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Original Article

Analysis of Variation in Total Airborne Bacteria Concentration to Assess the Performance of Biological Safety Cabinets in Microbial Laboratories

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ABSTRACT

Background: The purpose of this study was to compare the concentration of total airborne bacteria (TAB) in biosafety cabinets (BSCs) at universities and hospital microbial laboratories to assess the performance of BSCs.

Methods: TAB was determined by using the single-stage Anderson sampler (BioStage Viable Cascade Impactor). The samples were obtained three times (with the BSC turned off and the shield open; with the BSC turned off and the shield closed; and with the BSC tuned on and operating) from the areas in front of 11 BSCs.

Results: TAB concentrations of accredited and nonaccredited BSCs were determined. No significant differences were observed in the TAB concentrations of the accredited BSCs and the nonaccredited BSCs for the areas outside the BSCs in the laboratories ($p > 0.05$). TAB concentrations for the BSCs sampled with the shield open and the instrument turned off showed differences based on the sampling site outside the BSC in each laboratory.

Conclusion: These results imply that TAB concentration is not altered by the performance of the BSCs or TAB itself and/or concentration of TAB outside the BSC is not a good index of BSC performance.

1. Introduction

Biosafety means the application of knowledge, techniques, and equipment to prevent personal, laboratory, and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents. In order to safeguard the environment, the people involved in laboratory experiments, and those working outside the laboratory against infection, agents need to be stored in a contained environment to limit the chances of exposure to biological hazards. Nevertheless, the current domestic regulations do not completely ensure the worker's safety of health, unlike the National Institutes of Health/Centers for Disease Control and Prevention and the World Health Organization (WHO) regulations, while working in biosafety facilities [1]. In Korea, infectious agents are classified into four categories: Groups 1, 2, 3, and 4. However, there are differences in the exact definitions of the differentiation in classification by WHO [2]. The main difference between the South Korea classification and the WHO classification is that the latter also includes hazards to animals and the environment [3].

The characterization and measurement of the concentration of airborne infectious microorganisms in a laboratory is difficult because of the diversity of infectious microorganisms handled in a bio laboratory, variation in the efficiencies of air sampling

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Table 1
Characteristics of the institutions and the biological safety cabinets (BSCs)

Institution	Type of ventilation system	Size of the laboratory (m^3)	Type of laboratory	Type of BSC (ID)	Manufacturing country
Hospital	HVAC	444	Diagnostic	Class II A2 (A)	USA
Hospital	HVAC	810	Diagnostic	Class II A2 (B-1) Class II A2 (B-2)	USA USA
University	HVAC	99	Mouse	Class II A2 (I)	South Korea
University	General	173	Fish	Class II A2 (E)	South Korea
University	General	528	Microbial	Domestic BSC* (C)	South Korea
University	General	75	Microbial	Domestic BSC* (J)	South Korea
University	General	372	Microbial	Domestic BSC* (D)	South Korea
University	General	399	Microbial	Domestic BSC* (F)	South Korea
University	HVAC	173	Microbial	Class II A2 (H)	South Korea
University	HVAC	49	Microbial	Class II A2 (G)	South Korea

HEPA, high efficiency particulate air; HVAC, heating, ventilating, and air conditioning.
* Domestically identified as a type of BSC, but down-flow velocity and the efficiency of the HEPA filters could not be tested because of the faulty BSC design.

equipment, different viability of each infectious microorganism, and lack of a standardized method for measuring individual microorganisms in the air. The measurement of total airborne bacteria (TAB) has been used by indoor environment experts as an index of microbial contamination in the air. The TAB test method has not been demonstrated to be a successful indicator of the performance of biosafety cabinets (BSCs); however, the assessment of total airborne fungi has been evaluated to be an indicator of BSC performance [4]. BSCs are used to control the spread of hazardous microorganisms handled in a laboratory, whereas the source of total bacteria could be diverse, and most of it is believed to come from sources other than the BSCs and the surrounding work area.

Until recently, in comparison with exposure monitoring of chemical, physical, and biotechnological facilities, research on biosafety facilities has been relatively neglected [5–8]. A limited number of studies have been carried out on the subject of infection in hospitals and biological [9], but there have been few studies focusing on the evaluation of biosafety facilities [10].

The purpose of this study was to determine if there are any performance differences between the BSC (Class II, Type A) facilities that were accredited and passed the four requirement tests such as downflow, intake velocity, high efficiency particulate air (HEPA) filter leak, and the airflow smoke pattern test, and those that were nonaccredited and failed at least one among the four tests in accordance with the standard procedures at university laboratories and microbial laboratories in hospitals [11,12]. We also aimed to measure the TAB concentration outside BSCs in laboratories where workers routinely handle various infectious agents during the course of daily activities.

2. Materials and methods

2.1. Characteristics and assessment criteria for BSC

A BSC is not a chemical fume hood. Fume hoods are designed to remove chemicals and aerosols from the work area, whereas BSCs are designed to provide both a clean work environment (product protection) and protection for employees who work with materials that could be biological hazards. BSCs use vertical laminar airflow, HEPA filtration, and negative air exhaust to create a barrier against infectious airborne entities such as microorganisms. They use HEPA filters to clean the air that goes into the work area and out into the environment. The air in BSCs is recirculated over the work area through the HEPA filter. The HEPA filter removes airborne particles from the air, but does not remove gas or vapor. In this study, domestic BSCs that were sold as a type of BSC, but where downflow velocity and the efficiency of the HEPA filters could not be tested because of faulty BSC design structure and A2 types of BSC Class II were tested. In the Class II A2 type, 70% of the HEPA-filtered exhaust air is recirculated within the cabinet; the remaining 30% is released as exhaust. The filtered exhaust air may safely recirculate into the workroom area.

During 2009, this study was performed on 11 BSCs at two different institutions, including two biological test laboratories at general hospitals and nine different laboratories at three universities with accredited and nonaccredited BSC facilities in accordance with the standard procedures [11,12].

2.2. TAB sample collection and analysis

The concentration of total airborne bacteria (TAB) in the area was determined within 50 cm in front of the 11 BSCs. None of the BSCs were connected in series; each BSC used an air foil and had the same sash configuration. The BioStage Viable Cascade Impactor (SKC Inc., Eighty Four, PA, USA) with 400 holes connected to a QuickTake 30 pump (SKC Inc.) with a flow rate of 28.3 L/minute was used. The 30 pump was calibrated between each test and was charged completely, and the connection tubes were of appropriate sizes throughout the entire study period. The sampling time selection was 5 minutes, and the tests were performed separately on each occasion, considering whether the shield was open or closed prior to the sampling procedure and even during the operation. When testing a BSC during operation, we tried to maintain enough distance from the BSC (1 m) to ensure that any influence resulting from the proximity of the personnel performing the test on the results was excluded. We also took extra cautionary measures to avoid any possible contamination by sterilizing the sampler with alcohol between the replacements of the agar plates. Tryptic soy agar media (Hanil Komed Co., Seongnam, Korea) in petri dishes (diameter, 100 mm) placed on the impactor were used to sample the TAB. There were 33 sampling heads, all operating simultaneously from each BSC, and a total of 11 samples were collected from the center of each room housing the BSCs, to compare the TAB concentrations of BSCs with the background concentration of TAB; care was taken such that there was no person in the room during the background sampling. After sampling, the samples were stored in an icebox while transporting them to the incubator, and were incubated in a bacterial incubator for 2 days at 35 °C. The TAB concentration was determined and expressed as colony forming units (CFU)/m^3 of air.

2.3. Statistical analyses

For BSCs performance test, simple descriptive statistics was used to present the pass rate. The Shapiro-Wilk test confirmed that TAB concentration was normally distributed. Analysis of variance

and *t* test were applied to evaluate the TAB difference according to the type of laboratory, prior to and after the operation and the performance of the BSCs. SPSS software package (version 12.0; SPSS Inc., Chicago, IL, USA) was used.

3. Results

Table 1 summarizes the characteristics of the laboratories investigated with respect to the institutions and the BSCs. Ventilation systems at all the hospitals were heating, ventilating, and air conditioning (HVAC) systems and were manufactured in the USA. However, the ventilation systems at the university laboratories were not HVAC systems; they performed general ventilation, except in the case of laboratories I, H, and G. Table 2 shows the results of TAB determined at the laboratories with accredited and nonaccredited BSC facilities at universities and hospitals. The mean concentrations of TAB when the BSCs were turned off during sampling ranged from 21 CFU/m^3 to 702 CFU/m^3, with an overall mean value of 164 CFU/m^3 when the shield was opened prior to sampling. The average concentrations of TAB when the BSCs were turned off ranged from 28 CFU/m^3 to 543 CFU/m^3, with an overall mean value of 182 CFU/m^3 when the shield was closed prior to sampling. During the sampling procedure, the results showed the lowest CFU concentration at 57 CFU/m^3 and 205 CFU/m^3 for institutions A and E, respectively. The background concentrations of TAB sampled in the center areas of the laboratories ranged from 14 CFU/m^3 to 386 CFU/m^3, with a mean value of 146 CFU/m^3 (Table 2). The ratio of mean TAB concentrations for areas in front of BSCs/TAB concentrations for the background areas ranged from 0.6 to 3.4, with a mean value of 1.2. There was no significant difference between TAB concentrations for areas in front of BSCs and those for the background areas. No significant differences were also observed between the TAB concentrations for accredited BSCs and the nonaccredited BSCs ($p > 0.05$).

4. Discussion

Our results show that all the BSCs that had been accredited by the National Science Foundation (NSF) of the USA and/or the Korean Industrial Standards (KS) of Korea were well above the standard of performance, regardless of their origins [11,12]. The accredited BSCs are those that have already cleared the performance test and those that have been maintained regularly and periodically, and the maintenance includes adjustment of velocity and replacement of the HEPA filters. If periodic regular maintenance is not performed, even the accredited BSCs can be exposed to the risks of contamination. For the domestic BSCs (made in Korea) that were not accredited by either the NSF or KS, there was no existing standard drafted by the manufacturer to test their performance with respect to the downflow velocity and efficiency of the HEPA filters because of the faulty design of the BSCs (Fig. 1).

Fig. 1. Example of the faulty design of biosafety cabinets due to the fluorescent light, which was disturbing the tests for the downflow velocity and efficiency of the HEPA filters. HEPA, high efficiency particulate air.

TAB concentrations determined with the BSC shield open and the BSC turned off showed great differences that were based on the measurement site outside the BSC in each laboratory (Table 2). These differences can be attributed to the indoor environment of the laboratories such as cleanliness and differences in temperature and relative humidity [13]. The reason for no detection of TAB in laboratory I (Table 2) on both occasions—prior to and after the operation—was due to the effects of the air conditioning facilities in the laboratory, which was a laboratory for animal experiments with negative pressure in the atmosphere. A limitation of this study was the lack of repeat samples to estimate the mean TAB values for each laboratory. We also could not test the interior parts of the BSCs and the workspace interior, such as the HEPA filter condition and the condition of the laboratory room door, which should have been closed during the sampling.

All the BSCs purchased after 2008 had been accredited, except for those in laboratory A, whereas all the BSCs purchased prior to

Table 2
Concentrations of accredited and nonaccredited TAB facilities at different institutions when sampling was performed with the biosafety cabinets (BSCs) turned on or off

Institution	BSC	TAB concentration (CFU/m^3)					M/B ratio*	Accreditation
		BSC turned off		BSC turned on	Mean (SD)	Background		
		Open	Close					
Hospital	A	198	138	57	131 (70.8)	86	1.5	Yes (NSF)
University	E	702	543	205	483 (253.8)	NA	NA	Yes (KS)
	I	ND	ND	ND	ND	ND	ND	Yes (KS)
Hospital	B-1	175	213	220	203 (24.2)	115	1.8	No
	B-2	93	86	93	91 (4.0)		0.8	No
University	C	182	93	266	180 (86.5)	205	0.9	No
	F	93	123	337	184 (133.1)	153	1.2	No
	G	21	28	93	47 (39.7)	14	3.4	No
	D	50	337	266	218 (149.5)	386	0.6	No
	H	108	115	71	98 (23.6)	108	0.9	No
	J	153	220	220	198 (38.7)	251	0.8	No
Mean (SD)		164	182	182	176 (10.4)	146	1.2	

NA, not applicable; ND, not determined; SD, standard deviation; TAB; total airborne bacteria.
* The ratio of mean TAB concentrations for areas in front of BSCs/TAB concentrations for the background areas.

2008 had not been accredited. The laboratory personnel lacked basic understanding about biosafety and BSC prior to 2008 in South Korea [1]. This finding does not necessarily explain the fact that all the BSC facilities had been accredited since 2008, because this study was not carried out for all the national BSC facilities, even though it appears that the manufacturers had begun to consider the specific requirements of BSCs and recognize the importance of BSC accreditation only recently. BSCs may not protect the operator from inhaling infectious airborne particles that may be released during microbiological manipulations if using nonaccredited BSC due to improper standard procedures.

TAB concentrations for the areas inside the laboratories were within the specified range as per the guidelines of the American Conference of Government Industrial Hygienists [14]. A previous study [15] suggested that concentrations of airborne bacteria that could be cultured exceeding 600 CFU/m^3 are associated with insufficient ventilation or abnormal sources of microorganisms. The mean TAB concentration (162 CFU/m^3) determined in this study was higher or similar to those determined in previous indoor environmental studies, which reported TAB concentrations of 104 CFU/m^3 in a sawmill factory, 135 CFU/m^3 in office buildings, 50 CFU/m^3 in a museum, and 176 CFU/m^3 in an Italian office building equipped with an HVAC system [16–19].

In conclusion, no significant differences were observed between the TAB concentrations for accredited BSCs and the nonaccredited BSCs ($p > 0.05$). This can be attributed to the differences in the indoor environment of each laboratory, such as cleanliness, occupants, temperature, and relative humidity. Therefore, the TAB concentration is not related to the performance of the BSCs or TAB itself and the concentration of TAB outside the BSC is not a good index of BSC performance.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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