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Evaluation of a Stereotactic (6-direction) Ultraviolet Irradiating Pass Box

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ABSTRACT

Ultraviolet (UV) irradiation is a simple, effective, and widely accepted method for disinfection against microorganisms. We have developed a stereotactic pass box that has UV lamps installed for irradiation from 6 directions and evaluated its efficacy with physical and biological methods. We evaluated inactivation profiles for bacteria, fungi, and viruses. UV irradiation was performed for 5, 10, 15, and 20 seconds for bacteria and T4 bacteriophages and for 5, 10, 20, and 30 minutes for the human immunodeficiency virus and fungi. The biological efficiency of irradiation was measured on the basis of the reduction of infectivity. Bacteria and T4 bacteriophages were highly susceptible to UV, but the human immunodeficiency virus and fungi were less sensitive to UV and required more energy for inactivation. We also evaluated the efficacy of irradiation in proportion to the number of irradiation sources and observed a strong correlation with the UV radiant energy at each point. Six-directional (3-dimensional) UV irradiation was effective for sterilizing microorganisms at all positions tested in the irradiation area. We have evolved the new dimensions of the pass box, which is convenient, efficient, and reliable for decontamination. (Jikeikai Med J 2011 ; 58 : 7-15)

Key words : ultraviolet ray, microorganism, disinfection, sterilization, pass box

INTRODUCTION

Effective sterilization and decontamination techniques are needed to kill contaminating microorganisms[1]. A procedure is needed for disinfection which is efficient while also being inexpensive, quick, simple, nontoxic, practical, and suitable for a wide range of materials.

Ultraviolet (UV) irradiation has been widely used to kill microorganisms[2,3]. The maximum damage to cells is achieved at a wavelength of 260 nm[4]. The germicidal effect of UV irradiation on living cells is well documented and widely used for bacteria, viruses, molds, yeasts, protozoa, and endotoxins[5-8]. The mechanism of disruption by UV is rearrangement of the molecular structure of DNA and RNA, especially by cross-linking nucleotides, leading to changes that block replication of nucleic acids[9,10]. In particular, UV has been used to disinfect the surfaces of materials (e.g., laboratory notes) transferred from biological containment areas to clean rooms (e.g., administrative area and offices). Unlike most other disinfection methods, UV irradiation does not employ chemicals or heat and, therefore, can be used to sterilize materials those could otherwise be

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damaged under harsher conditions[11]. However, UV irradiation of materials with notable 3-dimensional structure can be affected by shadowing, which results in nonuniform irradiation and incomplete sterilization, especially when irradiation is performed from a single direction.

In the present communication, we describe a novel apparatus that generates UV light from 6 directions. Furthermore, we evaluated its efficacy for disinfection against several microbes and have shown its superiority to conventional pass boxes.

MATERIALS AND METHODS

1. Description of the stereotactic UV irradiation system

The stereotactic UV irradiation system was developed by Nippon Muki Co. Ltd. (Ibaraki). The chamber is made of stainless steel (SUS-304, polished by buffing with #400 abrasives to a mirror finish) and has 7 luminaries : 4 mounted in the centers of the 4 side walls, 1 hung from the center of ceiling, and 2 on the base. All lamps in this pass box are low-pressure models, rated as 15 W of germicidal (253.7 nm) radiation, and 30 cm in length. A diagram of the box is shown in Fig. 1(a).

The intensity of UV irradiation at 253.7 nm was measured to confirm the amounts of radiant energy in these experiments. A radiometer (UVP254-01, Iwasaki Electric Co., Ltd., Tokyo) placed in the center of the pass box was used to measure UV irradiant intensity from each direction perpendicular to the detector (Fig. 2). Irradiation was done from 1 direction to 6 directions, and the total energy was calculated as the percentage of the energy compared to that irradiated from all directions as 100%.

2. Preparation of microorganisms

The bacteria used in this study were obtained from the American Type Culture Collection (Bethesda, MD, USA) : *Staphylococcus aureus* (#12600), *Escherichia coli* (#11775), *Pseudomonus aeruginosa* (#10145), and *Klebsiella pneumoniae* (#13883). They were grown aerobically in Luria-Bertani (LB) medium at 37°C. *Candida albicans* and *Aspergillus niger* were isolated from patients, cultured on Sabouraud agar plates at 25°C, and biologically identified at The Jikei University Hospital. Human immunodeficiency virus (HIV)-1 IIIB was obtained from the AIDS Research and Reagent Program (National Institutes of Health, Rockville, MD, USA). For virus stock preparation, lymphoblastoid H9 cells[12] were infected with HIV and cultured for 10 days in 10% fetal bovine serum/RPMI 1640. Cultures were vigorously shaken for 1 minute and centrifuged at 400 *g* for 5 minutes. Then, supernatant was aliquoted with an equal amount of fetal bovine serum and stored at −80°C until use. The median tissue culture infective dose ($TCID_{50}$)

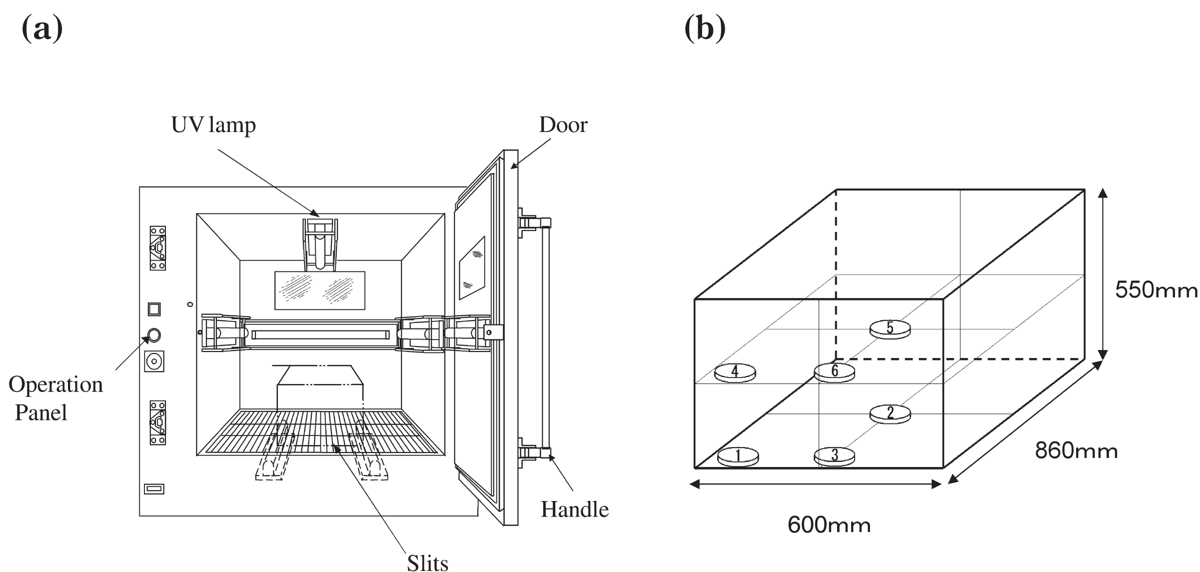
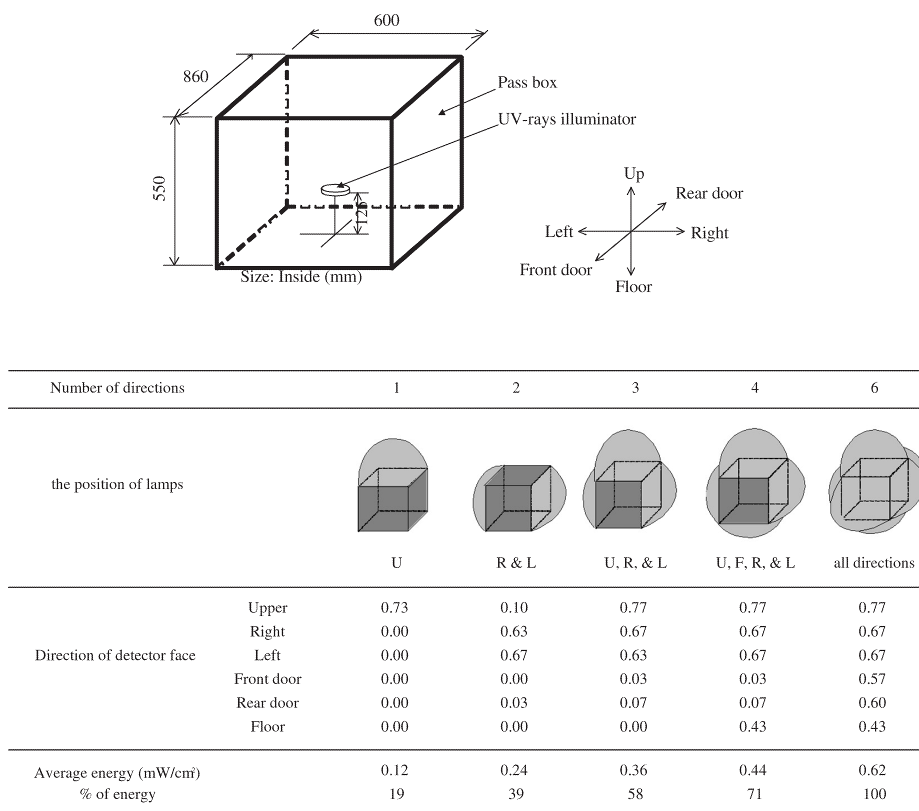


Fig. 1. (a) Diagram of the pass box. Description of the geometry of the pass box used in the experiments.
(b) Dimensions of the pass box and the positions where materials were placed



Number of directions		1	2	3	4	6
the position of lamps		U	R & L	U, R, & L	U, F, R, & L	all directions
Direction of detector face	Upper	0.73	0.10	0.77	0.77	0.77
	Right	0.00	0.63	0.67	0.67	0.67
	Left	0.00	0.67	0.63	0.67	0.67
	Front door	0.00	0.00	0.03	0.03	0.57
	Rear door	0.00	0.03	0.07	0.07	0.60
	Floor	0.00	0.00	0.00	0.43	0.43
Average energy (mW/cm²)		0.12	0.24	0.36	0.44	0.62
% of energy		19	39	58	71	100

U : Upper, R : Right, L : Left, FD : Front Door, RD : Rear Door, F : Floor

Fig. 2. UV energy measurements obtained inside the box when different UV lights were activated. The energy corresponding to the number of irradiation sources is listed.

was determined as described previously[13]. T4 bacteriophages were grown in indicator *E. coli* on a LB agar plate, collected with phosphate-buffered saline (PBS), extracted with chloroform, and stored in LB as described previously[14].

3. Bioassay method for bacteria

Liquid cultures of bacteria (16-20 hours) were diluted to approximately 1×10^5 colony forming units (cfu). Fifty microliters of culture was spread in an 80-mm-diameter area on a 100-mm-diameter LB agar plate and placed at each position in the pass box shown in Fig. 1(b). Each plate was irradiated for 5, 10, 15, or 20 seconds and then cultured at 37°C. Each exposure was tested in triplicate. The colonies obtained were counted after overnight incubation, and the mean of triplicate trials was used to calculate the surviving fraction.

Spores of *C. albicans* and *A. niger* were resuspended in PBS, and 0.1 ml of the sample was plated on Brain-Heart Infusion plates. Each plate was irradiated in the center of the apparatus (position 2) and then cultured at 25°C.

4. UV irradiation of HIV-1

Sixty microliters of viral stock (100 $TCID_{50}$) was placed in the center of a 100-mm-diameter plastic dish and dried for 30 minutes at room temperature until completely dry. Uncovered dishes were then placed at each position in the pass box (Fig. 1(b)) and irradiated from 6 directions for 1, 5, 10, 20, or 30 minutes. After irradiation, 60 μl of H9 cells (1×10^6 cells/ml) were added directly to each spot of virus and incubated at 37°C for 90 minutes, and then 20 μl of mixture was taken and added to each well of a 96-well plate containing 180 μl of fresh medium. After 10 days, the culture supernatant was harvested, and the number of viral particles was determined with a reverse-transcriptase assay as described previously[15]. All experiments were performed in triplicate.

5. UV inactivation of T4 bacteriophages

One hundred microliters of T4 bacteriophage (3.4×10^3 plaque-forming units [pfu]/ml) was evenly spread in an 80-mm-diameter area onto a 90-mm-diameter LB agar plate, placed at position 2, and irradiated for 2, 5, 10, 20, or 30 seconds. Soft top agar (0.6%) and log-phase *E. coli* as an indicator were added directly to the plate, which was then incubated at 37°C overnight. The number of plaques was counted to examine the survival of T4 bacteriophages.

6. The effect of the number of irradiation directions on inactivation

S. aureus was cultured in LB liquid medium at 37°C overnight and diluted to approximately 1×10^3 cells/ml. Fifty microliters of culture was spread in an 80-mm-diameter area on a 100-mm-diameter LB agar plate and then placed face up or face down at positions 1 to 6 of the pass box. Irradiation was performed for 20 seconds from 1 direction (upper), 2 directions (right and left), 3 directions (upper, right, and left), 4 directions (upper, lower, right, and left), or 6 directions (upper, lower, right, left, front, and rear). The plates were incubated at 37°C overnight, and the numbers of colonies were counted.

7. The effect of the presence of liquid on HIV inactivation

Sixty microliters of viral stock (100 $TCID_{50}$) was spotted at the center of a 100-mm-diameter plate. For dry conditions, the spotted viruses were dried at room temperature to complete desiccation and then irradiated. For wet conditions, the spotted viruses were irradiated immediately. The plates were placed at position 3 of the pass box and irradiated from 6 directions. After irradiation, 60 μl of H9 cells (1×10^6 cells /ml) was added to the virus directly and incubated at 37°C for 90 minutes, and then 20 μl of mixture was taken and transferred to each well of a 96-well plate containing 180 μl of fresh medium. After 10 days, the progeny viruses were measured with the reverse-transcriptase assay. Experiments were performed in triplicate.

RESULTS

1. Energy measurement

When irradiation was performed from 6 directions, enough energy was obtained at any direction in the pass box (Fig. 2).

2. Inactivation profiles by UV irradiation

For all bacteria, the face-down position was less effective than the face-up condition, and irradiation in the corners (positions 1, 3, 4, and 6) was less effective than irradiation in the center (positions 2 and 5) (Table 1). The bacteria most sensitive to UV irradiation was *K. pneumoniae*, followed by *E. coli*, *P. aeruginosa*, and *S. aureus*. The bacterium with the greatest resistance was *S. aureus*. The infectivity of *S. aureus* showed a 2 log (99%) reduction with 15 seconds' irradiation and a 3 log (99.9%) reduction with 20 seconds' irradiation; however, 5 seconds' irradiation proved ineffective for decreasing infectivity. At some irradiation positions, surviving bacteria were still present existed after 20 seconds' irradiation; however, none of the bacteria tested survived after 30 seconds' irradiation regardless of their position.

C. albicans was inactivated after 5 minutes' UV exposure, and detectable spores of *A. niger* remained until 30 minutes' irradiation, even at position 2 (data not shown).

For complete inactivation of HIV-1, approximately 30 minutes' irradiation was needed in the face-up position (Fig. 3(a)). The face-down position was less effective for inactivation, as in the bacteria experiments. Although HIV was less sensitive to UV irradiation, the positional relevance to inactivation was similar to that in the bacteria experiments.

In contrast to HIV, the T4 bacteriophage was highly sensitive to UV irradiation, and 10 seconds' irradiation was sufficient for complete inactivation, even in the corners of the apparatus (Fig. 3(b)).

3. The advantage of 6-direction irradiation

If a plate was placed on the bottom in the face-up position, bacteria were quickly inactivated, even with 1-direction (upper) irradiation (Table 2). However, if the plate position was parallel to the UV lamp (places 4, 5, and 6) and irradiated from 2 directions (right and left), almost no inactivation was observed. When a plate was placed face down, more bacteria survived, and inactivation was never complete at position 3, although the survival rate decreased according to the number of UV sources.

4. Irradiation of HIV in liquid

HIV in liquid with 50% serum was more resistant than HIV in the absence of liquid, and 30 minutes' irradiation was needed to completely inactivate HIV (Fig. 4).

Table 1. UV inactivation of Bacteria

Bacterium		Position	UV irradiation (seconds)				
			0	5	10	15	20
S. aureus	Face Up		1,500				
		1		2	0	0	0
		2		0	0	0	0
		3		2	0	0	0
		4		150	0	0	0
		5		3	0	0	0
		6		18	0	0	0
	Face Down		1,704				
		1		820	297	10	1
		2		115	2	0	0
		3		1,680	538	77	1
		4		759	1	0	1
		5		138	0	0	0
		6		581	2	0	0
E. coli	Face Up		240				
		1		0	0	0	0
		2		0	0	0	0
		3		0	0	0	0
		4		0	0	0	0
		5		0	0	0	0
		6		0	0	0	0
	Face Down		370				
		1		12	1	0	0
		2		0	0	0	0
		3		5	1	0	0
		4		1	0	0	0
		5		0	0	0	0
		6		1	0	0	0
P. aeruginosa	Face Up		270				
		1		0	0	0	0
		2		0	0	0	0
		3		0	0	0	0
		4		3	0	0	0
		5		0	0	0	0
		6		0	0	0	0
	Face Down		350				
		1		33	3	1	0
		2		3	0	0	0
		3		49	12	1	0
		4		7	0	0	0
		5		0	0	0	0
		6		6	0	0	0
K. pneumoniae	Face Up		1,080				
		1		0	0	0	0
		2		0	0	0	0
		3		0	0	0	0
		4		0	0	0	0
		5		0	0	0	0
		6		0	0	0	0
	Face Down		960				
		1		0	0	0	0
		2		0	0	0	0
		3		1	0	0	1
		4		0	0	0	0
		5		1	0	0	1
		6		0	0	0	0

The values indicate the number of colonies after overnight culture.
Experiments were performed three times and the most representative data is shown.

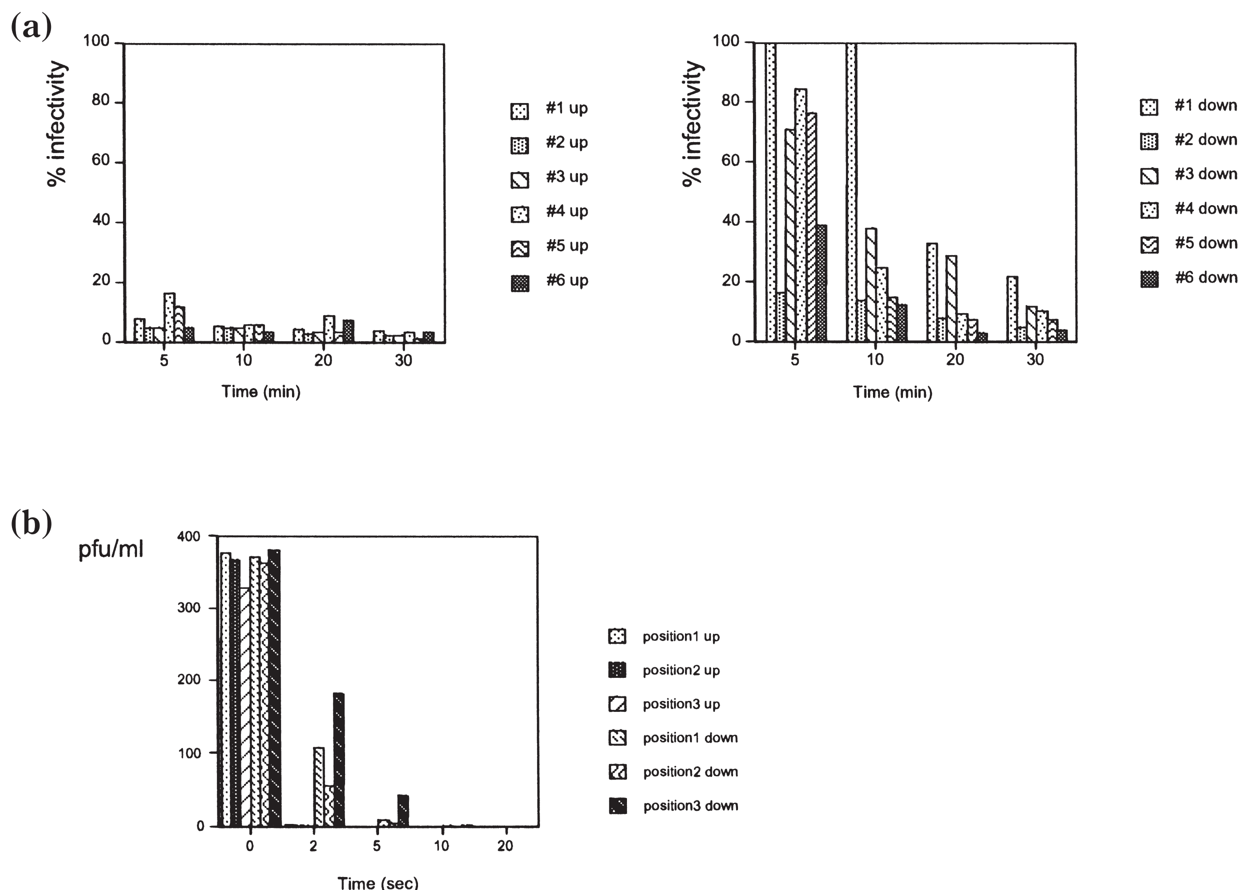


Fig. 3. UV inactivation of viruses as determined with cell culture infectivity assay
(a) Inactivation profile of HIV-1. Values represent the percent of control (no irradiation). Experiments were performed 3 times, and the means of the triplicate experiments are shown.
(b) Inactivation profile of the T4 bacteriophage. Values are the means of triplicate experiments.

DISCUSSION

In biological laboratories and industries, several apparatuses and methods are used to sterilize materials that are carried into and out of facilities ; sterilization methods include the application of chemical disinfectants and physical treatments, and their ability to inactivate microorganisms has been determined[16]. Although these methods are adequate to sterilize contaminating microorganisms, each method has its limitation. Chemicals can be toxic to life or be carcinogenic, and some objects cannot be heat sterilized or can be damaged by chemical disinfection.

The potential of UV treatment to inactivate microorganisms has been reported. Adenoviruses[17], *Aspergillus*[18], and *Mycobacterium avium*[19], which have significant resistance to standard UV disinfection, have been used to study the effectiveness of sterilization with UV light. Devine et al. have described a novel microwave-powered device for UV disinfection[20]. McDevitt et al. have evaluated a bench-top UVC exposure chamber for air disinfection for airborne smallpox transmission[21], and Mori et al. have developed a water sterilization device with UV light-emitting diodes[22]. UV light can directly damage proteins and nucleic acids and has germicidal effects. Moreover, UV disinfection is less expensive than other methods[23] ; therefore, it is practical for sterilization or disinfection of various materials.

In the present communication, we evaluated the germicidal effects of the stereotactic pass box to prove the superiority of this 6-dimensional UV irradiation system.

We first measured the intensity of UV irradiation at 253.7 nm in this apparatus and found it sufficient to extinguish the infectivity of microorganisms. However, disequilibrium of UV energy was still observed, even when irradiation was performed from 6 directions. To determine

Table 2. Inactivation profile by the number of irradiation directions

Position		The number of UV irradiation directions				
		0	1	2	4	6
Face Up		745				
	1		5	0	0	0
	2		6	0	0	0
	3		9	0	0	0
	4		0	145	0	0
	5		0	476	0	0
	6		0	603	0	0
Face Down		757				
	1		648	611	2	0
	2		279	177	0	0
	3		786	748	20	2
	4		516	569	0	0
	5		364	193	0	0
	6		365	470	0	0

The values indicate the number of colonies. Experiments were performed as two separate experiments in triplicate. The representative data is shown.

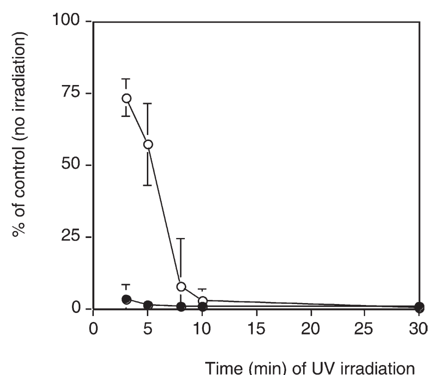


Fig. 4. Inactivation of HIV under wet and dry conditions
Each data point is an average of results for triplicate experiments and the error bars represent 1 standard deviation.
Open circles indicate values under wet conditions at position 3.
Closed circles indicate values under dry conditions at position 3.

the amount of energy available for killing microorganisms, when the irradiation is provided by several sources (omnidirectional) and the target is 3-dimensional, we performed the following experiments.

Ordinary bacteria were effectively inactivated, and they were fully inactivated by irradiation with this apparatus after a certain period of time. They required approximately the same dose of UV for 3 log units (99.9%) reduction in infectivity. *K. pneumoniae* was most sensitive to UV, followed by *E. coli, P. aeruginosa*, and *S. aureus* ; these results were consistent with previously reported levels of UV energy to destroy microorganisms[5]. Fungi proved highly resistant to UV irradiation ; in particular, *A. niger* exhibited resistance 100 times that of vegetative bacteria. HIV was 10 times as resistant, and the T4 bacteriophage showed sensitivity against UV similar to that of bacteria. UV has been reported to inactivate HIV[24, 25] and to have effects against RNA viruses[26–28]. UV irradiation induces activation of long terminal repeats which leads to enhancement of HIV replication in chronically infected cells[29]. However, direct treatment of HIV with UV inactivates virions and abolishes their infectivity. Moreover, UV rays are adsorbed by phenol red dye. A possible reason that UV was much less effective against HIV than the T4 bacteriophage is the presence of phenol red in the culture medium. A simple target theory proposes that the inactivation kinetics of UV irradiation is based on a single "hit," and accumulation of a certain critical number of "hits" in a viral DNA or RNA overwhelms repair processes and leads to failure of replication[30]. The inactivation of microorganisms by UV irradiation is based on its ability to cause irreversible damage to nucleic acids of the target organism[31].

We next examined the effect of the number of radiation sources for *S. aureus* and found that little sterilization effect

was observed with 1 or 2 sources, and even 4 sources was inadequate for inactivation at some positions. Because UV rays are emitted directly forward from each lamp and reflects against the stainless steel walls of the pass box, UV energy should be uniformly distributed within the apparatus. However, the 3-dimensional structure of target materials raises the possibility of shadowing. For this reason, it is difficult to ensure the uniform distribution of UV energy over the entire surfaces of target materials, even if the walls of this apparatus are made of stainless steel and polished for reflection.

UV is even effective for inactivating microorganisms in water. Sobsey has reported that a variety of factors influence the efficacy of bacterial disinfection in water by UV[32]. For the application of UV to the disinfection of clinical samples, we tried to evaluate HIV inactivation in the presence of 50% serum under dry and wet conditions. More energy was needed to kill HIV under wet conditions, but they could be inactivated after longer irradiation.

This stereotactic pass box, which has 6 sources of UV irradiation, is efficient for disinfection against various microorganisms, even if the target material has a complex structure or is in the corner of the irradiation area. Therefore, this stereotactic pass box might be used to disinfect a wide range of materials.

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