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G.N. GABRICHEVSKY MOSCOW RESEARCH INSTITUTE
OF EPIDEMIOLOGY AND MICROBIOLOGY
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TEST REPORT
on the decontamination effectiveness of pulsed UV radiation of continuous spectrum by UIKb-01-Alpha system against surfaces contaminated with hospital bacterial strains

Object of research: UIKb-01-Alpha system

Manufacturer: MELITTA Scientific and Production Company, Russia

Moscow, 2012

APPROVED
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22 May 2012

TEST REPORT
ondecontamination effectiveness of pulsed UV radiation of continuous spectrum by UIKb-01 Alpha system against surfaces contaminated with bacterial hospital strains

Test aim: to determine effectiveness of pulsed ultraviolet radiation of continuous spectrum against multiresistant hospital strains in order to determinehow pulsed Alpha system application should be used for open surfaces decontamination in health facilities.

Objectives of testing:

1. Determine effective application modes of pulsed UV Alpha-1 system against bacterial hospital strains with multiple drug resistance and acquired resistance to chemical disinfectants.
2. Study whether and how the effectiveness of pulsed ultraviolet radiation of continuous spectrum depends on the type of test surfaces, presence/absence of protein protection and placement of surfaces relative to UV radiation (horizontal, vertical).
3. Compare effects of pulsed ultraviolet radiation on hospital microorganisms and St. aureus 907 test strain, which is used to set effective modes of chemical disinfectant application.

Venue and time of testing: Federal Budgetary Scientific Institution G.N. Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology of Federal Service on Consumer Rights Protection and Human Well-being, February – April 2012

Materials and methods

Equipment:UIKb-01-Alpha pulsed xenon UV-bactericidal system for express air disinfection in rooms of category 1 and category 2 with no people present, manufactured by Melitta Scientific and Production Company, Russia (Registration Certificate No.ФСР 2010/06906 of 26.02.2010; GOST R Certificate of Conformity No. РОСС RU.ИМ04.В07590 of 08.03.2013). System application mode: distance between the system and vertical surfaces – 2 meters, horizontal surfaces – 1.2-1.7 meters.
Test objects: plastic (single use Petri dishes), metal plates (medical metal).
Characteristics of hospital bacterial strains (from the museum of G.N. Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology):
P. aeruginosa str.1098 – high resistance to beta lactam antibiotics and carbapenems. Resistance to 14 preparations out of 17. Resistance to colistin and piperacillin/tazobactam is preserved.

MRSA str.02840 – resistant to beta lactam antibiotics, benzylpenicillin and polysynthetic penicillins (to 7 out of 16 antibiotics tested).

St.aureus str.907 (test strain to determine the level of activity of chemical disinfectants).

Acinetobacterbaumannii str.1315 – hospital strain resistant to beta lactam antibiotics and carbapenems (resistant to 16 out of 19 antibiotics tested).

Pr. mirabilis epidemic strain – strain isolated from clinical material (urine) from patients during an outbreak of HAI. Has multiple drug resistance.

Enterococcus faecium(vancomycin-resistant str. No.4 obtained from the collection of Smolensk Research Institute of Antimicrobial Chemotherapy) – resistant to vancomycin, ampicillin, gentamicin, tetracycline.

Strains used in the tests were studied for resistance to surface disinfectants belonging to various chemical groups. Disinfectants were applied as suspensions in accordance with the Guide P. 4.2.2643-10 "Methods of laboratory research and testing of disinfectants to determine their effectiveness and safety", M. 2011. E coli 1257 and St. aureus 907 test strains were used to control the effectiveness of chosen disinfectant application modes.

Test findings are presented in Table 1.

Results of tests for sensitivity of hospital microorganism strains against disinfectants in modes recommended by the Guides for surface disinfection

Table 1

disinfectant	Composition	Application mode	E.coli 1257 teststrain	St. aureus 906 teststrain	Pr. mirabilis str.4	Acineto-bacter 1315	VRE, str.4	St. aureus 02840	Ps. aerug. 1098
Microzid RF Liquid	Propanols 62%	5 min	FS	FS	FS	4,00E+05	FS	FS	4,00E+05
Hexaquart Forte	Quaternary ammonium salts (27,9% total), surfactants	3.5%-30 min	FS	FS	FS	1,00E+05	FS	FS	FS
Newzhavel	50% Na salt of dichloroisocyanuric acid	0.1%-30 min	FS	FS	FS	FS	FS	FS	2,00E+05
Petroxin	18%H_2O_2 6% quaternary ammonium salts	3%-30 min	FS	FS	FS	FS	FS	FS	3,00E+05

Note: full sensitivity (FS)

As evident from the results, non-fermentative enterobacteria (Ps. aeruginosa and Acinetobacterbaumannii) showed resistance to surface disinfectants.

Methods used to study the effectiveness of surface disinfection: Vertically arranged cups (metal plates) contaminated with cultures of hospital strains were irradiated at the distance of 2 meters from the lamp. Time of irradiation was 5-10-20 minutes (Fig.1). System effectiveness at horizontal arrangement of test objects was tested at the distance of 1.2-1.7 meters from the lamp (Fig.2 and Fig.3).

Fig.1 Vertical arrangement of plastic test objects

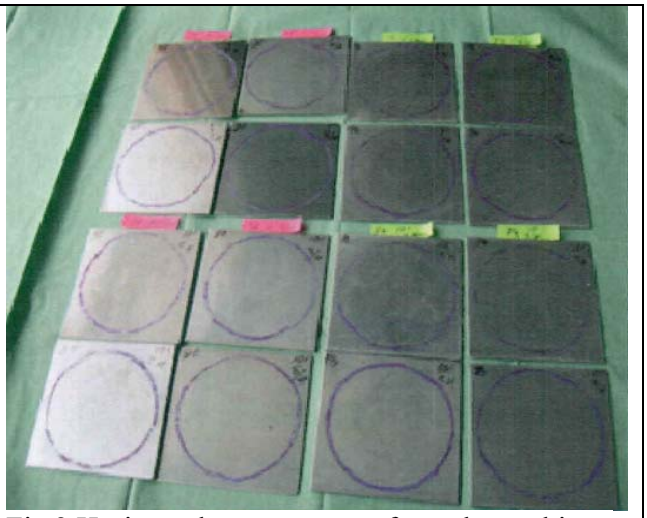
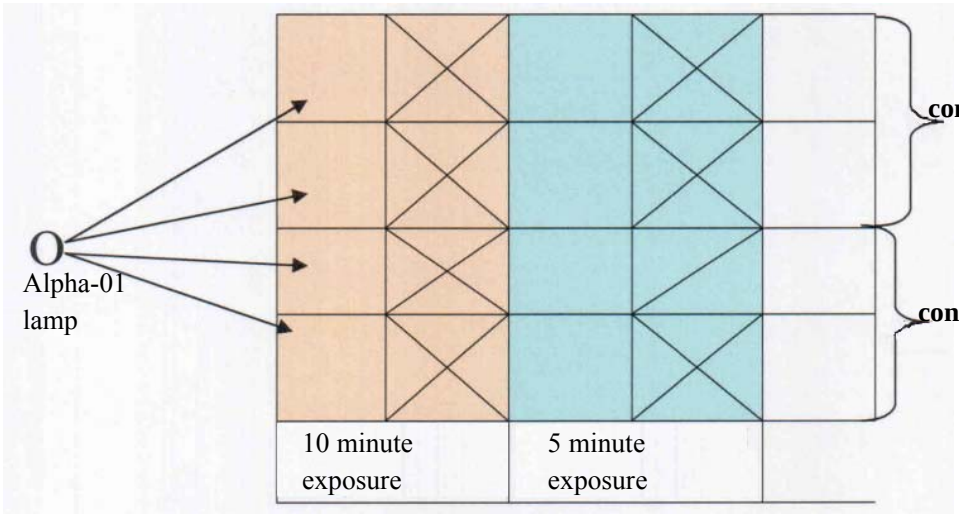


Fig.2 Horizontal arrangement of metal test objects

Fig.3 Scheme of the test to determine UIKb-01 Alpha effectiveness with horizontal arrangement of metal test objects contaminated with P.aeruginosa and MRSA



Conventions:

	plates with no biological load
	plates with biological load
	plates with no biological load
	plates with biological load
	plates with controlled density of cultures with no biological load and with biological load (not subjected to irradiation)

A one-day suspension of a hospital strain or a test strain was modeled on standard industry turbidity sample No.20 (9lg) with saline and saline with ram blood serum (6 ml of saline, 4 ml of serum). Blood agar was used to grow the cultures.

A micropipette was used to place 0.02 ml of microbial suspension on the bottom of a sterile Petri dish or a metal plate of the same surface area. Microdrops were uniformly spread around the surface of the test object.

The tests were conducted no later than 1 hour after microdrops with test microorganisms were spread.

Lids were removed from Petri dishes before the tests (irradiation) began. After the tests the dishes were filled with 10 ml of sterile saline while thoroughly stirring with circular motions, then filled with 10 ml of dense nutrient medium which had been melted then cooled to 45°C, and stirred with circular motions. After that the lids were put back on again and the liquid was left to congeal until it acquired dense consistency. This method is the most accurate one, as it permits to register even single viable colonies without inoculations of tenfold dilutions.

After the irradiation, the culture was wiped from the metal test objects onto a sterile drape which was then beaten in a retort with beads filled with 10 ml of sterile saline. Petri dishes with blood agar then received 0.1 ml of the resulting suspension each.

The dishes were placed into a thermostat for 48 hours at 37°C.

The tests were conducted 3-4 times for each time mode and each surface type. Viability of cultures was controlled 4 times before the tests.

The number of colonies of viable microorganisms on the dishes was counted. While counting the number of colonies of viable microorganisms after metal plates disinfection, the ones subjected to the method of 2 tenfold dilutions were counted as well.

Effectiveness of treatment was calculated according to the following formula:(average number of colonies on control plates – average number of colonies on test dishes): average number of colonies on control dishes x 100.

A criterion of application mode effectiveness 99.99% (4lg) was introduced for chemical disinfectants treating indoor surfaces and objects of hospital environment. Effectiveness criteria of preventive surface disinfection were not developed for the method under study, but they cannot be lower than the stated levels.

Test results.

Table 2 shows test results for effectiveness of UIKb-01 Alpha pulsed ultraviolet system against hospital and test strains of gram positive and gram negative bacteria applied to horizontal and vertical plastic and metal surfaces.The very first tests with P. aeruginosa showed that 20 minute treatment leads to death of almost 100% of microorganisms, which is why subsequent tests only involved 5 or 10 minute exposure.

Disinfection effectiveness of vertically arranged test objects with a UIKb-01 Alpha pulsed ultraviolet system against clinical and museum strains of microorganisms.

Table 2

Hospital strain 1098 of pseudomonas aeruginosa					
Distance between the test objects and the lamp (cm)	Test method	Factors	Irradiation time, minutes		
			5	10	20
		Microbial cell contamination density of test objects before irradiation	av.1.5x10^7 –metal plates av. 1.65x10^7 for dishes		
200.0	Dish with nutrient medium w/o serum	Number of surviving microbial cells	1. 35 2. 0 3. 30	1. 1 2. 1 3. 0	1. 0 2. 0 3. 0

			4. 0 av. 16	4. 2 av. 1	4. 0
		Disinfection effectiveness, %	6lg, 99.9999%	7lg, 99.999994%	100.00%
200.0	Dish with nutrient medium w/ serum	Number of surviving microbial cells	1. 10 2. 0 3. 0 4. 25 av.9	1. 0 2. 0 3. 1 4. 4 av.1	1. 0 2. 0 3. 0 4. 0
		Disinfection effectiveness, %	6lg, 99.99995%	7lg, 99.99999%	100.00%
200.0	Metal plates w/o serum	Number of surviving microbial cells	1. $1x10^2$ 2. $3x10^2$ 3. 0 4. 0 av.$1x10^2$	1. $1x10^2$ 2. 0 3. 0 4. 0 av.25	1. 0 2. 1 3. 3 4. 0 av.1
		Disinfection effectiveness, %	5lg, 99.9993%	5lg, 99.9998%	7lg, 99.99999%
Series 2. Pr. mirabilis, hospital str.4					
200.0	Dish with nutrient medium w/o serum	Number of surviving microbial cells	1. 4 2. 16 3. 0 av. 6.7	-	**Average contamination density of the object before irradiation $5x10^8$ KOE/cm^2**
		Disinfection effectiveness, %	7lg, 99.9999987%	-	
200.0	Dish with nutrient medium w/serum	Number of surviving microbial cells	1. 3 2. 3 3. 1 av. 2	no growth no growth no growth 0	
		Disinfection effectiveness, %	8lg, 99.9999995%	100%	
200.0	Metal plates w/o serum	Number of surviving microbial cells	1. $2x10^2$ 2. $2x10^3$ 3. 0 av.$7.3x10^2$	- - - -	**Average contamination density of the object before irradiation $6x10^8$ KOE/cm2**
		Disinfection effectiveness, %	5lg, 99.99988%		
200.0	Metal plates w/serum	Number of surviving microbial cells	1. $8x10^2$ 2. $8x10^2$ 3. $6x10^2$ av. $7.3x10^2$	- - - -	
		Disinfection effectiveness, %	5lg, 99.9998%		
St. aureus, hospital str.02840					
200.0	Dish with nutrient medium w/o serum	Number of surviving microbial cells	1. 50 2. 3 3. 32 av. 28	0 0 0	**Average contamination density of the object before irradiation $5x10^8$ KOE/cm^2**
		Disinfection effectiveness, %	7lg, 99.99999%	100.000	
200.0	Dish with nutrient medium w/serum	Number of surviving microbial cells	1. 48 2. 50 3. 50 av. 50	1 0 0	

		Disinfection effectiveness, %	7lg, 99.99999%	8lg, 99.999999%	
200.0	Metal plates w/o serum	Number of surviving microbial cells	1. 0 2. $2x10^2$ 3. 0 av. 67	- - - -	**Average contamination density of the object before irradiation $5x10^8$ KOE/cm^2**
		Disinfection effectiveness, %	6lg, 99.999987%	-	
200.0	Metal plates w/serum	Number of surviving microbial cells	1. $2x10^3$ 2. 0 3. $3.5x10^3$ av. $1.83x10^3$	- - - -	
		Disinfection effectiveness, %	5lg, 99.9996%		
St. aureus (museum str.907)					
200.0	Dish with nutrient medium w/o serum	Number of surviving microbial cells	1. 40 2. 3 3. 52 av. 32	- - -	**Average contamination density of the object before irradiation $7x10^8$ KOE/cm^2**
		Disinfection effectiveness, %	7lg, 99.999995%		
200.0	Dish with nutrient medium w/serum	Number of surviving microbial cells	1. 56 2. 45 3. 42 av. 48	no growth no growth no growth 0	
		Disinfection effectiveness, %	7lg, 99.999993%	100%	
Series 3. Acinetobacterbaumanni, hospital str.1315					
200.0	Dish with nutrient medium w/o serum	Number of surviving microbial cells	1. 25 2. 20 3. 15 av. 20	1. 8 2. 13 3. 25 av. 15	**Average contamination density of the object before irradiation $2.32x10^7$** KOE/cm^2
		Disinfection effectiveness, %	6lg, 99.9999%	6lg, 99.99993%	
200.0	Dish with nutrient medium w/serum	Number of surviving microbial cells	1. 30 2. 35 3. 40 av. 35	1. 12 2. 33 3. 35 av. 27	**Average contamination density of the object before irradiation $1.6x10^7$** KOE/cm^2
		Disinfection effectiveness, %	5lg, 99.9998%	5lg, 99.9998%	
Series4***Enterococcus faecium,*** str.4 (VRE)					
200.0	Dish with nutrient medium w/o serum	Number of surviving microbial cells	1. 24 2. 16 3. 22 av. 21	1. 8 2. 13 3. 25 av. 15	**Average contamination density of the object before irradiation $1.9x10^7$** KOE/cm^2
		Disinfection effectiveness, %	7lg, 99.99999%	100.000	
200.0	Dish with nutrient medium w/serum	Number of surviving microbial cells	1. 18 2. 20 3. 19 av. 19	1. 29 2. 20 3. 11 av. 20	**Average contamination density of the object before irradiation $2.3x10^7$**

					KOE/cm^2
		Disinfection effectiveness, %	6lg, 99.9999%	6lg, 99.9999%	
200.0	Dish with nutrient medium w/o serum	Number of surviving microbial cells	n/g 3x10^2 2x10^2 3x10^2 4x10^2 30x10^2 30x10^2 6x10^2 4x10^2 av.9.1x10^2	n/g n/g 3.0x10^2 n/g n/g 5.0x10^2 1.5x10^3 4.0x10^2 1.3x10^3 av. 4.4x10^2	**average contamination density of the object before irradiation 1.83x10^7** KOE/cm^2
		Disinfection effectiveness, %	4lg, 99.995%	4lg, 99.998%	
200.0	Dish with nutrient medium w/serum	Number of surviving microbial cells	4x10^2 9x10^2 4x10^2 10x10^2 n/g 3.0x10^2 13x10^2 n/g 11x10^2 av. 50	n/g n/g 11x10^2 6.0x10^2 14x10^2 n/g 8x10^2 n/g 16x10^2 av. 6.1x10^2	**average contamination density of the object before irradiation 1.83x10^7** KOE/cm^2
		Disinfection effectiveness, %	4lg,99.997%	4lg, 99.997%	

Test were also conducted to determine effectiveness of UIKb-01 Alpha pulsed ultraviolet system against hospital strains of P.aeruginosa and MRSA on metal plates which were arranged horizontally relative to the irradiation. The distance between the horizontal surface and the floor was 80 cm. Results are presented in Table 3.

UIKb-01 Alpha pulsed ultraviolet system effectiveness for disinfection of horizontally arranged metal test objects contaminated with clinical strains of microorganisms.

Table 3

Average distance to the object (m)	**Presence of biological load**	**Irradiation time**	**Average contamination density (KOE/cm^2)**		**Effectiveness lg/%**
			test	**control**	
Pseudomonas aeruginosa, hospital strain 1098					
1.6	w/o serum	5 min	1.0x10^2	1.15x10^7	5/99.9991
1.7	w/serum		1.6x10^2	1.4x10^7	4/99.9989
1.4	w/o serum	10 min	1x10^2	1.15x10^7	5/99.9991
1.5	w/serum		83	1.4x10^7	5/99.9994
St aureus, hospital (MRSA) strain 02840					
1.4	w/o serum	5 min	0	4.6x10^7	7/100.00
1.5	w/serum		33	4.7x10^7	6/99.99993
1.2	w/o serum	10 min	0	4.6x10^7	7/100.00
1.3	w/serum		0	4.7x10^7	7/100.0

Discussion.

Conducted tests showed high efficiency (99.99%-100%) of the pulsed ultraviolet system against multiresistant hospital strains and strains with multiple drug resistance, including P. aeruginosa and Acinetobacter baumannii with resistance to chemical disinfectants (Fig.4). This is especially important as it is difficult to solve the practical problem of rotation when isolating hospital bacterial strains with acquired resistance to various groups of chemical disinfectants (surfactants, oxidants, alcohols). This is due to a small choice of alternative groups of chemical disinfectants that meet the requirements of regulatory documents on toxicology, primarily for emergency rooms, neonatology departments, delivery wards and children's departments.

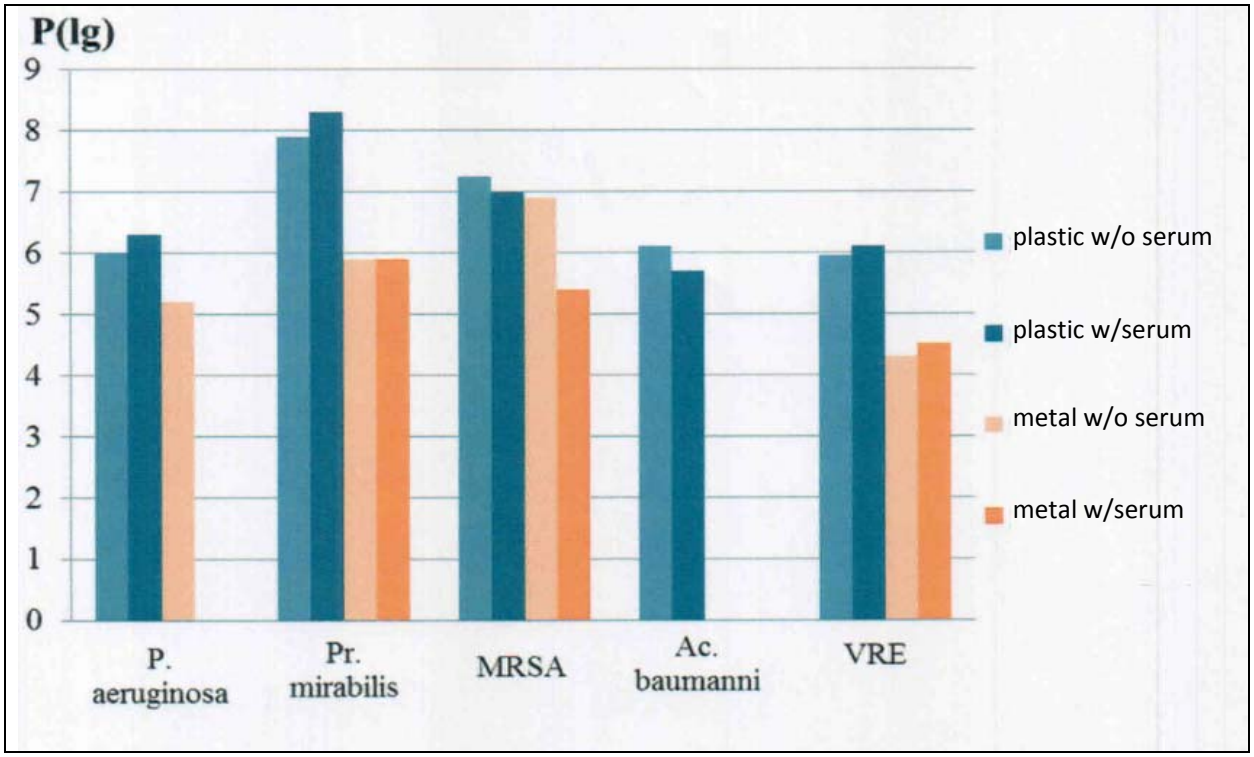


Fig. 4 Disinfection effectiveness of UIKb-01 Alpha system against vertically arranged test surfaces contaminated with hospital strains of microorganisms, at 5 minute exposure (lg).

Data in Tables 2 and 4 allows to conclude that effectiveness of pulsed ultraviolet irradiation of continuous spectrum against hospital strains of gram-positive and gram-negative microorganisms (except mycobacteria) does not depend on the presence of biological contaminants (40% serum) and ensures a guaranteed death of 99.99-99.999999% microorganisms. Difference of disinfection effectiveness of plastic and metal surfaces is not significant (1-2 lg).

Comparative tests with test strains of St aureus 907 and test strains of MRSa showed that results are completely identical (99.99999% effectiveness at 5 minutes of exposure). After 10 minutes of exposure only 1 colony survived on the three dishes contaminated with a hospital strain of staphylococcus aureus and none – on dishes with test strains. Fig 5.

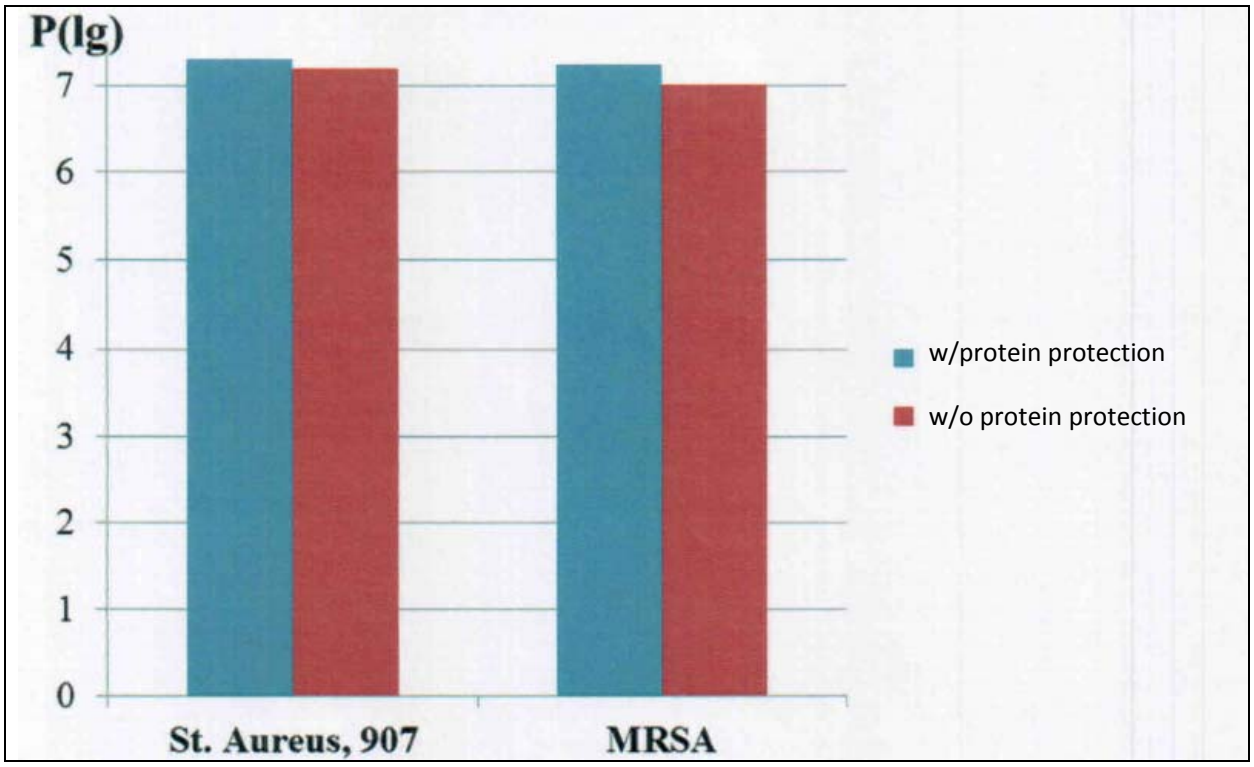


Fig.5. Disinfection effectiveness of UIKb-01 Alpha system against plastic test objects contaminated with a test strain of St aureus 907 and a hospital strain of MRSA, 5 minute exposure.

Microscopy and studies of biochemical properties of MRSA colonies still viable after 5 minute exposure shower that they lost coagulase activity, lecithinase and pigment.

Effectiveness of irradiation by UIKb-01 Alpha system against microorganisms spread on horizontally arranged metal test objects was studied on strains of P.aeruginosa and strains of St.aureus with multiple drug resistance to antibiotics and chemical disinfectants. The results are presented in Fig.6.

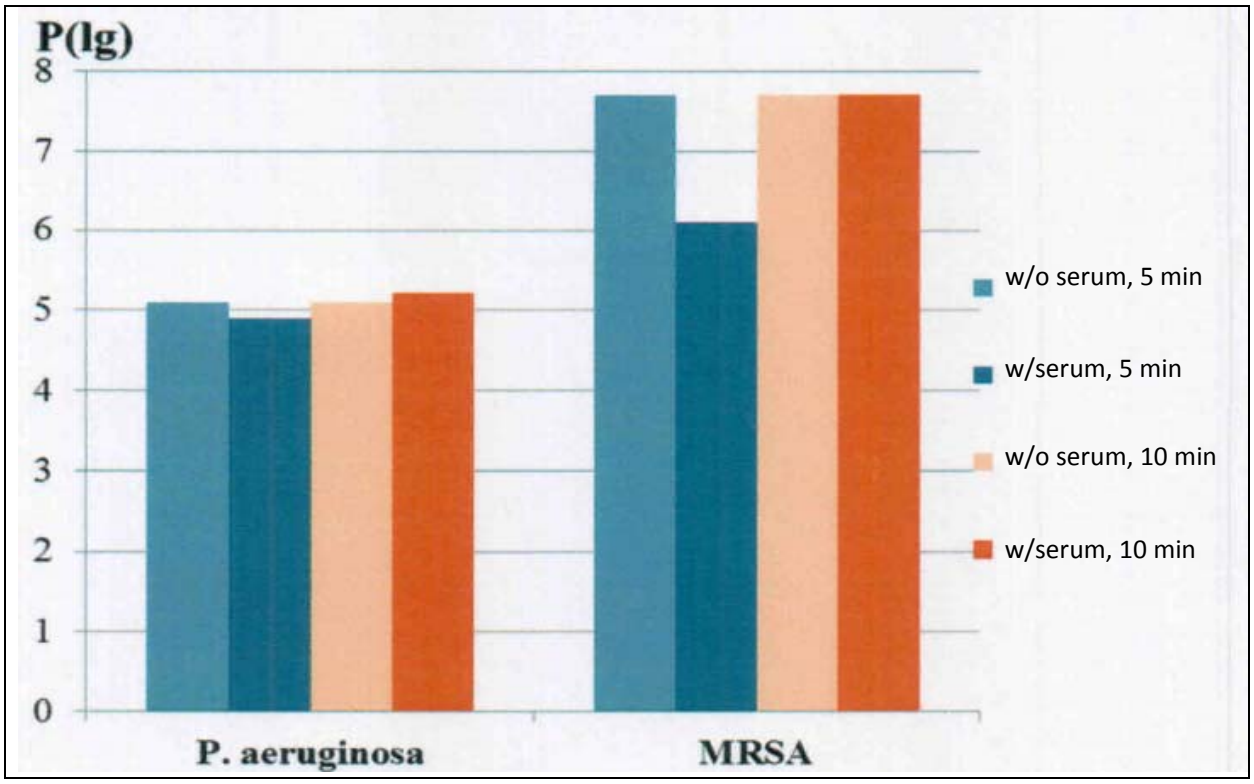


Fig.6. Disinfection effectiveness against metal plates contaminated with P. aeruginosa and MRSA, with horizontal arrangement and 5 minute exposure (in lg).

The obtained test data suggests that after only 5 minutes of irradiation the contamination destiny of metal plates contaminated with P. aeruginosa decreased by 4-5 lg (99.99-99.999%), and contamination density of plates contaminated with MRSA – by 6-7 lg (99.9999-100.00%). Such high effectiveness of surface disinfection method with short exposure (5 minutes) has a significant meaning for disinfection of rooms of A and B class (for example, between surgeries), when it is important to ensure highest effectiveness, short exposure time and lack of additional effort on the part of medical staff.

Laboratory experimental studies of plastic and metal test surfaces showed that it's staphylococci that are most sensitive to irradiation, both hospital strains and test ones. From 99.9999% to 99.99999% of staphylococci die after 5 minutes of irradiation. In two experiments on plastic and metal objects, all staphylococci died after 10 minutes of irradiation (one colony survived on 12 dishes with primary contamination density from $4x10^7$ to $5x10^8$KOE/cm^2). Considering that MRSA presents a significant problem to many health facilities, as it is the main etiological agent of purulent-septic infections, using UIKb-01 Alpha system to disinfect air and surfaces as part of disinfection measures presents a lot of interest.

CONCLUSIONS:

1. The tests showed that UIKb-01 Alpha pulsed ultraviolet system hashigh disinfection effectiveness for surfaces (99.99%-100.00%) against museum and hospital (multiresistant) bacterial strains under test, including the ones with high epidemic potential.
2. The tests proved that effectiveness of disinfection for plastic and metal surfaces against gram-positive and gram-negative bacteria under test with UIKb-01 Alpha pulsed ultraviolet system at

5-minute exposure is between 99.99% and 100% and does not depend on the orientation of test objects relative to the source of irradiation or presence/absence of biological protection.

3. The results of the tests make it possible to recommend health facilities to use Alpha pulsed ultraviolet systems as part of their disinfection measures, especially in A and B category rooms.

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<*Signature*> Alyoshkin V.A.
2 July 2012

LABORATORY TEST PROTOCOL
No. 005/12 of 27.06.2012

Name of the enterprise, organization (applicant): MELITTA Scientific and Production Company, Russia

Test aim: to determine effectiveness of pulsed ultraviolet radiation of continuous spectrum against multiresistant hospital strains of P. aeruginosa, MRSA, VRE in order to establish pulsed Alpha system application modes for open surfaces decontamination in health facilities.

Location and time of testing: Federal Budgetary Scientific Institution G.N. Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology of Federal Service on Consumer Rights Protection and Human Well-being, February – 21-26 June 2012

Materials and methods

Equipment: UIKb-01-Alpha pulsed xenon UV-bactericidal system with a remote control board and automatic operational time settings for express air disinfection in rooms of 1-5 categories up to 75 cubic meters in volume and with no people present, manufactured by Melitta Scientific and Production Company, Russia (Registration Certificate No.ФСР 2010/06905 of 26.02.2010; GOST R Certificate of Conformity No. РОСС RU.ИМ04.Н07593valid until 11.03.2013).

Test objects: plastic (single use Petri dishes).

Characteristics of hospital bacterial strains (from the museum of G.N. Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology):

***P. aeruginosa str.1098* –** high resistance to beta lactam antibiotics and carbapenems. Resistant to 14 preparations out of 17. Resistance to colistin and piperacillin/tazobactam is preserved. The strain showed resistance to 3 surface disinfectants used in health facilities in recommended modes.

***MRSA str.02840* –** resistant to beta lactam antibiotics, benzylpenicillin and polysynthetic penicillins (to 7 out of 16 antibiotics tested). Resistance to disinfectants not identified.

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Enterococcus faecium (vancomycin-resistant str. No.4 obtained from the collection of Smolensk Research Institute of Antimicrobial Chemotherapy) – resistant to vancomycin, ampicillin, gentamicin, tetracycline. Resistance to disinfectants not identified.

Methods used to study effectiveness of surface disinfection: A one-day suspension of a hospital strain or a test strain was modeled on standard industry turbidity sample No.20 (9lg) with saline and saline with ram blood serum (6 ml of saline, 4 ml of serum).

Vertically arranged cups (metal plates) contaminated with cultures of hospital strains with biological contaminant were irradiated at the distance of 1.5 meters from the lamp. Time of irradiation was 10 and 15 minutes.

Tests were conducted three times.

Effectiveness of treatment was calculated according to the following formula: (average number of colonies on control plates – average number of colonies on test dishes): average number of colonies on control dishes x 100.

Test results

microorganism	Factors	Results at irradiation time of		Average contamination density of the object before irradiation
		10 min	15 min	
MRSA str.02840	Number of surviving microbial cells	480 640 450 av.523	670 650 300 av.540	$8x10^8$ $9.4x10^8$ $8.2x10^8$ $av.8.5x10^8$
	Disinfection effectiveness, %	99.99994	99.99994	
Enterococcus faecium, str.4	Number of surviving microbial cells	180 120 60 av.120	160 112 115 av.129	$8.8x10^8$ $8x10^8$ $9.2x10^8$ $av.8.6x10^8$
	Disinfection effectiveness, %	99.99998	99.99998	
P. aeruginosa str. 1098	Number of surviving microbial cells	480 680 590 av.583	420 690 430 av.513	$9.0x10^8$ $6.5x10^8$ $8.5x10^8$ $av.8x10^8$
	Disinfection effectiveness, %	99.99993	99.99993	

Conclusion

1. Tests showed high effectiveness (**99.9999%**) of Alpha-05 pulsed ultraviolet system against multiresistant hospital strains of *Pseudomonas aeruginosa, Staphylococcus aureus (MRSA), vancomycin-resistant enterococcus (VRE)* applied to test surfaces,at 10 minute exposure and 1.5 meter distance from the object.
2. Alpha-05 pulsed ultraviolet systems can be recommended for use in health facilities as part of targeted preventive and anti-epidemic measures (disinfection of air and open surfaces,

including the ones contaminated with multidrug resistant hospital microorganisms), especially in rooms of A and B category, as the level of bactericidal effectiveness of the systems which was determined by the tests exceeds effectiveness criteria accepted for solutions of disinfectants used to treat surfaces of rooms and other objects in the hospital environment (99.9%). (Guide P 4.2.2643-10 ‘‘Methods of laboratory research and testing of disinfectants to determine their effectiveness and safety”, M. 2011).

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