Loss of Class II Type A2 Biological Safety Cabinet Containment in a BSL-3 Laboratory Due to Supply Air Outlet Temperature

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The purpose of this article is to discuss field data observations concerning the adverse impact of supply air outlet temperature on the containment performance of a class II type A2 biological safety cabinet in a particular BSL-3 laboratory environment. The data presented highlights the limitations of field performance testing of Class II biological safety cabinets in accordance with NSF/ANSI 49 – 2007 Class II (laminar flow) biosafety cabinetry, Annex F and suggests that consideration shall be given to implementation of in-situ quantifiable field performance testing requirements for critical laboratory applications.

Background

As a component of the commissioning of a newly constructed BSL-3 laboratory, B & V Testing, Inc., an east coast testing and certification firm, was contracted to perform in-situ containment testing of a Class II type A2 biological safety cabinet (BSC) with an installed liquid reagent handler and spectrophotometer. For comparative performance data, containment testing was also performed on an empty adjacent BSC in the same laboratory, Baker Company model SG403A with canopy connection to building exhaust. This article focuses on what was observed during the containment testing of the empty BSC.

Prior to containment testing performance, the laboratory air balancing was completed and the BSC was tested and certified in accordance with NSF/ANSI 49 – 2007 *Class II (laminar flow) biosafety cabinetry,* Annex F and manufacturer's specifications. It is important to note that the biosafety cabinets were positioned in a fairly narrow corridor with HEPA-filtered supply air in close proximity. See figure 1.

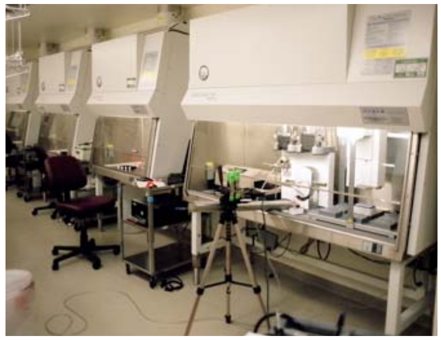

Figure 1. Newly installed Class II type A2 canopy-connected biological safety cabinets with HEPA-filtered supply air in close

Testing Methodology

The Bio-Analog test methodology was selected to quantify BSC containment performance[1]. This methodology, developed by the Baker Company, tilizes tracer gas sulfur hexafluoride (SF6), for containment testing of ducted BSCs. See Figure 2.

Figure 2. Bio-Analog Containment Test Set Up proximity to BSCs.

During the containment test, over a 5 minute run period, SF6 is passed through an NSF 6-jet collision nebulizer filled with 55 mil of water at 20 psi, positioned 14" above the work surface centerline and 4" back from the view screen.

For leak detection, an iTi Qualitek model 200 leak detector with a detection range of 0.01 - 60 ppm was utilized with the detector probe positioned outside of the cabinet, 6.5" above the center line of the BSC work surface and 1.5" from the plane of the BSC access opening. The NSF test arm was positioned along the cabinet center line.

BSL-3 Laboratory and Biosafety Cabinet Constant Conditions

During containment testing, the following constant laboratory conditions were in place: lab pressure: - .06"w.g.(-15 pascals), lab supply air: 1320 CFM via four 2' X 4' terminal ceiling HEPA filters and two diffusers (250 CFM per HEPA filter following re-direction of supply air to rear of lab), lab exhaust air: 1500 CFM via four BSCs, one exhaust register and autoclave exhaust, cooling mode supply air outlet temperature: 58 - 63 degrees F (14 -17 C) Note: outside air 24 C.

For the SG403A, the following constant operating conditions were in place in accordance with manufacturer's specifications: inflow velocity: 107 feet per minute (.54 m/s), downflow velocity: 54 feet per minute (.27 m/s), canopy duct connection: - .05" w.g. (-12 pascal). It is worth emphasizing that the BSC passed the airflow smoke patterns testing as per NSF/ANSI 49, Annex F, including the view screen retention test. As a reminder the test is described here:

F.4.3.2 View screen retention test: Smoke shall be passed from one end of the cabinet to the other, 1.0 in (2.5 cm) behind the view screen at a height 6.0 in (15 cm) above the top of the access opening.

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Results

For the purpose of discussion, the results are organized into two scenarios, consisting of ten 5-minute runs for each scenario: Scenario one is the warming mode with HEPA filter supply outlet temperature ranging from 64 – 70 degrees F (18-21 C) and scenario two is the cooling mode with HEPA filter supply outlet temperature ranging from 58 – 63 degrees F (14-17 C).

During initial containment test runs performed in the warming mode, no loss of containment performance was observed. See Figure 3 for results of a typical run in warming mode. Time is on the X axis and SF6 released in ppm is on the Y axis.

During initial containment test runs performed in the cooling mode, periodic repeatable loss of containment performance was observed. See Figure 4 for results of a typical run in cooling mode. See Figure 4 for results of a typical run in cooling mode.

Periodic containment loss was verified through visual smoke testing. When the smoke source was held in a constant position behind the view screen (4" behind view screen, 14"above the work surface along the cabinet center line) for an extended period of time, periodic smoke release from the top of the BSC access opening was observed. During the observed, periodic smoke releases, smoke could be seen briefly escaping from the top of the access opening before being quickly sucked back in.

In response to the observed containment breaches during cooling mode, steps were taken to investigate and remediate possible causes for containment loss. Remediation efforts included reduction of HEPA-filtered supply air velocity and volume for filters adjacent to the BSC and partial and complete blocking off of HEPA-filtered supply air in BSC proximity. During re-testing following these efforts, it was only when HEPA-filtered supply air was entirely blocked off that no loss of containment was observed.

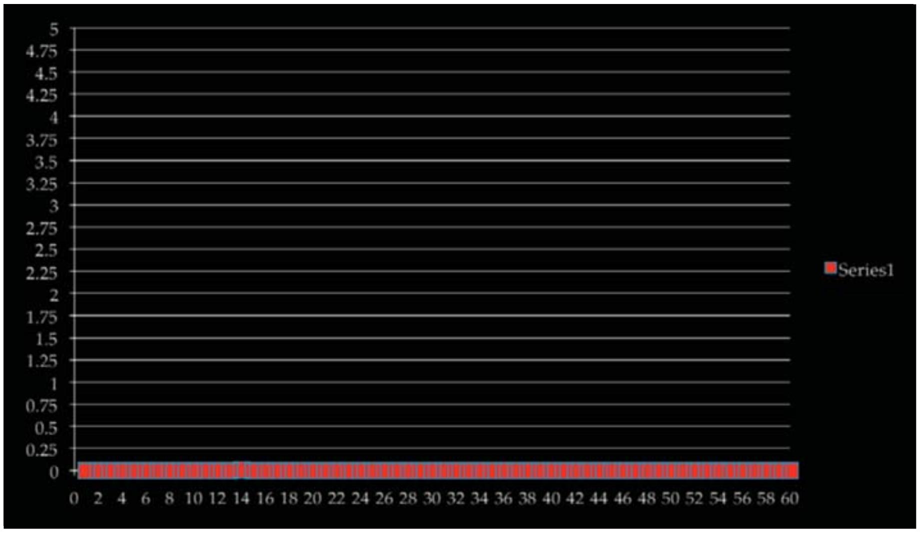

Figure 3. Typical run warming mode. Time (5 minutes)

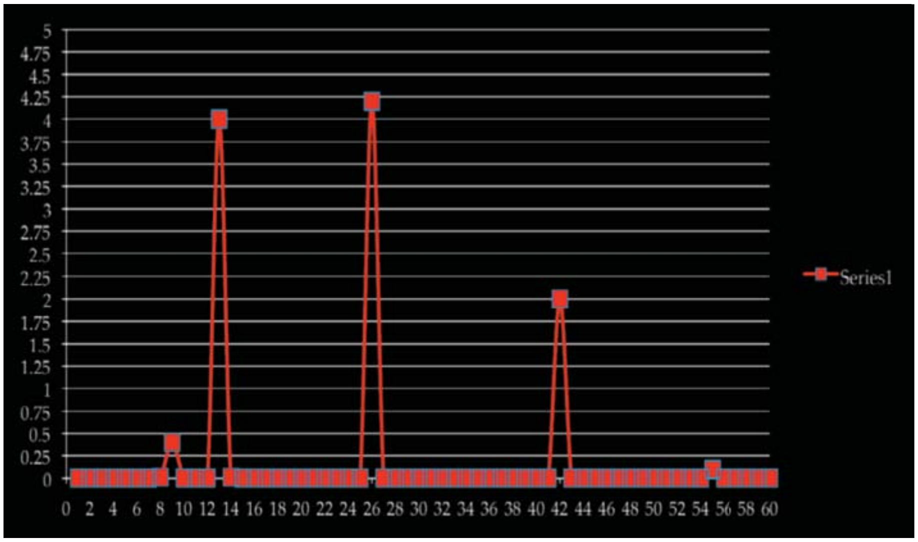

Figure 4. Typical run cooling mode Time (5 minutes)

Summary Results Table 1.

	Average SF6 Release Containment	Protection Factor	# of Peak Releases	Average SF6 Peak Release	Peak Release Containment Protection Factor
Warming Mode	< 0.01 ppm	6 logs	< 1peak release	0.3 ppm*	5.9 logs
Cooling Mode	0.13 ppm	4.4 logs	4.6 peak releases	1.73 ppm	3 logs

* one measurable peak release of 0.3 ppm during course of ten 5-minute runs

A summary of results is outlined in Table 1 (above) for the ten runs in cooling mode and ten runs in warming mode. Test results summarized include:

1. Average SF6 release concentration per 5 min test runs, averaged over 10 runs
2. Containment protection factor per run (interior SF6 concentration 10,000 ppm/average SF6 release concentration), averaged over 10 runs
3. Number of peak releases SF6 per run due to periodic loss of containment (peak release defined as SF6 release of > 0.10 ppm which results in loss of > one log protection factor), averaged over 10 runs
4. Average SF6 peak release concentration per run averaged over 10 runs
5. Peak release containment protection factor per run, average over 10 runs (interior SF6 concentration 10,000 ppm/average SF6 peak release concentration)

Results Discussion and Outcomes

When the laboratory was operating in cooling mode, it is theorized that the cold dense air exiting the HEPA filter in proximity to the BSC access opening was interfering with the inflow of air into the BSC access opening, resulting in periodic containment loss. Considering that the HEPA-filtered supply air exit velocity was relatively low following volume reduction and re-direction, approximately 35 feet per minute, it is also theorized that the narrowness of the corridor in which the BSCs were placed was a factor. Further investigation is warranted.

It is also worth pointing out that, had the testing occurred during a time of year, when the laboratory temperature controls were not calling for cooling mode, it is likely that the BSC containment performance loss would not have been observed.

As a result of the testing results, certification was voided on the lab BSCs until laboratory temperature controls could be modified to maintain supply air outlet temperature at greater than or equal to 17 C. Once proper temperature controls were implemented, re-testing was performed on all lab BSCs, and containment performance was verified.

Conclusions and Recommendations

The data demonstrates that the supply air outlet temperature of air sources in close proximity to Class II, Type A2 biological safety cabinets under certain laboratory conditions may adversely impact BSC containment performance. Secondly, airflow smoke patterns testing as described in NSF/ANSI 49 2007 may be inadequate to accurately assess BSC containment performance under certain laboratory conditions. It is recommended that for critical and/or questionable BSC installations, quantifiable field performance testing such as the Bio-Analog test or European KI discus test, should be performed to verify containment performance under as-installed conditions, and that field certification should be performed with supply air systems operating in "worse-case" scenarios (at coldest/max set-points).

Acknowledgements

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Footnotes

1. Field Test Quantifying Biological Safety Cabinet Containment Performance, R.L. Jones, The Baker Company, Presented at 1994 Annual Conference of American Industrial Hygiene Association