



Development of Particle Tracer Techniques to Measure the Effectiveness of High Containment Laboratories

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Abstract

Biosafety Level 3 (BL-3) laboratories are designed to prevent the escape of pathogenic microorganisms by operating at negative pressure so that if microorganisms become airborne, they remain within the laboratory. However, the authors are not aware of any published evidence or international guidance on the level of pressure differential required for BL-3 laboratories. This uncertainty was reflected in a survey of BL-3 laboratories in the United Kingdom where a range of pressure differentials between 30 and 100 Pascals (Pa) were found. In this paper, an attempt is made to address this issue. The authors have developed techniques to quantify the effectiveness of containment laboratories in preventing the egress of airborne microorganisms. A potassium iodide (KI) aerosol tracer method was adapted to measure the degree of containment in an experimental facility and in five working BL-3 laboratories. The level of laboratory containment was expressed as the laboratory protection factor (LPF). Using this technique, it was found that providing an anteroom increased the LPF by approximately one order of magnitude. No direct relationship was found between the magnitude of negative pressure and LPF. There was, however, a direct relationship between inflow velocity and LPF: A volumetric inflow of 10 m³/min into a laboratory through an anteroom gave a LPF of greater than 10⁵. The KI aerosol tracer method offers a simple and appropriate means of validating the performance of BL-3 laboratories in terms of the LPF.

Introduction

Manipulations utilizing category 3 pathogenic microorganisms that may lead to the generation of infectious aerosols must be carried out within primary containment such as microbiological safety cabinets and sealed centrifuge rotors. Secondary containment is also required; this involves protecting the surrounding rooms and environment in the event of the breakdown of the primary containment or after an accidental release. The construction, design, and operation of these facilities are described in a range of national and international guidelines (Advisory Committee on Dangerous Pathogens, 1995; US Department of Health and Human Services, 1999; World Health Organization, 1993). BL-3 laboratories need to be physically separated from the surrounding environment and specific rules govern entry to this facility. These can involve passage through two sets of doors that create an anteroom to accommodate personnel changing from their normal clothing into protective clothing.

BL-3 containment laboratories are designed to prevent the egress of pathogenic microorganisms by keeping the room at a negative pressure with respect to the outside environment and ensuring a high air-change rate. Negative pressure is maintained in containment rooms partially by increasing the air flow extracted from the room and partially by minimizing the leakage of air through gaps between the door and door frame and any vents in the doorleaf. In

addition, the ventilation is designed so as to replace the air at such a rapid rate that any aerosol generated within the room is quickly diluted and removed. The combination of these two factors should allow laboratory personnel to evacuate the laboratory safely after a breach of primary containment and return to the laboratory to deal with the accident after a given time, confident that any generated aerosol has been removed.

Due to the lack of experimental evidence, no information is provided in international and national microbiological containment guidelines on the magnitude of negative pressure required for effective containment of category 3 agents. Also, no documented evidence is available to show the effects of changing the magnitude of negative pressure on containment. Regulations are also vague on whether providing an anteroom between the laboratory and the outside environment is required to enhance the overall containment. This contrasts with the highly detailed national and international standards for the design of primary containment equipment such as centrifuges and microbiological safety cabinets (British Standards Institute (BSI), 1992; International Electrical Commission (IEC), 1993; National Sanitation Foundation (NSF), 1983). This also contrasts with the regulations pertaining to the design of cleanrooms for the manufacture of medical products and electronic components.

As part of this study, an informal survey of BL-3 laboratories in 10 institutions (including universities, research institutes, and pharmaceutical companies) throughout the United Kingdom (UK) showed that a wide range of pressure differentials—between 30 and 100—Pa were routinely used. Marked differences in laboratory design in the public and private sectors were noted. Anterooms were linked to all these laboratories and were used as changing and temporary storage facilities. In university and hospital laboratories, the BL-3 facilities generally comprised an anteroom and laboratory having no independent ventilation system but relying on a ducted Class I microbiological safety cabinet in the main laboratory to create a negative pressure, normally of approximately 30 Pa. In some instances, the practice was to ensure the cabinet was operating for an additional 10 minutes after the day's work was com-

pleted. In contrast, most pharmaceutical and research laboratories in commercial organizations had independent ventilation systems that were continuously monitored by sensors and alarm systems. Microbiological safety cabinets in these facilities were continuously operated 24 hours a day to maintain the desired air balance. In one facility, entry to the laboratory was achieved through a sequence of three anterooms. The total negative pressure gradient in this case was 100 Pa between the laboratory and the corridor.

The use of excessive negative pressures to ventilate containment facilities can create problems. These may include:

1. Increased energy costs due to heating losses
2. Structural damage to wall and ceiling surfaces and sealants
3. Significant quantities of airborne particulate matter that can be drawn in from the surrounding environment

The aim of the present study was to develop test methods to measure the degree of containment of BL-3 laboratories. These techniques may be used to investigate the effects of various design factors on the containment provided by these laboratories. The information gained should prove useful to the laboratory designer.

The study was comprised of two stages:

1. Initial studies were carried out in an experimental laboratory complex where the air flow and differential air pressures could be altered whenever required. This facility was sealable and so could be decontaminated readily after use.
2. Further studies were carried out in operating containment laboratories of various sizes and designs and having a wide range of airflows and pressure differentials.

The effectiveness of open-fronted microbiological safety cabinets is routinely tested in the UK, using monodispersed aerosols of potassium iodide (KI) (BSI, 1992). The KI methodology was originally developed to study movement of particulate contaminants between rooms in burns units in hospitals (Foord & Lidwell, 1972). The effectiveness of open-fronted microbiological safety cabinet performance is measured in terms of the operator protection factor (OPF), which is the ratio of KI particles gener-

ated within the cabinet to those detected outside the cabinet. An OPF of greater than 10^5 is regarded as being adequate (ACDP, 1995). Using the same principle, the OPF can also be determined using aerosolized bacterial spores. These procedures were adapted to assess the performance of the high-containment laboratories in this study.

The effectiveness of the containment of laboratories was assessed in terms of the laboratory protection factor (LPF), which is defined as the ratio of the number of particles generated in the laboratory to the number of particles collected in the area outside the laboratory. This protection factor was measured using airborne particle tracers containing either aerosol-stable microorganisms such as spores of *Bacillus subtilis* var niger (Cox, 1987) or KI.

Since the use of microbial tracers was impractical in working laboratories, only the KI tracer technique was used in this setting. The advantages of this technique over the microbial methods are rapid results and no contamination of the laboratory under test, avoiding any need for subsequent decontamination.

Materials and Methods

Experimental Laboratory

This laboratory was designed to allow volumetric inflows and pressure differentials to be easily adjusted independently of each other. The room complex (Figure 1) consists of three units that simulate

the laboratory, the anteroom, and a corridor. The dimension of the “laboratory” was 3.0 m x 3.1 m x 2.3 m high, the “anteroom” was 3.7 m x 1.7 m x 2.3 m high, and the “corridor” room was 3.0 m x 2.4 m x 2.5 m high. (Note that in the remaining portions of this paper, the environmental laboratory rooms are referred to within quotes to differentiate them from the working laboratory rooms.) The pressure differentials were monitored by calibrated Magnahelic (Michigan City, USA) manometers, and the volumetric inflows were calculated from the air flows measured by a rotating-vane anemometer as detailed below (under calculation of airflow). This “anteroom” and “laboratory” were operated at the following respective combinations of negative pressures—0,0; 15,30; 20,40; and 40,70 Pa—for microbiological tests. In experiments to assess the effect of inflow velocity on room containment using KI, the pressure differentials were held at -30 Pa, -60 Pa, while the volumetric inflow was varied between 6.1 and 14.7 m³min⁻¹. This was achieved by adjusting ventilation grilles incorporated in the laboratory doors.

Test Laboratories

Tests were carried out in five BL-3 laboratories with anterooms (Laboratories A-E) and one BL-2 laboratory without an anteroom (Laboratory F). The characteristics and experimental variables of the

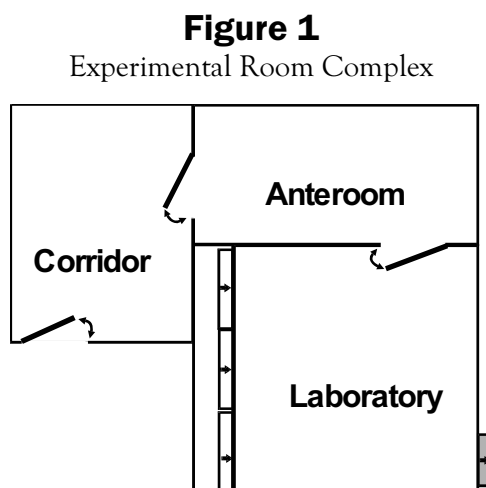


Table 1

BL-3 Laboratories Tested for Containment Using the KI Test Methodology

Laboratory	Anteroom ΔP^+ (Pa)	Laboratory ΔP (Pa)	Laboratory Inflow Through Door ($\text{m}^3 \text{min}^{-1}$)	Laboratory Volume (m^3)	Ach ¹
A	-40	-50	10.8	58.9	11
B	-20	-30	8.2	95.9	8.4
C ²	-25	-50	15.4	97.8	23.3
D	-50	-120	12.7	102.2	19.3
E	-25	-60	2.4	127.6	19.3
F	none	NA	4.9	158.4	5.5

⁺ Pressure differential, ¹ Air changes per hour, ² Ventilated anteroom 32.6 ach, NA—not applicable

laboratories are given in Table 1. The pressure differentials were measured on Magnahelic (Michigan City, USA) manometers. All the laboratory extract and supply air flows were measured using a rotating-vane anemometer (Model AV2, Airflow Developments, High Wycombe, England), and the laboratory inflows and air changes were calculated from these data and the room volumes as detailed below. Each laboratory was tested using the potassium iodide (KI) technique also detailed below.

Potassium Iodide Method-Principle

The potassium iodide (KI) method was developed by Foord and Lidwell (1972) to study the movement of particles in hospitals and was later adapted by Clarke and Goff (1981) to test microbiological safety cabinets *in situ*. In 1992, the method was adopted as the British Standard and is currently used in the UK (BSI, 1992). An integrated piece of equipment using these principles is commercially available for testing microbiological safety cabinets (Containment Technologies, Wimborne, UK). This equipment was adapted for use in this study. The principal to operate the equipment is as follows: A solution of 1.5% (w/v) potassium iodide in 95% (v/v) aqueous ethanol is fed dropwise at a regulated rate to a spinning disc at a fixed speed. Droplets of a defined size are flung off the spinning disc. The number and size of the droplets depend upon the injection flow rate and the speed and diameter of

the disc, respectively. The water and the ethanol instantly evaporate from the droplets formed, providing solid monodispersed particles of average size (7 microns). The aerosol generator is designed to produce 6.2×10^6 KI particles in a 10-minute period.

Air-containing particles that escape from the containment space are sampled by four centripetal membrane filter samplers drawing 100 liters min^{-1} through a 3.0-micron, 25 mm-diameter cellulose nitrate membrane filter (Whatman, Maidstone, England (cat. No: 7188 002)). After each test, the filters are immersed in a solution of 0.1% palladium chloride in 0.1 M hydrochloric acid which reacts with any KI to form brown spots that can be easily identified and counted using a hand lens.

Measurement of Laboratory Protection Factor Using the KI Method

The spinning disc aerosol generator was placed at a height of 0.8 m inside the laboratory and 1 m from the door of the environmental room or containment laboratory. Two of the centripetal sampling heads were placed in the anteroom, 0.3 m from the door and 1 m from the wall at heights of 0.3 m and 0.8 m. The other two sampling heads were placed in the corridor area at the same heights (1 m from the door and 0.8 m from the wall). Experiments were carried out for either 5-minute or 10-minute periods. This technique was also used in the working laboratories.

Measurement of Laboratory Protection Factor Using the Microbial Aerosols

Microbial aerosols were generated within the experimental laboratory using a Collison nebulizer (May, 1973) operating at a pressure of 180 kPa containing a suspension of *Bacillus subtilis* var niger spores in distilled water at a concentration of 3.16×10^7 spores ml⁻¹. The nebulizer was placed 1 m from the laboratory door at a height of 0.8 m from the floor. Two slit samplers constructed in the HPA workshops (BSI, 1992), operating at a flow rate of 25 liters min⁻¹ and containing tryptone soya broth agar (TSBA) plates that were placed in the anteroom and in the "corridor." Experiments were carried out for a period of 10 minutes. The nebulizer was weighed and the spore suspension was assayed before and after the experiment to calculate the number of spores aerosolised.

Microbial Tracer

Spores of *Bacillus subtilis* var niger (ATCC 9372) were used as the challenge microorganism. The spore suspension was prepared as described by Sharp et al. (1989) and washed three times in distilled water and heated to 60°C for 1 hour before using to kill any vegetative forms that were likely to be inactivated by aerosolisation (Sharp et al., 1989). The suspension was assayed by serial dilution and plating out onto TSBA plates (Oxoid, Basingstoke, England). The plates were incubated at 37°C for 18 hours and the distinctive orange colonies were then counted to determine the spore concentration.

Laboratory Test Conditions

The performance of the experimental room complex and the various test laboratories were assessed in three distinct operating modes.

1. With the laboratory doors closed as is the normal situation for a working laboratory
2. When an operator enters the anteroom during the tests and exits after 1 minute
3. When an operator enters the laboratory and exits after 1 minute. This is intended to model a situa-

tion where a laboratory worker enters the laboratory, discovers an accident situation, and leaves after removing his or her contaminated clothing.

In each laboratory, the pressure differentials and the volumetric airflow through the doors were measured and noted.

Calculation of Air Flow

The air flow in each room was measured using the rotating vane anemometer. The volumetric flow (m³ min⁻¹) passing through inlet and extract grilles was determined by multiplying the average of a minimum of four face velocity readings by the area of the grille. The volumetric inflow through the door was then calculated from the difference between the total volumetric outflow and inflow through any supply grilles. As all the rooms tested were sealed, it can be assumed that the bulk of the make up air volume was drawn through the door.

Calculation of the Protection Factor

The laboratory protection factor is a measure of the effectiveness of the laboratory to prevent release of aerosols into the rest of the premises. It is calculated according to the following formula:

$$LPF = P_a/P_s \quad (1)$$

Where,

LPF = laboratory protection factor

P_a = Number of particles aerosolised

P_s = Number of particles sampled outside the laboratory

The KI aerosol generator is designed to consistently generate 6.2×10^5 aerosol particles per minute. The number of spores aerosolised in the experimental room studies was calculated by weighing the Collison nebulizer and its contents before ($M_{o,g}$) and after use ($M_{i,g}$) (i.e., weight loss, assuming a density of 1g ml⁻¹; this allows the volumetric output ($V = M_o/M_i$) to be calculated). Since the concentration of spores in the suspension (C_s) is known, then the aerosol output of the spores (P_s spores) can be calculated:

$$P_s = V \times C_s \quad (2)$$

For the purpose of this study, it was assumed that all airborne particles that escaped from containment were collected by the air samplers. Hence, the number of spores or particles outside the test area represents the number released into the environment. Realistically, this is unlikely despite the fact that the samplers were placed at the same height as the aerosol generator where the highest aerosol concentrations would be found. However, it would be unrealistic to assume perfect mixing in the anteroom and, therefore, normalize the particle release for the room volume. Furthermore, this would be impossible for the corridor measurements. Therefore, the calculated LPF may be slightly higher than the actual LPF.

Results

Microbiological Tests

With the doors of the experimental room complex closed, no airborne spores were detected outside even when there was no pressure differential between the rooms. When an operator entered and exited the experimental room complex, there was a release of aerosol from the “laboratory” into both the “anteroom” and the “corridor” at all pressure differentials (Figure 2). However, no relationship between increasing differential and biological LPF (bLPF) in the “anteroom” or “corridor” could be identified. In all cases, the bLPF in the “corridor” exceeded 1×10^6 . For “anteroom” entry there was a

Figure 2

The effect of magnitude of pressure differential on biological laboratory protection factor during laboratory entry tests in the experimental room complex measured in the anteroom (●) and corridor (○).

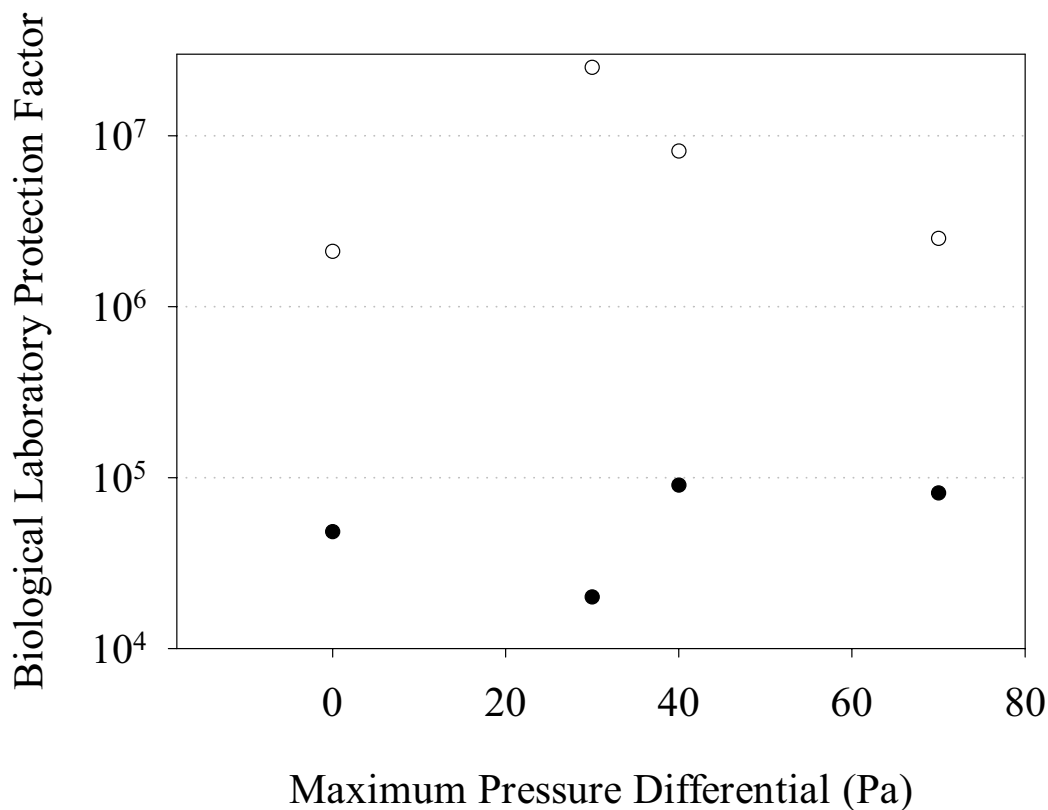


Table 2

Biological Laboratory Protection Factors in Experimental Room for Anteroom Entry

ΔP (Pa)		Laboratory Protection Factor	
Anteroom	Corridor	Anteroom	Corridor
0	0	3.4×10^5	1.0×10^7
-20	-40	2.4×10^7	$> 2.0 \times 10^9$
-40	-70	8.0×10^6	3.2×10^{10}

Table 3

Laboratory Protection Factor for Operating Laboratories

Laboratory	Laboratory Protection Factor		
	Doors Closed (Corridor)	Lab Entry and Exit (Anteroom)	Lab Entry and Exit (Corridor)
A	$> 3.1 \times 10^6$	$< 4.4 \times 10^2$	6.1×10^4
B	1.6×10^6	$< 4.4 \times 10^2$	1.5×10^4
C	1.6×10^6	$< 4.4 \times 10^2$	9.6×10^4
D	$> 3.1 \times 10^6$	$< 4.4 \times 10^2$	5.8×10^4
E	NA	$< 4.4 \times 10^2$	7.6×10^3
F (BL2)	$> 3.1 \times 10^6$	NA	1.5×10^3

NA—not applicable

lower bLPF at static pressure than when a pressure differential was applied (Table 2).

KI Tests in Working Laboratories

The KI tests were carried out in the working laboratories (described in Table 1) for static and for laboratory-entry tests. The results are shown in tabulated form in Table 3 and plotted as LPF vs. maximum pressure differential in Figure 3. There was minimal or no release of material from laboratories when the doors were left closed, even from the BL-2 laboratory without an anteroom. The LPF measured in the corridor during the laboratory entry test varied from 7.6×10^3 to 9.6×10^4 for the BL-3 laboratories (1.5×10^3 for the BL-2 laboratory). Figure 3 shows that the LPF was not dependent on the magnitude of pressure differential.

Table 4 lists the LPFs for anteroom entry tests for the BL-3 laboratories. Again, there was no direct

relationship between magnitude of pressure differential and LPF.

Effect of Volumetric Inflow

The KI test method was also used to measure the effect of altering the laboratory inflow rate in the experimental room complex. The pressure differential was kept constant at -30 Pa in the “anteroom” and -60 Pa in the “laboratory” while the inflow was altered. The results of these tests for “laboratory” entry are shown in Figure 4. The results of the operating laboratory studies are also shown in this figure. The results of the anteroom entry tests are shown in Figure 5 combined in the same way. Each of these graphs shows a direct relationship between volumetric inflow and LPF. The results obtained in the working laboratories and the experimental room are similar.

Figure 3

The effect of magnitude of pressure differential on laboratory protection factor during laboratory entry tests in the BL-3 laboratories.

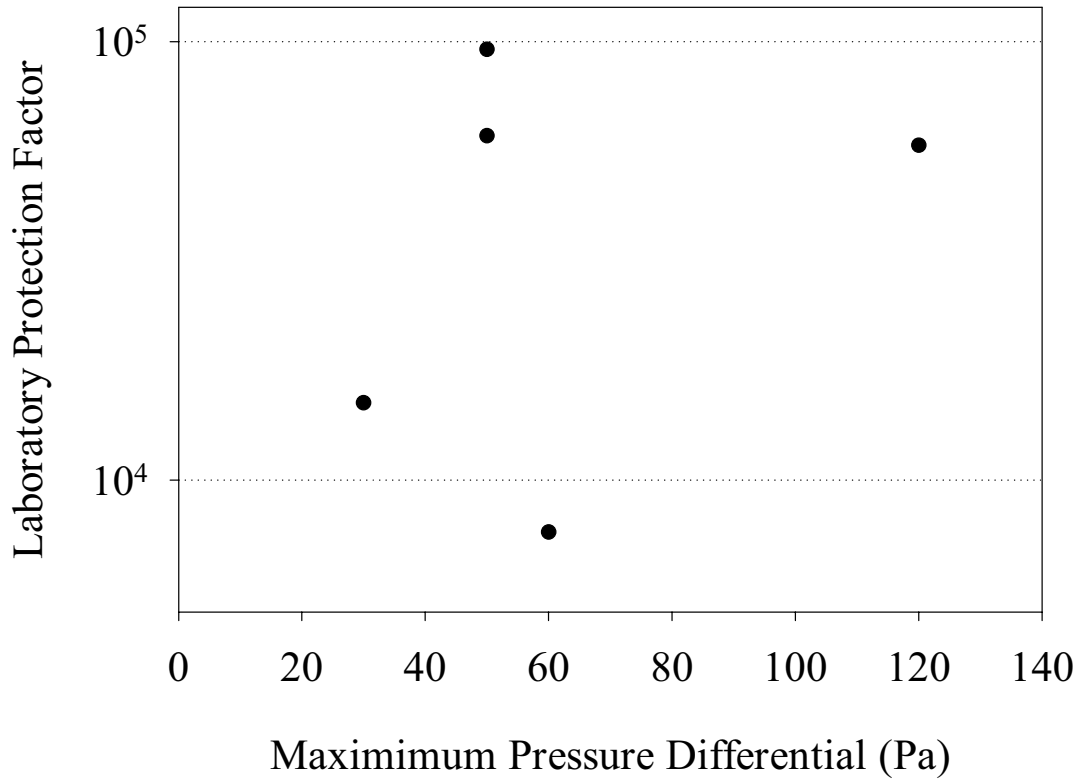


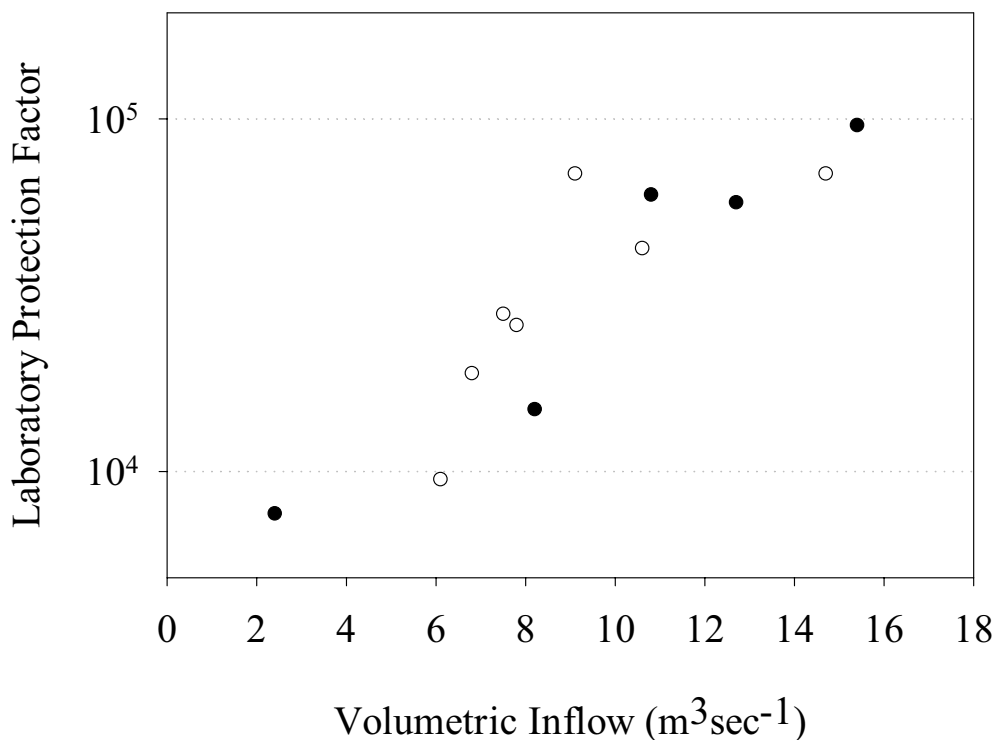
Table 4

Laboratory Protection Factors in Operating BL-3 Laboratories for Anteroom Entry

Laboratory	Laboratory Protection Factor	
	Anteroom	Corridor
A	9.1×10^4	$>3.1 \times 10^6$
B	3.6×10^4	1.0×10^6
C	9.1×10^4	5.2×10^6
D	7.8×10^5	2.1×10^6
E	$< 2.4 \times 10^3$	1.0×10^5

Figure 4

The effect of volumetric inflow on laboratory protection factors during laboratory entry tests in the BL-3 laboratories (•) and the environmental room complex (o)



Discussion

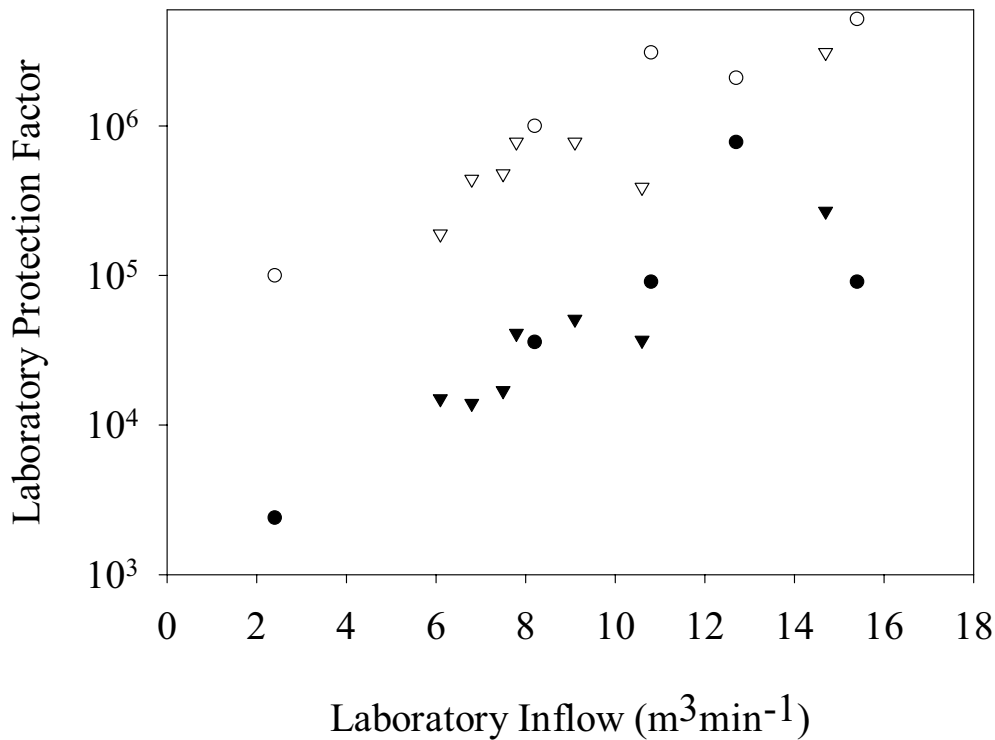
This study demonstrates that the KI method can be usefully applied to quantify the secondary containment provided by negative-pressure laboratories. This technique has been shown to be much more sensitive than the microbiological method described in the experimental room complex. The increased sensitivity is caused by at least two factors. First, the high sampling rate of the centripetal samplers allows more of the released aerosol to be collected. Second, the higher effective particle size of the KI particles (7 microns) makes them more penetrative against directional air flows than microbial spores (ca1 micron) due to their greater inertia. The KI method is also more sensitive and representative of microbial aerosol particles than various gas tracer methods that

have been used to measure air transfer from rooms (Keene & Sansone, 1984). Thus, the KI test method may be considered a useful technique for commissioning and regular validation of containment laboratories.

The significant feature of this study is the finding that the LPF, a measure of containment, is dependent on the laboratory inflow and not the magnitude of the pressure differential. Since negative-pressure laboratories are normally designed on the basis of magnitude of pressure differential, this finding has major implications for containment-laboratory designers. This result can be explained by analyzing the effects produced when the doors of negative-pressure laboratories are opened. At negative pressure, or even at equal pressure, particles will not leak from the room through door gaps when the

Figure 5

The effect of volumetric inflow on laboratory protection factors during anteroom entry tests in the BL-3 laboratories (circles) and environmental room complex (triangles) measured in the anteroom (closed symbols) and corridor (open symbols).



pressure differential is static (i.e., when the door is closed). Release of particles is possible only when a door is opened or when the room pressure becomes positive as a result of ventilation malfunction. The latter may be identified by sensors linked to alarmed manometers. However, if an accident occurred resulting in the release of an aerosol, the staff involved are likely to leave the laboratory immediately. When the occupants leave through the laboratory door, the pressure differential will be reduced and will eventually become zero. In fact, in some cases the room pressure may momentarily become positive. During the evacuation period, some airborne material may be carried in eddy currents created by the departing occupants. Without differential pressure this airborne material can be prevented from escaping from the laboratory only by providing sufficient air flow

into the room through the open door.

The effect of opening the laboratory doors when the occupants leave can be demonstrated by calculating the velocity through the open doors of the individual laboratories from the laboratory inflow data in Table 1. The inflow velocity through the laboratory door (area 1.9 m^2) of laboratory E, the laboratory with the lowest volumetric inflow, was only 0.02 m s^{-1} . In laboratory C, with the highest volumetric inflow, the inflow velocity was 0.14 m s^{-1} . All these inflow velocities are less than those required for Class 1 microbiological safety cabinets ($0.7\text{--}1.0 \text{ m s}^{-1}$). However, in safety cabinets these inflows are created over the face of the cabinet before, during, and after the entire working period. In the case of the containment laboratory, the doors will normally be open only for a very small portion of the working period,

albeit sometimes subsequent to accidents. Therefore, these smaller velocities may be just as effective as shown by the experimental results.

When an operator entered the anteroom from the corridor, a release of aerosol from the laboratory into the anteroom and a slight release of aerosol into the corridor occurred (Table 4). The likely mechanism is as follows: When the outside door was opened, there was a transient flow of air out of the laboratory caused by the pressure fluctuation that could be observed from the laboratory manometer. This would cause some of the airborne material to enter the anteroom and subsequently the corridor when the person left.

Containment laboratory ventilation design has traditionally been based on maintaining negative pressures. The pressure differential between a laboratory and its surroundings can be increased in two main ways. The first is to increase the amount of air being drawn into the room. This is the more expensive method and can cause structural problems and contamination problems by drawing dirty ambient air into the laboratory. The second and less expensive method to increase negative pressure requires a lower exhaust flow rate in association with tightly sealing the laboratory door. This method is adequate as long as the laboratory door is closed. However, when the door is opened the pressure differential is rapidly lost and particles released within the laboratory cannot be prevented from escaping, along with air flow going outward through the door. Therefore, the main design criterion to prevent release of material from a laboratory should be the inward air flow. Pressure differential indicators would only have a secondary purpose as a monitor of the correct performance of the laboratory since the magnitude of the pressure differential should be proportional to the laboratory in a fixed system.

These studies also highlight the benefit of including an anteroom in the laboratory suite as shown in Table 3. All the BL-3 laboratories had higher LPF than the BL-2 laboratory F without an anteroom. This was demonstrated even in laboratory E which had a lower inflow than laboratory F. The overall LPF is increased by approximately 10-fold when an anteroom is incorporated into the facility.

In many BL-3 laboratories, negative pressure in

the laboratory is achieved by using Class I microbiological safety cabinets. Assuming that the volumetric throughput into standard Class I cabinets is between 0.7 and 1 m s⁻¹ through a front aperture of approximately 0.2 m x 1.2 m (BSI, 1992; NSF, 1983), the flow through the laboratory door would be 10.1 to 14.4 m³ min⁻¹. This is within the higher range for the laboratories at the Health Protection Agency, Porton Down and therefore should allow the same degree of protection as long as all air is channelled through the laboratory door and there are no other major laboratory air supply ducts.

The UK design guidelines published for operating theatres are very relevant to the needs of laboratory design since they also have a requirement for secure directional air flow rooms (NHS Estates, 1994). In this standard, door sizes, door crack width, air velocity between rooms, and resultant pressure differentials are all defined. This allows the designer to build operating theatres and validate them to an accepted standard. This type of approach can be and should be used for containment laboratories to ensure adequate containment without entailing unnecessary extra costs. The following design guidelines are suggested from the results of this study.

- Anterooms should be provided for all BL-3 laboratories.
- Laboratories should be designed to have a volumetric inflow through a standard door of about 10 m³ min⁻¹ and have a fixed alarmed negative pressure of approximately 30 Pa.

It is important that this type of information is provided by the national and international standards bodies in order to enable architects, safety advisers, and laboratory users to ensure that high category laboratories are constructed with an adequate level of containment.

The LPF can also be used in an assessment of the risks of release of airborne microbial aerosols from laboratories after escaping primary containment. The LPF is a measure of the comparative risk of material release from a containment laboratory. The LPF can be used in conjunction with the “spray factor” concept originally reported by Dimmick (1973) to carry out risk assessments. The spray factor is the proportion of a suspension aerosolised in an accident. This is well illustrated by the following ex-

ample: If a flask is dropped, it is estimated that 1 microorganism in 10^6 may be aerosolised (ACDP, 2001). In this case the spray factor is 10^6 . When a similar flask containing 50 mls of a 10^{10} per ml suspension of a pathogen is dropped, then 5×10^5 microorganisms will be aerosolised ($50 \times 10^{10} \times 10^{-6}$). If the LPF is 1×10^4 , then 50 microorganisms will be released into the corridor. If the LPF is higher (i.e., 1×10^5), the release will only be 5 microorganisms.

The use of quantitative risk assessment for the design and operation of microbiology containment laboratories, as demonstrated in this paper, should lead to a rational basis for biocontainment that does not entail excessive cost for the operators.

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References

- Advisory Committee on Dangerous Pathogens. (1995). *Categorisation of biological agents according to hazard and categories of containment* (4th ed.). London: HSE Books.
- Advisory Committee on Dangerous Pathogens. (2002). *The management, design and operation of microbiological containment laboratories*. London: HSE Books.
- British Standards Institute. (1992). *British standard 5726:1992 microbiological safety cabinets*. Milton Keynes: BSI.
- Clark, R. P., & Goff, M. R. (1981). The potassium iodide method for determining protection factors in open-fronted microbiological safety cabinets. *Journal of Applied Bacteriology*, 51, 439-460.
- Cox, C. (1987). *The aerobiological pathway of microorganisms*. Chichester, England: John Wiley and Sons.
- Dimmick, R. L. (1973). Laboratory hazards from accidentally produced airborne microbes. *Developments in Industrial Microbiology*, 15, 44-47.
- Foord, N., & Lidwell, O. M. (1972). The control by ventilation of airborne bacterial transfer between hospital patients, and its assessment by means of a particle tracer. I. An airborne-particle tracer for cross-infection studies. *Journal of Hygiene*, 70, 279-287.
- International Electrical Commission. (1993). *IEC 1010-2-020. Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2. Particular requirements. Section 2.20 Specification for laboratory centrifuges*.
- Keene, J. H., & Sansone, E. B. (1984). Airborne transfer of contaminants in ventilated spaces. *Laboratory Animal Science*, 34, 453-457.
- May, K. R. (1973). The Collison nebuliser: Description, performance and application. *Aerosol Science*, 4, 235-243.
- National Sanitary Foundation. (1983). *Standard 49. Class II (laminar flow) biohazard cabinetry*. Ann Arbor, Michigan: Author.
- NHS Estates. (1994). *Health technical memorandum. 2025b. Ventilation in healthcare premises—Design considerations*. London: HMSO.
- Sharp, R., Scawen, M., & Atkinson, A. (1989). Fermentation and downstream processing of *Bacillus*. In C. Harwood (Ed.), *Bacillus*. (pp. 255-292). New York: Plenum Publishing Company.
- U.S. Department of Health and Human Services. (1999). *Biosafety in microbiological and biomedical laboratories* (4th ed.). Washington, DC: U.S. Government Printing Office.
- World Health Organization. (1993). *Laboratory biosafety manual* (2nd ed.). Geneva: Author.