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RESEARCH OF MYCOBACTERICIDAL ACTIVITY OF CONTINUOUS SPECTRUM PULSED ULTRAVIOLET LIGHT

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The article presents the experimental studies results of continuous spectrum pulsed UV light mycobactericidal activity against the laboratory strain of *Mycobacterium terrae* and clinical strains of *Mycobacterium tuberculosis* with multiple and extensive drug resistance. A pulsed xenon lamp of a mobile "Alfa-01" unit for air decontamination was used as ultraviolet light source. The experiments showed high activity of pulsed UV light against all studied strains, which does not depend on the distance to the treated surface (up to 4 m). The efficiency of contaminated surfaces disinfection reached 100%. Exposing contaminated objects to pulsed xenon UV lamps' light leads to multiple molecular genetic changes in DNA macromolecules with complete loss of drug resistance to rifampicin and partial loss of drug resistance to isoniazid.

Key words: Mycobacterium terrae, clinical strains of *Mycobacterium tuberculosis*, multiple and extensive drug resistance (MDR and XDR), continuous spectrum pulsed UV light, inactivation efficiency

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Disinfection policy at medical facilities is the principal prevention method of healthcare-associated infections (HAI). For almost every disinfection method there are different practices of air and surfaces disinfection with varying efficiency degree for all types of pathogens (bacteria, spores, viruses and fungi) [8, 11].

Tuberculocidal disinfection practices require a more detailed consideration. Use of disinfectants and decontamination equipment is one of the basic strategies for tuberculosis prevention. This disinfection type has strict requirements for preventing the Mycobacterium tuberculosis (MBT) introduction, its transmission inside or going out of the facility, as well as for working in tuberculosis outbreaks conditions [9]. Tuberculocidal disinfection mode must be present in operating instructions/manuals for disinfectants because it is a standard procedure not only at TB facilities, but also in general hospitals due to frequent cases of MBT introduction while hospitalizing of bacteriologically proven tuberculosis patients [9].

MBT, including its multiple and extensively drug resistant hospital strains, has a higher degree of resistance to disinfectants compared to vegetative bacterial forms [11]. The share of *M. tuberculosis* strains of the Beijing genetic family throughout Russian Federation has increased. This strain has high adaptability and drug resistance [10]. Most chemical disinfectants used for TB disinfection imply high concentrations and increased exposure time, which leads to increased toxicological hazard for patients and personnel, as well as to larger labor input and longer disinfection duration [8]. Quaternary ammonium compounds (most used at healthcare facilities) and guanidine derivatives have no expressed tuberculocidal effect [8].

In addition, the so-called human factor significantly reduces the efficiency of wiping with disinfectant solutions.

There are many ways and factors of tuberculosis transmission (airborn, air-dust, alimentary and contact-associated) that require both air and hospital surfaces disinfection.

Tuberculocidal disinfection poses the following requirements:

- Efficiency, confirmed by laboratory tests performed in certified centers, not only against approved archival microorganisms but also against clinical MBT strains, including strains with multiple and extensive drug resistance;
- Disinfection efficiency of at least 99.9% for indoor air and at least 99.99% for the surfaces;
- Low labor input and short disinfection cycle;
- Mitigation of the human factor impact.

The most efficient and meeting all the said requirements disinfection method is based on ultraviolet (UV) irradiation. For increased efficiency and reduced exposure time, powerful (over 1.5 kW) open-type UV units are used [15].

Currently, a fundamentally new disinfection technology is actively implemented in the world healthcare: exposure to high-intensity UV light of continuous spectrum (200-800 nm) generated by pulsed xenon lamps [2, 4, 14].

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The mechanisms of affecting the living matter of such lamps fundamentally differ from the widely-spread exposure to monochrome UV light of low-pressure mercury or amalgam lamps. A long-term implementation of pulsed UV units has shown high efficiency of air and surfaces disinfection with a short cycle duration [12, 13, 16].

Materials and methods

Research of mycobactericidal activity of continuous spectrum pulsed UV light against *Mycobacterium terrae*

In an accredited testing laboratory center of G.N. Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology of Rospotrebnadsor, an experimental study of continuous spectrum pulsed UV light efficiency against *Mycobacterium terrae* strains DSM 43227 ATCC 15755 has been performed. This strain is normally used for studying and estimating the tuberculocidal efficiency of disinfectants as per Procedural guidelines MU 3.5.2596-10.

A suspension of *Mycobacterium terrae* strain DSM 43227 ATCC 15755 dated June 2011, prepared according to the optical standard (109 CFU/mL), was used as a test microorganism for artificially contaminating the test surfaces (plastic Petri dishes).

A daily suspension of *Mycobacterium terrae* culture in normal saline was prepared according to industry standard turbidity samples of 1×10^9. Next, monitoring of the culture viability (at least three replications) was performed. In order to obtain a 1×10^7 suspension, two serial dilutions with culture viability control in three replications were performed [5].

Using a micropipette, 100 μL of the microbial mixture was applied to the bottom of a sterile Petri dish, rubbed up with a spatula, dried and placed vertically at a distance of 2 and 4 m from the lamp. The samples were irradiated for 2, 4, 8, 16, and 30 minutes. Then, 9.9 mL of sterile saline was added, the mixture was mixed with circular motions, and 3 serial dilutions were made. From each dilution, three inoculations were made onto slants with Löwenstein-Jensen medium. After 21-days' incubation at 37°C, all living cells were counted [5]. The control group followed the same scheme, but without irradiation.

Studies were performed in three replications for each time setting and distance from the source of radiation to the test surface.

Serially manufactured mobile pulse xenon UV bactericidal unit for emergency disinfection of air in 1st and 2nd category rooms in the absence of people UIKb-01-Alpha (hereinafter, Alpha-01) was used as a source of continuous spectrum pulsed UV light. Alpha-01 generated light flashes with a frequency of 2.5 Hz. The average electric power of the pulsed xenon lamp was 1 kW.

The mycobactericidal efficacy of pulsed UV irradiation (in %) with respect to *Mycobacterium terrae* was determined as the difference quotient between the average mycobacterial count in the control Petri dishes and Petri dishes exposed to irradiation, to the average mycobacterial count in the control dishes multiplied by 100 [3].

Research of mycobactericidal activity of continuous spectrum pulsed UV light against clinical strains of Mycobacterium tuberculosis with multiple drug resistance

The research was performed in the Moscow Scientific and Practical Center for Tuberculosis Control of the Moscow Healthcare Department. Ten multidrug-resistant M. *tuberculosis* strains (isoniazid- and rifampicin-resistant), freshly isolated from tuberculosis patients, were used for the research. Drug resistance of the test strains was pre-determined using the absolute concentration method as per Decree of the Ministry of Health of the Russian Federation No. 109 dated March 21, 2003 [7]. The studied strains were highly polymorphic (presence of flask-like and branching forms), accumulated chromosomal mutations and, as a result, had high drug resistance and higher capacity to resist external environmental factors when compared to sensitive strains [1, 12]. Besides, there are no efficiency studies of monochrome UV radiation of mercury lamps against such strains.

Due to high disease-inciting power of the strains, the research was performed in a laminar flow cabinet of the second biological safety degree, using a pulsed xenon lamp at 28 cm distance from the Petri dish with the treated mycobacterial suspension for 5 min. Mycobacteria dosage was standardized in accordance with standard No. 5 of the State Research Institute for Standardization and Control of Medicines (500,000,000 microbial cells per mL of sterile distilled water), then serially diluted to obtain the required microbial load (250 CFU/mL). The resulting suspension of each tested M. *tuberculosis* strain was divided into 2 groups. The first one deemed as control. It was transferred to a test tube with Shkolnikova's semisynthetic liquid medium with 10% of inactivated bovine serum, and was immediately transferred to another room (control) to avoid exposure to pulsed UV light. The second portion was put into a sterile Petri dish and exposed to pulsed UV light. At the end of the experiment, the treated suspension was transferred to the nearby room and immediately poured into a test tube with Shkolnikova's semi-synthetic liquid medium with 10% of inactivated bovine serum (experiment). Each test strain was irradiated separately in five repetitions. Upon completion of the experiments, the irradiated and control tubes were placed in a heat chamber for incubation. Results were obtained microscopically and macroscopically after expiration of optimal cultivation periods, and after *ad oculus* witnessing mycobacterial growth in control and test tubes.

To more fully understand inactivation mechanisms, molecular genetic changes in DNA of the same 10 strains, exposed to pulsed UV light, were studied. After exposure to pulsed UV light, DNA was isolated from MBT cells. DNA damages in MBT cells were determined using TB-BIOCHIP (MDR) biological microchips. These microchips were developed in the V.A. Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences. The results were recorded using the BIOCHIP-IMB portable biochip analyzer with the appropriate software.

The research was focused on the presence or absence of mutations that determine resistance to rifampicin in the RpoB gene (507-533 codons) responsible for the synthesis of RNA polymerase β-subunit, as well as in three genes responsible for isoniazid resistance: *kat*G gene (315-335 codons) responsible for catalase-peroxidase synthesis; *inh*A gene (promotor and structural regions) responsible for enol-ACP-reductase metabolism; and *ahp*C gene (promotor region) responsible for alkyl hydroperoxide reductase metabolism.

Research of mycobactericidal activity of continuous spectrum pulsed UV light against clinical Mycobacterium tuberculosis strains with extensive drug resistance (XDR MBT)

The research was also performed in the Moscow Scientific and Practical Center for Tuberculosis Control. For experiments were used control test-strains (*Mycobacterium terrae* (collection of G.N. Gabrichevsky Institute for Epidemiology and Microbiology), *Mycobacterium tuberculosis* H37RV), as well as 10 clinical *Mycobacterium tuberculosis* strains with extensive drug resistance (to isoniazid, rifampicin, aminoglycosides, ethambutol, pyrazinamide, capreomycin, ethionamide and fluoroquinolones). Drug resistance of the test strains was pre-determined using the absolute concentration method as per Decree of the Ministry of Health of the Russian Federation No. 109 dated March 21, 2003 [7] Mycobacteria dosage was standardized in accordance with Standard No. 5 of the State Research Institute for Standardization and Control of Medicines (500,000,000 microbial cells per mL of sterile distilled water), then serially diluted to obtain the required microbial load (250 CFU/mL). The resulting suspensions were divided into 2 groups (test and control), the same way as in the research of MDR *M. tuberculosis* strains. The microbial suspension in the control group was inoculated without irradiation. The second group (test) was exposed to pulsed UV light in a laminar flow cabinet at a 40 cm distance for 20 s, 1 min, or 3 min. After exposure, the second group was inoculated on liquid and solid media.

After inoculation, test and control tubes and Petri dishes were placed into a thermal incubation chamber and into a Bactec MGIT 960 automated incubation device. The results were obtained microscopically and macroscopically after the expiration of optimal cultivation periods (the same way as in research of MDR *M. tuberculosis* strains) and on the basis of Bactec MGIT 960 registration dates. In case of mycobacterial growth in the experimental group, drug resistance of *M. tuberculosis* after irradiation was re-evaluated. Mycobactericidal efficacy against MDR and XDR strains was evaluated by the absence (– no growth) or presence (+ growth) of MB CFU growth. The degree of test objects' contamination was expressed as follows: + presence of MB growth (1-20 CFU), ++ moderate MB growth (21-100 CFU); +++ heavy MB growth (> 100 CFU).

Study results

Results of *Mycobacterium terrae* contaminated objects' exposure to continuous spectrum pulsed UV light depending on the distance and irradiation time are shown in Fig. 1.

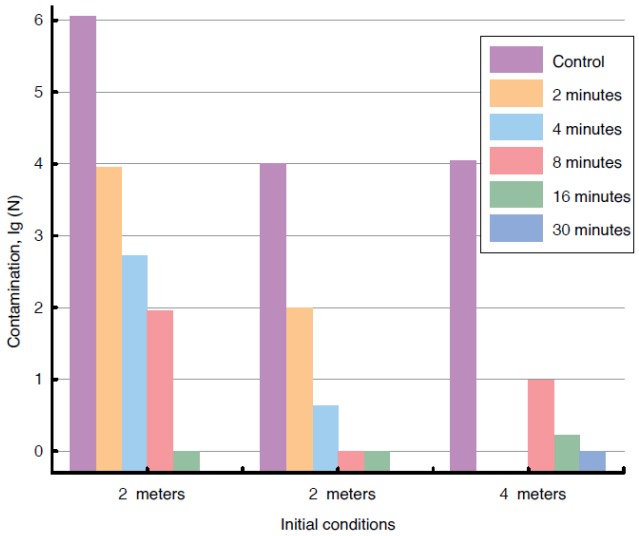

Fig. 1 *Reduction of test objects contamination (N) on a logarithmic scale under pulsed UV light impact at 2- and 4-meters' distance for Mycobacterium terrae.*

The obtained results show that the level of test objects' initial contamination has no influence on their disinfection efficiency. When the test objects were irradiated at 2-meters' distance from the lamp, the achieved disinfection efficiency was almost the same regardless of the initial contamination level. For 2-minutes' exposure at the initial contamination level of 10^9 CFU/mL, the efficiency reached 99.18%; at 10^7 CFU/mL it reached 98.9%. For 4-minutes' exposure, the disinfection efficiency was 99.95% and 99.96%, respectively; for 8-minutes' – 99.991% and 99.99%, respectively. This fact evidences the possibility to use pulsed UV units for surfaces disinfection room in high contamination conditions (preventive disinfection as per epidemiological indications, focal disinfection).

The experiments demonstrated, that 100% decontamination efficiency depends on the initial contamination. It was achieved in 16 and 30 minutes for samples with 10^7 and 10^9 CFU/mL contamination, respectively.

Obviously, the obtained decontamination efficiency results can be extrapolated for lower surface contamination levels of 100-200 CFU/cm^2, relevant for practical use at tuberculosis control facilities [5].

The obtained data allows to estimate the bactericidal surface dose (D90) of pulsed continuous spectrum UV light required for tenfold reduction of surface *Mycobacterium terrae* contamination: 30-32 J/m^2.

The results of disinfection efficiency research for test objects located at 2- and 4-meters' distance from the lamp with different exposure times are shown in Table 1.

Table 1. **Reduction of test objects' contamination with *Mycobacterium terrae* under pulsed UV light impact at various distances and with various exposure times**

Pulsed UV light impact at 2-meters' distance from the lamp						
Microbial suspension density 10^7 CFU/mL						
Dilution	Inoculum amount (CFU/mL)	Contamination density (CFU/mL), control	Surviving cells (CFU) after exposure to UV light for several minutes, test			
			2 min	4 min	8 min	16 min
10^{-3}	10,000	Mean 1.0×10^4	Mean 110	Mean 4.3	Mean 1.0	0
10^{-4}	1,000	Mean 0.9×10^3	Mean 0.3	0	0	0
10^{-5}	100	Mean 110	0	0	0	0
Efficiency, %			98.9	99.96	99.99	100
Pulsed UV light impact at 4-meters' distance from the lamp						
Microbial suspension density 10^7 CFU/mL						
Dilution	Inoculum amount (CFU/mL)	Contamination density (CFU/mL), control	Surviving cells (CFU) after exposure to UV light for several minutes, test			
			8 min		16 min	30 min
10^{-3}	10,000	Mean 1.06×10^4	Mean 9.3		Mean 1.7	0
10^{-4}	1,000	Mean 1.13×10^3	Mean 1.7		Mean 0.3	0
10^{-5}	100	Mean 113	0		0	0
Efficiency, %			99.8		99.97	100

The results show that 99.99% efficiency of continuous spectrum pulsed UV light against *Mycobacterium terrae* at 2-meters' distance from the lamp is achieved in 8 minutes; 100% effectiveness – in 16 minutes. The increase in the distance between the light source and contaminated surface to 4 m led to increase of exposure time for maintaining the required efficiency of continuous spectrum pulsed UV light: 99.98% efficiency was achieved in 16 minutes; 100% – in 30 minutes.

It is known that surface irradiance decreases in reverse proportion to the squared distance from the light source, which requires a proportional increase in the exposure time to ensure the similar decontamination efficiency level. The experiments showed, that the exposure time increase was proportional to the distance to the treated object (for example, 100% efficiency was achieved in 30 minutes, and not in 60 minutes according to the calculation), which evidences increased reflected light rate in the total UV light flux. Therefore, it can be stated with certainty, that with increasing distance, the continuous spectrum pulsed UV light activity remains unchanged.

Research of continuous spectrum pulsed UV light activity against multidrug-resistant M. tuberculosis strains showed 100% tuberculocidal effect on all 10 strains, newly isolated from patients (Table 2).

Table 2. **Evaluation of continuous spectrum pulsed UV light mycobactericidal activity against 10 clinical strains of *Mycobacterium tuberculosis* with multiple drug resistance**

No. of test culture	*M. tuberculosis* growth after exposure to pulsed UV light	
	test	control
1–10	-	++

Research of molecular genetic changes in DNA of the same 10 strains treated by pulsed UV light demonstrated (Table 3), that no fragments of DNA macromolecules were detected in 50% of cases (Nos. 3, 6, 7, 9, 10) after irradiation of MBT cells. DNA fragments containing information on the presence of mutations responsible for rifampicin- and partially to isoniazid-resistance were lost after irradiation in 40% of cases (Nos. 1, 4, 5, 8).

The obtained results allow for the conclusion, that deep photochemical oxidation occurs in the DNA macromolecule nitrogenous bases and nucleotides.

Moreover, in contrast to monochrome radiation of mercury bactericidal lamps, the oxidation is multichannel rather than selective: damage (mutations) is observed simultaneously in all nucleotides, including DNA segments responsible for multidrug resistance.

The results of continuous spectrum pulsed UV light activity research in respect to clinical XDR strains of *Mycobacterium tuberculosis* are presented in Table 4.

It is apparent, that absolute tuberculocidal effect was achieved after 3-minutes' exposure for all test strains. 100% disinfection efficiency was achieved after 20 s unit operation for 10 out of 12 strains. Clinical strains Nos. 8, 11 demonstrated highest resistance to pulsed UV light.

Table 3. **Changes in various DNA fragments' codons of MDR *Mycobacterium tuberculosis***

No.	Gene responsible for resistance to Rifampicin		Genes responsible for resistance to Isoniazid					
	RpoB		κatG		inhA		oxyR – ahpC	
	Present	Absent	Present	Absent	Present	Absent	Present	Absent
1	531 Leu	No	315Arg	315Arg	A8, G16	G16	No	No
2	No	No	315Ser	315Ser	G16	G16	No	No
3	516 Val	No	315Gly	No	G16	No	A10	No
4	516 Val	No	315Arg	No	G8	G8	No	No
5	531 Leu	No	315Thr	315Thr	No	No	No	No
6	531 Leu	No	No	No	No	No	No	No
7	526 Leu	No	315Thr	No	No	No	T10	No
8	511 Pro	No	315Arg	315Arg	G16	No	No	No
9	No	No	315Thr	No	No	No	No	No
10	No	No	315Thr	No	No	No	No	No

Table 4. **Continuous spectrum pulsed UV light mycobactericidal activity against test strains of *Mycobacterium terrae*, *Mycobacterium tuberculosis* H_{37}RV and clinical XDR strains of *Mycobacterium tuberculosis***

Test strains	Mycobacterial growth after light exposure					
	test			control		
	20 s	1 min	3 min	20 s	1 min	3 min
Mycobacterium terrae ATCC 15755	-	-	-	+++	+++	+++
Mycobacterium tuberculosis H_{37}Rv ATCC 25618	-	-	-	+++	+++	+++
Clinical strains Nos. 3, 4, 5, 6, 7, 9, 10, 12	-	-	-	+++	+++	+++
Clinical strain No. 8	+++	+++	-	+++	+++	+++
Clinical strain No. 11	++-	++-	-	+++	+++	+++

Notes: - no MBT growth; + MBT growth; ++- moderate growth (21-100 cfu); +++ heavy growth

Conclusions

1. Continuous spectrum pulsed UV light is highly efficient against all studied test strains: laboratory test strains (*Mycobacterium terrae, Mycobacterium tuberculosis* H_{37}Rv) and clinical strains of *Mycobacterium tuberculosis* with multiple and extensive drug resistance. The experiment showed the possibility of disinfecting contaminated surfaces with 100% efficiency from the most resistant and epidemiologically significant tuberculosis strains.

2. High-intensive UV light of pulsed xenon lamps triggers numerous molecular genetic changes in the DNA macromolecule with a complete loss of rifampicin-resistance and partial loss of isoniazid-resistance.

3. Mycobactericidal activity of continuous spectrum pulsed UV light does not depend on the distance between the light source and the treated surface (within the studied 0.3-4 m range).

4. The obtained results allowed for developing a tuberculocidal mode of pulsed units' operation, ensuring surfaces disinfection with 99.99% efficiency after 5-15-minutes' exposure.

5. The combination of proven high efficiency of room disinfection and minimum exposure time of pulsed UV units with xenon lamps allows for recommending their use in the disinfection measures complex (preventive and focal disinfection) at tuberculosis facilities and health facilities with high risks of introducing, spreading and transferring tuberculosis pathogens including their MDR and XDR strains.

Conflict of Interests. The authors state that they have no conflict of interests

References

1. Vishnevskiy B.I. Drug resistance of Mycobacterium tuberculosis. Lecture. Meditsinsky Alyans, 2017, no. 1, pp. 29-35. (In Russ.)
2. Goldshteyn Ya.A., Golubtsov A.A., Shashkovskiy S.G. Air and open surfaces decontamination with pulsed UV light at medical facilities. Poliklinika, 2014, no. 3 (2), pp. 51-54. (In Russ.)
3. Ispolzovanie ultrafioletovogo izlucheniya dlya obezzarazhivaniya vozdukha v pomescheniyakh. Rukovodstvo R 3.5.1904-04. [Use of pulsed UV light for indoor air decontamination. Guidelines R 3.5.1904-04]. Moscow, Federalny Tsentr Gossanehpidnadzora Minzdrava Rossii Publ., 2005, 46 p.
4. Kamrukov A.S., Kozlov N.P., Ushakov I.B., Shashkovskiy S.G. Development and implementation of pulsed plasma-optical technologies and units in space medicine and practical healthcare. Gazeta Vestnik MGTU Im. N. E. Baumana, 2011, pp. 107-119. (In Russ.)
5. Metody izucheniya i otsenki tuberkulotsidnoy aktivnosti dezinfitsiruyuschikh sredstv. Metodicheskie ukazaniya MU 3.5.2596-10. [Research and assessment of disinfectants tuberculocidal activity. Guidelines MU 3.5.2596-10]. Federalny Tsentr Gigieny i Epidemiologii Rospotrebnadzora Publ., Moscow, 2010, 54 p.
6. Mordovskojy G.G., Ponikarovskaya I.V., Yakushkina A.YUu., Putyrskiy V.P., Yampolskiy V.A., Panov G.V. Mycobacterium tuberculosis detection methods during sanitary-and-bacteriologic assessment of air and surfaces contamination at tuberculosis facilities. Journal of Ural Medical Academic Science (Vestnik Uralskoy Meditsinskoy Akademicheskoy Nauki), 2015, no. 2 (53), pp. 16-18. (In Russ.)
7. Decree of the Russian Ministry of Health No. 109 dated March 21, 2003. On improvement of tuberculosis control measures in the Russian Federation. (In Russ.)
8. Dezinfektologiya. Metody laboratornykh issledovaniy i ispytaniy dezinfektsionnykh sredstv dlya otsenki ikh effektivnosti i bezopasnosti. Rukovodstvo R 4.2.2643-10. 3.5. [Disinfectology. Laboratory research and testing of disinfectants to evaluate their efficiency and safety. Guidelines P 4.2.2643-10. 3.5.] Federal Center of Hygiene and Epidemiology of the Federal Service on Customers' Rights Protection and Human Well-Being Surveillance, Moscow, 2010, 615 p.
9. Sistema infektsionnogo kontrolya v protivotuberkuleznykh uchrezhdeniyakh. Rukovodstvo. [Infection control at tuberculosis facilities. Guidelines]. L.S.Fedorova, eds., Moscow, Tver, OOO Izdatelstvo Triada Publ., 2013, 192 p.
10. Situatsiya po tuberkulezu v 2016 g. Analiticheskie obzory po tuberkulezu. Tsentr monitoringa tuberkuleza. [TB situation in 2016. Analytic review of tuberculosis. Tuberculosis monitoring center]. State Budgetary Healthcare Institution “Central Scientific Research Institute of healthcare organization and informational support”, http://mednet.ru/index.php?Itemid=137 Moscow, 2016, 69 p.
11. Shestopalov N.V., Panteleeva L.G., Sokolova N.F., Abramova I.M., Lukichev S.P. Federalnye klinicheskie rekomendatsii po vyboru khimicheskikh sredstv dezinfektsii i sterilizatsii dlya ispolzovaniya v meditsinskikh organizatsiyakh. [Federal clinical practice guideline for choosing chemical agents for disinfection and sterilization at healthcare facilities]. Moscow, 2015, 67 p.
12. Chan-Ick Cheigh, Mi-Hyun Park, Myong-Soo Chung, Jung-Kue Shin, Young-Seo Park. Comparison of intense pulsed light- and ultraviolet (UVC)-induced cell damage in Listeria monocytogenes and Escherichia coli O157:H7. Food Control 25. 2012, pp. 654-659.
13. Guillou S., Leroi F., Orange N., Bakhrouf A., Federighi M., Elmnasser N. Pulsed-light system as a novel food decontamination technology: a review. Canad. J. Microbiology, 2007, vol. 53, no. 7, pp. 813-821.
14. http://www.thecleanzine.com/hospital_hygiene.php
15. https://www.melitta-uv.ru, https://www.xenex.com
16. Ren Zhuo Chen, Stephen A. Craik, James R. Bolton Comparison of the action spectra and relative DNA absorbance spectra of microorganisms: Information important for the determination of germicidal fluence (UV dose) in an ultraviolet disinfection of water. Water Research, 2009, vol. 43, pp. 5087-5096