Performance Testing of Large Biological Safety Enclosures

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Executive Summary

The use of robotics and automated equipment to perform repetitive tasks in the microbiology lab is becoming more prevalent with advances in technology. While this equipment has increased productivity and quality control while reducing worker stress, it has not eliminated the need for engineering controls to assure product sterility and to protect the lab worker from potentially hazardous bioaerosols. Cell sorting and pipetting are two examples of prevalent automated procedures that produce bioaerosols. The size of the automated equipment along with support and interface needs usually prevent the use of standard class II Biological Safety Cabinets (BSCs) as the engineering control. The development of large biosafety enclosures for this equipment has identified the need for unique design solutions and test procedures. The test results from this research shows that class II performance levels are attainable and verifiable for the various challenges imposed by the large automated equipment and their interface to ancillary equipment.

Automated Laboratory Equipment and Aerosol Generation

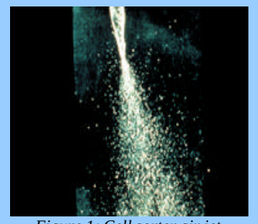

Figure 1: Cell sorter air jet

Cell Sorters (Cytometry)
Flow cytometry, in particular jet-in-air cell sorters, give rise to biological aerosols. (1, 2, 3, 4). Micro droplets in the 3-7 µm range are created in a consistent manner during normal operation. During system failures, such as syringe clogs, the rate and characteristics of the aerosol generation can be varied. Cell samples being analyzed or sorted may carry known or unknown human pathogens.

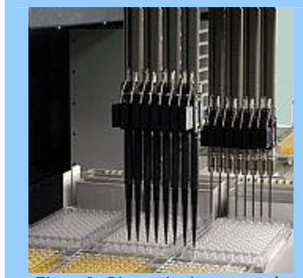

Figure 2: Pipette tips in automated liquid handling system

Liquid Handlers (Pipetting)
Automated pipetting robots have increased throughput and quality control while eliminating worker stress associated with repetitive manual operations.(7, 8) However, these systems have not eliminated the need for a sterile work space nor the need to contain potentially hazardous biological aerosols created during liquid transfer. Siphoning and positive pressure deposition of liquids from open tubes or open microarrays give rise to aerosols.

Given the possibility for aerosol generation, risk assessment may dictate that these operations be performed in a biological safety cabinet. (5, 6)

Performance and Design Requirements

Just like the manual processes that automated equipment replaces, contamination control is required for product sterility. Additionally, due to factors discussed above, containment of generated aerosols is required in order to protect laboratory workers and the environment.

Automated laboratory instrumentation has additional requirements due to the complexity of such systems. These requirements must be met, while still maintaining the product protection and containment:

- **Size:** equipment may be extremely large and is often placed in dedicated spaces/rooms. However, it must be easily accessible for maintenance.
- **User Interface**: access must be provided to allow laboratory personnel to monitor the equipment and perform routine tasks (e.g. loading/unloading samples, clearing jams).
- **System Interface:** the primary equipment is often integrated with a suite of complementary laboratory equipment to automate an entire process. For example, in a cell culture application, the liquid handler may receive plates from a plate stacker, then move the plates to an incubator.
- **Support:** automated equipment also requires utilities and other services to support its operation. This may include data ports, electrical lines, fluid tubing, and standard plumbing connections (gas, vacuum).

Design Solution

In order to provide product, personnel, and environmental protection, an enclosure that provides performance on the level of a Class II Biological Safety Cabinet (BSC) is called for. However, the need to support automated instruments within the enclosure necessitates design solutions that are adapted to these unique requirements. Simply creating an oversize version of a standard Class II BSC is not a viable solution.

The size of the automated equipment dictates a larger enclosure size. Even so, the equipment usually fills the enclosure to a much greater degree than BSCs used for manual procedures. Equipment size also requires the cabinet workspace to extend down to the floor level. Adjustable exhaust grilles are placed within the cabinet to manage the unique airflow balance for various types of large equipment.

Class II BSCs are unique among engineered controls in that they prevent contaminates from migrating in either direction across their boundaries. This performance is made possible by the placement of a negative pressure zone created by an exhaust grille along the bottom side of the front access opening. The access opening on conventional BSCs is generally of a standard size and a regular shape.

For automated equipment enclosures, system interfaces may require openings with different aspect ratios, unique shapes, and non-standard locations (i.e. sidewall instead of front access opening). An approach that provides contamination control, while allowing maximum design and fabrication flexibility, is a rectangular opening larger than the extreme dimensions of the transfer mechanism, with full perimeter ventilation, and baffles added to match the mechanism silhouette.

The front access opening of the enclosure is still primarily for operator interface. Since there is machinery in motion inside the cabinet, provisions may be necessary for operator safety. A number of systems for physical safety have emerged as equipment designs have evolved. These systems may consist of physical guards that prevent breaching of the boundary or light bars which stop equipment motion when the boundary is breached. Specific user intervention may require a key override of door locks with interlocks provided to put the machine in pause mode upon door opening.

Various electrical and plumbing utilities are required to support the equipment functions. Electrical power, data cables, vacuum lines and media feed lines often penetrate the shell of the enclosure. Iris ports (Figure 4, inset) are used to minimize the penetration open area, thus reducing the airflow required to maintain performance.

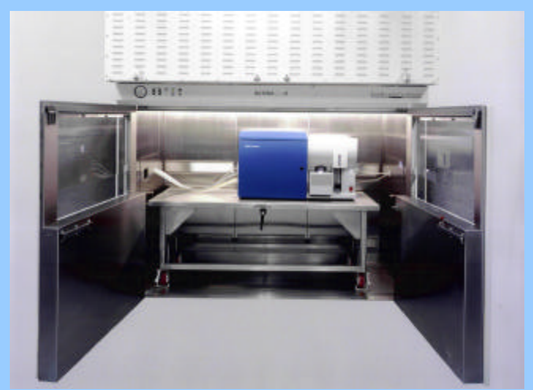

Figure 3: BD Bioscience FACSAria Flow Cytometer (located within a BioPROtect II biological safety enclosure)

Test Procedures

The microbiological test procedures and criteria for class II BSCs are defined in NSF International Standard 49. (10) However, strict adherence to the NSF procedures is sometimes not possible with larger specialized enclosures due to geometric interferences and sometimes not desirable when unique critical areas are defined by the process. The NSF procedure was adapted for each unique design, including conducting tests with the enclosed equipment and any transfer mechanisms in place.

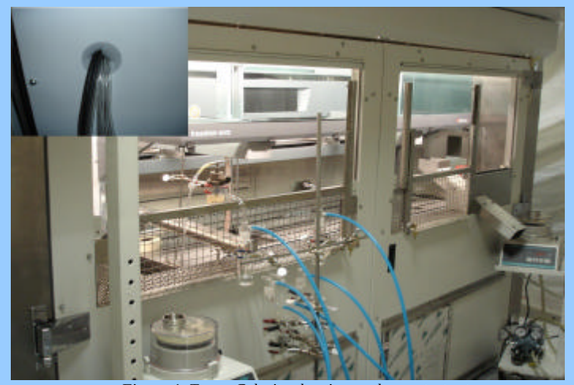

Figure 4: Tecan Celerity showing enclosure test set up (inset: "iris port" sidewall penetration)

Unique airflow balance may be required for each specific application. The balance set point is best chosen by imbalancing the cabinet towards conditions of failure and plotting test results to define the cabinet performance envelope. (9) As each enclosure is designed to accommodate a specific piece of automated equipment, it is not feasible to develop a statistically significant cabinet performance envelope. The NSF performance range concept is utilized to identify the limits of performance and properly chose the most forgiving airflow balance.

Just as with a conventional Class II BSC, field certification utilizes surrogate air flow readings to verify performance. Again the deviations from the NSF defined procedure are required due to equipment interferences with the downflow traverse plane and due to the number of openings through which air flows into the cabinet. Design provisions allow measurement of airflows without opening the enclosure and in a manner that provides reproducible results with the presence of the enclosed equipment.

Results and Discussion

Multiple types of equipment from different manufacturers have been tested. Results are presented for a flow cytometer (FACSAria, BD Biosciences, San Jose, CA) and an automated liquid handling system (Cellerity/EVO, Tecan Group LTD, Mannedorf/Zurich, Switzerland).

The BioPROtect II (The Baker Company Inc, Sanford, ME) was tested with the FACSAria flow cytometer in operation. This was important to establish that neither the geometry nor the functional components of the system (fans and laser) were detrimental to performance of the enclosure.

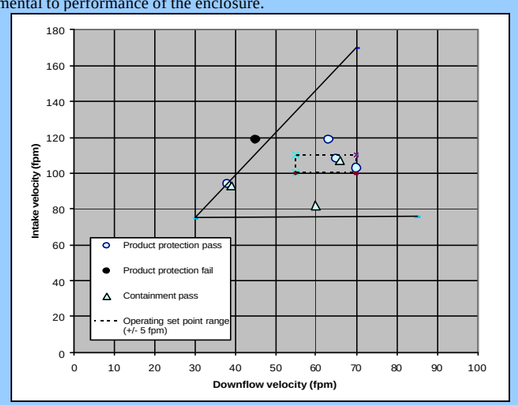

Figure 5: Performance Envelope for BioPROtect II (with BD Bioscience FACSAria Flow Cytometer)

Based on pass/fail results of the microbiological challenge testing (see Figure 5), a performance envelope was established for the BioPROtect II enclosing the BD FACSAria. The operating set point was chosen well within the bounds of the established performance envelope.

The solution developed for the Tecan Cellerity automated cell culture system had a number of unique modifications to accommodate system interfaces (see Figure 4). This included a modified front access opening to include a plate disposal chute, and a tunnel through the enclosure sidewall for the conveyor to bring materials in and out of the unit while running. Testing for this application focused on verifying the performance of these openings.

Test Type	Velocities (fpm)		Microbiological Test Locations			
	Down-flow	Intake	Left Access	Right Access	Waste Chute	Sidewall Tunnel
Product Protection	60	115	Pass			
	62	116	Pass	Pass	Pass	Fail
	60	110				Pass
	63	111				Pass
Contain-ment	60	110				Pass
	63	111				Pass
	69	96	Pass	Pass	Pass	Pass
	70	95	Pass	Pass	Pass	Pass

Figure 6: Microbiological Testing of BioPROtect II (with Tecan Cellerity Liquid Handler)

Results are shown in Figure 6. Testing product protection at the right side wall tunnel with high intake velocity settings was found to have a localized point of failure. This was deemed acceptable given the procedures to be used and the passing results obtained at set point. Based on these results, an operating set point was established.

Conclusions

1. Unique performance ranges/envelopes may exist for openings designed for specific interfaces with other equipment or the operator. Cabinet airflow set points must be developed and tested to satisfy the performance requirements of all openings simultaneously.
2. Unique air flow setting procedures may need to be developed on a case by case basis for each equipment configuration.
3. Class II performance can be obtained through proper cabinet design and airflow development.

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