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The Microbiological Validation of a New Containment Level 4 Cabinet Line

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Abstract

The aim of this study was to quantify microbiologically the operator protection factor (OPF) and measure the extent of cross-contamination provided by a new Advisory Committee on Dangerous Pathogens (ACDP) containment level 4 cabinet line system (glove box line). To accomplish this goal, the cabinet line was filled with microbial tracer aerosol, and microbial air samplers were used to detect any release of microbial tracer, both inside and outside the cabinet line when procedures were carried out. These procedures mimicked normal working conditions and realistic accident scenarios. The operator protection factors (OPFs) and internal cross-contamination ratios were calculated.

The cabinet line gave OPFs of between $>10^6$ and $>10^7$ in all tests. No cross-contamination was detected when internal doors remained closed between the individual cabinets and the spine. However, low levels of

microbial tracer were detected moving from the contaminated cabinet to the spine but not to other individual cabinets when cabinet doors were opened.

These experiments demonstrated that the cabinet line provides a high level of protection for the operator and that multi-agents can be manipulated simultaneously within the cabinet line without cross-contamination. In addition, data have been produced, within a large primary containment system, from which informed risk assessments and procedures can be written. Such data provide a basis with which to compare primary containment with alternative ways of working with dangerous pathogens, such as positive pressure-suited systems.

Keywords

Containment, level 4, biosafety, microbiological safety cabinet, protection

Introduction

A new microbiological containment building has been built at the Defence Science and Technology Laboratories (Dstl), Porton Down, which houses 12 Advisory Committee on Dangerous Pathogens (ACDP) containment level 3 laboratories (CL3), 8 CL3 animal laboratories, 2 containment level 4 laboratories (CL4), and 2 CL4 animal laboratories. The CL4 laboratories are configured such that work can be undertaken in either positive pressure-suited or primary containment (cabinet line) mode. ACDP CL4 is the United Kingdom equivalent to Biosafety Level 4 (BSL4) in the United States.

A new cabinet line was installed and commissioned within a CL4 laboratory (Figures 1-4). This cabinet line includes an L-shaped spine linking a dunk tank and autoclave (at opposite ends). Eight Class 3 microbiological safety cabinets (MSC) are attached to the spine. A refrigerator and two incubators are also integrated into the spine. Materials can be introduced and removed through a dunk tank containing an appropriate disinfectant at one end of the spine, and the cabinets are designed to be independently disinfected using formaldehyde. The cabinet line terminates in an interlocked, double-ended autoclave for the safe removal of waste. The spine of the cabinet line extends to and incorporates the area enclosing the toxic side of the autoclave.

Air is routed independently through both spine and MSCs and exhausted into an extract manifold running the length of the spine. Fans mounted on the extract manifold then exhaust the air (via eight thimble exhaust systems) out of the CL4 laboratory.

Tests on the cabinet line were performed using standard physical testing methodologies including a leak tightness pressure hold test (BS 5726-4:1992) and a Dispersed Oil Particulate (DOP) positive pressure scan test (BS 5726:1979, Appendix C.2). Before the first use of this facility, microbiological aerosols were used to challenge the system with a range of realistic procedures and accident scenarios, using *Bacillus atrophaeus* NCTC 10073 (formerly *Bacillus subtilis var niger*) spores. These tests assessed the degree of operator protection afforded by the cabinet line and measured the extent of potential cross-contamination within the system.

Materials and Methods

Microbial Aerosol Generation

A spore suspension (3×10^9 cfu/mL) of aerostable *B. atrophaeus* was used as the microbial tracer. Collison nebulisers (May, 1973) were used for aerosol generation within the system.

Microbial Air Sampling

- (i) Cyclone Sampler—Cyclone samplers, operating at 650 L min^{-1} , using sterile distilled water as a collecting

fluid, were used to measure the microbial aerosol concentration within the room and the cabinet line (Bennett & Parks, 2006; Decker et al., 1969).

- (ii) Casella Slit Sampler—Low-volume Casella slit samplers (Casella, London), operating at 30 L min^{-1} , containing Tryptone Soya Broth agar (TSBA) plates were used to measure the microbial aerosol concentration within the room and the cabinet line.
- (iii) All Glass Impingers—All glass impingers (May, 1957), operating at 11.5 L min^{-1} , containing 10 mL sterile distilled water as the collecting fluid, were used to measure the microbial aerosol concentration within the room and the cabinet line.
- (iv) Microbial Analysis—The collection fluid from the cyclone and the AGI samplers was diluted and plated out onto TSBA plates. All the TSBA plates were incubated for 24 hours at $37^\circ\text{C} (\pm 2^\circ\text{C})$ before being counted.

Tests were divided into Operator Protection tests and Cross-contamination tests (within the cabinet line).

Operator Protection Tests

Tests were carried out to measure operator protection factors (OPFs) under normal procedures and under accident scenarios.

During each test the nebuliser was run to generate a microbial aerosol within the test cabinet. An AGI sampler situated nearby was used to measure the challenge concentration. The Cyclone and Casella slit samplers were operated near to operator positions to determine any release and potential exposure to the operators.

The challenge level was determined by measuring the weight loss from the Collison nebuliser (as aerosol challenge would be rapidly removed by the high air change rate within the cabinets) and related to the measured challenge level sampled under static conditions within the cabinet.

The results of these tests have been expressed as OPF calculated as follows:

$$\text{OPF} = \frac{\text{Aerosol conc. within cabinet (cfu.m}^{-3}\text{) measured by AGI}}{\text{Aerosol conc. outside (cfu.m}^{-3}\text{) measured by Cyclone or Casella}}$$

An OPF of greater than 10^5 for all tests was regarded as an acceptable performance (BSEN12469:2000).

MSC-2 was used for tests 1-10 and MSC-8 was used for tests 11-16. A summary of the tests is shown in Table 1 and the cabinet positions are shown in Figure 1.

A number of background samples were taken during the tests without the nebuliser running to determine the ambient levels of tracer.

Cross-contamination Tests

Tests were performed to measure the transfer of aerosols within the cabinet line system, both from the cabinets to the main spine and any possible contamination from one cabinet to another along the spine. Various procedures were undertaken whilst the system was operating normally. The Collison nebuliser was used to generate

microbial aerosols within MSC-6 (measured using an AGI as before). Air samplers (AGI, Casella) were then operated within the spine and other cabinets to detect any cross-contamination during various procedures detailed in Table 2 and air sampler configurations shown in Figure 1.

Sampler Configuration for Cross-contamination Tests

Configuration One

Nebuliser and challenge AGI within MSC-6
 AGI 1 within spine, between MSC-4 and MSC-6
 AGI 2 within spine, next to pass-through door to MSC-2
 Casella sampler within MSC-4

Configuration Two

Nebuliser and challenge AGI within MSC-6
 AGI 1 within spine, between MSC-4 and MSC-6
 AGI 2 within spine, next to pass-through door to MSC-2
 Casella sampler within MSC-2

Configuration Three

Nebuliser and challenge AGI within MSC-6
 AGI 1 within spine, next to the pass-through door to MSC-4
 AGI 2 within MSC-4
 Casella sampler within MSC-4

Results

Operator Protection Factors (OPFs)

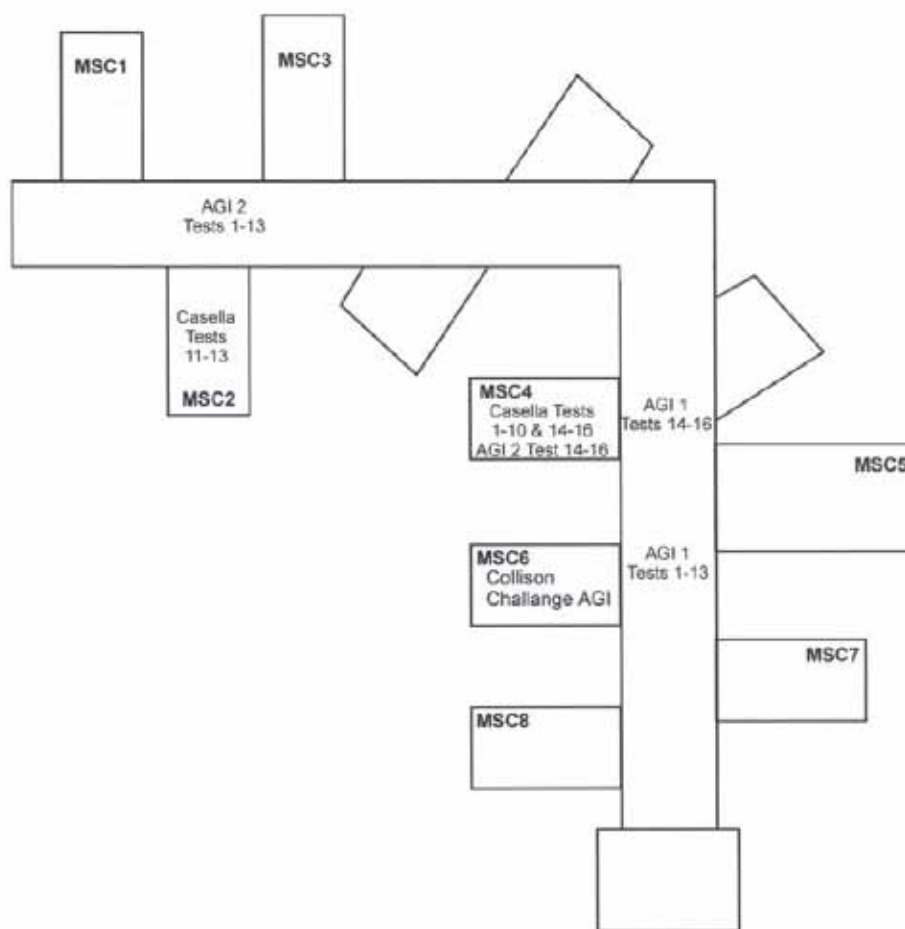
The cabinet line gave an OPF of between $>10^6$ and $>10^7$ in all tests, including glove removal and loss of ventilation which was significantly higher than the minimum required level of 10^5 . The OPFs for each of the OPF tests are listed in Table 1. The range of values is quoted in the table—rather than mean and standard deviation values—as in some tests. Recovery of bacteria was below the detection limit of the test.

Cross-contamination Tests

No microbial aerosol was released from MSC-6 when the door between the cabinet and the spine was kept closed (tests 1, 3, 4, and 10).

Figure 1

Schematic diagram of cabinet line and sampler positions for cross-contamination tests.



When the door to MSC-6 was opened, approximately 6% of the microbial aerosol was detected in the spine compared to the concentration in MSC-6, and a lower concentration was detected at the far end of the spine. No aerosol was detected in the adjacent cabinet, MSC-4 (tests 5, 6, and 7).

When the door to MSC-6 was left open, between 6% and 26% of the microbial aerosol compared to that in MSC-6 was detected in the spine. A lower concentration was detected in the far end of the spine (0.8-4.7% of

MSC-6). No aerosol was detected in the adjacent cabinet, MSC-4 (tests 2, 8, and 9).

When transfer of material from one cabinet to another was mimicked, approximately 15%-20% of the microbial aerosol compared to that in MSC-6 was detected. A lower concentration was detected in the far end of the spine (0.3% of MSC-6). Aerosol was transferred to another cabinet at the other end of the spine (MSC-2) when the door to the cabinet was opened (tests 11, 12, and 13).

When transfer from one cabinet to another was mim-

Table 1

Operator Protection factors for various procedures.

Test No.	Test Details	Challenge Level cfu/m ³	Range of Recovery, Casella, cfu/m ³	Range of OPF from Casella	Range of Recovery, Cyclone, cfu/m ³	Range of OPF from Cyclone
BG		N.A.	<6.7 - 13.3	N.A.	2.0 - 4.54	N.A.
1 - 3	Normal running conditions with manipulations of gloves	2.49x10 ⁸	<6.7 - 6.7	3.73x10 ⁷ - >3.73x10 ⁷	1.85 - 2.85	8.76x10 ⁷ - 1.35x10 ⁸
4 - 6	Normal running conditions, safe change of two gloves	2.36x10 ⁸	13.3 - 30	1.78x10 ⁷ - 7.87x10 ⁷	1.85 - 16.2	1.46x10 ⁷ - 1.28x10 ⁸
7 - 9	Normal running conditions, glove 'o' ring removed, glove forced off collar into cabinet	2.6x10 ⁸	13.3 - 53.3	4.89x10 ⁶ - 1.96x10 ⁷	4.55 - 16.95	1.54x10 ⁷ - 5.73x10 ⁷
10	Cabinet line turned off, exhaust dampers open, manipulation within cabinet, pressure monitored to ensure cabinet went positive (peaks of >25Pa).	2.83x10 ⁸	<6.7 (ND)	>4.23x10 ⁷	46.15	6.14x10 ⁶
11 - 13	All power turned off, exhaust dampers open, manipulation within the cabinet, pressure monitored to ensure cabinet went positive (peaks of >25Pa).	2.76x10 ⁸	<6.7 - 26.6	1.04x10 ⁷ - >4.12x10 ⁷	3.26 - 17.93	1.54x10 ⁷ - 7.74x10 ⁷
14 - 16	Cabinets turned off, exhaust dampers closed, manipulation within cabinet, pressure monitored to ensure cabinet went positive (peaks of >25Pa).	2.57x10 ⁸	<6.7(ND) - 6.7	3.84x10 ⁷ - >3.84x10 ⁷	2.54 - 3.69	6.97x10 ⁷ - 1.01x10 ⁸

BG = Background sample; N.D. = Not Detected

Table 2

Summary of cross-contamination tests.

Test No.	Sampler Configuration	Procedure Details	MSC6 Cabinet AGI 1 (range cfu/m ³)	Spine AGI 1 (range cfu/m ³)	Spine AGI 2 (range cfu/m ³)	MSC4 Casella (range cfu/m ³)
1,3,4,10	One	Static test, all samplers run, no doors opened	2.4 – 7.7x10 ⁷	ND	ND	ND
5,6,7	One	MSC6 internal door opened material moved in/out for 1 minute	3.7 – 4.2x10 ⁷	2.0 – 2.4x10 ⁶	3.8 – 7.7x10 ⁴	ND
2,8,9	One	MSC6 internal door left open material moved in/out for 1 minute	1.6 – 6.7x10 ⁷	1.1x10 ⁶ – 1.2x10 ⁷	4.1 x10 ⁵ – 1.7x10 ⁶	ND
11,12,13	Two	MSC6 internal door opened for 2 minutes, then shut. After 4 minutes MSC2 internal door opened material moved in/out for 1 minute.	4.5 – 5.0x10 ⁷	6.8x10 ⁶ – 1.2x10 ⁷	1.6 – 1.9x10 ⁵	1.5 – 2.2x10 ²
14,15,16	Three	MSC6 internal door opened for 1 minute. After 3 minutes MSC4 internal door opened material moved in/out for 1 minute	1.5 – 3.0x10 ⁷	2.5x10 ⁵ – 1.2x10 ⁶	ND	ND – 2.0x10 ¹

N.D. = Not Detected

Figure 2

Cabinet line dunk tank

**Figure 3**

Cabinet line and MSCs 2, 4, and 6



Figure 4
Microscope MSC



icked, about 15%-20% of the microbial aerosol compared to that in MSC-6 was detected. A lower concentration was detected in the far end of the spine (0.3% of MSC-6). Aerosol was transferred to another adjacent cabinet (MSC-4) when the door to the cabinet was opened (tests 14, 15, and 16).

The cross-contamination test results are summarised in Table 2.

During all of the above tests, the cabinets were running normally; hence, as the aerosol was generated within MSC-6, much of it is removed by the high air change rate within the system. One final test was run (test 17) within MSC-6, without the cabinet ventilation running to determine the true challenge level, which was found to be 1.4×10^8 cfu/m³.

Discussion

The cabinet line was designed to provide a system that enables diagnostic and research work to be carried out safely and efficiently with ACDP Hazard Group 4 microorganisms, such as the filoviruses Ebola and Marburg. The operator protection tests (OPFs) demonstrated that an exceptionally high level of protection was provided by the cabinet line system under normal working conditions and under a range of realistic accident scenarios. The tests demonstrated that the OPF was significantly higher than the minimum required OPF of 10^5 during scenarios where the containment barrier was breached, such as when a glove was removed and forced into the cabinet. During normal operation the cabinet line was designed to operate with an air flow of approximately 300 air changes an hour and, therefore, the high protection factors achieved during these tests were not surprising as microbial aerosols would be removed efficiently. During

the most stringent tests performed, where there was a total loss of ventilation and manipulations were undertaken within the cabinet creating a positive pressure, high operator protection factors were still achieved, giving a high degree of operator confidence.

All Hazard Group 4 microorganisms are viral in origin not bacterial. However, it was decided to use a bacterial tracer spore as the OPF tests undertaken in this study were designed to measure the protection afforded by the cabinet line system from a monodispersed, small-particle (approximately 1 μ m-3 μ m) aerosol challenge regardless of the agent used. Published aerosol infective doses for Ebola Zaire in rhesus macaques are as low as 400 plaque-forming units (pfu) (Johnson et al., 1995); Marburg virus has been shown to be lethal by the aerosol route in rhesus macaques (Lub et al., 1995) and African green monkeys (Bazhutin, 1992). It was shown that 1 pfu was approximately equivalent to 25-30 virions (Bray et al., 1998); therefore, the infectious dose for rhesus macaques could be between 10,000 to 12,000 virions. Filovirus-infected non-human primates represent an appropriate animal model for predicting human infectious doses of filoviruses (Geisbert et al., 2004); therefore, the aerosol-infectious dose for humans may be reasonably assumed to be similar. In a monodispersed aerosol (size range 1 μ m-3 μ m) of bacterial spores generated by a Collision nebuliser, each particle would usually contain one bacterium. A suspension of virus at an equivalent starting concentration would generate particles each containing a number of virions (up to 10-fold more, based on average filovirus dimensions of approximately 80nm x 800nm). Therefore, based on these theoretical calculations, an aerosol challenge of approximately 1.0×10^9 virions released from the cabinet line under the worst case scenario (test 9 - OPF of 4.89×10^6) would release approximately 200 virions into

the environment. This is equivalent to approximately 4-8 pfu. Therefore, even in the worst case scenario, this would be approximately 100 times less than the theoretical infectious dose for rhesus macaques (and by extrapolation humans).

Many laboratory-acquired infections have been reported in the scientific literature (Collins & Kennedy, 1998; Pike, 1979). Historically, procedures such as centrifugation or needle-stick injuries are the main cause of laboratory-acquired infections (Sewell, 1995). Procedures carried out within the cabinet line would not produce microbial aerosols in the concentrations described in this study. A centrifuge rotor leak, for example, can generate microbial aerosols with concentrations of 2.30×10^4 cfu/m³, and dropping a large bottle results in 1.37×10^4 cfu/m³ (Bennett & Parks, 2006). The data presented here, however, provide a level of confidence within which to base emergency accident procedures and safe working practices.

The basis of the cross-contamination tests was to establish a set of working procedures within the cabinet line upon which cross-contamination would not occur. The spine of the cabinet line is at a greater negative pressure than the individual cabinets. This is designed such that contamination within the spine is prevented from entering the cabinets. However, it presents the possibility that aerosol contamination could enter the spine from the cabinets. The microbiological tests presented here demonstrated the importance of allowing time for individual cabinets to vent, to remove any aerosols, before the internal pass-through doors are opened. If an aerosol was generated in the spine as a result of a spill or accident, individual cabinets would not be contaminated unless the internal doors were opened. Tests have shown that it would be possible to work on two separate microorganisms within two separate microbiological safety cabinets within the cabinet line, and that provided internal doors remained shut or cabinets were allowed to vent before the doors were opened, no cross-contamination would occur between cabinets.

In summary, the cabinet line system provides exceptionally good operator protection, even under accident scenarios, with the flexibility to work on more than one viral agent without cross-contamination of work.

Editors' Note

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