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Heat sources in a Biosafety Cabinet Compromise Experimental and User Protection

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| ABSTRACT

Keeping a contamination free environment in the laboratory has commonly been achieved by one of two ways: a flame or a biosafety cabinet (BSC). However, it has been frequently observed that the two practices have been combined, where a heat source has been used within the BSC. As flames require flammable gasses and cause hot air to rise, it was hypothesized that these could lead to a loss of BSC Containment. Here, these practices were tested with several heat sources (Bunsen burner, High Heat Bunsen Burner, Spirit Lamp

and Bacti-cinerator) in two sizes of BSC, using smoke for airflow visualization, particle counting for air cleanliness, and aerosol microbiological testing to show Containment. Large flamed burners were found to have the most detrimental effects on the ability of the BSC to maintain Containment, especially in the center of the work area, while the smaller heat sources were variable. Overall, it was determined that BSCs cannot operate safely while housing a heat source, as it could cause unexpected contamination of the work or the worker.

| INTRODUCTION

A Biosafety Cabinet (BSC) is a ventilated enclosure that is an essential piece of lab equipment for many procedures. They are the primary source of contamination removal and prevention, and are heavily relied upon for protection of the user (Personnel), the experiment (Product), and the room and building (Environmental). All three types of protection are known as Containment. BSCs use specifically directed airflow to control and entrain aerosols and particulates, and High Efficiency Particulate Air (HEPA) filtration to capture them, removing them from the airstream.

BSCs are grouped into three classes depending on their level of Containment and physical characteristics. The largest class is Class II. By definition, a Class II BSC must provide Containment with the

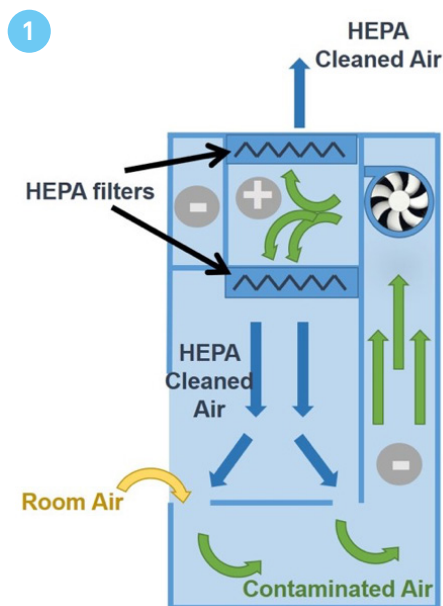
three types of protection (Personnel, Product, and Environmental), have a front access opening with inward flowing air, HEPA filters, and a motor/blower system (NSF International Standard 49, 2016). The inward flowing air provides Personnel protection, while the Supply HEPA filter provides unidirectional downward flowing contaminant-free air for Product protection, and Environmental protection through the Exhaust HEPA filter (**Figure 1**). Class II is then split into 5 BSC Types: A1, A2, B1, B2, and C1. The most common type found in laboratories worldwide is the Class II Type A2 cabinet, sometimes referred to as a “recirculating” type cabinet, as it has a portion of the air (~70%) recirculated within the BSC (**Figure 1**). This cabinet has a minimum 100 feet per minute (FPM) intake air through the front access opening.

FIGURE 1.

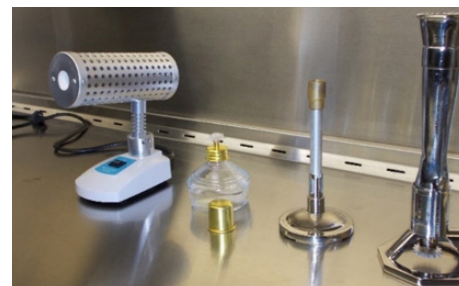
Sideview diagram of a Class II Type A2 BSC.

FIGURE 2.

Heat Sterilizers. From left to right, the Bacti-Cinerator, Spirit Lamp, Standard Bunsen Burner, and High Heat Bunsen Burner.



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Since these cabinets rely on strict airflow patterning and direction, any disturbance in that path may compromise Containment. In many microbiological studies, a flame is commonly used for sterilizing tools during routine practices. This technique works well, and has been widely used for decades, if not centuries and millennia. However, the flame will create hot air, which rises, counteracting the downward flowing air within the work area of the BSC and creating turbulence. Turbulence can allow for the possibility of a contaminant to be transferred into or throughout the BSC. The amount of

turbulence and how strongly it will affect Containment is currently unknown. Here the amount of turbulence in both a 4 foot and 6-foot wide Class II Type A2 BSC is demonstrated, as well as the level of Containment retained as a result of housing four standard types of laboratory heat sterilizers during BSC operation (**Figure 2**). Similarly, some burners require a flammable gas. Whether a flammable gas is safe within a BSC will also be addressed.

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 outfit with a 4-inch hose and attached to a 6-foot PVC pipe with holes drilled every 2 inches to provide a uniform curtain of smoke throughout the entire worksurface. The pipe was mounted just below the supply HEPA filter diffuser near the rear wall of the work area.

Airflow was then visualized under normal operating conditions and with each of the four heat sources shown in **Figure 2**: a standard Bunsen burner, a high heat Bunsen burner, a spirit lamp, and a Bacti-cinerator electric furnace.

Particle counting.

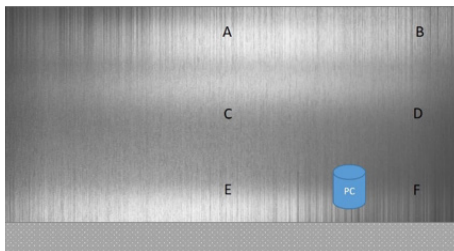
The worksurface of a 6-foot Class II Type A2 BSC (Baker SterilGARD SG604) was split into 6 locations (A-F as shown in **Figure 3**) of common heat source placement. Locations A and B were

| METHODS

FIGURE 3.

Heat source placement within the worksurface of a 6ft BSC (A-F). Particle counter nozzle placement shown as a blue cylinder.

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along the backwall, C and D were on the horizontal midline, and E and F were along the front intake grate. A, C, and E were placed on the front to back center line, and B, D, and F were 6" off the sidewall.

The nozzle of a MetOne particle counter (Model A2408-1-115-2) was placed 6" inward from the front intake grille 6" off the work surface, in between heat source placement locations, as denoted in **Figure 3**. Particles 0.3 μ m and 0.5 μ m in size were measured at standard operating conditions with no heat sources, and then with each heat source in each location. The number of particles was then compared to the ISO standard classification for air quality (EN ISO 14644-1) to determine if the BSC can continue to maintain ISO Class 5 air.

Aerosol Microbiological Challenge Testing.

The containment capability of the BSC was tested using microbiological aerosols as described in NSF International Standard 49 (NSF International, 2016). Two sizes of BSC were used for these experiments, a 4-foot wide and a 6-foot wide Class II Type A2 BSC (Baker SterilGARD SG404 and SG604, respectively). Testing was split into three types: Personnel, Product and Cross Contamination testing. The collision nebulizers contained a slurry of *B. subtilis* var. *niger* spores, and Tryptic Soy Agar petri dishes were placed as directed in the Standard (NSF International, 2016). After proper setup, the bacteria were nebulized into 1 μ m droplets (May, 1973) with the BSC running in the standard operating configuration, or with the heat sources in Locations A, B, C, or D for the Product and Personnel testing. Only Locations B and C were used for the Cross Contamination testing. After the tests were conducted, all petri dishes were covered and placed in a 37°C tissue culture incubator (Baker Cultivo Ultra Plus). Results were read after 24 hours of growth, and pass/fail was determined.

Flammable Gas calculation.

Since the most common flame sources within a BSC require natural gas or propane to function, the amount of gas a BSC can handle safely should be addressed. A BSC will have a hot motor/blower over which flammable gas can flow during standard operation. Using the formulas previously calculated for volatile chemicals (**Equation 1**; Stuart et al., 1983; Held et al., 2016), we can

determine that 10 and 20 mL/min of natural gas or propane, respectively can be emitted into the airstream of a 4-foot Class II Type A2 BSC and stay within 10% of the Lower Explosion Limit (LEL). By comparison, propane is known to be released from its tank at 0.12mL/min. This is well within the safe range.

| RESULTS

EQUATION 1.

$$ER = \frac{Q_i * MW * LEL * 473}{403 * SG * S_F * 100}$$

Similarly, the autoignition temperature for propane is 504°C. NSF International Standard 49 dictates that a motor/blower must not exceed 150°C (NSF International, 2014). Therefore, the motor/blower does not become hot enough to cause spontaneous ignition, even in the presence of flammable gas within the BSC. However, this does not take into account any potential sparks, faulty gas lines or valves, cracked or leaky tubing, etc., all of which would circumvent these calculations and cause an explosion.

Smoke Visualization.

During standard operation, smoke should be seen flowing in a steady, unidirectional pattern from the HEPA filter diffuser down to the worksurface as a smooth curtain (**Figure 4A**, [Supplemental Video 1](#)). When heat sources were placed in the work area, disturbances could be seen of varying severity. The high heat Bunsen burner showed the greatest fluctuations as shown in **Figure 4B** and [Supplemental Video 2](#), followed by the standard Bunsen burner (**Figure 4C**, [Supplemental Video 3](#)). Much smaller disturbances were observed with the spirit lamp and Bacti-cinerator where the flame was observed to shift to horizontal (**Figure 4D** and **E**, [Supplemental Video 4](#) and [5](#), respectively).

Particle Counting.

Under standard operating conditions, a Class II Type A2 BSC should maintain ISO Class 5 air (NSF International, 2016). Disturbances in the airflow may allow for contaminating particles to enter the work area through the front access opening. Particles of 0.3 and 0.5µm were measured at the front access opening with the heat sources placed in each of 6 locations described in **Figure 3**. As seen in **Table 1**, many of the locations were able to maintain ISO Class 5 air (green), however, both styles of Bunsen burners failed to maintain this air quality at the center Location C as well as along the front access opening, Location E (red). The High Heat Bunsen Burner also failed at the other front access opening position,

Location F. The taller flames seemed to affect the Momentum Air curtain and intake airflow much more strongly than the small Spirit Lamp flame or Bacti-Cinerator, allowing more particles to enter the BSC work area.

Aerosol Microbiological Containment Testing.

In order to test full Biosafety containment, the cabinet was subjected to aerosol microbiological testing as outlined in NSF International Standard 49, which is broken down into three specific tests: the personnel protection test, the product protection test, and the cross contamination test (NSF International, 2016). Each test has a specific configuration for placement of the nebulizer and air samplers, concentration of bacterial spores within the nebulizer, as well as pass/fail criteria. A passing result of all three tests is required in order to claim adequate Containment. All three tests were conducted in both a six- and four-foot wide Class II Type A2 BSC (Baker SterilGARD SG604 and SG404, respectively) for each of the four heat sources, in each of the back four locations (A, B, C, and D, shown in **Figure 3**). Overall, it was apparent that the six-foot BSC had a greater capability to overcome the microbiological challenge in the presence of heat (**Table 2**), whereas the four-foot BSC could not (**Table 3**). Three of the heat sources were able to maintain Containment in at least one location in the six-foot BSC: the Bunsen burner at Location A, the Bacti-cinerator at Location B, and the Spirit Lamp at Locations A, B, and D (**Table 2**). None of the heat sources were able to maintain Containment within the four-foot BSC (**Table 3**). As shown in **Tables 2** and **3**, certain locations were more prone to failures, especially Location C, the direct center of the work area, or the most commonly used area of a BSC. Interestingly, Location A and B failed in every location tested in the four-foot BSC, as well as 10 out of 12 tests in Location C, and 7 out of 8 tests in Location D (**Table 3**).

FIGURE 4.

Smoke visualization of airflow disturbances by heat sources within a BSC. Shown are: (A) Normal operation, (B) High Heat Bunsen Burner, (C) Standard Bunsen Burner, (D) Spirit Lamp, and (E) Bacti-Cinerator.

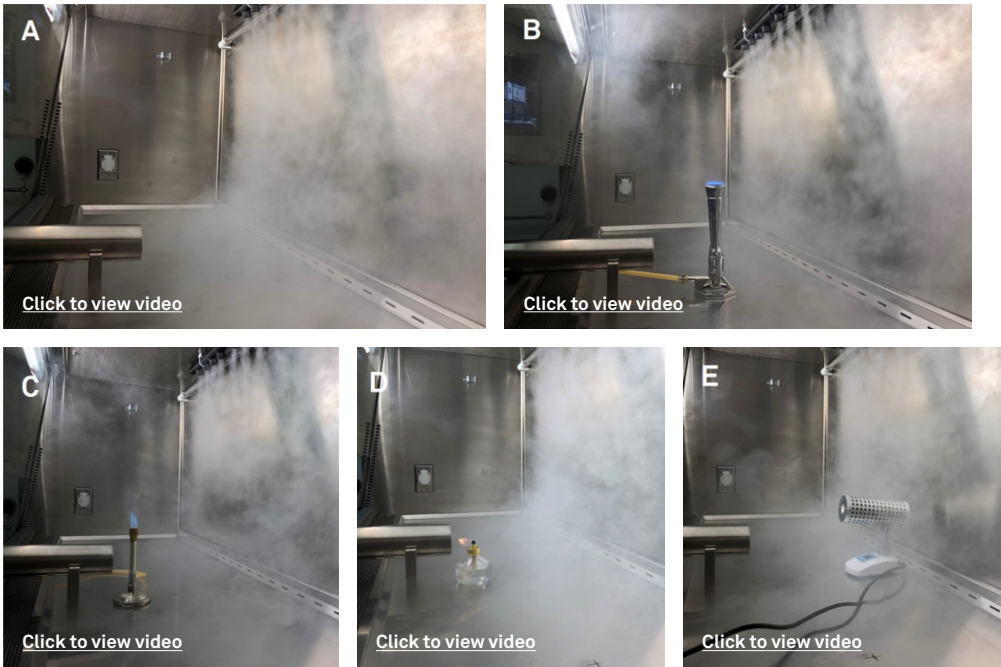


TABLE 1.

Particle Counts measured at 0.3 and 0.5 μm at each location. Green denotes meeting while red means failing to meet ISO Class 5 air.

Location	Bunsen Burner		High Heat Bunsen Burner		Bacti-Cinerator		Spirit Lamp	
	0.3 μm	0.5 μm	0.3 μm	0.5 μm	0.3 μm	0.5 μm	0.3 μm	0.5 μm
A	0	0	2153	388	600	71	35	0
B	0	0	0	0	282	35	35	0
C	19768	5507	63293	12814	883	247	35	0
D	1200	282	2400	424	530	0	71	0
E	92098	34594	505849	114019	1271	212	282	0
F	6495	1553	19062	3565	106	0	671	141

TABLE 2.

Aerosol Microbiological Containment testing results for the four heat sources in a 6-foot Class II Type A2 BSC. Pass (green) and Fail (red) criteria determined by NSF International Standard 49 (NSF International, 2016).

Location	Bunsen Burner			High Heat Bunsen Burner			Bacti-Cinerator			Spirit Lamp			Total Fails
	Personnel	Product	Cross	Personnel	Product	Cross	Personnel	Product	Cross	Personnel	Product	Cross	
A	Green	Green	Green	Green	Red	Green	Green	Red	Green	Green	Green	Green	2
B	Green	Red	Red	Green	Green	Red	Green	Green	Green	Green	Green	Green	3
C	Green	Red	Red	Red	Red	Red	Green	Green	Red	Green	Red	Red	8
D	Green	Red	Green	Green	Red	Green	Red	Green	Green	Green	Red	Green	3

TABLE 3.

Aerosol Microbiological Containment testing results for the four heat sources in a 4-foot Class II Type A2 BSC. Pass (green) and Fail (red) criteria determined by NSF International Standard 49 (NSF International, 2016).

Location	Bunsen Burner			High Heat Bunsen Burner			Bacti-Cinerator			Spirit Lamp			Total Fails
	Personnel	Product	Cross	Personnel	Product	Cross	Personnel	Product	Cross	Personnel	Product	Cross	
A	Green	Red	Red	Green	Red	Red	Green	Red	Red	Green	Red	Red	4
B	Green	Red	Red	Green	Red	Red	Green	Red	Red	Green	Red	Red	8
C	Green	Red	Red	Green	Red	Red	Green	Red	Red	Green	Red	Red	10
D	Green	Red	Green	Green	Red	Red	Green	Red	Red	Green	Red	Red	7

| CONCLUSIONS

The Primary Engineering Controls (PECs) known as Biosafety Cabinets protect against contamination of the user (Personnel Protection), the experiment or work being done (Product Protection), and the laboratory or facility (Environmental Protection) through the use of HEPA filters and specifically controlled airstreams. Any disruption in this airflow allows for potential contaminants to enter the BSC or travel throughout the work area within the BSC, also known as cross contamination. Heat sources, such as flames, cause air to rise counteracting the standard downflow air needed within the work area. Contrasting airflow directions lead to eddies, swirling, and the potential to move contaminants from one area to another within the BSC. While calculations revealed that the BSC under standard operation would be able to handle a surplus of flammable gas being supplied to the burners, it is not advisable to use flammable gasses within a BSC due to the risk of unintended sparks or gas leaks that may occur, leading to unintended ignition and potential explosion.

Smoke visualization revealed large vortexes that moved throughout the whole work area, especially with large flames (**Figure 4, Supplemental Videos 2-5**). Smaller heat sources had variable results, where sporadic upward currents of air could be seen leading to inconsistent contamination control. At times, the smaller burners had rapidly waving and horizontal flames.

Similarly, the use of a particle counter to determine if the BSC maintains ISO Class

5 air showed that both Bunsen burners with larger flame heat sources could not maintain ISO Class 5 classification, while the smaller heat sources (Bacti-cinerator and Spirit Lamp) could (**Table 1**).

However, when tested more stringently with accordance to NSF International Standard 49 Aerosol Microbiological testing, differences with heat source placement throughout the BSC work area (**Figure 3**) were made apparent.

It was also noted that BSC size, or nominal width (4- vs. 6-feet) made a large difference in whether the cabinet could overcome the heat disturbances created by the burners. As shown in **Tables 2 and 3**, the heat sources in almost all locations caused a loss of containment by failing at least one of the three tests: Personnel, Product, or Cross Contamination. Interestingly, the most common placement for a burner (center of the work area, Location C) was prone to the most failures. Moving the burner to the center position along the back wall (Location A) resulted in the least amount of failures, and even maintained Containment with the Bunsen burner and Spirit Lamp in the 4-foot BSC (**Table 3**).

Due to the highly variable results from location to location, and between burner types, using a heat source within a BSC cannot be recommended. There are too many instances where Containment may be lost, as well as the potential for unintended spark generation or leaked flammable gas to lead to explosion.

| REFERENCES

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