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Comparative study of product contamination rates in class II biological safety cabinets with and without ultraviolet light disinfection

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ABSTRACT

Class II biological safety cabinets (BSCs) are equipment necessary in microbiological laboratories to protect workers from laboratory-acquired infections. The recommendation has dismissed the use of ultraviolet (UV) light in BSCs. Nevertheless, >80% of class II type A2 BSCs manufactured in the U.S. in the past decade were installed with UV light. This study aimed to determine the contamination rates of products in BSC working surfaces disinfected with UV light irradiation for 15 min and subsequently with 70% ethanol, and compared with those disinfected using only 70% ethanol. Results showed that the numbers of contaminated plates with bacterial and fungal colonies after disinfection with 70% ethanol alone were not significantly different from those after disinfection with UV light plus 70% ethanol. No significant difference in the numbers of contaminated plates was observed between BSCs in air-conditioned and window-ventilated rooms. Finally, the benefits and limitations of using a UV light system for the disinfection of working surfaces in class II BSCs were discussed.

Keywords: ultraviolet light; class II biological safety cabinets; contamination; disinfection; working space

1. INTRODUCTION

Biological safety cabinets (BSCs) were originally designed in 1909 by the W. K. Mulford Pharmaceutical Co., Glenolden, Pennsylvania, to prevent laboratory workers from being infected with *Mycobacterium tuberculosis* during the preparation of tuberculin, *M. tuberculosis* protein used in tuberculosis skin test (Kruse et al., 1991). Microbiological cabinetry is now regarded as important equipment in the microbiological laboratory due to its ultimate containment efficacy for preventing laboratory-acquired illnesses (Kruse et al., 1991). Three classes of safety cabinets have been developed to reduce risks to laboratory workers handling pathogens in the microbiology laboratory. Class II BSCs are the most commonly used BSCs worldwide.

The ultraviolet UV bandwidth was discovered in 1801 following the determination to eradicate almost all bacterial activities using some wavelengths of UV irradiation. Compared with chemical sterilization methods, e.g. chlorination and ozonation, the UV light system has been widely applied in industries during the last few decades

because it lacks toxic chemical consequences (NuAire, 2016). UV light has been introduced as a component of BSCs in an attempt to reduce cross contamination to the product in the working space and to prevent the pathogen-associated infections of laboratory workers (Marra et al., 2018). However, the utilization of UV light in BSCs has been widely discussed in the research community of the U.S. (Meechan and Wilson, 2006) because UV radiation is harmful to the skin and eyes due to its ability to be transmitted through the BSC glass. UV light is no longer a required feature of BSCs. Nevertheless, most manufacturers continue to provide UV light as an optional feature of BSCs (Meechan and Wilson, 2006). More than 80% of class II type A2 BSCs manufactured in the U.S. in the past decade were installed with UV light (NuAire, 2016). This study aimed to investigate the contamination rates of products prepared in BSCs with and without UV light decontamination. Other parameters, e.g., air-conditioned rooms and window-ventilated rooms were also examined. Evidence and discussion that will be useful for researchers deciding to install UV-C light in their BSCs have also been included.

2. MATERIALS AND METHODS

This study was conducted in the microbiological laboratories at Department of Microbiology, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand. Four class II BSCs were used in the experiments: two cabinets, No. 1 (Esco, LA2-6A1) and No. 2 (Scanlaf, Mars), were located in air-conditioned rooms and the other two cabinets, No. 3 (Esco, LA2-6A1) and No. 4 (EHRET, V-190), in the rooms with opened windows for ventilation. All the BSCs were tested for proper functioning in accordance with National Sanitation Foundation (NSF)/ American National Standards Institute (ANSI) 49-2020.

Two types of media, namely, plate count agar (PCA) and potato dextrose agar (PDA) were used to test any contamination that may occur in the working space of BSCs. The following experiments were designed to compare the contamination rates of products prepared under short wavelength ultraviolet (UV-C) light and non-UV-C light:

- 70% ethanol disinfection: the blower of BSCs was turned on for 5 min before cleaning the surface of working space with 70% alcohol.
- UV-C system plus 70% ethanol disinfection: UV lamp was switched on for 15 min, the blower of BSCs was then turned on for 5 min and the surface of working space was cleaned with 70% alcohol.

After the disinfection step, PCA or PDA plates without lid were placed in five different positions inside the working space of the BSCs for 45 min (Figure 1). Incubation was conducted at 37°C for 24 h for PCA and at 25°C for 5 days for PDA. Contamination was determined by observing microorganism growth on PCA and PDA, e.g., bacterial or fungal colonies. Numbers of contaminated plates and numbers and morphology of colonies were recorded. All experiments were carried out in duplicate on different days.

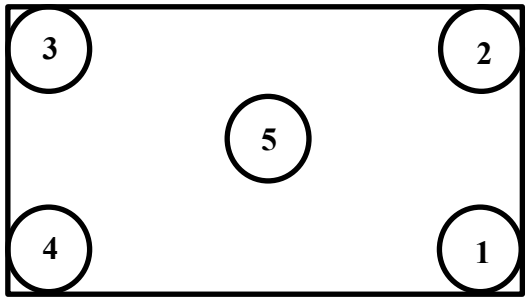

Figure 1. Position of plate count agar (PCA) and potato dextrose agar (PDA) plates in the working space of class II biological safety cabinets tested

Statistical analysis was performed using SPSS (IBM, Armonk, New York; version 21). Significant difference in the numbers of contaminated plates between disinfections with UV-C plus 70% ethanol and 70% ethanol alone in the four BSCs were tested using univariate analysis of variance. Significance was considered at P-value ≤ 0.05.

3. RESULTS

Table 1 shows the numbers of PCA and PDA plates with microorganism growth after being incubated at 37°C for 24 h and at 25°C for 5 days, respectively. For PCA, the number of agar plates with contamination (containing colony) from the experiments with UV-C light (3/40 plates; 7.5%) was slightly higher than that from the experiments without UV-C light (2/40 plates; 5.0%). All contaminated agar plates contained only one colony that was either 0.2 or 1-cm diameter white colony (Figure 2A and 2B, respectively).

Table 1. Numbers of plate count agar (PCA) and potato dextrose agar (PDA) plates with microorganism colony growth prepared in class II biological safety cabinets

BSC number/ room condition	PCA (contaminated plates/total plates)		PDA (contaminated plates/total plates)	
	UV-C & ethanol disinfection	Ethanol disinfection	UV-C & ethanol disinfection	Ethanol disinfection
No. 1/ air-conditioned room	1/10	0/10	2/10	3/10
No. 2/ air-conditioned room	0/10	0/10	2/10	1/10
No. 3/ window-ventilated room	0/10	0/10	1/10	0/10
No. 4/ window-ventilated room	2/10	2/10	0/10	0/10
Total	3/40	2/40	5/40	4/40

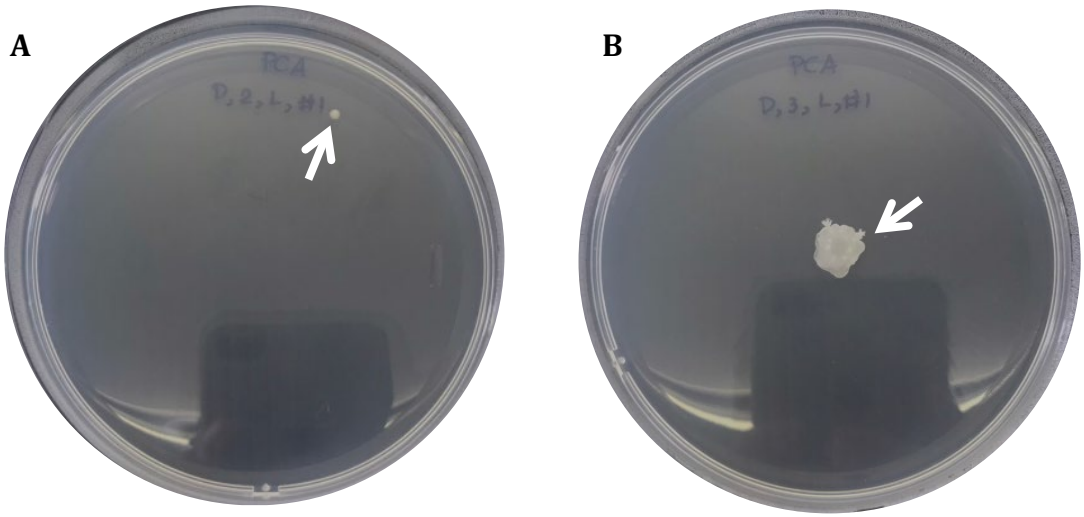

Figure 2. Observation of bacterial colony (arrows) on PCA plates from BSC No. 4 with the use of UV-C disinfection at positions 2 (A) and 3 (B)

Statistical analysis revealed no significant difference in the numbers of contaminated agar plate between UV-C plus ethanol and ethanol sterilizations from the four BSCs (P-value > 0.05).

The numbers of fungal-contaminated PDA plates after incubation at 25°C for 5 days are also listed in Table 1. Similar to those of PCA, the numbers of contaminated PDA plates from the experiments with UV-C light (5/40 plates; 12.5%) was slightly higher than those of from the experiments without UV-C light (4/40 plates; 10.0%). The maximum colony number observed was three colonies on one plate from the experiment with UV-C light. The other eight contaminated agar plates (four plates each from with and without UV-C light) contained only one colony each. The smallest colony size found was approximately 0.3 cm and the largest colony size was 5 cm (Figure 3). Statistical analysis indicated no significant difference in the number of contaminated plates between the usage and non-usage of UV-C light in the four BSCs (P-value > 0.05).

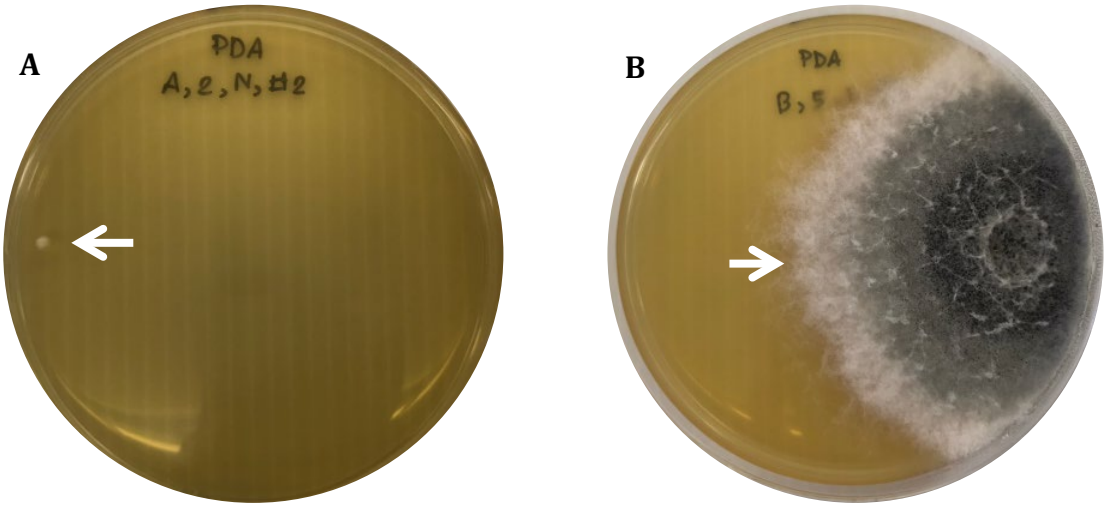

Figure 3. Observation of fungal colony (arrows) on PDA plates from (A) BSC no. 1 without UV-C utilization at position 2 and (B) BSC no. 2 with UV-C disinfection at position 5

The positional effects on the working space in BSCs were observed and high numbers of contaminated plates were observed at position 2 (Figure 1) for PCA and PDA plates (three and five plates, respectively) (Table 2). Contamination on agar plates under with and without UV-C disinfection was also determined in the BSCs located in air-conditioned and window-ventilated rooms (Table 1). The numbers of PCA plates with microorganism colony contaminated in the BSCs disinfected without and with UV-C light placed in the air-conditioned room (zero and one plate, respectively) were lower than those in the BSCs placed in the window-ventilated room (two plates each in with or without UV-C light). By contrast, the numbers of contaminated PDA plates in the BSCs placed in the air-conditioned room from with and without UV-C light experiments (four plates each) were higher than those in

the BSCs placed in the window-ventilated rooms (zero and one plate from without and with UV-C disinfection, respectively). Statistical analysis of contaminated plate numbers revealed no significant difference (P-value > 0.05) between air-conditioned and window-ventilated rooms for PCA and PDA.

Table 2. Numbers and position of contamination in agar plates (number of contaminated plates/total agar plates tested)

Medium	Position # 1		Position # 2		Position # 3		Position # 4		Position # 5	
	Non UV-C	UV-C	Non UV-C	UV-C	Non UV-C	UV-C	Non UV-C	UV-C	Non UV-C	UV-C
PCA	0/8	0/8	1/8	2/8	0/8	1/8	0/8	0/8	1/8	0/8
Total (n=80)	0/16		3/16		1/16		0/16		1/16	
PDA	1/8	0/8	2/8	3/8	0/8	0/8	1/8	0/8	0/8	2/8
Total (n=80)	1/16		5/16		0/16		1/16		2/16	

4. DISCUSSION

In this study, experiments were conducted to compare the contamination rate of products prepared in working surface that was previously disinfected with or without UV-C light. The numbers of contaminated PCA and PDA plates under only 70% ethanol disinfection were comparable with those under UV-C plus 70% alcohol disinfection (Table 1); however, no significant difference in contamination rate was observed. This finding was in agreement with the publication of the Centers for Disease Control and Prevention and National Institutes of Health, (2020) and Burgener (2006) that UV light is not required in BSCs to disinfect the interior surface for protection against contamination on work space. Nevertheless, >80% of class II type A2 BSCs manufactured in the U.S. in the past decade were requested by researchers to be installed with a UV light system (NuAire, 2016). According to the NSF/ANSI guidelines, for the installation of UV germicidal system in BSCs, the UV lamp has to be cleaned once a week because any dust and dirt can affect germicidal effectiveness, checked approximately every 6 months to ensure the appropriated UV light intensity for germicidal activity and turned off when the laboratory is occupied because UV exposure can damage the cornea and skin and lead to cancer (Richmond and McKinney, 2000). For the checking of germicidal activity, known bacterial strain cultures under appropriate conditions, e.g., exposure times to kill and the position of culture plates, can be used to monitor the UV light installed in BSCs (Harrington and Valigosky, 2007). Plate irradiation testing could also be employed annually to monitor the efficiency of equipment with $\geq$40 $\mu W/cm^2$ UV lamps.

For the efficient operation of BSCs, one major parameter of concern is cabinet location and room environment to avoid the disruption of airflow patterns. For example, all windows in the room should be closed (Richmond and McKinney, 2000). However, the results revealed that the numbers of contaminated PDA plates from BSCs in air-conditioned rooms were higher than those from BSCs in window-ventilated rooms. For PCA, the numbers of contaminated plates from BSCs in air-conditioned rooms were slightly lower than those from BSCs in window-ventilated rooms (Table 1). This phenomenon occurred because the BSCs were placed in the corner of the rooms. As a result, the air flow pattern inside the BSCs was not significantly affected by the environment, thus leading to low contamination rate.

The contamination rate in the PDA plates was higher than that in the PCA plates (Table 1). One explanation is that the surface disinfectant used in the study, i.e., 70% ethanol, can prevent fungal spore spreading but has no sporicidal activity (Gilpin and Powitz, 2020). Several disinfectants active against bacterial and fungal spores, including iodophores, peroxides, peracetic acid, hypochlorous acid and chlorine dioxide can be used (Gilpin and Powitz, 2020). However, for chlorine bleach oxidizing disinfectants, the agents may accelerate stainless corrosion and thus must be neutralized with sodium thiosulfate or rinsed with sterile water.

The UV light system has been widely used as an effective germicide but is only appropriate for the sanitation of lightly contaminated surface (Kayani et al., 2021). According to the standard 50% tissue culture infectious dose assay, 222 nm UV-C radiation at 3 mJ/cm^2 can reduce 99.7% of viable SARS-CoV-2 but not SARS-CoV-2 RNA genome (Kitagawa et al., 2021). However, the benefit of using UV light for disinfection purposes has been reported by several publications. For example, when personal protective equipment (PPE), e.g., N95 respirators, must be reused due to shortages during the COVID-19 crisis, UV light in BSCs can be employed to decontaminate the mask surface under the proposed protocol (Weaver et al., 2021). The effect of no-touch disinfection method to reduce healthcare-associated infection has been determined by systematic review and meta-analysis (Marra et al., 2018). Compared with hydrogen peroxide vapor systems, UV light systems can be efficiently used in reducing the numbers of *Clostridium difficile* and vancomycin-resistant enterococci; however, no difference was observed for methicillin-resistant *Staphylococcus aureus* and Gram-negative multidrug-resistant bacteria. UV light in BSCs can also be used to decontaminate *Bacillus anthracis* spores on hard surfaces (Turnbull et al., 2008). In addition to its germicide function, UV light is considered as an ideal disinfectant to prevent cross contamination among PCR samples (Meechan and Wilson, 2006). The preclusion of researchers who require BSCs for the preparation of PCR templates might occur if UV light installation is prohibited.

5. CONCLUSION

This study provides evidence that the efficacy against germicidal activity of chemical disinfectant, e.g., 70% ethanol, is comparable with that of UV light plus 70% ethanol to prevent the contamination of products in class II BSCs. Researchers may need to consider all the benefits

and limitations of UV light when deciding to install it in their equipment.

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