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G.N. GABRICHEVSKY MOSCOW RESEARCH INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY

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SCIENTIFIC REPORT

on the results of efficiency studies of pulsed continuous UV light generated by the **UIKb-01-Alpha unit** against antimicrobial-resistant spores of clinical **C. difficile** strain

Object of research: UIKb-01-Alpha unit

Manufacturer: Scientific and Industrial Enterprise «MELITTA», Ltd., Russia

Moscow, 2016

APPROVED
Director of FBSI G.N. Gabrichevsky
Moscow Research Institute
of Epidemiology and Microbiology
of Rospotrebnadzor
Academician of RAS, Professor
_____ V.A. Alyoshkin
April 20, 2016

SCIENTIFIC REPORT
on the results of efficiency studies of pulsed continuous UV light generated by the UIKb-01-Alpha unit against antimicrobial-resistant spores of clinical *C. difficile* strain

Test objective: activity study of pulsed continuous UV radiation generated by the UIKb-01-Alpha unit against antimicrobial-resistant spores of *C. difficile* clinical strain

Research work objectives:

1. Studying the activity of pulsed UV radiation against the spores of clinical ***C. difficile*** strain.
2. Developing the operating modes of UIKb-01-Alpha unit for disinfecting rooms, potentially contaminated with spores of clinical ***Clostridium difficile*** strains.
3. Developing practical (clinical) recommendations for disinfecting the surfaces contaminated with ***Clostridium difficile*** spores at healthcare facilities.

The work was performed under Agreement No. 62 dated 10.02.2015

Location and time of the tests: FBRI G.N. Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology of Rospotrebnadzor, March-April 2016.

Materials and methods

Equipment:

- **UIKb-01-Alpha pulsed xenon UV bactericidal unit for urgent air disinfection in category 1 and 2 rooms in the absence of people**, produced by SIE "Melitta", Ltd., Russia (Registration Certificate No. FSR 2010/06906 dated 26.02.2010; Certificate of Conformity GOST R No. ROSS RU.IM04.V07590 dated 08.03.2013); Treatment was performed at different exposure times (2 min, 4 min, 8 min, 16 minutes) and different distances from the radiation source (2 and 4 meters).

- **Bactron Anaerobic chamber** (Sheldon Manufacturing Inc., USA)

Test surfaces: single-use sterile plastic Petri dishes.

Growth media:

- Anaerobic Agar (Becton Dickinson, USA) with 7% (v/v) of whole lamb blood
- Clostridium difficile Agar Base (HiMedia Labs, India)

Study object characteristics: the clinical ***Clostridium difficile*** strain 503/16 isolated from a subject with a clinical pattern of a Clostridium difficile associated infection.

API 20A, ATB ANA test systems (bioMerieux, France) were used to identify and evaluate antibiotic sensitivity (resistance). In addition, MALDI-TOF mass spectrometry was used for identification. The sensitivity of anaerobic bacteria was studied in accordance with the NCCLS/CLSI recommendations by using agar dilution method (Brucella agar, Becton Dickinson, USA) with addition of hemin (5 μg/ml), vitamin K1 (1 μg/ml) (Becton Dickinson, USA) and lysed lamb blood (final concentration: 5%) for MIC calculation The spores are oval subterminal, fermenting glucose, mannitol, salicin, mannose, melecytosis, lactoses, and sucroses, maltoses; not fermenting xylose, arabinose, glycerol, cellobiose, raffinose, sorbitol, rhamnose, trehalose; not producing indole or urease, hydrolyze gelatin and esculin. The strain is to metronidazole-, cefepime-, and clindamycin-resistant; vancomycin-sensitive.

Research methods:

1) Preparation of *C. difficile* biomass: 100 μl 1 McF of *C. difficile* culture was inoculated on Petri dish; Clostridium difficile Agar Base (HiMedia Labs, India) selective culture medium was used, the inoculations were incubated in an anaerobic atmosphere (nitrogen: 80%, hydrogen: 5%, carbon dioxide: 10%) at 37 °C for 48 hours, then for another 48-72 hours at 22 °C;
2) On Days 4 and 6 of incubation in anaerobic atmosphere at 22 °C, the culture was tested for sporogenesis intensity which reached 96% of total cells number in FOV (a sufficient number – at least 90% spores of total cells number in FOV);
3) After sporogenesis completion, the culture was carefully washed from the dense culture medium surface into vials with sterile isotonic sodium chloride solution;
4) The spore culture was subjected to heat shock for 10 minutes at 75 °C;
5) Bacterial suspension with 1 McF density was prepared;
6) The spore cells number in 1 McF was verified by studying 10 FOV of smears, stained as per Aujeszky method – 95% of spore cells of total cells number in FOV were received.
7) 100 μl of microbial suspension were applied to sterile plastic Petri dishes surface, spread over the surface, and dried.
8) The dishes were attached vertically to the wall with double-sided adhesive tape along the line of UV light exposure at 2 or 4 meters' distance.
9) Test dishes were exposed to lamp irradiation in air; the control dishes were not exposed to the lamp irradiation for the same time, but were under aerobic conditions.

Before the start of irradiation, covers were removed from Petri dishes, contaminated with spore culture. After exposure to UV light, the dishes were immediately placed in anaerobic atmosphere; their contents were sterilely washed off with an isotonic solution to 1000 μl. Then the obtained suspension was titrated to -1, -2, -3 dilution and 100 μl of each dilution were inoculated to culture medium in 90 mm Petri dishes. The inoculations were incubated at 37 °C for 24 hours in oxygen-free environment. After incubation, the number of colonies grown on each Petri dish was counted, and the cells (spores) number per 1 ml with 1 McF density was calculated using specific formulas.

The **efficiency of UIKb-01-Alpha against *C. difficile* spores** (%) was calculated by the formula: (average number of spores on control dishes – average number of spores on test dishes): the average number of spores on control dishes multiplied by 100.

The studies were performed in triplicate for each exposure time and each distance from the radiation source. For n = 3, the average number of spores on control dishes and the average number of surviving spores on test dishes were calculated.

RESULTS

Table 1 shows the results of efficiency study of the of pulsed ultraviolet light generated by UIKb-01-Alpha unit against sporous forms of clinical *C. difficile* strain applied on vertical plastic test surfaces at 2 and 4 meters' distance from the light source at different exposure times.

Table 1. Disinfection efficiency of test surfaces contaminated with sporous forms of clinical *C. difficile* strain with pulsed UV light from the UIKb-01-Alpha unit at 2 and 4 meters' distance from the radiation source at different exposure times.

	Exposure time (min)	Number of surviving spores (1 ml, 1 McF)					
		2 meters			4 meters		
		CFU/ml	Efficiency		CFU/ml	Efficiency	
			%	lg		%	lg
experiment	2	8.02E+03	93.84	1.21	-		
	4	2.01E+03	91.16	1.05	9.83E+04	91.06	1.05
	8	0.00E+00	100.00	4.31	3.87E+03	97.41	1.59
	16	0.00E+00	100.00	4.25	0.00E+00	100.00	4.08
control	2	1.30E+05			-		
	4	2.28E+04			1.10E+06		
	8	2.04E+04			1.50E+05		
	16	1.79E+04			1.20E+04		

Note: 3.8 x 10*8 CFU/ml, 1 McF

DISCUSSION

The research results showed that the pulsed continuous ultraviolet light generated by the UIKb-01-Alpha unit has a pronounced sporicidal effect. At 2 meters' distance from the radiation source, the efficiency against sporous forms of clinical *C. difficile* strain reached 100 % after 8 minutes' exposure. At 4 meters' distance from the radiation source to contaminated surfaces, the time required to achieve 100% efficiency increased to 16 minutes.

CONCLUSIONS:

1. High activity of UIKb-01-Alpha pulsed ultraviolet units for disinfecting vertical test surfaces, contaminated with sporous forms of clinical *C. difficile* strain with high epidemic potential, was shown by experiments.
2. We have determined the operating mode of UIKb-01-Alpha pulsed ultraviolet units that provides an efficiency of over 99.99% (≥4lg) when disinfecting surfaces contaminated with spores of clinical *C. difficile* strains at 2 and 4 meters' distances from the radiation source. The exposure time is 8 and 16 minutes, respectively.
3. The efficiency levels of pulsed ultraviolet units obtained in the studies meet the performance criteria adopted for disinfectants used in the disinfection mode of surfaces in hospital environment (99.99%) (Guideline R 4.2.2643-10 Methods of Laboratory Studies and Testing of Disinfectants for Assessing their Efficiency and Safety, M. 2011).

Practical recommendations for healthcare facilities.

1. UIKb-01-Alpha ultraviolet pulsed units should be included in disinfection procedures (preventive and local surfaces disinfection) in the structural subdivisions of healthcare facilities with high risks of clostridial infections incidence and transmission. The ground is their high efficiency (over 99.99% in 16 and 8 minutes' exposure at 4 and 2 meters' distances from the radiation source, respectively).

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