Bigfoot Biosafety Systems Marketing Primer

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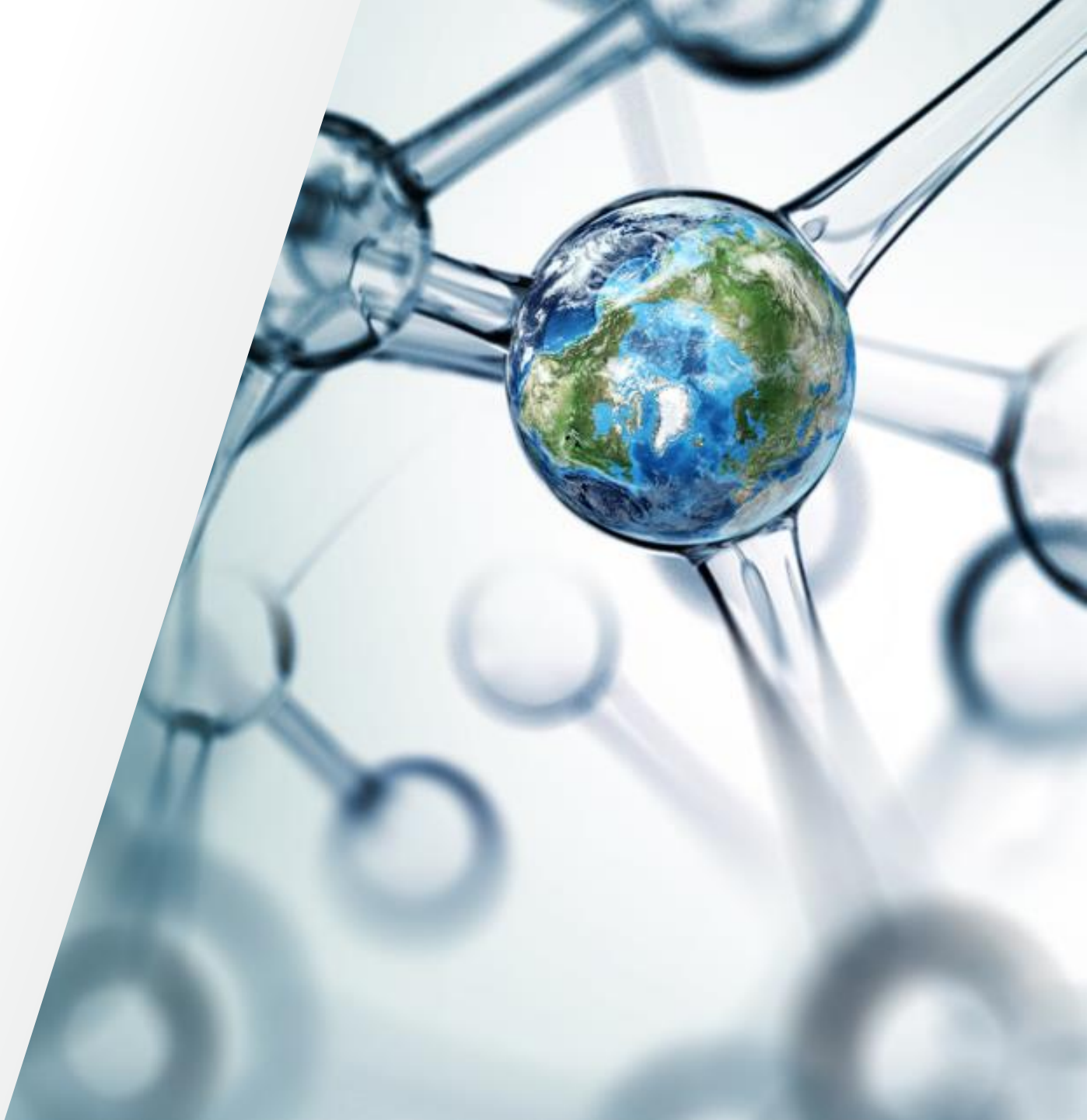

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Revisions

Revision	Change Description	Changed By/Date
A	New Release	LG 02/15/24
B	Added more clear “For internal use only” disclaimer	LG 04/04/24

Purpose

For Internal Use Only

- This slide deck is intended to be an internal resource for our Bigfoot sales team
- This slide deck describes the design, testing, installation, and ongoing service of the Bigfoot biosafety systems.
- The Bigfoot Biosafety Enclosure is a novel approach to Cell Sorter biosafety. The system is well-validated, but the testing and service of the systems are different compared to typical Biosafety Cabinets. This document answers the most common questions related to Bigfoot Biosafety.

Background and Definitions

- Biosafety Cabinet (Microbiological Safety Cabinet)
  - Devices that use partial barrier systems that rely on the movement of air to provide personnel, product, and environmental protection.
- Biosafety Level (Containment Level)
  - BSL1 (CL1) - Suitable for work involving well well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. Work is typically conducted on open bench tops using standard microbiological practices.
  - BSL2 (CL2) - Suitable for work involving agents that pose moderate hazards to personnel and the environment. Laboratory personnel have specific training. Access to the laboratory is restricted. All procedures in which infection aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.
  - BSL3 (CL3) - Applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with agents that may cause serious or potentially lethal disease through inhalation route exposure. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infection aerosols from the laboratory.
  - BSL4 (CL4) - Required for work with agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission.
- Primary Containment Device
  - A contained workspace designed to provide protection to its operator, the laboratory environment and/or the work materials for activities where there is an aerosol hazard. Protection is achieved by segregation of the work from the main area of the laboratory and/or through the use of controlled, directional airflow mechanisms. Primary containment devices include biological safety cabinets (BSCs), isolators, local exhaust ventilators and ventilated working spaces.
  - Primary containment devices are the more generic category of biosafety cabinet. All Biosafety Cabinets are Primary Containment Devices. Not all Primary Containment Devices are Biosafety Cabinets.

[1] NSF49, NSF/ANSI 49-2018: Biosafety Cabinetry – Design, Construction, Performance, And Field Certification
[2] Laboratory biosafety manual, 4th edition

Background and Definitions

- NSF49
  - One of the two primary international standards that defines the design, construction, performance, and validation methods of biosafety cabinets.
  - NSF49 is more United States-centric. Biosafety Cabinets sold in the U.S. typically adhere to NSF49.
- EN12469
  - One of the two primary international standards that defines the design, construction, performance, and validation methods of biosafety cabinets.
  - EN12469 is more European Union-centric. Biosafety Cabinets sold in Europe typically adhere to EN12469.
- Other Standards
  - There are other standards that define the testing and performance of biosafety cabinets, but they are essentially identical to EN12469. These include the Chinese Industrial Standard, YY 0569 – 2011, Class II Biological Safety Cabinets, and the Japanese Industrial Standard, JIS K 3800, Class II Biological Safety Cabinets
- Personnel Protection
  - Refers to protecting the user of the biosafety cabinet.
- Product Protection
  - Refers to protecting the sample being manipulated or stored in the biosafety cabinet from being contaminated from inflowing air from the lab environment or from the spread of aerosol within the cabinet itself (cross contamination protection).
- Environmental Protection
  - Refers to the greater environment outside of the laboratory. The exhaust of a Biosafety Cabinet must be HEPA filtered to prevent contamination of the surrounding environment if the output of the cabinet is exhausted outside the facility.

Background and Definitions

- Class I Biosafety Cabinet (NSF49 definition)
  - Provides personnel and environmental protection without product protection
  - Minimum inflow air velocity of 75 feet per minute through the user access opening
  - Exhaust is HEPA filtered
- Class II Biosafety Cabinet (NSF49 definition)
  - Partial barrier systems that rely on the movement of air to provide personnel, environmental, and product protection
  - Side-to-side cross contamination of product is minimized by the internal downflow of HEPA filtered air moving towards the work surface
  - Exhaust may be either recirculated into the lab or discharged from the building via a canopy connection
  - Appropriate for work involving procedures at BSL1, BSL2, and BSL3. A Class III BSC is not required to perform BSL 3 work.
- Class III Biosafety Cabinets (NSF49 definition)
  - Designed to work with highly infectious or hazardous agents
  - A gas-tight enclosure (glove box)
  - Passage in and out of enclosure requires a decontamination pass-through
  - Both supply and exhaust must be HEPA filtered. Exhaust must pass through two HEPA filters in series.
- Class II, Type A2 Cabinets (NSF49 definition)
  - A subcategory of Class II cabinets. Meets all the class II requirements above. Most class II cabinets are Class II, Type A2.
  - “A minimum average inflow velocity of 100 feet per minute through the work access opening”
  - “Have HEPA filtered downflow air that is a portion of the mixed downflow and inflow air from a common exhaust plenum”
  - “May exhaust HEPA filtered air back into the laboratory of the environment through an external exhaust system connected to the cabinet with a canopy connection”
  - “Have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums”.

Background and Definitions

- Microbiological Safety Cabinet Class I (EN12469 definition)
  - “Safety cabinet with a front aperture through which the operator can carry out manipulations inside the cabinet and which is constructed so that the worker is protected and the escape of airborne contamination generated within the cabinet is controlled by means of an inward airflow through the working front aperture and filtration of the exhaust air.”
- Microbiological Safety Cabinet Class II (EN12469 definition)
  - “Safety cabinet with a front aperture through which the operator can carry out manipulations inside the cabinet and which is constructed so that the worker is protected, the risk of product and cross contamination is low and the escape of airborne particulate contamination generated within the cabinet is controlled by means of an appropriate filtered internal airflow and filtration of the exhaust air.”
  - A minimum aperture protection factor of 1 x 10^5.
  - A leaktightness index of LI-C, or a leakage of a target micro-organism tested under defined conditions and leakage below detection limit or threshold value.
  - A product protection of less than 5 Colony Forming Units per test.
  - A cross contamination of less than 2 Colony Forming Units per test.
- Microbiological Safety Cabinet Class III (EN12469 definition)
  - “Safety cabinet in which the working area is totally enclosed and the operator is separated from the work by a physical barrier (i.e. gloves mechanically attached to the cabinet).
- NSF49 Class II Type A2 vs. EN12469 Class II cabinets
  - Although there are small differences between these two definitions, from the perspective of a user a Class II Type A2 cabinet as defined by NSF49 is equivalent to a Class II MSC as defined by EN12469.

[3] EN 12469:2000, Biotechnology. Performance criteria for microbiological safety cabinets

Background and Definitions

- Vaporized Hydrogen Peroxide
  - VHP is trademarked by Steris, therefore technically this should only be used when refereeing to the Steris product, however the acronym is commonly used to describe all hydrogen peroxide decontamination methods
  - The generic term is VPHP (Vapor Phase Hydrogen Peroxide)
  - The only approved method to decontaminate the Bigfoot biosafety systems
- Inflow
  - The velocity or volume of air that flows from the room into the front access opening, providing an air barrier to prevent the escape of aerosols generated in the cabinet's work zone.
- Downflow
  - The velocity or volume of air that flows down from the top of the interior of the work area to the work surface. The uniform or laminar downflow HEPA filtered air is the mechanism that provides Product Protection by establishing an air barrier that prevents the mixing of inflow (laboratory) air from the HEPA filtered downflow air that washes over samples placed inside the cabinet.
- Canopy Connection (Thimble Connection)
  - The required method to connect a Class II Type A2 (Class II MSC) to a facility exhaust system to be discharged outside the building
  - A BSC exhaust connection where there are one or more openings of gaps in the connection between the BSC and the external exhaust system.

Bigfoot Biosafety Systems Overview

- Bigfoot contains two biosafety systems
  1) Biosafety Enclosure
     - A Primary Containment Device similar to a Class II Type A2 BSC or Class II MSC
     - Encloses the sample input system (Loader), Nozzle, Sort Output Chamber, and User Work Surfaces
  2) Aerosol Management System (AMS)
     - A secondary system that draws air from inside the Sort Chamber. Aerosol Management Systems are a flow cytometry community accepted system that is defined by an ISAC biosafety committee.
- System Components
  - Both the Biosafety Enclosure and Aerosol Management Systems have one fan and one HEPA filter. Therefore, the instrument as whole contains two biosafety systems fans and two HEPA filters. Unlike a traditional biosafety cabinet that requires two HEPA filters for the BSC alone, the Bigfoot system uses a single HEPA filter to supply both the recirculated air and exhaust. See airflow diagrams later in document for details.
- Redundancy
  - The two systems are redundant with respect to each other for biohazardous aerosols created in the Sort Chamber. If there is a fault with the Biosafety Enclosure, personnel protection is maintained by the Aerosol Management System alone. If there is a fault with the AMS, personnel protection is maintained by the Biosafety Enclosure alone.

Classification

- The main Bigfoot biosafety system we refer to as the Biosafety Enclosure. It is classified as a Primary Containment Device not a Biosafety Cabinet.

- The Bigfoot Spectral Cell Sorter was designed with two biosafety systems which function in parallel to provide excellent personnel, product, and environmental protection. The first of the two biosafety systems is the flow cytometry specific Aerosol Management System (AMS) which evacuates biohazardous aerosols from the Sort Chamber – the most contaminated space in the instrument. The second of the two biosafety systems is a Primary Containment Device that we refer to as the “Biosafety Enclosure”. The Biosafety Enclosure was designed to function as a Class II Type A2 Biosafety Cabinet (Class II MSC), and, due to the novel design of the highly integrated flow cytometer-biosafety system combination, we cannot classify the product as a traditional BSC. The definition of a BSC is strict and refers to an empty stand-alone product that typical biohazardous sample preparation and workflows take place within. Because the product is highly-integrated, and the Bigfoot cell sorter is inseparable from the biosafety systems, the product does not fall under the classification of a “Biosafety Cabinet” or “MSC”. The technically correct term to describe our system is a Primary Containment Device. This is a more generic classification that is used to describe a variety of custom products such as biosafety robotic lab automation enclosures. However, we can still demonstrate performance similar to a Class II BSC by subjecting our Primary Containment Device to the same functional tests that are described in NSF49 and EN12469.

Primary and Secondary Containment

- Primary Containment Device is a term that encompasses Microbiological Safety Cabinets. All MSCs are a subset of Primary Containment Devices. Not all Primary Containment Devices are MSCs.
- Primary Containment Devices can also be described less rigorously in different combinations such as Primary Physical Containment, Primary Containment, and Primary Containment Measures.
- Primary Containment “provides protection of the worker and the immediate environment and can be achieved through a combination of good microbiological practices or techniques and the use of appropriate containment devices or safety equipment, eg MSCs. Further protection may include procedural controls and use of other safety equipment, and may be supplemented by immunization.” [9] Section 5, Reference 134, Page 37.
- Secondary Containment “provides measures to protect those outside the immediate facility and can be achieved by a combination of laboratory design and operating procedures, eg restriction of access, air handling and safe disposal of waste.” [9] Section 5, Reference 134, Page 37.
- Do not use Primary Containment Device as an acronym. The term PCD is not used in industry, the standards, and is not widely used. When talking about the Primary Containment Device in communications, either use the term biosafety enclosure or spell out the full Primary Containment Device.

Similarities Between Typical BSC and Bigfoot Enclosure

- Design and Construction
  - Although the Bigfoot Integrated Biosafety Enclosure can't be classified as a Biosafety Cabinet, it was designed to meet the functional requirements of and be validated like a Class II Type A2 biosafety cabinet (Class II MSC). Here are the ways in which the Bigfoot Integrated Biosafety Enclosure is similar to a typical BSC.
  - It is a partial barrier system that rely on the movement of air to provide personnel, environmental, and product protection
  - Side-to-side cross contamination of product is minimized by the internal downflow of HEPA filtered air moving towards the work surface
  - Exhaust may be either recirculated into the lab or discharged from the building via a canopy connection
  - Appropriate for work involving procedures at BSL1, BSL2, and BSL3.
  - A minimum average inflow velocity of 100 feet per minute through the work access opening
  - Has HEPA filtered downflow air that is a portion of the mixed downflow and inflow air from a common exhaust plenum
  - May exhaust HEPA filtered air back into the laboratory of the environment through an external exhaust system connected to the cabinet with a canopy connection"
  - All biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums
  - Ducts and interior surfaces are stainless steel. Sealant used is specified in the NSF49 standard.

Similarities Between Typical BSC and Bigfoot Enclosure

- Factory-Performed Validation
  - The Bigfoot integrated Biosafety Enclosure was challenged to the standardized microbiological testing defined in NSF49 and EN12469. See “Microbiological Testing” section for details.
  - The Personnel Protection of Class II MSCs is defined in EN12469:2000, 5.4, Retention at the Front Aperture. A nominal average inflow air velocity of 105 fpm (0.53 m/s) is maintained whenever the instrument is in a running state. The Bigfoot biosafety enclosure was verified at the same level as a Class II MSC with the use of both microbiological and proxy methods (KI Discus test). The Bigfoot biosafety enclosure has an Aperture Protection Factor of greater than 1 x 10^5.
  - The Environmental Safety of Class II MSCs is defined in EN12469:2000, 7.3, Environmental safety. The HEPA filters of the Bigfoot biosafety enclosure can be verified at the same efficiency as typical Class II MSCs. Both use H14 HEPA filters with a listed efficiency of 99.995% for 0.3 µm particles. When measuring the efficiency, upstream test ports are provided to directly measure the concentration of the challenge aerosol for both the biosafety enclosure and AMS HEPA filters.
- User-Performed Validation
  - The Biosafety Enclosure can be validated from the same testing services that would validate typical BSCs. A test protocol to validate the biosafety performance of the product is provided. This testing is recommended to be performed at the first installation, and every 12 months after or in accordance with the safety requirements of the institution. See “User-Performed Validation” section for details.

Similarities Between Typical BSC and Bigfoot Enclosure

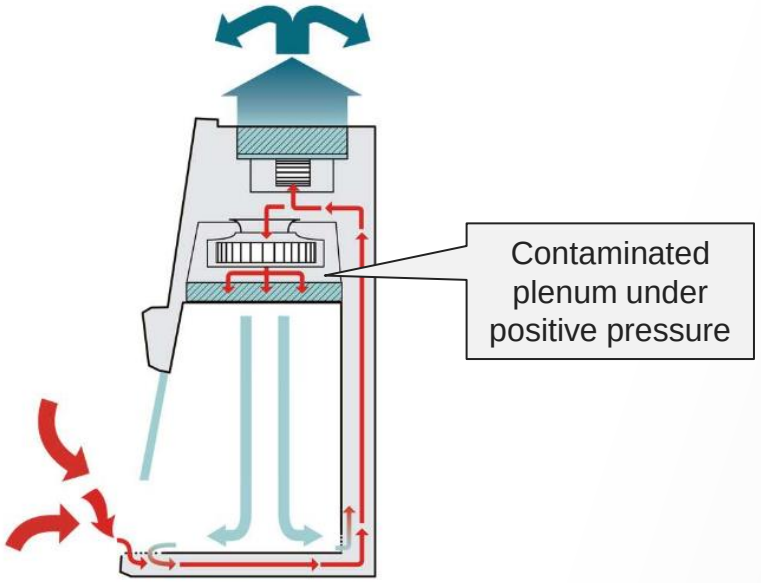
- Cleaning
  - Interior surfaces are designed with large internal radii to allow for easy cleanup
  - A drain spillage trough is provided to safely contain liquid spills below the work surfaces
- Decontamination
  - The biosafety enclosure intake and exhaust can be sealed with an optional kit and be decontaminated with Vapor Phase Hydrogen Peroxide. See “Decontamination” section for details.
- Canopy Connection Compatibility
  - The biosafety enclosure exhaust can be connected to a facility exhaust system with an optional canopy connection. See “Canopy Connection” section for details.
- UK Night Door Requirements
  - It is a Health and Safety requirement that the sash of an MSC is closed at night. The sash of the Bigfoot Biosafety Enclosure can not be normally closed. To adhere to this H+S requirement an optional accessory is required to plug the work access opening at night.
- Alarms
  - When the inflow air velocity is reduced to less than 95 feet per minute, an audible and visual low air velocity alarm will trigger.
  - For instruments that exhaust through a canopy connection, a separate alarm warns if there is a facility exhaust failure.

Differences Between Typical BSC and Bigfoot Enclosure

- Leaktightness
  - The Bigfoot biosafety enclosure is not a leak-tight enclosure by design. In contrast to a typical Class II BSC, the order of the HEPA filters and fans that move air through the HEPA filters are switched. This allows all contaminated air to be filtered prior to pressurization by the fan. This approach allows all contaminated ducts and plenums to remain at negative pressure with respect to the lab environment. If a small leak were present, it would be a “safe” leak as lab air would leak into the duct rather than contaminated air escaping the duct. This approach is detailed in the patent US 11,808,689 B2, Integrated Biocontainment Cell Sorter.
- Number of HEPA Filters
  - In typical biosafety cabinets there are two HEPA filters. One for the recirculated air and one for the exhaust air. In the Bigfoot Biosafety Enclosure there is only one HEPA filter for the “Biosafety Cabinet” portion of the instrument. There is a separate Aerosol Management System HEPA filter unrelated to the Biosafety Enclosure. This is possible because the air is HEPA filtered prior to the recirculation/exhaust branch. Uniform downflow air is established with the use of a three-stage diffuser instead of a HEPA filter.
- Order of Fans and Filters
  - In a typical biosafety cabinet the HEPA filters are placed downstream of the fans. Air travelling through the Cabinet passes through the fan and is pressurized prior to being HEPA filtered. Since this creates contaminated air that is under positive pressure with respect to the surrounding environment, according to NSF49 and EN12469, this requires this contaminated air to be surrounded by an even bigger duct under negative pressure. This is one of the reasons why typical biosafety enclosures are so large. In contrast, the Bigfoot Biosafety Enclosure filters the air prior to pressurization at the fan. There is no contaminated air under positive pressure in the system. See “Airflow Diagrams”.
- Sliding Sash
  - A typical biosafety enclosure will have a sliding sash that can fully closed and fully opened. This consequences of this approach are that the height of the sash is important to biosafety. Only at the operational height is personnel protection maintained. If a user needs to lift the sash more to change a nozzle tip, personnel protection is lost. In contrast, the Bigfoot Biosafety Enclosure sash operates with a constant-area opening. Regardless of the position of the sliding sash personnel protection is maintained. This was validated during the instrument’s microbiological testing.

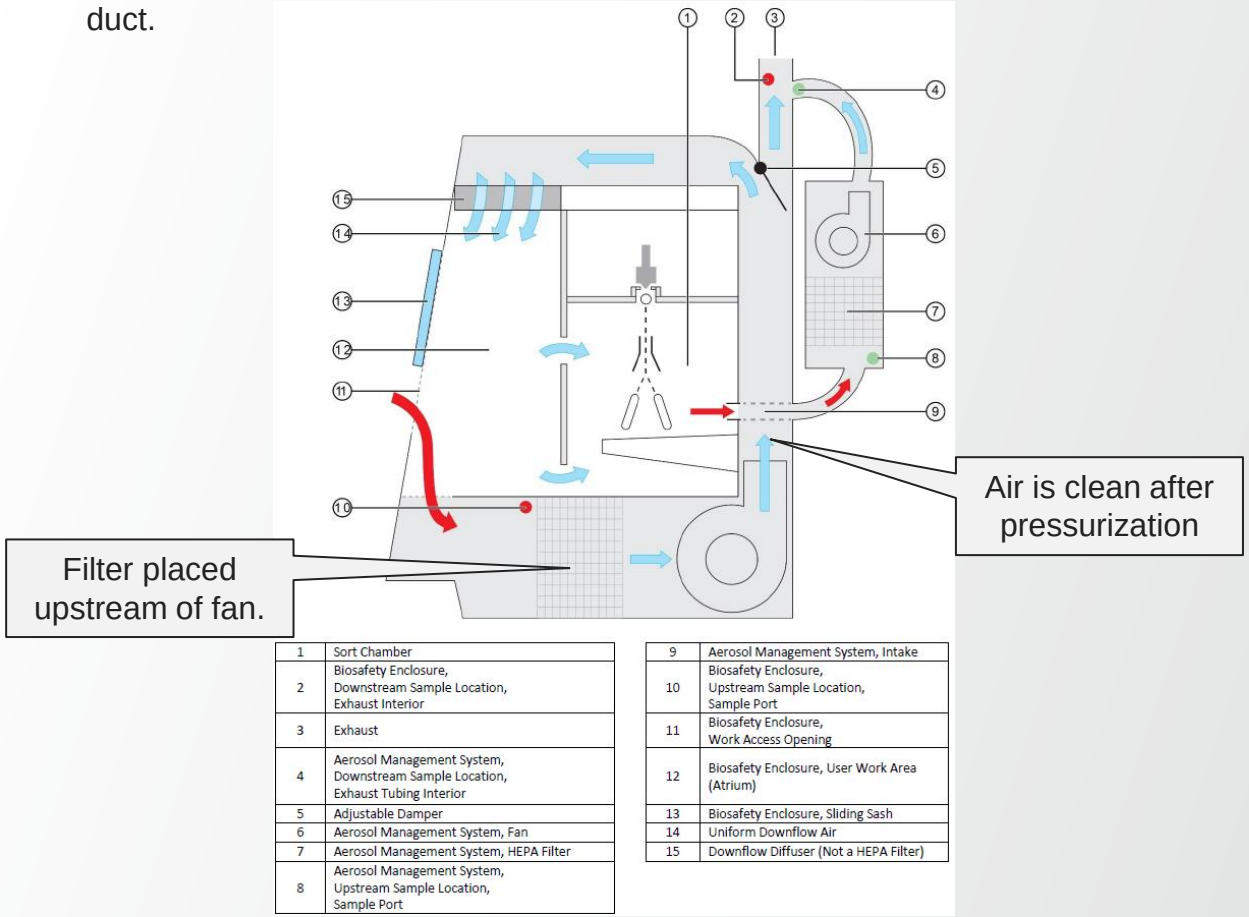
Airflow Diagrams

- Typical Biosafety Cabinets pressurize contaminated air prior to HEPA filtration. This leads to plenums that are contaminated and under positive pressure. To provide adequate personnel protection, these contaminated plenums under positive pressure must be surrounded by a larger duct or plenum under negative pressure. This requires a large volume.
- Typical Biosafety Cabinets require at least two HEPA filters: one for the recirculated air, and one for the exhaust air.



Typical Class II Type A2 Biosafety Cabinet

- In contrast, the Bigfoot Biosafety Enclosure HEPA filters contaminated air prior to pressurization. In this way there are no contaminated ducts under positive pressure with respect to the lab. If a small leak were present, it would be a “safe” leak as lab air would leak into the duct rather than contaminated air escaping the duct.



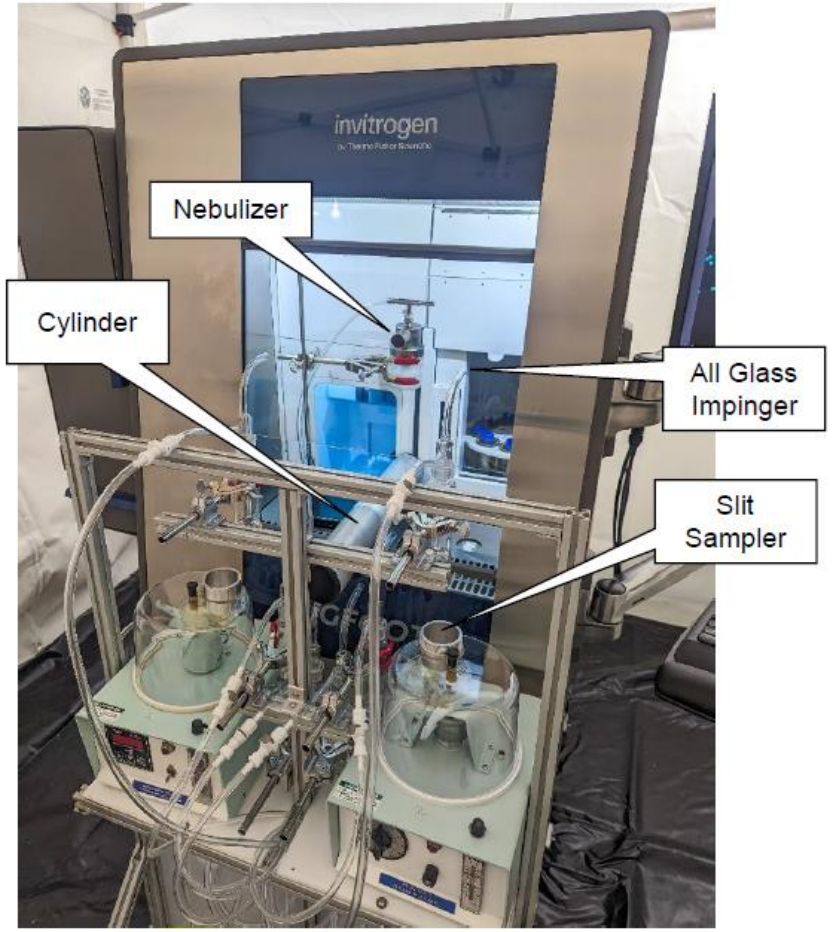
1	Sort Chamber
2	Biosafety Enclosure, Downstream Sample Location, Exhaust Interior
3	Exhaust
4	Aerosol Management System, Downstream Sample Location, Exhaust Tubing Interior
5	Adjustable Damper
6	Aerosol Management System, Fan
7	Aerosol Management System, HEPA Filter
8	Aerosol Management System, Upstream Sample Location, Sample Port

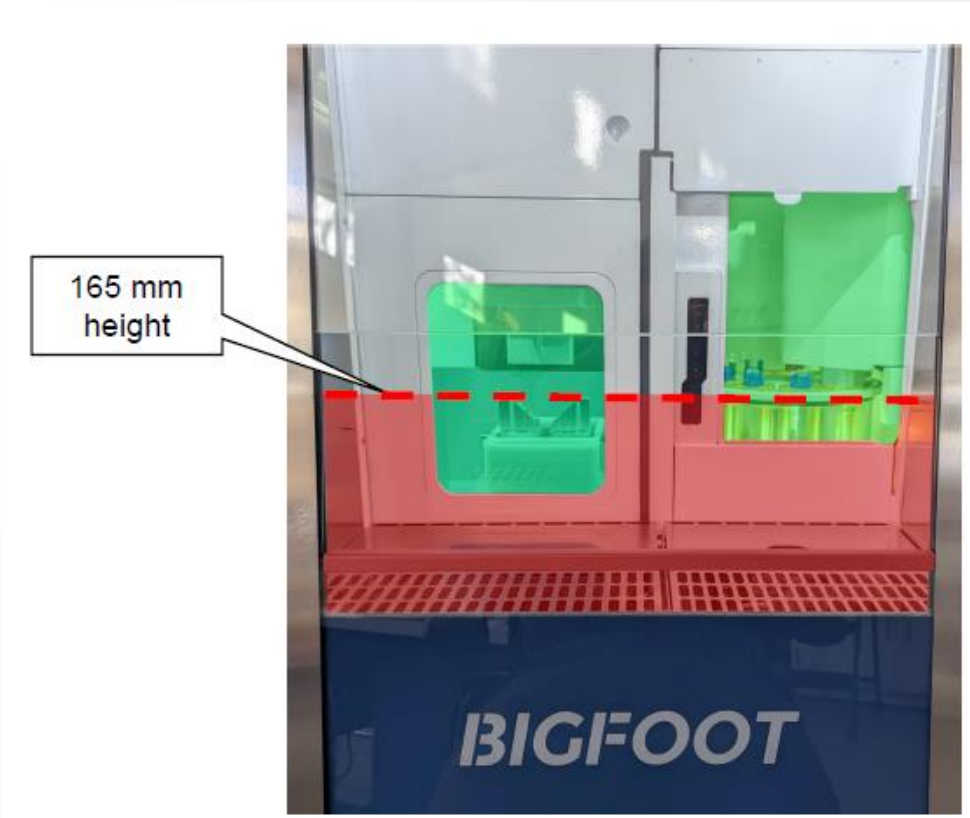
9	Aerosol Management System, Intake
10	Biosafety Enclosure, Upstream Sample Location, Sample Port
11	Biosafety Enclosure, Work Access Opening
12	Biosafety Enclosure, User Work Area (Atrium)
13	Biosafety Enclosure, Sliding Sash
14	Uniform Downflow Air
15	Downflow Diffuser (Not a HEPA Filter)

Bigfoot Primary Containment Device and Aerosol Management System

Microbiological Testing

- Most importantly, the Bigfoot Biosafety Enclosure was functionally validated with the rigorous NSF49 and EN12469 microbiological test methods. The test report is DT00346 and is available in the Knowledge Base. At a minimum, read the Summary and Discussion. The biosafety enclosure meet all the functional requirements for personnel protection. Product protection was not met at the elevation of the work surfaces, but it was maintained at an elevation of 165mm above the work surfaces.





User-Performed Validation

- A test protocol is provided for users to validate the performance of the biosafety enclosure and Aerosol Management System on an ongoing basis.
- This test protocol was developed by Technical Safety Services (TSS) and adapted by Thermo Fisher Unity Clean Air for use in EMEA.
- The test protocol was designed to use industry-standard tools and techniques.
- It is most typical to for a user to hire a third-party testing service or an organization's shared resource testing service to perform this testing. A testing service that can validate typical BSCs should be able to use the protocol DT00149.
- It is recommended that the first time this procedure is performed that Technical Support is contacted to be on stand-by if anything in the procedure needs to be clarified. The procedure involves using SQS software, which can be confusing for some Field Service Technicians.
- It is recommended that the procedure DT00149 is performed once every 12 months.
- In the UK and some parts of EMEA it is typical for an additional functional test call the "KI Discus" test to be required. DT00149 includes instructions for how to perform a KI Discus test to determine the instrument's Aperture Protection Factor.

Selling Points of Bigfoot Biosafety Enclosure

- All BD, Sony, Cytek, and Beckman Coulter cell sorters take similar approaches to biosafety. All competitor products are more-or-less existing flow cytometers that are placed inside a much larger biosafety cabinet. This approach requires the use of large, cumbersome and loud biosafety cabinets to completely enclose the cell sorter.
- In contrast, the Bigfoot Cell Sorter was designed from the beginning to seamlessly combine the functions of a biosafety cabinet with a cell sorter. Since the air volume enclosed by the Bigfoot Cell Sorter is significantly less than competitor products, the ducts, fans, and HEPA filters required to provide personnel protection are also much smaller.
- The large biosafety HEPA filter is placed below and to the rear of the instrument which reduces vibrations near the sensitive optics and allows for improved service access.
- Bigfoot uses a novel sliding sash to allow users to perform routine maintenance tasks such as swapping nozzle tips without breaking biocontainment.
- When competitor Cell Sorters need to be serviced, it requires removing the Cell Sorter from the Biosafety Cabinet. In contrast, on Bigfoot, most of the instrument can be serviced without breaking biocontainment.
- Some competitor sorters have a microbiological test report that use NSF49 and EN12469 test methods to demonstrate biosafety performance. We match this testing.

  The test report DT00346 is available for distribution.

ISAC test method and kit

- ISAC Method to validate Aerosol Management System
  - The community-accepted method to validate Aerosol Management Systems is a functional test that is commonly called the “Cyclex-D” test.
  - For this method a user creates a worst-case aerosol generation scenario by running a 1:100 dilution of 1 micron Dragon Green Beads through the instrument on a 70 micron tip at 60 psi (greatest sheath pressure possible), and purposefully impacts the stream on a hard surface to create a plume of aerosols.
  - A vacuum pump an aerosol collection cassettes are used to sample the air outside the Sort Chamber. If beads are detected outside the Sort Chamber, then that is a failure of the AMS.
  - All instruments are provided with a kit in a padded case with all required hardware except the beads. **We do not provide the Dragon Green Beads**.
- Cyclex-D Cassettes are obsolete
  - Cyclex-D cassettes were obsoleted by the manufacturer in 2023.
- Allergenco
  - The ISAC biosafety committee recommended replacement is to use Allergenco-D cassettes.
  - From March 2024 onward instruments will ship will Allergenco-D cassettes.
- Software Wizard and Companion PDF Procedure
  - In the Maintenance Tab of SQS there is a wizard that guides users through the procedure.
  - An accompanying PDF procedure is available as well in the Knowledge Base.

[4] ISAC Biosafety Standards
[5] International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards
[6] International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards, Revisions
[7] Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated in a Flow Cytometer Cell Sorter

Decontamination

- We do have a validate biosafety enclosure decontamination
  - See procedure DT00156. An additional document that details Steris' validation of the method is available.
  - Steris was contracted to develop and validate a VHP decontamination method.
  - Optional kit PL00349 must be purchased by the customer to perform this decontamination
  - The biosafety enclosure intake and exhaust must be sealed. The sealing hardware and fittings to connect to the Steris or Bioquell VPHP machines.
- We do not offer a validated "whole-instrument" decontamination method
  - The only validated decontamination method that we offer is for the biosafety enclosure in isolation. This is everything inside the sash (Loader, Output, Nozzle Chamber, HEPA Filters and Biosafety Fans. To decontaminate the entire instrument requires exposing the computer and optics to the H2O2 sterilant. This has not been formally validated, although some customers have done this. With repeated VPHP cycles it is known that the black anodized aluminum surfaces such as the detection covers will turn a faint orange/pink hue. This is only a cosmetic issue. If the personnel that perform the whole-instrument decontamination are properly trained and have the equipment to control the temperature and humidity this method can be performed while limiting the condensation of pure liquid H2O2, and thereby reducing the risk to the instrument.
- Optional low-cost VPHP method (U.S. instruments only)
  - Technical Safety Services, which only operated in the U.S., offers a low-cost VPHP decontamination method that is a fraction of the cost of hiring Steris. This procedure is DT00187. This procedure is stored in the employee-only FSE section of the knowledge base. TSS is the only company approved to perform this procedure

Decontamination Prior to Service

- Requirements
  - To protect the health and safety of our Bigfoot Field Service Engineers it is required that Bigfoot instruments are decontaminated prior to some service procedures such as HEPA filter replacements. It is the user's responsibility to schedule, pay for, and document the decontamination prior to service.
  - There are two types of decontamination.
  - 1) Fluidic decontamination. Decontaminating the surfaces where liquid customer sample can contact the internal plumbing: bulk fluids, sample paths, fluidic module. The decontamination method is bleach. The Bigfoot Field Service Engineers will take care of this aspect of the decontamination.
  - 2) Biosafety enclosure decontamination. This is where the intake and exhaust of the biosafety enclosure is sealed and VPHP sterilant is used to decontaminate the Loader, Sort Chamber, Nozzle Chamber, and HEPA filters. This portion of the decontamination is the customer's responsibility. TSS, Steris, and Bioquell are example VPHP providers.
  - There are two documents that define when decontamination is required. There is a simplified customer-facing document DF00214. The more complete internal document that includes more edge-cases is DT00288.
- Documentation
  - There are two forms that the customer is required to sign after decontamination. DF00214 and FRM0005274. Both are available on the Knowledge Base.

HEPA Filters

- HEPA filters are not user replaceable. The cost of HEPA filter replacement is included in the service contract. However, decontamination is required to HEPA filter replacement. The decontamination costs are the customer's responsibility.
- Expected HEPA filter lifespan. Depends on the air quality of the lab.
  - Biosafety Enclosure HEPA filter: 5 Years
  - AMS Filter: 10 Years

Specifications:

Region	European (EN 1822-1:2019) Filter Definition	Actual HEPA Filter Efficiency	HEPA filter efficiency that we advertise to our customers	Acceptable leak rate when tested with an aerosol photometer
U.S.	Not defined, Somewhere between H13 and H14	99.990%	99.970%	0.0150%
Non U.S. EMEA APJ	H14	99.995%	99.995%	0.0100%

Acceptable HEPA Filter Leak Rate, U.S. Instruments	**0.0150%** The Biosafety Enclosure filter efficiency of this product is listed as 99.97%. Access is not available for scan testing HEPA filters. The total leakage method is used to determine average filter performance. A 0.015% integral penetration corresponds to a 0.030% listed leak rate (99.97% HEPA filter efficiency). See NSF/ANSI 49 – 2018, A.2.3.2, Filters that cannot be scanned.
Acceptable HEPA Filter Leak Rate, Non-U.S. Instruments	**0.0100%** The Biosafety Enclosure is fitted with an H14 HEPA filter. The filter has been efficiency tested to 99.995% at the MPPS at the HEPA filter manufacturing facility. Access is not available for scan testing HEPA filters. The integral value is measured to determine average filter performance. EN 12469:2000, Annex D.5 defines the maximum acceptable local penetration as 0.01% for an H14 HEPA filter when integrity tested. EN12469 does not define an acceptable integral penetration when measured with an aerosol photometer. For instruments sold outside of the United States, the 0.01% value shall be used as the maximum allowable integral penetration when measured with an aerosol photometer.

BSL3/CL3 Notes

- Decontamination
  - Full-instrument decontamination is likely required. It is currently unknown how full instrument VHP exposure will affect the instrument.
  - There are elevated decontamination requirements for these instruments prior to service.
  - Bigfoot FSE access to BSL3 labs is restricted. In some cases these labs offer BSL2 antechambers where our FSEs are permitted to perform maintenance.
- AMS Validation
  - According to the ISAC guidelines, it is a requirement that the Biosafety Enclosure fans are turned off during testing of the AMS. This conflicts with guidelines for BSL3 labs. There is no ISAC approved method to test the AMS with the Biosafety Enclosure fan on. This problem would be present on any cell sorter/biosafety cabinet combination product.
- UK Requirements
  - Must double filter exhaust. We do not offer an integrated additional exhaust HEPA filter (AEF). Therefore, the only viable option is to use a canopy connection in combination with a facility exhaust system equipped with HEPA filters.

Required Communications with Customer

- Validation
  - Validation with a third-party testing service requires sending that testing service procedure DT00149. They will be unable to test the instrument without this procedure. This is available from the Knowledge Base, or by logging into the testing mode by entering in “biotest” for the username and password in SQS.
  - Bigfoot Field Service Engineers are not in the business of performing Biosafety Systems validation. To have the system tested according to the manufacture’s recommended test protocol (DT00149) requires hiring a third-party testing service.
- Financial Responsibility
  - Decontamination is required when replacing HEPA filters and some service visits. It is the user's responsibility to schedule, pay for, and filling out the forms demonstrating that the decontamination has been performed.
- Site Assessment
  - Not all locations within a lab are appropriate for a biosafety cabinet. Close proximity to anything that can generate significant air velocity can be problematic and lead to frequent warnings or a loss of personnel protection. This includes proximity to doors, walkways, HVAC supply/return ducts or wall-mounted AC units.
  - For a thorough assessment, recommend that the customer purchase a copy of BS 5726:2005
  - Here is a good summary of BS 5726

[8] Biosafety Cabinet (BSC) Placement Requirements for new Buildings and Renovations

Canopy Connection Site Requirements

- Canopy Connection
  - The exact minimum facility exhaust pressure can't be known ahead of time since connecting the canopy connection will change the airflow balance and static pressure of the facility exhaust. Our instruments requires less air volume, but more static pressure compared to typical biosafety cabinets. Some HVAC extract systems do not generate enough static pressure to be compatible with the canopy connection. Below are estimated minimum requirements.

Bigfoot Canopy Connection Facility Exhaust Requirments

Average Inflow Air Velocity	78 cfm (133 m^3/hr)
Average Inflow Air Velocity	78 cfm (133 m^3/hr)
Required Facility Exhaust Airflow Volume With Canopy Connection	160 cfm (272 m^3/hr)
Required Facility Exhaust Static Pressure With Canopy Connection	0.16 in H_2O (40 Pa)

Canopy Connections – UK Specific References

- Although the Subject Matter Expert Marc Dunn advised that Canopy Connections are not required at CL2 in the UK, the existing Health and Safety Executive guidance has not caught up to the more recent guidance. In many labs in the UK it is still common practice to require direct exhaust into the "laboratory air extract system" (requiring a canopy connection) or the use of two HEPA filters in series. A sample of labs with published SOPs is shown in the next page, all that specify the use of a canopy connection. Ultimately the decision is up to the customer and their biosafety committee based on their own risk assessment. We can provide a canopy (thimble) connection. We cannot provide an Additional Exhaust Filter that is integrated into the instrument. Below are the pertinent standards references.
- "At CL2, if the biological agents can be spread by aerosol and can cause human disease, procedures that are likely to create aerosols, eg vigorous shaking or sonication of liquids, must take place within an MSC or similar containment. Class I or II MSC's may be used depending on which is deemed the most appropriate. Extract air from MSCs must always be HEPA filtered. (For further information on the use of MSCs refer to Appendix 2.)" [9], Appendix 1, Reference 38, Page 55.
- "At CL3 all work with infectious materials that can be spread by aerosol and can cause human disease must take place in an MSC or similar containment. Normally a Class I or Class II MSC will be used, but a risk assessment may indicate a Class III cabinet is required for work with biological agents with an airborne route of transmission that can cause serious human disease, eg multi-drug resistant TB. Where re-circulating MSCs are used, exhaust air should be passed through two HEPA filters in series and consideration given to heat and humidity build-up and fumigation procedures." [9], Appendix 1, Reference 39, Page 55-56.
- "MSCs must exhaust through a HEPA filter or equivalent, preferably direct to the outside air or, if this is not practicable, via the laboratory air extract system. The HEPA filter works by removing particulates (generally called aerosols) such as microorganisms, from the air. There is a requirement within BS EN 12469 that the minimum grading of filtration in MSCs is equivalent to H14 as defined within BS EN 1822, ie with a collection efficiency of 99.995% of 0.3 µm to 0.5 µm sized particles. The HEPA filter should ideally be part of the MSC, but if not, they should be located as close to the cabinet exhaust as possible, to avoid accidental contamination of the building exhaust system with biological agents." [9], Appendix 2, Reference 10, Page 60.
- "If it is not practical for the MSC to exhaust to open air, either directly or indirectly via the laboratory exhaust, re-circulation of exhaust air through two HEPA filters in series may be considered as an alternative. While this method will remove biological agents from the air it may be difficult to remove chemicals, eg fumigant, when the cabinet has been fumigated. Therefore, the local fumigation protocol should include information on safe methods to remove the fumigant when the MSC is to be decontaminated" [9], Appendix 2, Reference 11, Page 61.
- Optional resource: The document "Additional Exhaust Hepa Filtration and Class II BSC" authored by Marc Dunn.

[9] Management and operation of microbiological containment laboratories, Advisory Committee on Dangerous Pathogens © Crown copyright 2018
[10] Additional Exhaust Hepa Filtration and Class II BSC, Note from Marc Dunn

Canopy Connections – UK Specific References, Continued

Examples of UK SOPs that would require a canopy connection for Bigfoot at CL2:

- Queen Mary University of London
  - "At Containment Level 2, the current British Standard (BS EN 12469:2000, Performance criteria for microbiological safety cabinets) specifies only a single HEPA in the exhaust with the caveat that the risk assessment may demand additional requirements. Within QMUL, it is recommended that all recirculating Class II cabinets be installed with double HEPAs on the exhaust to ensure they are suitable for work with all types of micro-organisms. This will need to be specified when the cabinet is ordered."
  - "If laboratories are faced with a major problem because of difficulties in arranging for the cabinet to exhaust to open air, recirculation of exhaust air through two HEPA filters in series may, in exceptional circumstances, be considered as an alternative."
- University of Cambridge, Department of Oncology
  - "Safety cabinets must exhaust through a HEPA filter or equivalent to the outside air or into the laboratory air extraction system, and in other respects such as siting, performance in use, protection factor and air filtration, should comply with the performance specifications detailed in British Standards. If laboratories are faced with a major problem because of difficulties in arranging for the cabinet to exhaust to open air, recirculation of exhaust air through two HEPA filters in series may, in exceptional circumstances, be considered an alternative."
- University of Birmingham
  - "Cabinets should be ducted wherever possible but if this cannot be achieved they should be fitted with a double HEPA filter in the exhaust."

Canopy Connections – Recommended Communication

Like any other MSC, there are requirements that govern their appropriate use. However, the most current EN standards and British standards disagree on the requirement of an additional exhaust HEPA filters. EN12469 permits the use of a single exhaust HEPA filter. This guidance replaced the now obsolete British Standard BS5726. However, the guidance published in the Health and Safety Executive “Management and operation of microbiological containment laboratories, Advisory Committee on Dangerous Pathogens” has not yet been updated to reflect the more current guidance and does require an additional exhaust HEPA filter at CL2. Due to this disagreement, different institutions in the United Kingdom have different requirements with respect to additional exhaust HEPA filters. Therefore the decision about whether you should use an additional exhaust HEPA filter should be made on the basis on your own institution’s requirements. If the other MSCs in your facility are required to use an additional exhaust HEPA filter or a thimble connection, then it would be best to use a thimble connection on your Bigfoot instrument. If the other MSCs in your facility are not required to use an additional exhaust HEPA filter, then you should be able to use your Bigfoot instrument as any other MSC without an additional filter or exhausting into your facility HVAC system. Bigfoot offers a thimble connection option, but does not offer an additional exhaust HEPA filter option.

Biosafety Accessories

- VHP Biosafety Cabinet Decontamination Sealing Kit – PL00349
  - This kit is required for a VPHP decontamination unless they are in the U.S. and have access to Technical Safety Services.
  - This kit includes the Intake Sealing Plate PL00354. Do not quote both PL00349 and PL00354 for the same customer.
- Intake Sealing Plate, Biosafety Enclosure Night Door – PL00354
  - This a part of the larger kit PL00349. This kit is not recommended as it's limited in use. It is recommended to purchase the entire kit PL00349. The kit PL00354 is only useful if a customer wants a "night door" but will never choose to decontaminate their own instrument.
- Bigfoot Canopy-Connection Biosafety Instrument Configuration - PL00329
  - For customers that know they want a canopy connection when first ordering the instrument.
- Field Upgrade Kit, Canopy Connection, Biosafety Enclosure Option- PL00384
  - For customers that want to upgrade to a canopy connection for an instrument that is already installed.

References

[1] NSF49, NSF/ANSI 49-2018: Biosafety Cabinetry – Design, Construction, Performance, And Field Certification
https://webstore.ansi.org/standards/nsf/nsfansi492019

[2] Laboratory biosafety manual, 4th edition, World Health Organization
https://www.who.int/publications/i/item/9789240011311

[3] EN 12469:2000, Biotechnology. Performance criteria for microbiological safety cabinets
https://www.en-standard.eu/bs-en-12469-2000-biotechnology-performance-criteria-for-microbiological-safety-cabinets/?gclid=EAIaIQobChMIro7si8C4_QIVzsmUCR1XvgCwEAAYASAAEgLMpfD_BwE

[4] ISAC Biosafety Standards
https://isac-net.org/page/Biosafety

[5] International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards, https://cdn.ymaws.com/isac-net.org/resource/resmgr/docs/2014_isac_cell_sorter_biosaf.pdf

[6] International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards, Revisions, https://cdn.ymaws.com/isac-net.org/resource/resmgr/docs/revision_to_isac_cell_sorter.pdf

[7] Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated in a Flow Cytometer Cell Sorter, https://cdn.ymaws.com/isac-net.org/resource/resmgr/docs/perfetto-2018-novel_impactor.pdf

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https://orf.od.nih.gov/TechnicalResources/Bioenvironmental/Documents/BiosafetyCabinetBSCPlacementRequirements_508.pdf

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https://www.hse.gov.uk/biosafety/assets/docs/management-containment-labs.pdf

[10] Additional Exhaust Hepa Filtration and Class II BSC, Note from Marc Dunn
In Bigfoot Knowledge Base