. If aerosols are detected outside of containment, then the cell sorter must be modified such that no aerosols are detectable. Contact the manufacturer of the cell sorter for instructions and information necessary to make these instrument modifications. Testing must also be done whenever changes are made to the cell sorter that may affect escape of aerosols, e.g., installation of a new drive head or flow cell, replacement of the sort chamber door, or alterations in the aerosol management system.

For instruments that are placed in biological safety cabinets it is imperative that laboratories validate the efficiency of aerosol containment of the cabinet before any potentially biohazardous sorting experiments are performed. Periodic re-testing and monitoring the proper functioning of the cabinet is mandatory.

Since every live infectious sort has the potential to create infectious aerosols, it is highly recommended to verify aerosol containment prior to every infectious cell sort and to maintain a record of the results. This practice will assure validation of the aerosol management system to contain aerosols containing potentially infectious pathogens.

Conclusions and Novel Applications

Laboratories involved in basic or clinical research are faced with increasing demands for sorting of unfixed samples. Biohazardous sorting is often performed for infectious disease studies to separate leukocyte subsets on the basis of cell surface expression patterns. Sorted cell populations can then be examined to determine their response to the pathogen of interest, to discover the cellular mechanisms of its pathogenesis, or to identify or characterize cells infected with the pathogenetic organism (Giorgi, 1994). Recent sorting applications include studies of gene expression in cells that either carry a pathogen or have been transfected with fluorescent vectors that contain genetic sequences from an infectious agent (Herzenberg et al., 2002). Novel applications involve preparative cell sorting of clinical samples for therapeutic interventions (Leemhuis and Adams, 2000; Lopez, 2002).

Clinical cell sorting requires not only protection of instrument operators from known or unknown pathogens contained in the patient samples, but also use of Good Manufacturing Practices under clean-room conditions to prevent contamination of the sorted product to be re-infused into the patient (Keane-Moore et al., 2002; Ibrahim and van den Engh, 2003; Jayasinghe et al., 2006).

The prevention of exposure of laboratory personnel to biohazards is of great importance. The recommendations set forth here represent a timely effort by ISAC to provide standard practices for sorting of unfixed cells, including known biohazardous samples. These recommendations may also aid laboratories in obtaining institutional (e.g., Institutional Biosafety Committee, Institutional Review Board) and/or regulatory agency approval for sorting of such unfixed cells.

Furthermore, it is hoped that the principles outlined here will continue to prompt cell-sorter manufacturers to design any new instruments with operator safety in mind.

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