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Hypoxia Workstation with HEPA-Filtration Option Demonstrates Enhanced Containment to Protect User

Bob Lloyd · Samir Patel · Becky Metivier

ABSTRACT

Hypoxia workstations are utilized by researchers who require stable and accurate low oxygen environments for their research. Oxygen, carbon dioxide, temperature and humidity are precisely controlled within the work area of the workstation to simulate an in vivo environment and create optimal conditions for the experiment.

Hypoxia workstations are closed glove boxes. They allow air from inside the workstation (which is under positive pressure relative to the room) to be released directly to the laboratory by design, and do not typically provide protection to personnel from potential contaminates in the exhausted air. If biological agents may be aerosolized within the workstation, it is important that biosafety officers have containment performance data to perform a complete risk assessment.

An enhanced containment option was developed for the Ruskinn Invivo$_2$ hypoxia workstation. This option was microbiologically tested at The Baker Company in order to quantify the release of a microbiological aerosol from the workstation to the laboratory environment. This paper describes the test procedure and presents results that can then be used to determine the risk to the laboratory researcher associated with various types of laboratory work.

INTRODUCTION

Ruskinn hypoxia workstations are designed to replicate low-oxygen in vivo physiology to provide the ideal research platform for cell biologists and cancer researchers. The Ruskinn Invivo$_2$ workstation (Figure 1) offers a positive pressure work area for closed culture research whereby oxygen, carbon dioxide, temperature, and humidity are precisely controlled.

In order to maintain the desired gas mixture inside the workstation, the pressure within the system is higher than that of the surrounding environment. This prohibits atmospheric gases from entering the workstation and upsetting the desired gas mixture. Typical hypoxia workstations are not specifically designed to provide containment (personnel protection), so the atmosphere inside the workstation has the potential to carry contamination when exhausted (or if any leaks occur).

Biosafety professionals evaluate laboratory equipment, including hypoxia workstations, to determine the risk level from potential biohazards. In some cases, limiting an operator's exposure to microbiological aerosols that may be generated in their workstation is desired.

For this reason, Ruskinn, a wholly-owned subsidiary of The Baker Company (Baker), designed an enhanced

Figure 1: The Ruskinn Invivo$_2$ hypoxia workstation.

containment option for its line of hypoxia workstations.

The enhanced containment option consists of the following modifications to a standard Invivo$_2$ workstation:

1. Two in-line HEPA filters - one is installed on the discharge side of the exhaust valve (Figure 2) and the other is installed on the discharge side of the interlock purge valve.
2. Single-piece rubber gloves are used in lieu of the typical arm sleeves, and must be tightly sealed to the glove ports. In the following test, Hypalon rubber gloves are used with a rubber "O" ring and a stainless steel pipe clamp to ensure proper sealing.
3. The Single Plate Entry System (SPES) (Figure 3) must be sealed off. In this test, the SPES was adequately sealed by tightening the screws to the maximum level.

To provide a biosafety professional with the data needed to assess the containment capabilities of this enhanced containment option, the solution was tested in the Baker laboratory. Drawing on their experience with containment equipment, Baker engineers adapted test methods commonly used for biological safety cabinets (ANSI/NSF 49[1]) and other containment equipment.

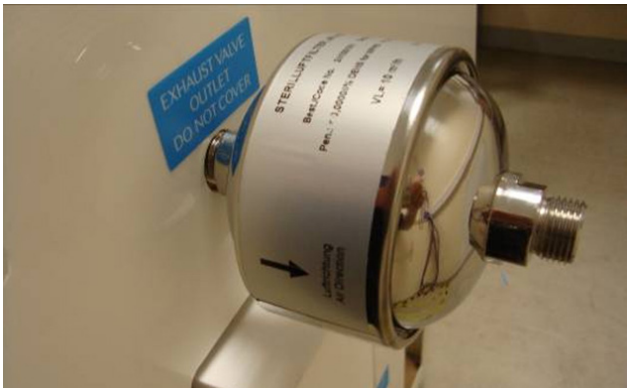

Figure 2: An in-line HEPA disc filter installed on the discharge side of the exhaust valve of the Invivo$_2$ workstation.

Figure 3: The SPES (shown above) is a mailbox-like slot that allows quick side entry or exit of individual plates and bypasses the interlock cycling process.

As a result of this work, biosafety professionals can consider this data as part of their risk assessment, and make an informed decision about whether the workstation with the enhanced containment option meets their laboratory's biosafety requirements. This paper describes the test methods employed and presents the results of the work performed in order to help quantify potential loss of an aerosol.

TEST METHODOLOGY

Purpose

This test demonstrates the level of exposure a user could potentially have when microbiological aerosols are generated inside the Ruskinn hypoxia workstation with enhanced containment option.

While no standard containment test protocol exists for hypoxia workstations, containment equipment is typically evaluated by aerosolizing spores inside the containment device using a calibrated nebulizer and sampling air within the laboratory environment using glass impingers and slit samplers. This method is employed in the following procedure.

Test Set-Up

The enhanced containment option was installed on the Invivo$_2$ 400. First the in-line HEPA filters were installed - one on the discharge side of the exhaust valve and the other on the discharge side of the interlock purge valve. The fittings were tested with Helium to ensure that they were not leaking.

Then black Hypalon rubber glove box gloves were installed on the arm port and sealed in place with a rubber "o" ring and stainless steel pipe clamp.

The tubing to supply the compressed air to the nebulizer was installed through the left wall of the cabinet (Figure 4).

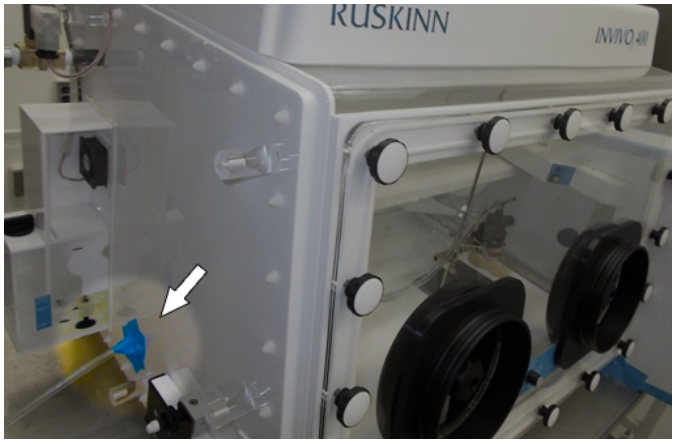

Figure 4: Tubing inserted into the cabinet to supply the necessary compressed air to the nebulizer. Please note that this would not be necessary during regular operation.

Six glass impingers were arranged so that they would be in the breathing zone of the cabinet user (Figure 5).

Three slit samplers were arranged within the laboratory. One was placed to the left of the workstation, and the other was placed to the right of the workstation directly in front of the interlock area. The third slit sampler was placed in the corner of the laboratory (Figure 6).

A nebulizer (calibrated as stated in NSF 49, appendix C) was placed in the center of the cabinet, six inches above the work surface and facing the rear of the cabinet to simulate where an actual aerosol may be created in normal operation (Figure 7).

A small fan was added to circulate and mix the laboratory air in order to better simulate actual laboratory conditions, where work would be taking place.

The pass through doors of the workstation were closed and remained closed for the duration of the experiment to simulate conditions during the time work would be carried out within the work area.

Test Procedure

The workstation was challenged with 7.2×10^4 *Bacillus subtilis* spores over a five minute period.

The slit samplers and impingers collected samples for a total of 30 minutes. The samples were then analyzed as stated in NSF 49 to determine the number of colony forming units (CFUs) present. The test was done three times, and results recorded.

Figure 5: Six impingers were placed in the area where the user would typically work with the Invivo$_2$. See top inset for a close-up of the glass impingers, similar to those used in this test.

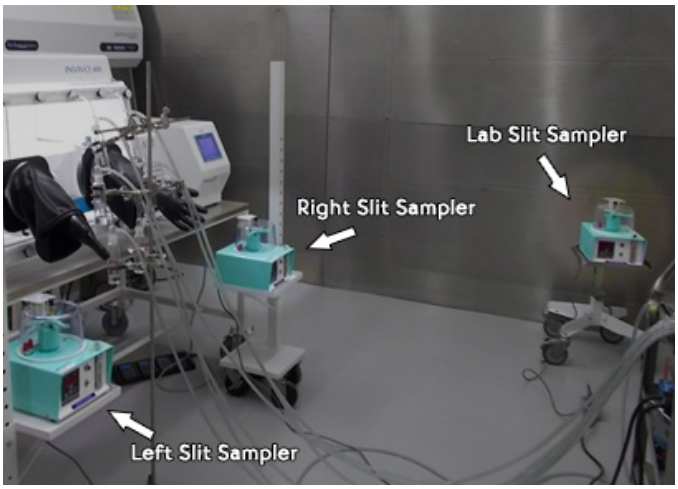

Figure 6: Placement of the three slit samplers during the test.

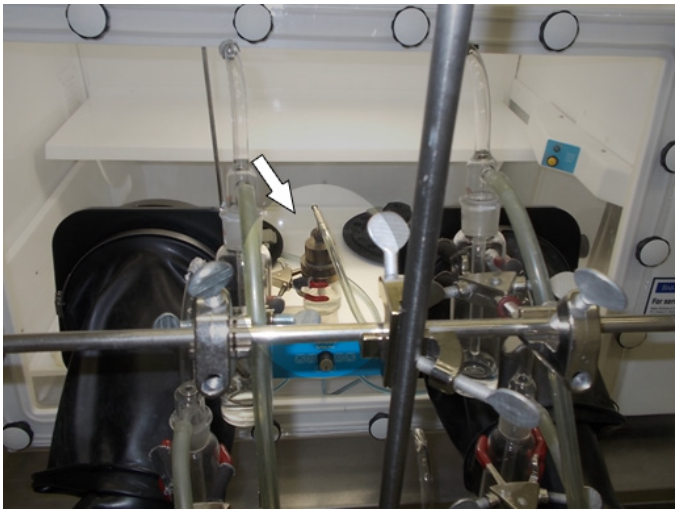

Figure 7: View of the nebulizer within the Invivo$_2$ 400.

RESULTS

The results from the three replicate tests are shown in Table 1. The Invivo$_2$ workstation with enhanced containment option provided measurable containment.

Fewer CFUs were collected directly in front of the workstation by the impingers than in the lab by the slit samplers, which means that there is a relatively high level of protection to the operator in their immediate breathing zone. The highest number of CFUs collected by the slit samplers was in the sampler directly in front of the interlock (right sampler). From this it can be deduced that there is some leakage from the interlock. This is likely due to the relative pressurization of the work area and pass through interchange, and because the door seals are designed to leak out in order to maintain the low O_2 levels.

A protection factor was determined by calculating a log reduction. Log reduction is a term used to express the reduction in number of live bacteria. Log (short for logarithm) is the exponent of 10; therefore a 1 log reduction is a 10-fold (or 90%) reduction in the number of live bacteria.

First, the highest numbers of CFUs found at each location in any of the three tests were added together to determine total CFUs collected (Table 2). Then the percent reduction of the initial challenge to total CFUs collected and log reduction were calculated (Table 3). A log 3 protection factor (99.9% reduction) was determined.

DISCUSSION

The results show that with an enhanced containment solution, the Ruskinn hypoxia workstation is capable of providing containment.

Evaluating containment, or personnel protection, is routinely performed on biological safety cabinets. Unlike hypoxia workstations, biosafety cabinets are operated with the work area under slightly negative pressure and aggressive airflows are required for containment. During testing an extremely high spore challenge is employed - much higher than what would be expected in typical laboratory procedures. The aerosol is this case is used to trace the direction of air streams flowing into and under the cabinet. In a biosafety cabinet it is assumed that there would be a loss of aerosol in normal use due

Test 1

Test Location	Total # of Spores Generated	Sampler Location	CFUs Collected	Total CFUs Collected
Laboratory (Slit Samplers)	7.2×10^4	Right	10	12
		Left	0	
		Lab	2	
Operator Work Area (impingers)	7.2×10^4	In Front of Work-station	5	5

Test 2

Test Location	Total # of Spores Generated	Sampler Location	CFUs Collected	Total CFUs Collected
Laboratory (Slit Samplers)	7.2×10^4	Right	9	16
		Left	0	
		Lab	7	
Operator Work Area (impingers)	7.2×10^4	In Front of Work-station	5	5

Test 3

Test Location	Total # of Spores Generated	Sampler Location	CFUs Collected	Total CFUs Collected
Laboratory (Slit Samplers)	7.2×10^4	Right	17	27
		Left	0	
		Lab	10	
Operator Work Area (impingers)	7.2×10^4	In Front of Work-station	3	3

Table 1: CFUs collected in the three replicate tests.

Test Location	Sampler Location	Test #	Highest # of CFUs Collected
Laboratory (Slit Samplers)	Right	3	17
	Left	1, 2, 3	0
	Lab	3	10
Operator Work Area (impingers)	In Front of Workstation	1, 2	5
		Total CFUs Collected:	**32**

Table 2: Largest CFU count detected, with total calculated.

Total # of Spores Delivered	Total CFUs Collected	Percent Reduction	Log Reduction
7.2×10^4	32	99.9%	Log 3

Table 3: Protection factor of the Invivo$_2$ workstation.

to an operator's movements, incursion from drafts within the lab space, and other disruptions to the lab air environment.

The Invivo$_2$ workstation is a closed system, so none of these disruptions are present. Therefore a lower spore challenge was used during these tests.

Preliminary testing in the Baker laboratory showed that the highest concentration that provided meaningful and quantifiable results was a challenge of 7.2×10^4 *Bacillus subtilis* spores over a five minute period. A much larger challenge resulted in CFUs too numerous to count.

A study on the generation of aerosols from a few typical laboratory procedures that may be performed within a hypoxia workstation found that the highest number of CFUs generated was 4,838[2]. Therefore, a challenge of 7.2×10^4 is significantly higher than what should be aerosolized within the workstation during typical laboratory operation.

CONCLUSION

Hypoxia workstations, like Ruskinn's Invivo$_2$, provide an ideal low-oxygen environment for cell biologists and cancer researchers to perform their work. While the primary goal of the workstation is to precisely control oxygen, carbon dioxide, temperature, and humidity within the work area, in some cases, a level of containment, or personnel protection, may be required by biosafety professionals.

The Invivo$_2$ with enhanced containment option can provide personnel protection. When challenged with a spore concentration higher than what should be present within the work area, a log 3 reduction was achieved. A user performing typical laboratory procedures within the workstation that generates a concentration of aerosols less than 7.2×10^4, can expect limited exposure when the enhanced containment option is utilized with the Invivo$_2$.

When determining which equipment is most appropriate for a laboratory's biosafety needs, a risk assessment should always be prepared by a biological safety professional. Armed with the data presented in this paper, they can better understand the containment capabilities of the enhanced containment option for the Ruskinn Invivo$_2$ hypoxia workstations, and make a more informed decision.

REFERENCES

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THE BAKER COMPANY

P.O. Drawer E, Sanford, Maine 04073
(207) 324-8773 · 1-800-992-2537 · FAX (207) 324-3869
www.bakerco.com

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THE BAKER COMPANY