

Viral Penetration of High Efficiency Particulate Air Filter of Biological Safety Cabinet in Microbiological Laboratory

YU Long¹, DUAN Wei², WANG XiaoDong¹, WEN ZhanBo³, LI Na³ and LI JinSong^{3*}

¹Center for Disease Control and Prevention of Navy, Navy General Hospital of PLA, Beijing, 101113, China

²Institution of Endemic Control, Beijing Center for Disease Control and Prevention, Beijing, 100013, China

³Department of Biosafety, State key laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, 100071, China

Abstract

Objective: Biological Safety Cabinets are the primary containment device used to protect the worker, environment and product from exposure to infectious agents within the laboratory. Spores of *Bacillus subtilis* are used as tracer agents to test Class II biological safety cabinets in NSF/ANSI Standard 49 and others. However, none of these standards characterizes the viral challenge tests.

Methods: One model bacterium and two different phages were selected to challenge two new Class II biological safety cabinets strictly conforming to the NSF/ANSI Standard 49 requirements.

Results: The two tested biological safety cabinets met the requirements of personnel, product, and cross-contamination protection test no matter which agent was used to challenge the system. However, the high efficiency particulate air filter leak testing results indicated that viral aerosol might penetrate through filter while bacterial could not.

Conclusions: The penetration ability of viral aerosol through HEPA filter might be superior to bacterial. This viral testing method might be a potential way used for the exhaust HEPA filter leak certification due to being able to reflect the containment performance of biological safety cabinet truly and intuitively.

Keywords: Biological safety cabinet; Biological tests; High efficiency particulate air filters; Biological aerosol; Viral penetration

Introduction

The clinical microbiology laboratory takes a critical role in infection control and health care epidemiology [1,2] which contributes to preventing transmission of infectious diseases in health care settings by promptly detecting and reporting epidemiologically important organisms, identifying emerging patterns of antimicrobial resistance, and assessing the effectiveness of recommended precautions to limit transmission during outbreaks [2]. As concerns about emerging pathogens and bioterrorism grow, the role of the clinical microbiology laboratory assumes ever-greater importance [3]. Laboratory workers who encounter or handle cultures of these organisms such as *Mycobacterium tuberculosis*, *Bacillus anthracis*, SARS virus, influenza virus and Hantavirus etc are at high risk for disease if exposed. It may be assumed that most laboratory infections which do not result from poor technique or accidents are generally connected with inhalation of infectious material [4]. The early reviews recognized that the primary route of transmission of many of the causative agents was by aerosol, and they led to the development of laminar-flow biological safety cabinets [5,6].

Biological safety cabinets are the primary containment device used to protect the worker, environment and product from exposure to infectious agents within the laboratory. There are three classes of biological safety cabinets, designated as Class I, Class II, and Class III [7]. Class II biological safety cabinets are the most widely used and accepted. A properly functioning cabinet is essential for working with infectious agents. NSF/ANSI Standard 49, EN12469:2000 Standard and China's YY0569-2005 Standard define the tests that all Class II biological safety cabinets must comply with to be certified. These standards require personnel, product and cross-contamination protection tests challenged by *Bacillus subtilis* var. *niger* (*B. subtilis*) spores. These tests determine if aerosols will be contained within the cabinet, outside contaminants will not enter the cabinet work area,

and aerosol contamination of other equipment in the cabinet will be minimized [8-10]. However, spore cultivation is a time-consuming task. The colony colour is too mutable to be distinguished and counted. And spores are hard to decontaminate bringing inconvenience to the operators according to our actual experiences and related researches [11, 12]. Furthermore, none of the tests in the above standards covers the viral challenge while the laboratory-acquired viral infections are very common [5]. Literature on the viral aerosol containment of class II cabinets is absent.

In order to investigate if the spores could be replaced by other model microorganisms and to observe if there is a testing difference between bacterial and viral models, we carried out these biological tests on two Class II biological safety cabinets strictly conforming to the NSF/ANSI Standard 49 requirements except that *Serratia marcescens* and two different phages were used as challenge models.

Materials and Methods

Study design

Two unused Class II biological safety cabinets were carried out personnel, product, and cross-contamination protection tests according to the procedure in NSF/ANSI 49-2002.8. Before the biological tests, the minimum required non-biological tests (Down-flow velocity test,

***Corresponding author:** LI JinSong, Professor, Department of Biosafety, State key laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, 100071, China, E-mail: lij-s@163.com

Received March 13, 2012; **Accepted** April 10, 2012; **Published** April 16, 2012

Citation: Long YU, Wei D, XiaoDong W, ZhanBo W, Na L, et al. (2012) Viral Penetration of High Efficiency Particulate Air Filter of Biological Safety Cabinet in microbiological Laboratory. *Biosafety* 1:102. doi:10.4172/2167-0331.1000102

Copyright: © 2012 Long YU, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Inflow velocity test, Airflow Smoke Patterns test, HEPA filter test) had been performed on the two biological safety cabinets and they met the NSF/ANSI 49 requirements. Cabinet A (Bio-II-A) was tested again with *Serratia marcescens* and phage SM702. Cabinet B (Class II-A2) was tested with three different agents: *Serratia marcescens*, phage PhiX174, and phage SM702 consecutively. For the commercial interests, the cabinet brands were omitted here. All interior work surfaces and exposed exterior surfaces were decontaminated with 75% ethanol and then disinfected with UV light before biological tests and after each agent. The cabinet was run with the air inflow velocity set at 0.55 ± 0.025 m/s at least 30 mins before the start of biological tests and operated continuously throughout all tests. All the tests were carried out in biosafety level 2 laboratory and experimenters were protected properly.

Bacterial and viral surrogates

One different bacterium from the reference agent in NSF/ANSI 49–2002 was utilized. The referred agent in NSF/ANSI 49–2002 is spores of *Bacillus subtilis* var. *niger* (*B. subtilis*, ATCC 9372), while what we used here was *Serratia marcescens* and two different phages in the biological tests. *Serratia marcescens* was selected for the smallest size in bacteria and the feature of colony. This environmental isolate of *S. marcescens* characteristically produces a red pigment, prodigiosin, and it is a nonpathogenic one belonging to biosafety level 1. SM702 was a newly isolated lytic bacteriophage specific for this isolate. SM702 has an isometric polyhedral head (about 64 nm in diameter) and a long noncontractile tail (about 143 nm long). The plaque of SM702 is transparent and 2-3 mm in diameter at the 12th hour. PhiX174 (ATCC13706-B1) is a tiny spherical virion having no envelope with a diameter of 27 nm, and contains a single piece of circular ssDNA. This small ssDNA virus infects *Eshcherichia Coli* (ATCC13706). Bacterial plates were incubated at 30°C for 24 hrs. Double-layer plate method was used to determine the plaque forming units from sampler and settling plates incubating at 37°C for 24 hrs. Bacterial colonies or phage plaques on the plates were counted after incubation [13].

Personnel protection test and biological test of HEPA filter integrity

The personnel protection test was performed using a suspension concentration of $5-8 \times 10^8$ CFU/mL (or PFU/mL). The nebulizer was centered between side walls of the cabinet facing the front opening, above a metal cylinder penetrating the air curtain and representing a user's arm. A single petri dish was placed beneath the cylinder but above the front air grille (supported by a metal ring) as the control. Six AGI-30s were positioned around the cylinder just outside the cabinet to capture agents that passed through the air curtain. Two slit-type air samplers were placed at the sides of the cabinet opening. The nebulizer was calibrated to deliver more than 8×10^8 viable agents during the 6.5 mins of nebulization for the personnel protection test. This test was repeated three times for every agent. If more than 10 agents were captured by the impingers or more than five by the slit samplers during any one of the three replicate tests, the cabinet failed the performance requirements.

Furthermore, the outlet filter integrity was detected by an Anderson-6 impinger to estimate if there is a leakage while the personnel protection test was being undergoing. The impinger was located on the top surface of the cabinet near the outlet and sampled the filtered air with 10 mins at a speed of 28.3 L/min. Negative control sampled the filtered air before aerosol was nebulized. It was noted that the NSF testing procedure does not include this process which was

carried out first in this paper. The process should be performed before product protection, test to minimize contamination of indoor air.

Cross-contamination protection test

The cross contamination protection test was fulfilled using a suspension concentration of $5-8 \times 10^4$ CFU/mL (or PFU/mL). The aerosol nebulizer was positioned against each side wall facing the opposite side wall. Two rows of control open agar settling plates were placed on the bench with the centerline under the outlet of the nebulizer; two rows of plates with their centers on and outer 36 cm line drawn front to back. The nebulizer was started for 5 mins. After 15 minutes' settlement, the plates were taken out and incubated as above description. Three replicate tests were run from each side of the cabinet for every agent. If more than two agents were captured by the agar plates located beyond the 36 cm line during any one of the six replicate tests, the cabinet failed the cross contamination protection test.

Product protection test

The aerosol nebulizer containing agent suspension with a concentration of $5-8 \times 10^6$ CFU/mL (or PFU/mL) was positioned outside the cabinet facing in. The cylinder (this acts as an artificial arm to simulate normal operating conditions, airflow disturbance) penetrating the air curtain was placed in the center of the cabinet below the nebulizer. Open agar plates were spread over to the work surface, with the intent of capturing any agents that entered the cabinet. A single Petri dish was placed beneath the cylinder but above the front air grille (supported by a metal ring), this serve as the control. Three replicate tests were fulfilled with the nebulizer operating for 5 mins per time, followed by a 5 mins settling period. If more than five agents were captured by the plates inside the work zone during any one of the three replicate tests, the cabinet failed the product protection test.

Results

Personnel protection test and biological test of HEPA filter integrity

The outcomes of personnel protection test and biological test of HEPA filter integrity are presented in Table 1. After 15 minutes settlement, the plates were taken out and incubated as above description. All the control plates were positive with no less than 300 CFUs or PFUs for different surrogates. Less than 10 agents were collected by the impingers and less than 5 by the slit samplers during any one of the three replicate tests for both biological safety cabinets no matter which agent was used. The two biological safety cabinets could fulfill the certification requirements in this test. However, the biological testing results of HEPA filter integrity were different between bacterium and virus. Anderson-6 sampled 0 or did not detect viable *S. marcescens* during any one of the three replicate tests for both biological safety cabinets while it captured 1 viable SM702 for biological safety cabinet A and 4 viable PhiX174 for biological safety cabinet B in one repeat respectively.

Cross-contamination protection test

Table 2 shows the certification results of cross-contamination protection test. All the control plates were positive with no less than 151 CFUs or PFUs for different surrogates, which indicated that this item of biological tests was meaningful [8]. Total number of agents recovered on agar plates with centers on and greater than 36 cm did not exceed 2 for each test no matter which indicator was utilized for the two biological safety cabinets.

Biological safety cabinet	Replicate	Agents sampled by slit sampler			Agents sampled by AGI-30s			Agents sampled by Anderson-6			Agents on positive control plates		
		S.marcescens (°CFU)	SM702 (°PFU)	PhiX174 (°PFU)	S.marcescens (°CFU)	SM702 (°PFU)	PhiX174 (°PFU)	S.marcescens (°CFU)	SM702 (°PFU)	PhiX174 (°PFU)	S.marcescens (°CFU)	SM702 (°PFU)	PhiX174 (°PFU)
A	1	ND	ND	—	ND	ND	—	ND	1	—	>300	>300	—
	2	ND	ND	—	ND	ND	—	ND	ND	—	>300	>300	—
	3	2	ND	—	ND	ND	—	ND	ND	—	>300	>300	—
B	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	>300	>300	>300
	2	ND	ND	ND	ND	ND	ND	ND	ND	4	>300	>300	>300
	3	ND	ND	ND	ND	8	ND	ND	ND	ND	>300	>300	>300

ND, non detected; “—” indicates that this item was omitted; °CFU, total colony forming unit on two plates; °PFU, total plaque forming unit on two plates; °CFU, total colony forming unit in 6 AGI-30; °PFU, total plaque forming unit in 6 AGI-30.

Table 1: Personnel protection test and biological test of HEPA filter integrity.

Bio-logical safety cabinet	Repli- cate	Agents on control plates (2×5)						Agents on plates (36 cm row) (1×5)						Agents on plates (>36cm row) (2×5)					
		S.marcescens (CFU)		SM702 (PFU)		PhiX174 (PFU)		S.marcescens (CFU)		SM702 (PFU)		PhiX174 (PFU)		S.marcescens (CFU)		SM702 (PFU)		PhiX174 (PFU)	
		LR	RL	LR	RL	LR	RL	LR	RL	LR	RL	LR	RL	LR	RL	LR	RL	LR	RL
A	1	1123	582	843	778	—	—	ND	ND	ND	ND	—	—	ND	ND	ND	ND	—	—
	2	856	590	954	536	—	—	ND	ND	ND	ND	—	—	ND	ND	ND	ND	—	—
	3	974	477	590	761	—	—	ND	ND	ND	ND	—	—	ND	ND	ND	ND	—	—
B	1	2398	2118	1986	1999	300	199	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2	2008	2095	1407	1482	448	324	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	2458	2324	1826	1710	459	151	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

“—”omitted item; LR: from left side to right side; RL: from right side to left side; 2×5:two lines×five plates=10 plates;1×5:one line×five plates=5 plates; ND, non detected.

Table 2: Cross-contamination protection test.

Product protection test

The result of product protection test was shown in Table 3. All the control plates were positive with no less than 300 CFUs for bacterium *S. marcescens* or 300 PFUs for phage SM702 and PhiX174. Nothing was captured by the plates during any one of the three replicate tests no matter which model was used for the two biological safety cabinets. The two biological safety cabinets met the product protection test requirement in NSF/ANSI 49 standard.

Discussion

The two biological safety cabinets met the requirements of personnel, product, and cross-contamination protection test no matter which agent (*S. marcescens*, SM702, or PhiX174) was used to challenge the system. However, the difference occurred when the exhaust HEPA filter was challenged by bacterial and viral aerosol, respectively. The latter could penetrate exhaust HEPA filter, but the former could not. Viral sampling results of split samplers at the sides of the cabinet opening and AGI-30s around the cylinder were 0, which could exclude the likelihood that viruses escaped from the opening and backward reasoned that the sampled viable virus by Anderson-6 might leak from the exhaust filter. Many investigators also have demonstrated that viable viruses could penetrate HEPA filters when challenged with viral aerosol [14, 15].

To determine if microbes could penetrate the HEPA filters, four characteristics of microbic aerosols should be considered: 1) Filtration velocity (flow rate), 2) Microbe concentration, 3) Duration of the biological challenge, and 4) Particle size [16]. There were no significant differences in filtration velocity, microbe concentration (same order of magnitude), and biological challenge time between virus and bacterium in our tests. Particle size should be the primary factor that causes the penetration difference. *S. marcescens* is the smallest bacterium (length about 0.9~2.0 µm; diameter about 0.5~0.8 µm), but we should not expect that it can penetrate the HEPA filter easily because the bacterial

size is larger than 0.3 µm not mention the larger aerosols containing one or poly-bacteria and broth components (aerodynamic count mean diameter about 1.23 µm detected by TSI3321). The sizes of phage SM702 and PhiX174 are about 64 nm and 27 nm, respectively [17,18]. The challenged viral aerosol sizes for SM702 and PhiX174 were about 0.844 µm and 0.831 µm, respectively (aerodynamic count mean diameter detected by TSI3321). The evaporation effect of airflow on the viral aerosols could make more amount of particles become the most penetrating particles (0.1~0.3 µm), which might be the reason why the viral aerosols could penetrate HEPA filter while bacterial could not.

High efficiency particulate air filters are defined as filters with a filtration efficiency of 99.97% for 0.3 µm diameter polydisperse dioctylphthalate (DOP) particles. Three would escape through the exhaust filter out of 10,000 particles, theoretically. The nebulizer could deliver more than 8×10⁸ viable agents during the 6.5 mins of nebulization, but it was hard to figure out clearly through that relation how many viable agents would leak from the filters because the biological particles were more sophisticated than the physical ones such as DOP particles which were stable and invariable. The droplets containing water and microbes should be shrinking until without water. The aerosolized live agents carried by the airflow would be trapped in HEPA filters and then decay or penetrate alive. The advantage is that using the biological particles could reflect the containment performance of biological safety cabinet truly and intuitively. The threat viruses are not routinely handled in BSL-2 laboratories, but class II microbiological cabinets may be used in some high containment facilities such as BSL-3 or BSL-4 laboratory with the addition of operator protection via personal protective clothing or positive pressure personnel suits in. The median infectious dose (MID50) for all the threat viruses is very low. Most believe that the MID50s are less than ten virions although the absolute figures are not available [19]. Hence, personnel working in laboratory should adhere to safety regulations and use adequate containment procedures in terms of biosafety level [20].

Biological safety cabinet	Replicate	Agents on plates			Agents on positive control plates		
		S.marcescens (CFU)	SM702 (PFU)	PhiX174 (PFU)	S.marcescens (CFU)	SM702	PhiX174 (PFU)
A	1	ND	ND	—	>300	>300	—
	2	ND	ND	—	>300	>300	—
	3	ND	ND	—	>300	>300	—
B	1	ND	ND	ND	>300	>300	>300
	2	ND	ND	ND	>300	>300	>300
	3	ND	ND	ND	>300	>300	>300

“—” indicates that this item was omitted; ND, non detected.

Table 3: Product protection test.

It is well known that approximately 70% of the cabinet air (class II, type A) is recirculated through a HEPA filter into the work area from a common plenum, while approximately 30% of the air is exhausted from the cabinet through an exhaust HEPA filter. We can expect that viruses could also penetrate downflow HEPA filter and be kept alive, since viable ones penetrating HEPA filter with the same rated efficiency were sampled in 30% exhaust air. Theoretically, more number of viruses would penetrate the downflow HEPA filter due to more number of resource agents carried by 70% downflow air, but these penetrating viruses would be delivered to the downflow air splitting as close to the work surface and exit the work area through grilles located at both the rear or front of the work surface, respectively. The splitted laminar airflow above the work area provided a relatively clean interspace. So in order to protect the product from contamination, manipulations should be finished at the center of the work area as possible as you can. Thus, for safety it is essential to wear laboratory coats and gloves and limit the movement of arms and hands to reduce staining and escaping.

Conclusions

Two biological safety cabinets (Class II) were carried out personnel, product, and cross-contamination protection tests according to the procedure in NSF/ANSI 49-2002 using three different biological indicators (*S. marcescens*, phage PhiX174, and phage SM702). Results revealed that the two biological safety cabinets met the requirements in the standard no matter which agent was used to challenge the system. However, the HEPA filter leak testing results indicated that the penetration ability of viral aerosol through HEPA filter might be superior to bacterial, which was possibly due to the size of viral aerosol even more near the most penetrating particles. This viral testing method might be a potential way used for the exhaust HEPA filter leak certification due to being able to reflect the containment performance of biological safety cabinet truly and intuitively, instead of complicated and costly-equipment-dependent Dioctyl phthalate (DOP) or poly- α -olefin (PAO) method [8,21]. This report might also give a clue to laboratory acquired viral infections and cause the laboratory workers to emphasize the importance of safety regulations and adequate containment procedures in clinical, research, teaching, public health, and production facility laboratories using Class II biological safety cabinets.

References

- Peterson LR, Hamilton JD, Baron EJ, Tompkins LS, et al. (2001) Role of clinical microbiology laboratories in the management and control of infectious diseases and the delivery of health care. *Clin Infect Dis* 32: 605-611.
- Pfaller MA, Herwaldt LA (1997) The clinical microbiology laboratory and infection control: emerging pathogens, antimicrobial resistance, and new technology. *Clin Infect Dis* 25:858-870.
- Siegel JD, Rhinehart E, Jackson M, Chinarello L Health Care Infection Control Practices Advisory Committee. (2007) 2007 guideline for isolation precautions: preventing transmission of infectious agents in health care settings. *Am J Infect Control* 35: s65-s164.
- Sulkin SE (1961) Laboratory-acquired infections. *Bacteriol Rev* 25: 203-209.
- Pike RM (1979) Laboratory-associated infections: incidence, fatalities, causes, and prevention. *Annu Rev Microbiol* 33: 41-66.
- Sewell DL (1995) Laboratory-associated infections and biosafety. *Clin Microbiol Rev* 8: 389-405.
- (2004) Laboratory biosafety manual (3rd edn) pp.51-64 World Health Organization, Geneva, Switzerland.
- (2002) NSF/ANSI49- Class II (laminar flow) biosafety cabinetry NSF International standard/American National Standard.
- (2000) European Committee for Standardization. Biotechnology-performance criteria for microbiological safety cabinets. Standard No. 12469, Brussels, Belgium.
- State Food and Drug Administration. Medical and Pharmaceutical Industrial Standard: Biological Safety Cabinets. Standard No. 0569, China; 2005.
- Setlow P (2006) Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *J Appl Microbiol* 101: 514-525.
- Amant DCS, Carey LF, Guelta MA (2003) An improved method for the production of *Bacillus subtilis* var. *niger* spores for use as a simulant for biological warfare agents-quality analysis. *Dtic. mil.*
- Adams MH (1959) Bacteriophages. Interscience Publishers, Inc. New York.
- Decker HM, Buchanan LM, Lawrence BH, and Goddard KR (1963) Air Filtration of Microbial Particles. *Am J Public Health Nations Health* 53: 1982-1988.
- Rapp ML, Thiel T, Arrowsmith RJ (1992) Model system using coliphage PhiX174 for testing virus removal by air filters. *Appl Environ Microbiol* 58: 900-904.
- Helmbuch BK, Wu CY, Hodge J, Wander JD (2007) Viral penetration of HEPA Filters. Technical report AFRL-ML-TY-TP-2007-4512. Air Force Research Laboratory, Tyndall AFB, FL.
- Yu L, Wen Z, Li J, Yang W, Wang J, et al. (2010) Effects of different sampling solutions on the survival of bacteriophages in bubbling aeration. *Aerobiologia* 26:75-82.
- Ackerman HW (1999) Bacteriophages. In J P Lederberg (Ed.) *Encyclopedia of microbiology* (2nd ed) pp.398-411. Academic Press, New York.
- Woods JBLC, MC, USAF (2005) USAMRIID's Medical Management of Biological Casualties Handbook. (6th ed) pp. 61-74 United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.
- Burnett LC, Lunn G, Coico R (2009) Biosafety: Guidelines for working with pathogenic and infectious microorganisms. *Curr Protoc Microbiol* chapter 1:unit 1A.1.
- Barbeito MS, Abraham G, Best M, Cairns P, Langevin P, et al. (1995) Recommended biocontainment features for research and diagnostic facilities where animal pathogens are used. *First International Veterinary Biosafety Workshop. Rev Sci Tech* 14:873-887.