Bigfoot Spectral Cell Sorter

A new approach to cell sorter safety

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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 µm, 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 a 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 laboratory 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 AMS testing method using the recently developed Cyclex D air sampler and 1 µm Dragon Green fluorescent microspheres 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.

Integrated aerosol containment

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

The NSF49 and EN12469 [3,4] standards require certification while the hood is empty, which is not the normal use case for this application of biocontainment. The Bigfoot sorter is a containment system and AMS, which can be certified while in operation to meet the safety and airflow requirements of these standards and cell sorting guidelines.

Although not an actual BSC, the Bigfoot Spectral Cell Sorter provides personnel and product protection similar to a Class II BSC. Test procedures and criteria laid out within NSF49 and EN12469 standards can be utilized to demonstrate performance. Specifically, this means the containment system:

- Maintains an average air velocity of 100 ft/min (NSF49) or >79 ft/min (EN12469) through the work access opening
- Provides high-efficiency particulate air (HEPA)-filtered downflow air that is mixed with the 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 biosafety enclosure 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 containment and product protection chamber. Pressure sensors independently monitor containment in both systems 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 enclosure. In the unlikely event hazardous aerosols leak out of the sort chamber, the greater enclosure 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).

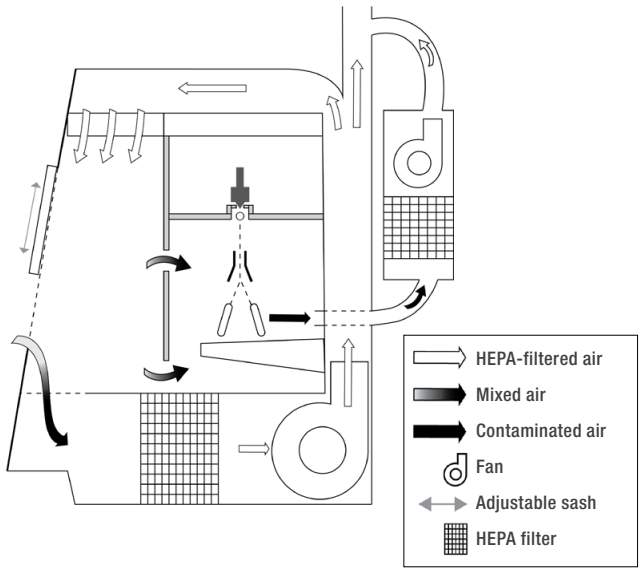

Figure 1. Airflow diagram.

Product and personnel protection are achieved using H14 HEPA filters and air velocity relationships similar to a BSC. H14 HEPA filters used with the Bigfooter sorter:

- Provide 99.995% collection efficiency at the most penetrating particle size
- Have localized collection efficiency of ≥99.975%
- When placed in local exhaust ventilation (LEV) and containment devices, have no leaks greater than 0.01% when scanned with an aerosol photometer

During a sort, the separate Bigfoot 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. During this time, the greater enclosure continues operating independently to maintain containment.

For improved containment of potential hazards, the integrated system of the Bigfoot Spectral Cell Sorter filters the air before a fan pressurizes it for recirculation or exhaust. Unidirectional 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 uniform 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 enables 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.

Laboratory 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 enclosure 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 enclosure 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 enclosure to operate the system (Figure 4).

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

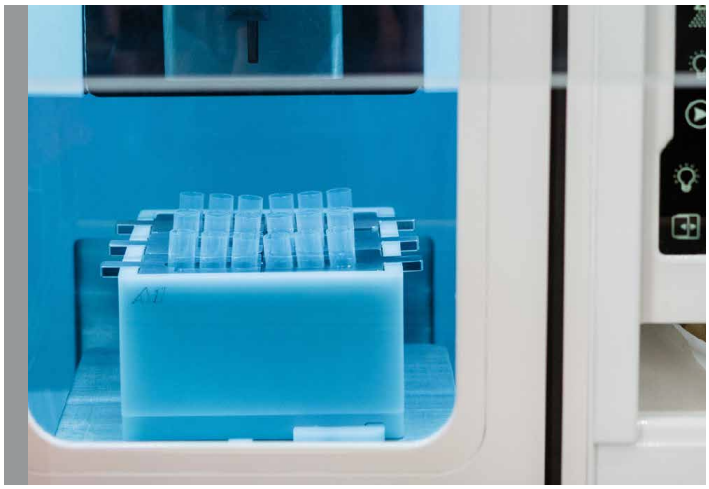

Figure 3. The sort collector with numerous sort tubes.

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

Low noise

Due to the integrated design of the biosafety enclosure, the Bigfoot Spectral Cell Sorter is far more compact compared with other currently available models. The smaller containment area and thoughtful fan placement have resulted in a system that produces less noise during operation. Therefore, the Bigfoot 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.

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 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 containment system 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 certification. This SQ software has been developed specifically to run the instrument and the integrated containment system together, which helps the operator seamlessly follow all biosafety precautions while running the Bigfoot Spectral Cell Sorter.

Conclusion

We have reinterpreted BSC design by enclosing only components that are potentially exposed to pathogens 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 [5]. 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.

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

References

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