Analysis of the Class III Biological Safety Cabinet Integrity Test

David Stuart, David Eagleson*, Robert Lloyd, Christopher Hersey, and Dennis Eagleson

The Baker Company, Sanford, Maine

Abstract

The Class III Biological Safety Cabinet (BSC) provides maximum personnel protection when it is adequately field certified and properly used. One of the major criteria for this protection is proper performance of the Class III BSC integrity (tracer gas) test as described in the National Institutes of Health Laboratory Safety Monograph (NCI, 1979). This article deals with the origins, appropriateness, and overall understanding of this essential Class III BSC factory acceptance and field certification test.

Introduction

The Class III Biological Safety Cabinet (BSC) provides maximum personnel protection when it is adequately field certified and properly used (Stuart et al., 2004). The NSF/ANSI Standard 49 covers Class II Biological Safety Cabinets only (NSF, 2010). In the absence of such a standard for Class III BSCs, the Laboratory Safety Monograph (LSM) is the recognized federal reference for the testing of Class III BSCs in the United States (NCI, 1979). The LSM is rather dated (1979), and over the years considerable confusion has arisen concerning how to define the Class III BSC and how to apply the cabinet integrity tracer gas leak test requirements found in the LSM. This article is intended to answer some frequently asked questions, such as:

1. What is a Class III Biological Safety Cabinet (BSC)?
2. Why use the Laboratory Safety Monograph as the reference for testing Class III BSCs?
3. Why is the cabinet integrity test so important to the Class III BSC?
4. Where was the acceptance criterion for the Class III BSC cabinet integrity test established?
5. Why are there two criteria in the literature for the tracer gas cabinet integrity test?
6. Is the acceptance criterion for the Class III BSC cabinet integrity test meaningful and appropriate?
7. Since the Class III BSC is a “glovebox,” why not use the glovebox pressure decay integrity test from the American Glovebox Society (AGS, 2007)?
8. Why not use the NSF/ANSI Standard 49 Class II BSC cabinet integrity pressure decay test?
9. Since Freon R-12 can no longer be used as a tracer gas because of its impact on the ozone layer, how can the Class III BSC cabinet integrity test now be performed?

Methods

These questions were examined in terms of history and leak rate principles using calculations. The amounts of exposure resulting from various forms of the integrity test were calculated and used to help evaluate perceived inconsistencies and to understand what is meant by “no exposure to infectious doses of biohazards.”

Results and Discussion

A Brief History of the Class III BSC

The Class III Biological Safety Cabinet (Class III BSC) was developed to protect laboratorians from germ warfare agents at Fort Detrick in the 1940s (Barbeito, 2002). It is clear from the following quote in Design Criteria for Microbiological Facilities at Fort Detrick, Volume II. Design Criteria (Dick & Hanel, 1970), that the goal for the Class III BSC was to be the ultimate design in eliminating personnel exposure when dangerous biohazards were used:

“Required Use: Every infectious disease building shall have at least one laboratory equipped with biological safety cabinets, Class III, (gastight), so that any agent without restriction may be used safely in at least one place in the building.” (Dick & Hanel, 1970)

The term “gastight” is immediately stated in conjunction with Class III BSCs virtually every time they are named in this document. The design and test criteria for the Class III BSC are intended to ensure no exposure to infectious doses of biohazards. Passing the tracer gas cabinet integrity test with an acceptance criterion of very low leakage (gastight) is the defining test for the Class III BSC, just as passing the microbiological aerosol tracer test is the criterion that defines a Class II BSC (NSF, 2010).

The LSM Leak Rate Criterion

As mentioned in the history above, the leak rate criterion in the 1970 document was very low leakage. No leakage was preferred, but in the real world, that is nearly impossible to achieve and document. Fort Detrick was willing to accept the best results that could be achieved with the leak-testing equipment of the day:

“Ordinarily no leakage should be accepted. In terms of the presently available equipment (General Electric Co. Halogen Leak Detector Model 11-2) it is considered that readings up to 0.5 milliampere on the high sensitivity setting are insignificant when testing at 4 inches water gage pressure.” (Dick & Hanel, 1970)

The Laboratory Safety Monograph was prepared, and revised, in 1979 to make such information available to the scientific community at large (NCI, 1979). In dealing with
Concerns about the LSM Leak Rate Criterion

Concerns have been expressed about perceived inconsistency and/or change of the LSM’s leak rate criterion from \(1 \times 10^{-5}\) cc/s at 3” w.g. to \(1 \times 10^{-7}\) cc/s at 3” w.g. This confusion began with a leak rate for Class III BSCs published in the Federal Register, the official daily publication of rules, proposed rules, and notices of Federal agencies, as well as Executive Orders and other presidential documents (OFR, 1976). It appeared in Table 1:

| Biological safety cabinets, safety performance requirements, and specifications, June 1976 as: |
| "Gastight; leak rate < 1 by \(10^{-6}\) cm/s at 3-in water gage pressure." |
| OFR, 1976 |

This statement of leak rate omitted the tracer gas concentration in the cabinet at the time of the test. Statement of a leak rate without including the tracer gas concentration is meaningless. Was this intended for testing with 100% tracer gas in the cabinet or with pressurizing the cabinet to 3” w.g. with the tracer gas yielding 0.75% tracer gas in the cabinet? This practice of omitting the tracer gas concentration from the statement of leak rate has been continued in some specifications and criteria, perpetuating the confusion.

There is only one leak rate criterion for Class III BSCs in the LSM. It is difficult, and often impractical, to perform the test while assuring that 100% tracer gas is in the cabinet. Therefore, the LSM recommends testing the cabinet with 1% tracer gas in the cabinet at 3” w.g. pressure as long as the test acceptance leak rate of \(1 \times 10^{-7}\) cc/s is used to account for the dilution of the tracer gas in the cabinet. This results in the same total leak rate as that of the criterion.

When using 100% tracer gas in the cabinet, 100% tracer gas comes out through a leak. The total leak \((1 \times 10^{-5}\) cc/s) is measured by the leak meter. When using 1% tracer gas in the cabinet, what is coming out through the leak is only 1% tracer gas. The total leak is made up of 1% tracer gas and 99% air. Since the leak meter is seeing only 1/100 of the total leak, the acceptance criterion of the test leak rate must be adjusted by a factor of 100 to \(1 \times 10^{-7}\) cc/s. This results in a total leak of \(1 \times 10^{-5}\) cc/s, which is the one and only criterion.

Definition of a leak rate must include the concentration of tracer gas in the cabinet for the leak rate statement to be complete and meaningful.

Justification for Using Helium (He) as an Alternative to R-12 in the LSM Leak Rate Criterion

The LSM recommends that the tracer gas test be performed at a concentration of 1% gas inside the cabinet. Therefore, analysis of the performance of He compared to Freon R-12 should also be carried out at this concentration.

A leak rate in the \(1 \times 10^{-7}\) cc/s range will be governed by the viscosity of the tracer gas and air mix (Varian Associates, 1989). Since the difference in viscosity between 1% R-12 in 99% air \((1.795 \times 10^{-10}\) bar s) and 1% He in 99% air \((1.8013 \times 10^{-10}\) bar s) is only \(0.0063 \times 10^{-10}\), the results from using either R-12 or He in this test are substantially the same.

Is This Test Leak Rate of \(1 \times 10^{-7}\) cc/s with 1% Tracer Gas in the Cabinet at 3 Inches Pressure Appropriate for the Purpose of the Class III BSC Cabinet Integrity Test?

To demonstrate whether or not the LSM leak criterion is appropriate for the purpose of the Class III BSC, which is no exposure to infectious doses of biohazards, the amount of leakage can be calculated based on rates from lab accidents in the range of 1,500 microorganisms per cubic foot (ft\(^3\)) of air (Kenny, 1968). These microorganisms were bacteria (Serratia marcescens). However, 0.1\(\mu\)-size viruses expected to diffuse throughout the cabinet work space to equilibrium concentration will be used here. It is also given that the viruses will pass through the leak as easily as the mixture of tracer gas and air molecules do.

Imagine that a Class III BSC user is startled to find that the exhaust system has failed. This results in an accident with enough energy to uniformly disperse 2,000 plaque forming units (PFU) of virus per cubic foot of air (70,000 per cubic meter of air) throughout the interior of a 1 cubic meter size Class III BSC (Calculation A1). This results in 0.07 PFU/cc of air in the cabinet (Calculation A2).

The double door autoclave has been running and automatic dampers in the duct work have failed closed. The heat has raised the pressure in the cabinet to 3” w.g. which results in a total leak rate of \(1 \times 10^{-5}\) cc/s. At this rate the cabinet will leak 0.00252 PFU per hour, or 1 PFU every 400 hours (Calculation A3).

When the leak enters the room, the ventilation system is designed to carry it away before the worker is exposed to it. British Standard 5726 (BSI, 1979) estimates this effect to be a factor of 10, which results in exposure to 1 PFU every 4,000 hours. This is virtually no exposure. Even with several leaks of this size, the worker would have ample
time to leave the lab before being exposed to an infectious dose. With the Class III BSC being depended upon for safety, the user of highly hazardous agents cannot afford to accept anything less.

The Class III Biological Safety Cabinet as a Glovebox

Even though this cabinet is a glovebox, the Class III BSC is a highly specialized unit. It is designed and tested for a specific purpose, and is not interchangeable with just any glovebox. Therefore, the Class III BSC should not be categorized simply in this manner. It should be referred to properly as a Class III Biological Safety Cabinet, thus eliminating the confusion which has caused it to be confused with other types of gloveboxes. This sometimes results in the assumption that the cabinet integrity test from the American Glovebox Society (AGS) (AGS, 2007) is a reasonable test to use for validating Class III BSC performance. This is not so.

The AGS integrity test (AGS, 2007) is a pressure decay test with an acceptance criterion of 0.5% of the box volume leakage per hour at a pressure of -1.5" w.g. Theoretically, no outward leakage would occur under negative pressure. The positive equivalent, however, would amount to 1.38 cc/s. This results in 345 PFU per hour compared to the 1 PFU every 400 hours for the LSM test. For special gloveboxes, the AGS integrity test also has a lower limit of 0.02% of the box volume that results in a leak rate of $5.56 \times 10^{-5}$ cc/s and 14 PFU per hour (Calculation A4).

How many LSM failing leaks can occur in a cabinet that has passed the AGS leak test? The LSM criterion is no total leaks greater than $1 \times 10^{-5}$ cc/s with 100% tracer gas at 3" w.g. pressure in the cabinet. Calculation A5 shows that there can be 125,454 unacceptable LSM leaks in the standard AGS allowable leak and 5,054 in the more stringent AGS leak rate for special gloveboxes. Even the special AGS leak criterion is not equivalent to the LSM leak rate criterion.

The real problem is that the pressure decay test is a measure of overall leakage. It is impossible to know whether the overall leakage is made up of many small leaks, or a few relatively large leaks.

The Class III Biological Safety Cabinet Is Not a Class II Biological Safety Cabinet

Continuing with an approach similar to that above, since the Class III cabinet is a BSC, the NSF/ANSI Standard 49 cabinet integrity test should be able to be used to validate the Class III cabinet. The NSF test is also a decay test. It is performed at 2" w.g. pressure with no more than a 10% drop in pressure allowed over 30 minutes. By just raising the pressure to 3" w.g. and using it as a Class III test, this approach has the same problems as the AGS test. In this scenario, it does not even pass the AGS test. Using the same calculations as in Calculation A5, the NSF/ANSI pressure decay test allowable leak has the equivalent of 1,449,621 LSM allowable leaks in it.

A Practical Use for the Pressure Decay Test

When a Class III BSC is to be cabinet integrity tested, performing a pressure decay test first is ideal to ensure that no leaks will be large enough to create a tracer gas background problem during the LSM test.

Conclusions

The purpose of the Class III Biological Safety Cabinet is to provide a system that prevents operator exposure. Gastight, as validated by the LSM cabinet integrity test, is one of the foundations of no exposure. The Laboratory Safety Monograph is the federally accepted authority in the United States and should be used for testing Class III BSCs. The gastight criterion was established by Fort Detrick and was refined in the LSM. There is only one LSM gastight criterion: $1 \times 10^{-5}$ cc/s with 1% tracer gas under 3" w.g. pressure in the cabinet. The LSM test requirement is $1 \times 10^{-7}$ cc/s with 1% tracer gas under 3" w.g. pressure in the cabinet. Both of these two statements define essentially the same size leak.

The LSM tracer gas cabinet integrity test is a rigorous test designed to maintain high containment of infectious agents. It is an appropriate test to validate the performance of the Class III BSC and ensure no operator exposure. It is a misleading oversimplification to refer to Class III Biological Safety Cabinets as gloveboxes. As such, pressure decay tests are not appropriate for use in validating Class III BSC performance.

Helium is an appropriate alternative to Freon R-12 as a tracer gas for the LSM Class III BSC cabinet integrity test.

Acknowledgments

The authors wish to thank Dan Ghidoni and Eugene Lockhart for their questions and ideas during the early part of this work. *Correspondence should be addressed to David Eagleson at davideagleson@bakerco.com.

References

Appendix I: Mathematics Used

**Calculation A1**
Calculate the number of PFU in the 1 cubic meter ($m^3$) air in the cabinet. There are 35.3 ft$^3$ in 1 m$^3$. Therefore, 35 ft$^3$/m$^3$ × 2000 PFU/ft$^3$ = 70,000 PFU/m$^3$.

**Calculation A2**
Calculate the number of PFU per cubic centimeter (cc) in the leak. There are (100 cm)$^3$ per 1 meter$^3$ = $1\times10^6$ cc/m$^3$. 70,000 ($7\times10^4$) PFU/m$^3$ + $1\times10^6$ cc/m$^3$ = 0.07 PFU per cc of air.

**Calculation A3**

\[1\times10^{-5} \text{ cc/s} \times 3600 \text{ seconds/hour} = 0.036 \text{ cc/hr}\]

0.07 PFU/cc × 0.036 cc/hr = 0.00252 PFU/hr

This is 1 PFU every 400 hours. (1 PFU ÷ 0.0025 PFU/hr = 400 hr).

**Calculation A4**
Given a 1 meter$^3$ box, (100cm)$^3$ = $1\times10^6$ cc/m$^3$ (cc/m$^3$)

Standard glovebox test:

0.5% of the box volume/hr = 0.005 × $1\times10^6$ cc = $5\times10^3$ cc/hr

**Calculation A5**

**Standard glovebox test:**

The number of unacceptable LSM leaks in the allowable AGS leak is:

\[5\times10^3 \text{ cc/hr} ÷ 3600 \text{ s/hr} = 1.38 \text{ cc/s}\]

1.38 cc/s × 3600 s/hr = 4968 cc/hr × 0.07 PFU/cc = 345 PFU/hr

**Special glovebox test:**

0.02% of the box volume/hr = 0.0002 × $1\times10^6$ cc = 200 cc/hr

200 cc/hr ÷ 3600 s/hr = 5.56×10⁻² cc/s

5.56×10⁻² cc/s × 3600 s/hr = 200 cc/hr × 0.07 PFU/cc = 14 PFU/hr

**Microbial Risk Assessment Guideline: Pathogenic Microorganisms with Focus on Food and Water**

Scientists from the Environmental Protection Agency (EPA), U.S. Department of Agriculture’s Food Safety and Inspection Service (FSIS) and scientists from other federal agencies developed the Guideline for Microbial Risk Assessment: Pathogenic Microorganisms with Focus on Food and Water. This document provides a common framework to perform microbial risk assessment (MRA). It lays out a flexible set of approaches, methods, and tools for use to conduct their microbial risk assessment and provide more transparency to the process and results. This guideline addresses issues specific to microbial risk, e.g., secondary transmission, immune status, and growth and die-off of organisms, as well as addressing concepts generic to classical chemical risk assessment. To learn more, visit [www.epa.gov/raf/microbial.htm](http://www.epa.gov/raf/microbial.htm).