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APPROVED BY
Head of the FSBI "48th CRI"
of the Russian Ministry of Defense
Doctor of Biological Sciences, Professor,
Corresponding Member of RAS

S.V. Borisevich

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TEST PROTOCOL
on “Alfa-06” and “Alfa-09” units’ disinfection efficiency on metal surfaces
contaminated with the SARS-CoV-2 coronavirus

1 TEST OBJECT

1.1 The test objects are the “Alfa-06” and “Alfa-09” units.

2 TEST OBJECTIVES AND PURPOSES

2.1 Test objective is to determine the disinfection efficiency on metal surfaces contaminated with the SARS-CoV-2 coronavirus (hereinafter “coronavirus”) by “Alfa-06” and “Alfa-09” units.

2.2 Test purposes:

2.2.1 Determining the initial contamination level of the test surfaces before irradiating them with “Alfa-06” and “Alfa-09” units;

2.2.2 Determining the residual contamination level of the test surfaces after irradiating them with “Alfa-06” unit (sample No. 1);

2.2.3 Determining the residual contamination level of the test surfaces after irradiating them with “Alfa-09” unit (sample No. 2).

3 GENERAL PROVISIONS

3.1 The tests were performed in accordance with the main requirements of Sanitary-Epidemiological Guidelines SP 1.3.3118-13 “Safety of works with the microorganisms of I-II risk groups” and the Guideline R 3.5.1904-04 “Germicidal ultraviolet light use for indoor air disinfection”.

3.2 Estimated factors and design ratios

3.2.1 For the tests were used 5×5 cm stainless steel (grade 12X18H10T) surfaces.

The test objects were placed at 1-meters’ height from the floor and at 2-meters’ distance from the “Alfa-06” unit, or 1.5-meters’ distance from the “Alfa-09” unit.

The units were placed in accordance with the schemes provided by the ordering customer.

3.3.2 The coronavirus biological amplification (biological potency, contamination levels) was determined with help of mattresses with a monolayer of day-old Vero C1008 cells (25 cm² surface) with agar overlay medium by the negative selection method.

3.3.3 The biological potency was calculated as per formula 1:

$$A = \frac{a_{cp} \cdot b_n}{c}, \quad (1)$$

where A is the biological potency, PFU·ml⁻¹;

a_{cp} – the weighted average of negative colonies count on a mattress calculated as per formula 2, PFU;

b_n – the highest dilution ratio;

c – the inoculated volume, ml.

$$A_{cp} = \frac{a_1 + a_2 + \dots + a_n}{b_n \left(\frac{1}{b_1} + \frac{1}{b_2} + \dots + \frac{1}{b_n} \right)}, \quad (2)$$

where a_1 - a_n is the mean negative colonies count for dilutions of the test material numbers 1 through n ;

b_1 - b_n – the dilution ratio of the test material.

3.3.4 The test objects contamination levels for initial and secondary contamination, correspondingly, were calculated as per formula 3:

$$A_s = \frac{A \cdot V}{S}, \quad (3)$$

where A_s is the contamination level, PFU·cm⁻²;

V – the sorbing liquid volume used for wipe sampling of the biological material from the surface, ml;

S – the test-object contamination area, cm².

3.3.5 The statistical analysis of the obtained data was performed by calculating the standard deviation following the results of 5 tests.

4 MATERIALS AND METHODS

4.1 Devices and equipment required for the tests:

- “Alfa-06” unit;
 - “Alfa-09” unit;
 - MIR 254-PE thermostat, 240 L, manufactured by Panasonic;
 - electric medical instruments sterilizer, GOST 19596-89E;
 - utility-type refrigerator “Samsung”, 2 compartments, model RL44ECPS;
 - automatic thermostat “Binder”, 20-60 degrees’ temperature control, 53 dm³ volume, manufactured in the USA;
 - biosafety cabinet of II class, A2 type, BAVp-01 “Laminar-S” (220.150) “Lamsystems”, ZAO “Laminarnye sistemy”;
 - utility-type electric hot plate, GOST 14919-83E;
 - bacteriologic test tubes, GOST 23932-82E;
 - rubber cone-type corks, GOST 7852-76;
 - measuring pipettes 1.0, 5.0, and 10 cm³, accuracy class 2, GOST 20292-74E;
 - conical glass vessels, 100 and 250 cm³, thermally and chemically resistant, GOST 25336-82;
 - glass spreaders, FSBI "48th CRI" of the RF Ministry of Defense;
 - ophthalmic pointed scissors, twisted, Interrepublican Technical Regulations 42-64-66;
 - medical forceps, GOST 21241-75;
 - laboratory alcohol lamp, GOST 25336-82E;
 - laboratory clamp stand, SILB type, Technical Regulations 79 RF 265-72-5;
 - rubber ball syringe, Technical Regulations 38.106-141-80;
- The measuring equipment and devices used for the tests are metrologically checked and accredited as per GOST PB 8.576-2000 and GOST B 008.002-2013.
- ### 4.2 Consumables and chemical reagent required for the tests:
- reference SARS-CoV-2 virus culture (isolate B) dated March 16, 2020 with biological potency 5.8·10⁶ PFU·ml⁻¹;
 - Vero 1008 cells culture;

- bio agar (manufactured by Difco);
- fetal calf serum (manufactured by HyClone);
- antibiotics for cell cultures (manufactured by HyClone);
- neutral red (vital red for cell cultures);
- Hanks', Earle's, VKA, sodium bicarbonate, glutamate solutions (manufactured by the procedure of the FSBI "48th CRI" of the RF Ministry of Defense);
- saline solution as per PR-07-320-35, prescription of the FSBI "48th CRI" of the RF Ministry of Defense;
- rectified ethyl alcohol, GOST 18300-72;
- distilled water, GOST 6709-72;
- glass pencil, Technical Regulations 46-22-904-78;
- medicated cotton wool, GOST 5556-81;
- medical gauze, Technical Regulations 388-23-501-79;
- surgical gloves, GOST 3-88;
- technical-grade A grade hydrogen peroxide, Technical Regulations 2123-002-25665344-2008.

4.3 The personnel performing the tests with microorganisms of I-II risk groups must have working and protective clothing, personal protective equipment and personal hygiene means as per standardized regulations.

4.4 The tests were performed in the “contagious” area in maximally isolated laboratory conditions.

5 TESTS CONDITIONS AND PROCEDURE

5.1 The tests were performed in a “contagious” area workroom at (24±1) °C ambient temperature and (42±3) % relative humidity.

The initial biological potency of the SARS-CoV-2 virus culture (isolate B dated March 16, 2020) was $5.8 \cdot 10^6$ PFU·ml⁻¹.

5.2 For the tests were used 5×5 cm test objects contaminated with the coronavirus.

5.3 The units were allocated as follows:

- “Alfa-06”: the unit was situated sideways, at 2-meters’ distance against the test objects placed vertically at 1-meter’s height. The modes “Surface/bactericidal” (hereinafter “mode No. 1”), “Surface/sporicidal” (hereinafter “mode No. 2”) and “Surface/tubercular” (hereinafter “mode No. 3”) were used in sequence;

- “Alfa-09”: the unit was situated sideways, at 1.5-meters’ distance against the test objects placed vertically at 1-meter’s height. The modes “Surface/bactericidal” (hereinafter “mode No. 4”), “Surface/sporicidal” (hereinafter “mode No. 5”) and “Surface/tubercular” (hereinafter “mode No. 6”) were used in sequence.

For each unit, each treatment mode was used in sequence.

5.3 The tests were performed as per standard procedure developed by the FSBI "48th CRI" of the RF Ministry of Defense and as per Guideline R 4.2.2643-10.

5.5 During the first stage of the tests, the monolayer of day-old Vero cells culture was prepared on the matrass. The growth medium was then poured off from

the matrasses with the cells monolayer, selected for testing, and the matrasses were labeled.

5.6 0.1 ml of the coronavirus stock culture with the biological potency of $1.0 \cdot 10^6$ PFU·ml⁻¹ was applied onto test objects. The amount was evenly spread on the test object surface with a glass spreader.

5.7 The test objects were divided into test and control groups. The control test objects were used for control at the moment of applying the coronavirus and during the decontamination. For this purpose, 5 test objects were taken.

5.8 Samples were taken from the test objects by swabbing with two gauze tampons (moistened and dry). Each sample was hand shaken for 3 minutes in 10 ml of sorbing liquid.

5.9 To determine the coronavirus biological potency, 0.5 ml of the correspondingly diluted sorbing liquid were added to each matrass with the monolayer. The inoculate was evenly spread on the monolayer by swaying the matrass.

5.10 The matrasses were placed horizontally with the infected cells monolayer facing down. The matrasses were incubated for 60 minutes in the thermostat at (37.0 ± 0.5) °C.

5.11 After incubating, the inoculate was removed with a pipette and 10 ml of primary agar overlay medium, brought to the temperature of (42.0 ± 0.5) °C, were added to each matrass. Then the matrasses were placed horizontally with the infected cells monolayer facing down.

5.12 After the overlay medium solidified (10-15 min), the matrasses were turned with the monolayer up and placed in the thermostat at (37.0 ± 0.5) °C for 48 hours.

5.13 After the incubation, 10 ml of secondary agar overlay medium with neutral red were added to the matrasses for staining the monolayer. After that the matrasses were incubated for another 24 hours at (37.0 ± 0.5) °C. Then the negative colony counts were calculated.

5.14 The tests were performed under the following controls:

- control of the initial coronavirus biological potency;
- control of the diluting (sorbing) liquid;
- control of the Vero 1008 cells cultures.

6 RESEARCH RESULTS

6.1 The results of determining the initial and residual coronavirus contamination level of the test objects as well as their disinfection efficiency by the “Alfa-06” unit for the modes No. 1-3 are given in Table 1. The exposure time was 2.0 minutes for the mode No. 1; 3.0 minutes for the mode No. 2; and 6.5 minutes for the mode No. 3.

Table 1 – Disinfection efficiency of test objects by the “Alfa-06” unit, n=5, $\bar{x} \pm \bar{\sigma}$

Surface type and size of the test object	Mean initial contamination level of the test object at the time point..., $n \cdot 10^3 \text{ PFU} \cdot \text{cm}^{-2}$		Residual contamination level for the mode..., $\text{PFU} \cdot \text{cm}^{-2}$					
			No. 1		No. 2		No. 3	
	0 min	6.5 min	single	mean	single	mean	single	mean
Stainless steel 12X18H10T, 5×5 cm	3.62±0.28	2.95±0.16	0.8 1.6 0 0.8 0.4	0.72±0.60	0 0 0 0 0	0	0 0 0 0 0	0

6.2 The results of determining the initial and residual coronavirus contamination level of the test objects as well as their disinfection efficiency by the “Alfa-09” unit for the modes No. 4-6 are given in Table 2. The exposure time was 3 minutes 39 seconds for the mode No. 4; 6 minutes 45 seconds for the mode No. 5; and 14 minutes 03 seconds for the mode No. 6.

Table 2 – Disinfection efficiency of test objects by the “Alfa-09” unit, n=5, $\bar{x} \pm \bar{\sigma}$

Surface type and size of the test object	Mean initial contamination level of the test object at the time point..., $n \cdot 10^3 \text{ PFU} \cdot \text{cm}^{-2}$		Residual contamination level for the mode..., $\text{PFU} \cdot \text{cm}^{-2}$					
			No. 4		No. 5		No. 6	
	0 min	14.0 min	single	mean	single	mean	single	mean
Stainless steel 12X18H10T, 5×5 cm	3.62±0.28	2.63±0.21	0.4 0.8 0.4 0 0.8	0.48±0.44	0 0 0 0 0	0	0 0 0 0 0	0

6.3 The results given in Table 1 show that the irradiation generated by the “Alfa-06” unit leads to decontamination of metal test objects contaminated by the coronavirus (3.62 ± 0.28) $\text{PFU} \cdot \text{cm}^{-2}$. At that, the disinfection efficiency reaches 99.98 % for the “Surface/bactericidal” mode; and 100 % (complete decontamination) for the “Surface/sporicidal” and “Surface/tubercular” modes.

6.3 The results given in Table 2 show that the irradiation generated by the “Alfa-09” unit also leads to decontamination of metal test objects contaminated by the coronavirus (3.62 ± 0.28) $\text{PFU} \cdot \text{cm}^{-2}$. At that, the disinfection efficiency reaches 99.99 % for the “Surface/bactericidal” mode; and 100 % (complete decontamination) for the “Surface/sporicidal” and “Surface/tubercular” modes.

7 CONCLUSION

7.1 The “Alfa-06” unit ensures the disinfection of metal surfaces contaminated by the SARS-CoV-2 coronavirusom with 99.98 % efficiency when using the “Surface/bactericidal” mode; and with 100 % efficiency when using the “Surface/sporicidal” and “Surface/tubercular” modes.

7.2 The “Alfa-09” unit ensures the disinfection of metal surfaces contaminated by the SARS-CoV-2 coronavirusom with 99.99 % efficiency when using the “Surface/bactericidal” mode; and with 100 % efficiency when using the “Surface/sporicidal” and “Surface/tubercular” modes

7.3 The obtained results allow for recommending the “Alfa-06” and “Alfa-09” units’ use for surfaces disinfection as part of epidemiological response to combat the coronavirus infection spread.

Head of Research unit 10

lieutenant colonel

Senior research scientist, Research unit 10

Senior research scientist, Research unit 10

Senior research scientist, Research unit 1

Research scientist, Research unit 1

Research scientist, Research unit 10

Research scientist, Research unit 10

Research scientist, Research unit 10

Junior research scientist, Research unit 10

A. Zverev

D. Masyakin

N. Chepurenkov

I. Shatokhina

N. Boyarskaya

V. Bykov

I. Androshchuk

E. Kovalchuk

V. Trufanova