

## Biosafety Tips

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Biosafety Tips brings you practical approaches to biosafety or “news you can use.” If you are looking for a useful and sensible solution to a biocontainment problem, or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column, I share biosafety insights for managing a variety of workplace situations. I welcome feedback and suggestions for future topics. Please e-mail any comments or suggestions to karen\_byers@dfci.harvard.edu or to Co-Editor Barbara Johnson at barbara\_johnson@verizon.net.

### Cell Sorters Present Containment Challenges

The risk assessment for cell sorting is even more challenging with new high-speed cell sorters and “user friendly” cell sorters appearing in BSL-2 and BSL-3 laboratories, where the users may not be as experienced as flow cytometry technicians in dedicated facilities. Fortunately, the International Society for Analytical Cytometry (ISAC) has published new biosafety guidelines for unfixed cells that are run on a jet-in-air flow cytometer or a cell sorter that combines a flow cell with jet-in-air sorting (Schmid et al., 2007). ISAC provides this comprehensive biosafety reference on its web site at [www.isac-net.org/committees/biosafety.htm](http://www.isac-net.org/committees/biosafety.htm). The following URL contains the document “Standard Safety Practices for Sorting of Unfixed Cells” [www.mrw.interscience.wiley.com/emrw/9780471142959/cp/cpcy/article/cy0306/current/pdf](http://www.mrw.interscience.wiley.com/emrw/9780471142959/cp/cpcy/article/cy0306/current/pdf). This version replaces the 1994 ISAC biosafety guidelines and addresses the increased risk of some instruments as well as the improved aerosol control methods available for live cell sorting. The document includes a new recommendation for testing the aerosol containment of a given cell sorter with fluorescent beads before doing a potentially infectious sort.

### Fixed Cell Sorting

Demand for unfixed cell sorting has increased and assays involving cytokines or apoptosis, live DNA or RNA staining, and various membrane studies cannot be done with fixed cells (Schmid et al., 2007). However, if it is feasible for the experimental goals, fixation would be the biosafety recommendation. ISAC cautions that fixative concentrations should be verified as effective against potential infectious agents. An example cited describes fixation of cells infected with Human Immunodeficiency Virus (HIV) with 1% paraformaldehyde. The titer of HIV in infected cells, or in infected blood, was greatly reduced

by the 1% paraformaldehyde; however, cell-associated virus was not completely inactivated. It was possible to recover HIV from the fixed blood cells up to 18 hours post-fixation (Aloisio, 1990). In addition, even if the samples are completely inactivated by fixation, aerosol control may be a concern since the stains used may be toxic or carcinogenic (Schmid et al., 2007).

### Guidance on Risk Assessment

Researchers are under tremendous pressure to answer pressing biomedical research questions and may challenge safety decisions prohibiting infectious cell sorts in shared cytometry facilities. The ISAC biosafety guidelines provide a framework for discussion of these conflicts. The risk assessment sections refer staff to the biosafety professional for review of the infectious, carcinogenic, or recombinant hazards of the samples, and review issues such as the cell sorting instruments to be used and the potential for discharge or aerosols and exposure to the operator or bystander. Engineering controls, personal protective equipment, medical surveillance, and the level of flow cytometry and laboratory experience required for risk minimization are also covered. In my experience, researchers balk when denied access to a cell sorter in a shared facility and point to the fact that a given BSL-2 infectious agent is not transmitted by aerosol in the general population. This can be countered only with the argument that cultures have a higher concentration of the infectious agent, and cell sorting requires the deliberate formation of droplets. Examples of agents, which can be transmitted by a different route in a laboratory setting, include airborne transmission of scrub typhus (Oh et al., 2001) and ingestion transmission of HIV (Ruprecht et al., 1999).

### How are the aerosols formed?

A general explanation of the cell sorting process is available on many flow cytometry web sites such as [www.unsolvedmysteries.oregonstate.edu/flow\\_cytometry\\_06.shtml](http://www.unsolvedmysteries.oregonstate.edu/flow_cytometry_06.shtml). Aerosol generation occurs when a cell suspension is drawn up and pumped through a vibrating nozzle for the purpose of breaking the cell stream into individual droplets that fall between high voltage plates. The ISAC guidelines describe the creation of droplets and aerosols during cell sorting in detail. High-speed sorters operate under greater pressure and produce more small droplets as compared to older instruments that operate at slower speeds. However, the guidelines state clearly that “all sort-

ers also generate microdroplets, i.e., satellite droplet 3 to 7  $\mu\text{m}$  (Schmid et al., 2007), and additional droplets of all sizes are produced when a nozzle becomes partially clogged or the stream is accidentally deflected onto a hard surface, such as the waste catcher.” Excellent photos of the aerosol/droplet stream created when the cell sorter is not operating optimally on the web can be viewed at [www3.interscience.wiley.com/cgi-bin/fulltext/104084100/HTMLSTART](http://www3.interscience.wiley.com/cgi-bin/fulltext/104084100/HTMLSTART) (Perfetto, 2003). For this reason, the ISAC guidelines include the following statement:

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 located in a BSL-2 facility (modified as described in Environmental Controls, described below) using practices and containment equipment for BSL-3 by the CDC. 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.

For BSL-2 sorts, ISAC recommends enclosing the cell sorter in a biosafety cabinet or enclosure. When that is not possible, the recommended environmental controls are:

- Cell sorters used for BSL-2 sorts should be located in a separate, lockable room where no other lab activity is performed.
- The room exhaust should discharge to the outside away from occupied areas or be HEPA filtered.
- Airflow in the room is balanced to no less than 10 changes of air per hour.

### Advising Researchers on Purchase or Upgrade of Aerosol Containment Capability

Some flow cytometers have been specifically designed to fit in a biosafety enclosure, and researchers ordering these devices may not be aware of this fact. In addition,

containment improvements are available for many other flow cytometers (example in Figure 1). A careful review of instrument containment should be included in the risk assessment of cell sorting, and upgrades should be considered where appropriate. The “aerosol containment features” are auxiliary vacuum pumps to reduce aerosols in the chamber before the door is opened to address clogged nozzles or deflected streams. Some cell sorters are set up with a remote camera to allow the technician to observe the sorting streams away from the sorting area.

### Containment Enclosures

The BD FACSAria (Becton Dickinson Biosciences, San Jose, California) and JSAN (Bay Bioscience Co., Ltd. (Kobe, Japan) were designed to fit in a biocontainment biological safety enclosure (Baker Co., Sanford, Maine). A detailed explanation of this biosafety enclosure is presented in an ABSA Anthology (Ghidoni et al., 2006). Since the entire unit fits inside the Bioprotect II, some splash protection is also provided from the tubing that leads to the waste collection bottles. There are also high-speed sorters designed to be integrated into a biosafety cabinet: InFlux (Cytospeia, Seattle, Washington) and the Reflection (iCyt Visionary—Bioscience, Champaign, Illinois). Older units with water-cooled lasers (such as FACS Star, FACS Vantage, and FACSDiVa) may be too large to fit in a standard biosafety enclosure. However, a removable containment device (Cytek Development, Fremont, California) is commercially available. The Cytek unit draws air from the cell sorting and sample uptake areas and exhausts the air into the room through a HEPA filter. A custom enclosure would also solve an aerosol problem; Bigneat Containment Technology (Hampshire, United Kingdom) Flow Sciences, Inc. (Leland, North Carolina) and NuAire (Plymouth, Minnesota) have exhibited custom designs at ABSA conferences. One paper describes adapting the cell sorter to fit in a biosafety cabinet to allow sorting of peripheral blood cells in a BSL-2 laboratory (Lennartz et al., 2005). Becton Dickinson also recently purchased Dako Colorado, Inc. (Fort Collins, Colorado), which developed a Class I-type attachment for

**Figure 1**

Example of biosafety options available for one model of flow cytometer, the Becton Dickinson FACS DiVa™, from the web site at [www.bdbiosciences.com/immunocytometry\\_systems/products](http://www.bdbiosciences.com/immunocytometry_systems/products)

#### Closed Flow System

- Closed flow channel for analysis of biohazardous samples
- Autoclavable waste container with audible overfill alarm

#### BD™ Aerosol Management

- Aids in aerosol management for high-speed sorters
- Supplies distributed vacuum at the sort chamber when sort chamber door is closed
- Displays visible filter integrity display
- Is compatible with the BD FACSDiVa™ option

**Note:** Although these aids help with aerosol management, they do not replace good laboratory practices. All routine laboratory biological hazards protection should be followed in conjunction with the aerosol management system.

**Sample Splash Shields:** Provide additional droplet containment around the sample inlet port and nozzle assembly area

the MoFlo high-speed cell sorter. However, please note that while these “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 sorting any potentially infectious samples” (Schmid et al., 2007).

### Auditing Safety Measures

This new ISAC Standard emphasizes the importance of audits to verify that containment measures are effective. ISAC advises:

- Testing the sort chamber with bottled smoke and sealing any leaks found
- Using splash shields for the sample uptake area and/or the sort chamber
- Accessing and inspecting fluidic tubing that is under pressure before infectious sorts
- Using sizing nozzles appropriate for the cells to be sorted. At a minimum, the aperture should be four times greater than the cell size; six times the size of the cells to be sorted is listed as the ideal (Schmid et al., 2007).

Some manufacturers recommend cleaning the nozzles by sonication—biosafety professionals will have opinions on where that is done! Details such as proper disinfection of the equipment according to manufacturer’s directions, sink discharge of disinfected waste, and the use of personal protective equipment and handwashing after removal of personal protective equipment should all be evaluated during an audit. If a risk assessment warrants the wearing of respiratory protection, staff should be enrolled in a respiratory protection program. The ISAC standard contains a great deal of substantive, updated information that will be of assistance to biosafety professionals whose responsibilities require them to review the

sorting of unfixed biohazardous samples. Sharing the ISAC guidelines with staff performing this procedure is an important first step for safe cell sorting.

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## Molecular Biosafety

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The molecular biology and biotechnology fields are growing by leaps and bounds. Molecular Biosafety aims to shed light on how these cutting-edge techniques impact safety. Please e-mail your insights and questions to Margy Lambert at [mlambert@fpm.wisc.edu](mailto:mlambert@fpm.wisc.edu) or Co-Editor Barbara Johnson at [barbara\\_johnson@verizon.net](mailto:barbara_johnson@verizon.net) or Co-Editor Karen B. Byers at [karen\\_byers@dfci.harvard.edu](mailto:karen_byers@dfci.harvard.edu).

### A Reassessment of Adeno-Associated Virus Vector Risks That Takes New Information on Insertional Mutagenesis into Account

A recent *Science* article implicated insertional mutagenesis as a potential mechanism for induction of liver cancer in mice by adeno-associated virus (AAV) vectors (Donsante et al., 2007). The results of this study, as discussed by Kay 2007, have resurfaced concerns that AAV vectors may not be as safe as previously thought and may influence how AAV and recombinant AAV will be handled in research laboratories in the future.

AAV is a parvovirus that can be aerosol-transmitted and a dependovirus that normally requires another virus such as adenovirus or herpes simplex virus (HSV) to supply factors that support replication (Hamilton et al.,