



Multiple users in a Biosafety Cabinet compromise containment

WITH

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ABSTRACT

Biosafety Cabinets (BSCs) are primary containment devices used to help reduce the risk of contamination to the work, the worker, and the surrounding environment. These devices have rigorous testing and certification to ensure their functional capabilities of contaminant reduction according to NSF International Standard 49 but these BSCs are always tested empty without users present in front of them for a functional baseline. However, BSC are never

used in this manner. There are known commonly performed actions within BSC operation that may compromise BSC containment. Here, we address these BSC “myths”, specifically whether two or more people can work within one BSC and still maintain their intended capacity for particulate containment as measured through visual smoke demonstration and NSF International Standard 49 Microbiological Aerosol Testing.

INTRODUCTION

Primary Containment for most biological laboratories starts with the Biosafety Cabinet (BSC). These ventilated enclosures are built and tested to rigorous specifications dictated by NSF International Standard 49 to provide containment of particulates, aerosols and biohazards through three mechanisms: Personnel protection, Product protection, and Environmental protection.

This ensures that the user, the experiment or work being conducted, as well as the laboratory and building, are protected. This is achieved through the use of specifically directed and controlled velocities of air and High Efficiency Particulate Air (HEPA) filtration technology to remove particulates from the airstream.

How the air flows through a BSC will determine which class and type of cabinet it is. The three main cabinet classes are determined based on level and type of containment, or protection from biohazards, it will provide. Class I cabinets do not provide Product Protection and are generally referred to as “powder hoods”. Class III BSCs are gas-tight, closed glove boxes that operate under negative pressure, designated primarily for high-risk biohazard work.

Class II cabinets make up the greatest population of BSCs found worldwide. This class includes 5 Types of BSCs: A1, A2, B1, B2, and C1. Class II Type A cabinets are recirculating, allowing for reduced energy usage and the A2 classification make up the vast majority of all BSCs (airflow flow patterning shown in Figure 1).

Type B cabinets must be hard-ducted or directly connected to a facility’s exhaust system and provide fully exhausted air out of the BSC. Type C1 is a relatively new hybrid of the Type A and B cabinets allowing for flexibility of ducting outdoors or venting the cabinet to the room using dedicated zones in the work area and different installation protocols (NSF International, 2018).

The ability for a BSC to provide Containment is dependent on unobstructed airflow, as well as the velocity of the air coming in through the front access opening; however, when a BSC is in use materials, tip boxes, pipettes, even users’ arms will restrict airflow. Larger front access openings also require more air to be moved, with the blower compensating to keep the cabinet balanced.

All NSF International certified BSCs must be able to overcome at least some of these restrictions. The microbiological aerosol testing as described in NSF International Standard 49 accounts for some of this by directly testing how much aerosolized bacterial spores will exit the BSC (Personnel Protection test), enter the BSC (Product Protection test), or travel across the worksurface (Cross Contamination test) (NSF International, 2018).

The NSF Standard 49 provides testing criteria, usage recommendations for how to operate a BSC safely, but interestingly never mentions how many operators can sit at the front access opening at once.

It is generally assumed by BSC manufacturers that there shall be a single user at a time, yet BSCs are constructed in many sizes, with common widths varying from 3 to 6 feet. With that much work area, it could be assumed that multiple users could sit side by side and maintain the same level of protection as a single user. However, this had not yet been tested. Here we shall determine what level of protection multiple users would experience in a standard 6 foot Class II Type A2 BSC with both an 8 inch and 10 inch front access opening.

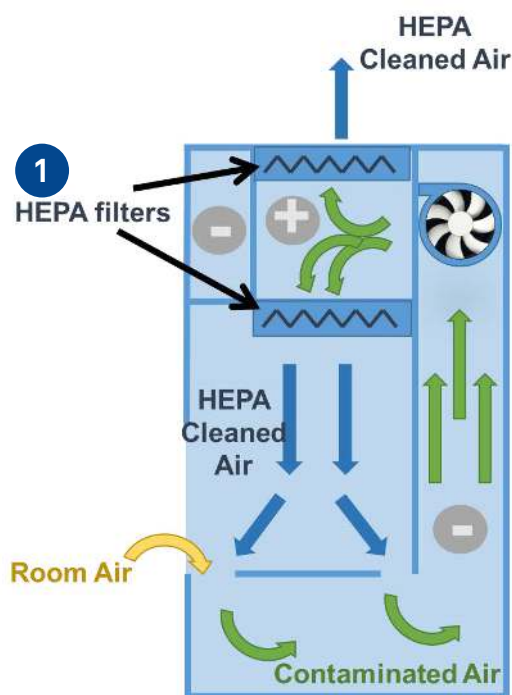


Figure 1
Sideview diagram of a Class II Type A2 BSC.

METHODS

Smoke visualization

To visualize airflow patterning within a Class II Type A2 6-foot wide BSC (Baker SterilGARD SG604), a Rosco Fog Machine (Model 1700) was outfitted with a 4 inch hose attached to a 6 foot PVC pipe with holes drilled every 2 inches to provide a uniform curtain of smoke.

This pipe was installed at eye level along the outside glass sash above the front access opening to visualize potential inward flowing air (Figure 2a).

2a



Figure 2a

Construction of a smoke curtain at the front access opening with the mannequins present.

Airflow measurements

Airflow into the cabinet was measured with a Direct Inflow Measurement (DIM) device (Shortridge Airdata Multimeter ADM-850L) on a capture hood attached to the front access opening when no mannequins were present. With mannequins at the front opening, a modified capture hood was created for the exhaust filter.

Two to three mannequins were equally spaced along the front access opening to simulate multiple users at the BSC (Figure 2b). The stainless steel challenge cylinder used for NSF International Standard 49 Aerosol Microbiological testing to help break the front barrier was also included.

2b



Figure 2b

Placement modification of the NSF International Standard 49 Aerosol Microbiological testing set up in the presence of three users.

The exhausted volume of air could then be used to calculate the inflow velocity using the standard equation (Equation 1), where Q = volumetric flowrate, V = velocity, and A = cross sectional area of where the airflow is being measured, and $Q_{\text{intake}} = Q_{\text{exhaust}}$.

Equation 1. $Q = V \cdot A$

The velocity of single streams of air were measured using a thermal anemometer or “hotwire” (TSI VelociCalc Plus Model 8385), such as around the mannequins or the downward flowing air in the work area.

Aerosol Microbiological Challenge testing

The containment capability of the BSC was tested using microbiological aerosols as described in NSF International Standard 49 (NSF International, 2018). Testing was split into three types: Personnel, Product and Cross Contamination testing. Both the sidewall and center Cross Contamination configurations were used (NSF International, 2016; NSF International,

2018). The collision nebulizers contained a slurry of *B. subtilis* var. *niger* spores (May, 1973); both nebulizers and Tryptic Soy Agar petri dishes were placed as directed in Standard 49 (NSF International, 2018), or as close to as written as possible.

The presence of the mannequins blocked some of the standard locations, and modifications were required. After the tests were conducted, all petri dishes were covered and placed in a 37°C cell culture incubator (Baker Cultivo Ultra Plus). Results were read after 24 hours of growth, and pass/fail was determined according the Standard (NSF International, 2018).

RESULTS

Smoke visualization

The air patterning within a Class II Type A2 BSC has a standard airflow, where room air enters the front access opening at a minimum velocity of 100 feet per minute (fpm). This strong inward flow prevents escape of hazards from the work area creating Personnel protection. The air is then drawn up the back or side plenums by a motor/blower system and pushed into an equalizing plenum. Here a portion of the air is exhausted through the exhaust HEPA filter, providing Environmental protection. The majority of the air is pushed through the supply HEPA filter in a unidirectional downward manner so as to completely and uniformly cover the work area. This creates Product protection. Disruption in the airflow in any kind can lead to loss of protection.

The mannequins were set up in front of the BSC and the changes to baseline air patterning was observed. With 2 mannequins, minimal disruption to the air flow was seen, mostly around the NSF stainless steel rod and the mannequin hands.

The further into the cabinet the hands were placed, the greater chance of some external air entering the work area. The greatest effect was seen with 3 mannequins placed at the front access opening. Great bursts of smoke were seen entering the work area which lead to the potential for contamination. Again, the most air flow disruptions were seen around the hands near the NSF stainless steel rod (Figure 3 and Supplemental Video 1).

3



Figure 3

Smoke penetrating the work area in the presence of three users' hands indicating a loss of Product Protection.

V1



Video Link: bit.ly/3b9KGdk

Supplemental Video 1:

Loss of containment by smoke visualization in the presence of three users in front of a 6 foot Class II Type A2 BSC.

Airflow measurements

The overall airflow through the BSC can be determined by measuring the volumetric inflow of air through the front access opening and comparing that to the total volume of air coming out the exhaust filter. The exhaust measurement is commonly greater than the inflow through the front access opening because this will include any additional air seeping in from around the viewscreen, through cable ports, and other unsealed areas as designed. All inward flowing air helps with Containment of potential biohazards.

The inward volume flow of air was shown to not be restricted by placing 2 or 3 mannequins in front of the cabinet front access opening where inflow was measured at 410 cubic feet per minute (cfm), and exhaust measured at 453 cfm at set point with no mannequins in front. This exhaust measurement was maintained with 2 mannequins at the front access opening and was only altered slightly to 450 cfm with the addition of the third mannequin (Table 1).

	Users in front of BSC	Inflow	Exhaust
8" front access opening	0	410 cfm	453 cfm
	2	n/a	453 cfm
	3	n/a	450 cfm

Table 1

Volumetric Airflow measurements through the front access opening (Inflow) or out the exhaust HEPA filter (Exhaust) of a 6 foot Class II Type A2 BSC when user bodies are located at the front access opening.

Aerosol Microbiological Containment testing

The 6 foot Class II Type A2 BSC (Baker SterilGARD 604) was first tested empty to the full criteria dictated by NSF International Standard 49 (NSF International, 2018) at both the 8" and 12" window sash opening with no mannequins in front to show a passing baseline for the cabinet. Once the BSC was determined to be properly functional, the BSC was balanced at an 8" sash opening and tested with 2 mannequins spaced equally in the front access opening.

The BSC was able to pass the Personnel, Product and Sidewall Cross Contamination test; when the Center Cross Contamination test (NSF International, 2018) was conducted, however, there was a loss of Protection.

When a third mannequin was added to the front access opening, the Personnel Protection was maintained, but Product and both Cross Contamination protection were lost (Table 2).

		Personnel	Product	Sidewall Cross	Center Cross
8" front access opening	2 users	PASS	PASS	PASS	FAIL
	3 users	PASS	FAIL	FAIL	FAIL
12" front access opening	2 users	FAIL	FAIL	PASS	FAIL
	3 users	FAIL	FAIL	PASS	FAIL

Table 2

Aerosol Microbiological Containment testing results for multiple users in a 6-foot Class II Type A2 BSC at both an 8" and 12" window sash. Pass (green) and Fail (red) criteria determined by NSF International Standard 49 (NSF International, 2016).

A wider front access opening will lead to a greater volume of air entering, which can be calculated using Equation 1. This greater volume of inflowing air can lead to potential loss of Containment if the BSC cannot properly control the air. When the 6 foot Type A2 BSC (Baker SterilGARD 604) was rebalanced with a 12" window sash, the 2 mannequins at the front access opening caused a loss of Personnel, Product, and Center Cross Contamination protection within the BSC. Adding the third mannequin yielded the same result (Table 2). This is evidence strongly supporting the NSF International committee decision to change the Cross Contamination testing method from the sidewall to the center of the BSC work surface (Test configuration shown in Figure 4).

4



Figure 4

Center Cross Contamination test (left) set up in the presence of three users.

CONCLUSIONS

Biosafety Cabinets (BSCs) have historically been tested by certifying agencies such as NSF International to ensure they provide the intended level of Containment or protection from biohazards. The current tests performed require that the BSC be empty and free of obstructions with the intention that this will determine the BSC's peak level of performance. It is known that common laboratory practices may impede this peak BSC performance. One such practice would be the presence of multiple users at the front access opening. Here the magnitude of that impact and the subsequent consequences were determined.

While the volume of air entering the BSC was not impacted by the presence of bodies blocking the front access opening (Table 1), it was visually observed that the mannequin hands breaking the front access air barrier caused potential influxes of smoke into the work area of the BSC, which could lead to potential contamination and loss of containment (Figure 3 and Supplemental Video 1).

This was then confirmed with the microbiological aerosol testing, where failures in maintaining protection were widely observed (Table 2). When set with the most common configuration of an 8" window sash opening, 2 users at the front access opening successfully maintained Containment per the NSF International Standard 49 prior to 2018 (NSF International, 2016). However, with the addition of the new Center Cross Contamination test, a potential for spreading biohazards across the cabinet was uncovered.

This phenomenon was again observed with a 12" window sash opening, but with the addition of a loss of Personnel and Product Protection. As the window sash is raised and the front access opening area increased, the speed or velocity of air needs to be maintained at the required 100 fpm, so the volume of air entering the cabinet is increased.

With a greater volume of air comes the greater risk of contaminants entering the work surface as demonstrated with the results (Table 2).

The greatest Containment loss was observed by adding the third user at the front access opening. By having the third body blocking the directionality of air into the cabinet and the added two arms breaking the front access opening air barrier, there was a much greater risk of contaminants entering the worksurface (loss of Product Protection) as well as a greater risk of biohazards exiting the cabinet (loss of Personnel Protection, Table 2). Here again was observed the passing result in the Sidewall Cross Contamination test and a failing result in the Center Cross Contamination test supporting the NSF International committee's decision to change the test recommended in the latest edition (NSF International, 2018).

There are some laboratory practices that may require multiple people to work within the same biosafety cabinet at the same time, however the risks associated with such procedures should be appropriately known to both users and biosafety officers. It is recommended that only a single user operate a BSC at a time; if it must be done, a maximum of two users in a 6 foot BSC operating at least 14 inches away from each other is the next best option, assuming they take extra precaution to prevent Cross Contamination to each other and have conducted a Risk Assessment with the appropriate Biosafety Officer.

REFERENCES

- NSF International. (2018) NSF/ANSI 49 – 2018 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification. Ann Arbor, MI.
- NSF International. (2016) NSF/ANSI 49 – 2016 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification. Ann Arbor, MI.
- May, K. R. (1973) The Collision Nebulizer: Description, Performance and Application. Aerosol Science. 4: 235-243.



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