

# Bigfoot Spectral Cell Sorter

## A new approach to cell sorter safety

Authors: Karen Helm, University of Colorado Cancer Center, Aurora, CO; Angie Goldfain, Thermo Fisher Scientific; Lincoln Gulley, Thermo Fisher Scientific

### Introduction

Cell sorting continues to be a powerful contributor in the quest to improve human health, allowing researchers to make timely advancements in drug and vaccine development, as illustrated by the newly released SARS-CoV-2 sorting protocols [1]. However, cell sorting is also known to produce aerosols that can expose the operator to both known and unknown pathogens. When cell sorters are run at high pressures, they can produce aerosols with high particulate content as well as extremely small particles. Such particles, especially those smaller than 2  $\mu\text{m}$ , are prone to deposition in both the upper airway and the alveolar spaces in the lungs, with potential to harm the operator [2]. This hazard necessitates protective barriers and containment apparatus to be incorporated into the cell sorter.

In the early 2000s, manufacturers sought to address these concerns by adding aerosol management systems (AMSs), which continuously evacuate the sorting chamber to remove aerosols. In the same period, the first attempt was made to fit a cell sorter into a Class II biosafety cabinet (BSC). Though less than ideal, it served to protect the sort sample and to some extent the operator. Current International Society for Advancement of Cytometry (ISAC) safety regulations require that all cell sorters now be equipped with an AMS [2]. These standards also state that most types of cell samples and lab spaces



necessitate the sorter to be enclosed in a BSC. Despite these regulations, AMSs and BSCs are frequently marketed as optional accessories when purchasing a sorter and, as such, frequently fall victim to funding shortfalls. Several newer sorters include an AMS integrated with a standard BSC, but they continue to resemble an ad hoc solution with manual operation of air-handling controls and poor access for service manipulation, which can lead to inconsistent results and an unsafe environment.

The referenced ISAC regulations require periodic testing of AMS and BSC systems. While the recently developed Cyclax-D air sampler and 1  $\mu\text{m}$  Dragon Green fluorescent microspheres AMS testing method is an improvement over previous procedures, the process remains cumbersome and not clearly defined for many sorters. The literature describes the testing using only one manufacturer's cell sorter, and while general guidelines are included to modify the procedure for other vendors' equipment, such modifications are not always clear-cut and frequently require burdensome manual overrides and manipulations. An instrument designed with a well-integrated AMS and BSC will likely solve most if not all of these common concerns.

## BSC and AMS design reinterpreted

The Invitrogen™ Bigfoot Spectral Cell Sorter is designed with an integrated BSC and AMS. Sample-related subsystems are segregated inside the BSC for optimal safety, sanitation, and performance. Sealed optical windows surround the nozzle, defining the barrier between the inside and outside of the BSC. This separation allows lasers, excitation optics, and scatter objective lenses to remain outside the BSC yet close to the interrogation point, which maintains the superior performance of a jet-in-air sorter. All other systems, such as detection, electronics, and fluidics, are also outside the containment area. This allows better service access and temperature regulation as compared to other sorters.

The Bigfoot Spectral Cell Sorter BSC system has been verified to meet personnel and product protection standards for a Class II Type A2 biosafety cabinet per the National Sanitation Foundation (NSF) International Standard 49, Section 3.8.2.3. Specifically, this means the BSC:

- Maintains an average air velocity of 100 ft/min through the work access opening
- Provides high-efficiency particulate air (HEPA)-filtered downflow air that is mixed with the downflow and inflow air
- Exhausts HEPA-filtered air into either the laboratory or, via an optional canopy connection, through an external exhaust system
- Holds all biologically contaminated ducts and plenums under negative pressure

The AMS and BSC portions of the system can be operated, and are monitored, independently. The system houses two independent exhaust fans and two independent HEPA filters; one fan and filter for the AMS, and one fan and filter for the BSC. Pressure sensors independently monitor containment in both the AMS and the BSC for redundant biosafety containment.

Aerosols in the segregated sort chamber are entrained in air that is ducted to a HEPA filter in the AMS. The door to access the sort chamber is inside the greater BSC. In the unlikely event hazardous aerosols leak out of the sort chamber, the greater BSC offers a secondary biosafety system to capture aerosols. As with all Class II Type A2 cabinets, a portion of the filtered air is recirculated to

deliver uniform downflow air, and the remaining filtered air is exhausted to the laboratory environment, or through an external exhaust system connected to the optional cabinet canopy connection (Figure 1).

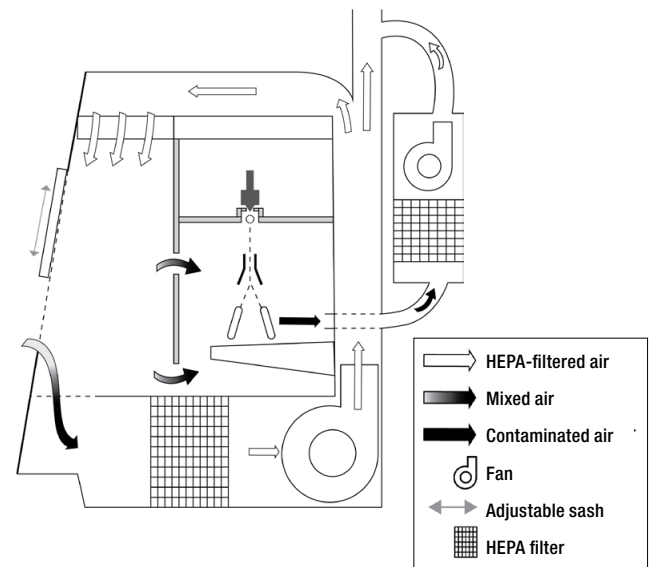


Figure 1. Airflow diagram.

Recirculated and exhausted air is HEPA filtered to remove 99.97% of particles greater than 0.3  $\mu\text{m}$ . Inflow air velocity is regulated to a minimum of 100 ft/min through the work access opening. During a sort, the AMS continuously draws air from the sort chamber and through a HEPA filter. If a clog is detected, software notifies the operator; stops the sort, sample, and sheath; and automatically increases the speed of the AMS fan to quickly purge the sort chamber of aerosols prior to allowing the user access to the affected area. All the while, the BSC portion of the system continues operating independently to maintain containment.

In contrast to standard BSCs, the Bigfoot Spectral Cell Sorter's integrated BSC filters the air before a fan pressurizes it for recirculation or exhaust. Uniform downflow air is established with a low-profile, three-stage diffuser. Therefore, all ducts under positive pressure contain only filtered air, reducing the risk of leaks containing aerosols. The fans and sensitive HEPA filters are located below the work surfaces and to the rear of the instrument. Thus, HEPA filters are protected from inadvertent damage during daily operation, noise is kept to a minimum, and vibrations are isolated from the optical path, which helps to maintain the superior optical stability of the Bigfoot Spectral Cell Sorter.

### User experience innovation

In addition to the effort invested in technical ingenuity, significant resources were also devoted to user experience innovations. Inside the laminar airflow barrier there is ample deck space for sample racks and plates, as well as access to a built-in sample vortex mixer, tube rack, and biohazard bag. This makes it possible for the operator to complete common tasks without breaching the safety barrier multiple times during the normal workflow (Figure 2). Interior work surfaces are made with nonporous, durable 304 stainless steel for easy cleaning.

Lab personnel in biohazardous environments must work efficiently and make use of techniques that reduce interactions with hazards in order to maximize safety. The Bigfoot Spectral Cell Sorter includes four major innovations to help operators limit hazardous contact:

- The sample loader holds multiple controls or samples, which reduces the need for the operator to enter and exit the BSC continuously for sample loading
- The sort collection rack accommodates numerous sort tubes to collect multiple sorts from different runs or one long run, which reduces the need for the operator to enter and exit the BSC to unload sorted samples (Figure 3)
- Plates can be sorted in less than 20 sec; therefore, the time the operator interacts with the biohazardous environment is reduced
- The internal control panel limits the need for the operator to reach in and out of the BSC to operate the system (Figure 4)

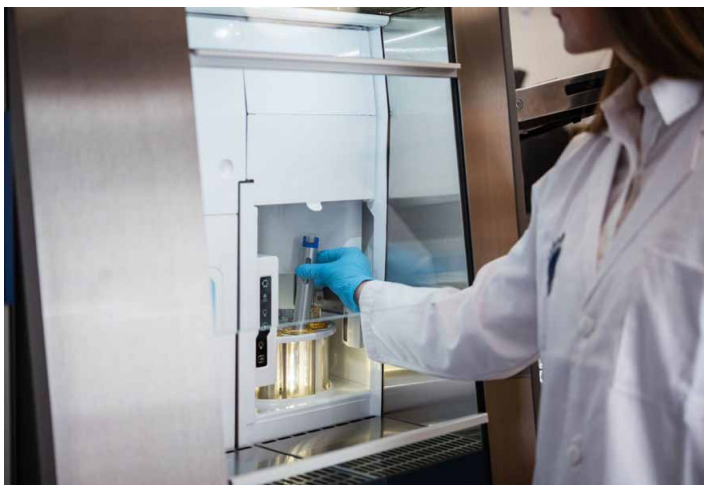


Figure 2. Adjustable sash in the up position for sample access.

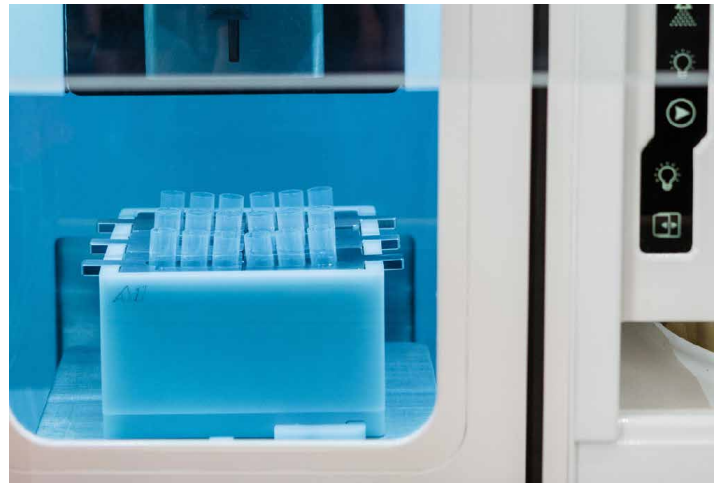


Figure 3. The sort collector accommodates numerous sort tubes.



Figure 4. The internal control panel limits the need for the operator to reach in and out of the BSC.

## Low noise

Due to the integrated design, the Bigfoot Spectral Cell Sorter is far more compact compared to other models currently available. The smaller containment area and thoughtful fan placement have resulted in a system that produces less noise during operation. Therefore, the Bigfoot Spectral Cell Sorter can be more comfortably housed near other laboratory instrumentation and operators, which is a valuable consideration for space-limited facilities. Furthermore, by minimizing materials use, integrated containment reduces the overall cost of the system so that safety is not compromised due to limited funding.

## Conclusion

We have reinterpreted BSC design by enclosing only potentially pathogen-exposed components and leaving the rest of the instrument accessible to operators and service personnel. The recent SARS-CoV-2 crisis has focused global attention on the importance of biosafety, which should prompt scientific laboratories to reevaluate existing biosafety measures, reinforce old procedures, and implement improvements. Biosafety is increasingly critical in sorting facilities and is mandated as a condition of obtaining some grants and funding [4]. The software-guided containment testing protocol simplifies and streamlines mandated safety assessments. The Bigfoot Spectral Cell Sorter provides containment and operator protections that meet the need for modern cell sorter safety without sacrificing performance or laboratory space.

## Persistent containment

Unlike traditional biosafety cabinets applied to flow cytometry applications, the sliding sash on the Bigfoot Spectral Cell Sorter allows improved operator access to the instrument while still maintaining aerosol containment. When the sash is in the up position, the operator has safe access to the sample area (similar to traditional cabinets). Uniquely, when interaction with the nozzle is necessary, the operator slides the sash down to access a BSC-contained upper opening for ergonomic interaction. Upon opening the nozzle door, the nozzle automatically moves forward where it can be easily cleaned or changed (Figure 5).

The BSC maintains personnel safety protections for total system operation regardless of the sash position.

## Seamless laboratory integration

The previously referenced ISAC standard was used to develop an integrated airflow-and-containment software wizard that guides personnel through the recommended protocols for periodic AMS testing and yearly BSC certification. This integrated software has been developed specifically to run the instrument and the BSC together, which helps the operator seamlessly follow all biosafety precautions while running the Bigfoot Spectral Cell Sorter.

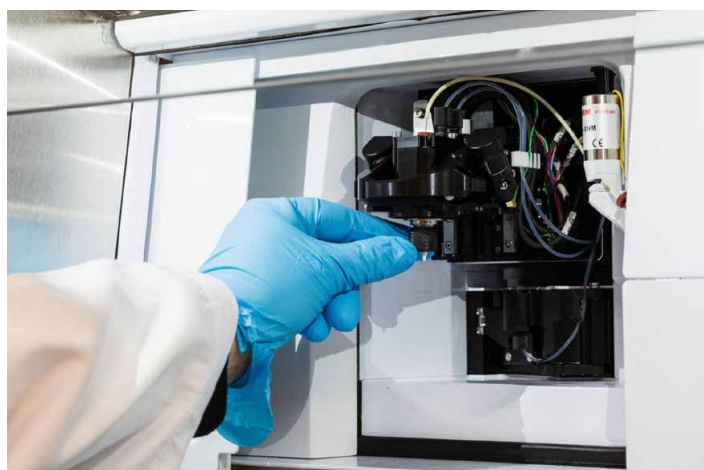


Figure 5. Adjustable sash in the down position for nozzle access.

## References

1. Perfetto, Stephen P. and Ben Fontes. New SARS-CoV-2 Sorting Protocols Released. ISAC, 26 March 2020, <https://isac-net.org/news/497501/NEW-SARS-CoV-2-SORTING-PROTOCOLS-RELEASED.htm>.
2. Holmes K, Fontes B, Hogarth P, Kunz R, Monard S, Pletcher C, Wadley R, Schmid I, Perfetto S. International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards Cytometry A. May;85(5):434–53. doi: 10.1002/cyto.a.22454. Epub 2014 Mar 13. PubMed PMID: 24634405; PubMed Central PMCID: PMC4117398.
3. The NFS Joint Committee on Biosafety Cabinetry. NSF/ANSI 49 - 2018 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification. NSF International/American Standards Institute 2019;3.8.2:5–6.
4. Office of Biotechnology Activities. NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules. Office of Biotechnology Activities; 2013. [Online: the most current version can be found at <http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines/>]

Find out more at [thermofisher.com/bigfoot](https://thermofisher.com/bigfoot)

**ThermoFisher**  
SCIENTIFIC