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MEDICAL BIOTECHNOLOGY

The Effect of Disinfectants on the SARS-CoV-2 RNA Detection in Swabs from Various Surfaces

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Abstract—The COVID-19 pandemic has spread rapidly around the world; some countries have introduced controls on imported products, including testing for viral nucleic acids. In this work, the influence of disinfectants for treating various SARS-CoV-2-contaminated surfaces on the detection of viral RNA fragments in swabs from these surfaces was analyzed using quantitative RT-PCR. Quaternary ammonium salt, hydrogen peroxide, 1-propanol, and sodium salt of dichloroisocyanuric acid, as well as ultraviolet irradiation, were tested as such disinfecting agents. Our results show that without exposure to disinfectants, viral RNA can be detected on the surface of all examined materials for at least 3 days. UV irradiation or irrigation with a disinfectant containing 0.2% active chlorine had the greatest effect on the decontamination of nonporous surfaces as measured by RT-PCR of swabs from these surfaces. Irrigation with disinfectants of porous surfaces (cardboard) had practically no effect on the detection of SARS-CoV-2 RNA by RT-PCR.

Keywords: SARS-CoV-2, viral RNA, RT-PCR, disinfectants, UV irradiation, surface swabs

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INTRODUCTION

The *Coronavirus* infection dated 2019 (COVID-19) is a respiratory infection, whose etiological agent is the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which appeared in China at the end of 2019 and then rapidly spread around the globe. The person-to-person transmission of the virus occurs as a result of direct or indirect contact, as well as by airborne droplets. The summary of the research of the World Health Organization (WHO) from July 9, 2020 provides information on the possibility of virus transmission through surfaces located in the immediate vicinity of a patient with subsequent contact of these surfaces with mucous membranes of another person (WHO official website https://apps.who.int/iris/bitstream/handle/10665/333114/WHO-2019-nCoV-Sci_Brief-Transmission_modes-2020.3-rus.pdf). However, this document emphasizes that despite the evidence of contamination of surfaces by SARS-CoV-2 and its ability to survive on certain surfaces, there are no publications yet that directly describe cases of virus transmission in this way, which is primarily due to the fact that it is difficult to distinguish between two paths of virus transmission, that is, by airborne droplets and through contaminated surfaces. Nevertheless, this route of SARS-CoV-2 spreading is believed to be possible, given the fact that this is a path through which coronaviruses and other respiratory viruses can be transmitted.

From the beginning of the pandemic, numerous laboratories have studied the viability of SARS-CoV-2 on various surfaces, but have not made unambiguous conclusions. In some works, the stability of SARS-CoV-2 and SARS-CoV-1 (Coronavirus that caused atypical pneumonia (SARS) in 2002–2004) was compared; it was established that both can remain infectious on a plastic surface for up to 3 days [1, 2]. The effect of temperature on the inactivation rate of SARS-CoV-2 has been shown; at 4°C, the virus infectivity decreased insignificantly within 14 days, while at 70°C, it was completely inactivated within 5 min [3]. With an initial viral load equivalent to that detected in sick people, SARS-CoV-2 remained viable for 28 days at 20°C on surfaces such as glass, stainless steel and paper banknotes [4]. At 40°C, the virus retained its viability for less than 24 h on most of the studied surfaces. In the work of M.A. Nikifirova et al. [5], it was shown that the virus completely lost its viability 120 min after drying on any surface. It is important to note that viral RNA continues to be detected by RT-PCR on all surfaces even after SARS-CoV-2 inactivation [3, 5].

WHO officials have reported that the likelihood of COVID-19 transmission through food products or

Abbreviations: C_t, threshold cycle; dNTP, deoxyribonucleoside triphosphate; E, amplification efficiency; *M*–slope; R^2, correlation coefficient; RT-PCR, reverse transcription polymerase chain reaction; UV, ultraviolet irradiation; WHO, World Health Organization.

packaging is extremely small and cannot be regarded as an obstacle to international trade (official website of Rosselkhoznadzor https://fsvps.gov.ru/fsvps/download/attachment/199798/282_Cov_HPAI_ASF_Rab_-21-12-2020_ALERT_282.pdf); however, certain countries have special requirements for imported goods, in particular for food products. As an example, in November 2020, the Government of the People's Democratic Republic of China ordered the state customs authorities to strictly control imported frozen food (website of the TASS news agency, Russia, https://tass.ru/obschestvo/10154431?nw=1606978459000). According to documents released by the State Council of China, the stimulation of research aimed at the detection of SARS-CoV-2 RNA in food cold chain products is recommended for COVID-19 prevention and control. In January 2021, information appeared that in the northeast of the Chinese territory SARS-CoV-2 was founded in a batch of ice cream, whose components were purchased in New Zealand and Ukraine. In early February 2021, the PDRC Customs Administration reported that Guangdong province authorities identified the SARS-CoV-2 genome in 13 batches of products from three Russian enterprises that manufacture poultry meat and by-products, which were certified for export to China (website of the TASS news agency, Russia, https://tass.ru/obschestvo/10617867).

In view of the current situation, the laboratories of the Rosselkhoznadzor are analyzing swabs from exported food products to identify the SARS-CoV-2 genome.

At present, disinfection of surfaces is carried out in accordance with the recommendation of the Research Institute of Disinfectology, Rospotrebnadzor (http://niid.ru/press/public/145866/), taking the temporary WHO instruction on cleaning and disinfection of environmental surfaces in the context of COVID-19 into account (WHO official website https://www.who.int/publications/i/item/cleaning-and-disinfection-of-environmental-surfaces-inthe-context-of-covid-19).

The goal of this work was to assess the effect of various disinfecting agents used for surface treatment on the detection efficiency of SARS-CoV-2 RNA fragments.

MATERIALS AND METHODS

Materials

A thermally inactivated swab containing the SARS-CoV-2 virus supplied by the Smorodintsev Research Institute of Influenza (St. Petersburg, Russia) was used as the initial sample. The RNA concentration was determined using parallel amplification of the sample and 10-fold dilutions of the positive control. The latter contained a gene-engineered plasmid vector with the target SARS-CoV-2 genomic material, fragments of the *N* and *E* genes obtained by amplification with specific primers recommended by the WHO (https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287-a1b17c111902.pdf).

The RNA concentration in the initial inactivated sample was 10^7 copies per mL (cop/mL).

RNA Isolation and Quantitative RT-PCR

Total RNA from the initial sample and swabs from various surfaces was extracted using a RIBO-prep kit for the isolation of nucleic acids (Central Scientific Research Institute of Epidemiology, Rospotrebnadzor, Russia).

PCR was carried out according to the technique developed in the All-Russian State Center for Quality and Standardization Medicines for Animals and Feed, which allowed the detection of fragments of the SARS-CoV-2 *N* and *E* genes using primers and probes recommended by the WHO (https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287-a1b17c111902.pdf). Amplification was performed within the framework of multiplex RT-PCR with real-time hybridization fluorescence detection using a Rotor Gene Q 6 Plex device (Qiagen, Germany). The procedure had the following parameters: 52°C for 30 min; then 5 cycles according to the scheme 95°C for 10 s, 55°C for 20 s and 72°C for 10 s, followed by 40 cycles according to the scheme 95°C for 10 s, 50°C for 20 s (with fluorescent signal measurement), and 72°C for 10 s.

The reaction mixtures contained RNA template (10 μL), PCR mix 1 (10 μL) (6 pmol of each of specific primers, 3 pmol probes, 4.4 pmol of each of dNTPs, and deionized water) and 5×One Step RT-PCR Mastermix solution (BelBioLab, Russia) (5 μL). The efficiency of extraction of nucleic acids was assessed by the amplification of an internal reference sample (a gene-engineered construct containing an artificially synthesized DNA fragment). The results of RT-PCR were interpreted in terms of the presence or absence of the intersection of the fluorescence curve with the threshold line set at the appropriate level, which indicated the presence or absence of the threshold cycle value (C_t).

Surface Contamination and Swabbing

To evaluate the effect of the structures of various materials on the detection of SARS-CoV-2 RNA, the initial virus sample was used to contaminate commercial packaging materials: glass, food grade plastic (polypropylene), styrofoam, aluminum, tin, and cardboard. A sample was applied to the surface with a sterile probe with a cotton swab and kept at room temperature until completely dry.

After drying, the surface was swabbed in triplicate. Each sample was taken from an area of 100 cm^2 with a saline-moistened sterile probe. The probe working surface was then immersed in a tube with sterile saline (500 μL). RNA was extracted with 100 μL of liquid.

Contaminated surfaces were swabbed 15, 60, 180 min, 24, and 72 h after contamination.

Treatment with Disinfectants

The tolerance of SARS-CoV-2 RNA to disinfection agents was determined by irrigation of surfaces contaminated with the initial sample with the following solutions: Dezital (Deznet, Russia) at a concentration of 1.5%; BabyDez Ultra (Gigiena Plus, Russia) (15%); Trilox-Spray (Bozon, Russia) (100%); and Sanivap-R (Meditsinskaya Dezinfekstiya, Russia) (0.2%). The surfaces treated with disinfectants were left for complete drying for at least 60 min.

The effect of ultraviolet irradiation (UV) on the detection in RT-PCR of SARS-CoV-2 RNA was also assessed. For this, the contaminated surfaces were irradiated for 30 min with a PhilipsTUV 15W/G15 T8 bactericidal lamp (Philips, Netherlands) at a distance of 0.3 m.

After the treatment, each surface was swabbed in triplicate. RNA was isolated from each sample, and virus-specific regions were amplified. This procedure was carried out in duplicate by different operators.

Data Processing

ΔC_t was determined as the difference between the average C_t value obtained for swabs from untreated surfaces and from surfaces treated with disinfectants or exposed to UV irradiation. C_t deviation was measured using the sample standard deviation and the median of some C_t values was also determined.

The amplification efficiency (E), correlation coefficient (R^2) and slope (M) were calculated using the software of the RotorGeneQ device according to the formula:

$$E = [10^{(-1/M)}] - 1).$$

We note that the results we obtained can only be applied to the materials studied in this work. They may differ from data on the effect of other disinfectants on the detection of SARS-CoV-2 RNA in swabs from other surfaces with different properties or data obtained using other PCR protocols with different amplification criteria.

RESULTS AND DISCUSSION

Viral RNA was found on surfaces of all tested materials contaminated with SARS-CoV-2: styrofoam, food grade plastic, aluminum, glass, tin, and cardboard. The difference between the C_t of the initial sample and the average C_t of swabs from all nonporous surfaces (glass, plastic, styrofoam, aluminum, and tin) was 5.76, which corresponds to a decrease in RNA concentration by 1.9 log(cop/mL). The maximum difference between C_t values for all analyzed swabs from nonporous surfaces was insignificant and equaled 1.38. From the nonporous materials, plastic was chosen for further experiments, since it is most frequently used for commercial packaging.

The difference between the C_t values for the initial sample and swabs from cardboard was 9.2. In this case, the RNA content decreased by approximately 3 log(cop/mL). This effect is most likely due to the high absorbency of the material.

The effect of the time of the presence of the virus on various surfaces on its RNA detection in RT-PCR for all swabs was evaluated at 15, 60, 189 min, 24, and 72 h after the application of the virus. Similar C_t values were obtained in all variants with a maximum difference of 0.89.

The influence of disinfectants on the detection of SARS-CoV-2 RNA was studied in swabs from plastic and cardboard. The following disinfection agents were used: Dezital preparation based on quaternary ammonium salt; BabyDez Ultra containing stabilized hydrogen peroxide as an active substance; Trilox-Spray based on 1-propanol; and Sanivap-R with dichloroisocyanuric acid as an active substance. In these experiments, the concentrations of the analyzed disinfectants were selected on the basis of recommendations in the Working Regulations for the Comprehensive Prophylaxis and Disinfection of Foods Exported to China provided by the Chinese.

Figure 1 shows the C_t values obtained for swabs from plastic treated with various disinfectants that were UV irradiated. The lowest decrease in the RNA concentration in disinfectant-treated compared to intact swabs (0.32 log(cop/mL)) (Table 1) was observed when using Trilox-Spray. BabyDez Ultra and Dezital caused a reduction of the viral RNA concentration by 1.42 and 1.54 cop/mL, respectively. UV irradiation resulted in the detection of the gene *E* fragment of the SARS-CoV-2 gene in only four out of six replicates with a decrease in the RNA concentration by 3.72 log(cop/mL), while the gene *N* fragment was not found at all. The use of Sanivap-R at a concentration of 0.2% led to the maximum reduction of the viral RNA content, by 4.95 log(cop/mL). The gene *N* fragment was not detected in all replicates, and the gene *E* fragment was found in two out of six replicates.

Thus, it was shown that irrigation of nonporous surfaces with a preparation containing dichloroisocyanuric acid salt as an active substance at a concentration prescribed by the above regulations, as well as UV irradiation caused a significant decrease in the SARS-CoV-2 RNA content in swabs from various surfaces.

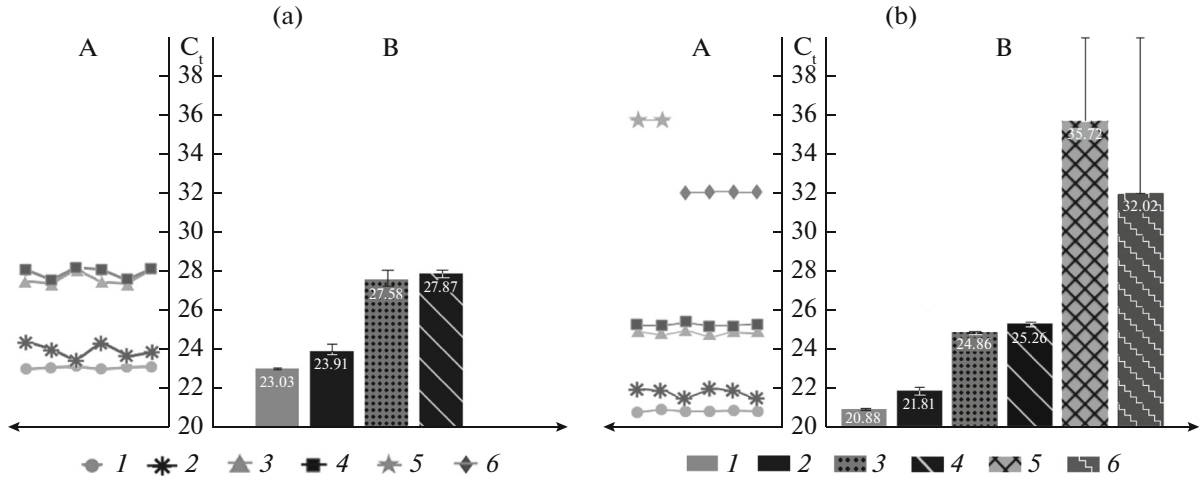

Fig. 1. The C_t values obtained by amplification of the SARS-CoV-2 *N* (a) and *E* (b) genes in swabs from contaminated plastic surfaces after treatment with various disinfectants: A, C_t values for six replicates; B, median of C_t values for the following options: (*1*) without treatment; (*2*) after irrigation with Trilox-Spray; (*3*) after irrigation with BabyDez Ultra; (*4*) after irrigation with Dezital; (*5*) after irrigation with Sanivap-R; and (*6*) after UV irradiation. The height of the columns is inversely proportional to the viral RNA concentration.

The analysis of swabs from cardboard surface treated with various disinfectants or UV irradiation are shown in Fig. 2. In this case, the decrease in the content of viral RNA was not significant (Table 2); the maximum value was observed when using Sanivap-R (by 1.80 log(cop/mL)), while the minimum effect was provided by Dezital (by 0.92 log(op/mL)). UV light caused the reduction of the viral RNA concentration by 1.48 log(cop/mL). When using various disinfectants, the maximum difference in the viral RNA content was 0.88. UV irradiation was largely not effective in reducing SARS-CoV-2 RNA in swabs from the cardboard surface.

Unfortunately, we cannot compare our results on the decrease in the SARS-CoV-2 RNA concentration after disinfection with the corresponding data of other researchers who studied the virus viability and infectivity [3, 5] but did not analyze the SARS-CoV-2 RNA fragments by RT-PCR.

It is important that our results on the detection of the viral genetic material on untreated surfaces are consistent with the data on the high stability of SARS-CoV-2: the virus was found on the surfaces of plastic, stainless steel, copper, and cardboard within 72 h after its application [2].

Table 1. Reduction of the SARS-CoV-2 RNA concentration in swabs from the plastic surface after disinfection

Disinfectant	ΔC_t[a]		Decrease in RNA concentration, log(cop/mL)[b]
	SARS-CoV-2 gene *E* fragment	SARS-CoV-2 gene *N* fragment	
Trilox-Spray	1.01 ± 0.19	0.88 ± 0.41	0.32
BabyDez Ultra	3.98 ± 0.06	4.55 ± 0.46	1.42
Dezital	4.38 ± 0.09	4.84 ± 0.39	1.54
Sanivap-R	14.84 (in 1 of 3 swabs)	–	4.95
UV light	11.14 ± 0.06 (in 1 of 3 swabs)	–	3.72

[a] Amplification parameters were: $R^2_{\text{gene } E} = 0.99$; $R^2_{\text{gene } N} = 0.98$; $E > 0.94$ for each of the detected genes. [b] The average decrease in RNA concentration for two genes in swabs from surfaces treated with a disinfectant is shown.

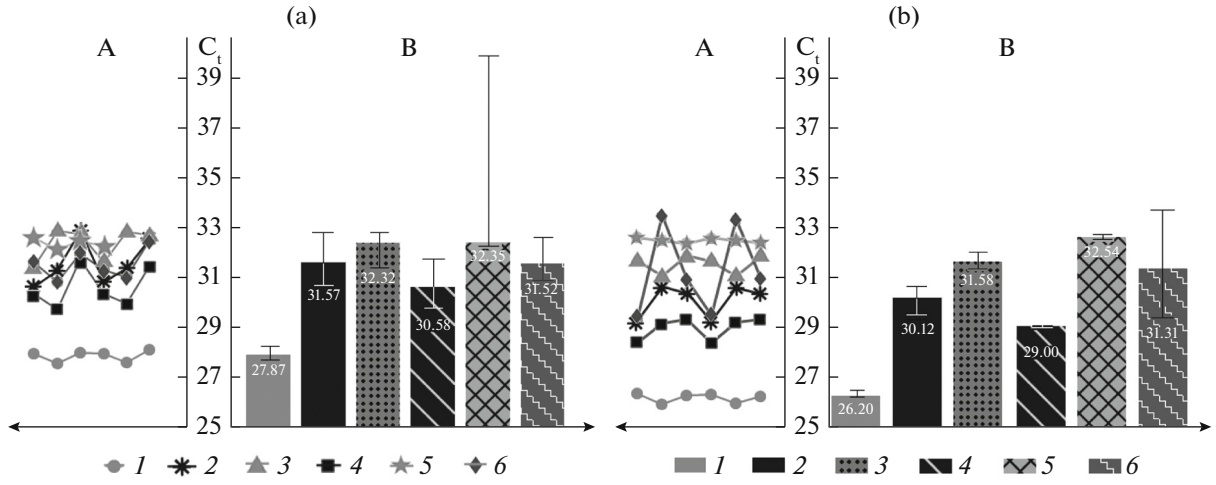

Fig. 2. C_t values obtained by amplification of the SARS-CoV-2 *N* (a) and *E* (b) genes in swabs from contaminated cardboard surfaces treated with various disinfectants: A, C_t values for six replicates; B, median of C_t values for the following options: (*1*) without treatment; (*2*) after irrigation with Trilox-Spray; (*3*) after irrigation with BabyDez Ultra; (*4*) after irrigation with Dezital; (*5*) after irrigation with Sanivap-R; and (*6*) after UV irradiation. The height of the columns is inversely proportional to the viral RNA concentration

CONCLUSIONS

Thus, the detection of infectious SARS-CoV-2 or viral RNA on the surfaces of various materials remains an urgent task whose solution is important, in particular, for international trade. The RT-PCR method detects viral genetic material, but cannot evaluate its viability and infectivity. According to these results, SARS-CoV-2 RNA was preserved on the surfaces of all the studied materials within the observation period, 3 days after application; the RT-PCR results did not significantly change during this time. It was shown that UV irradiation or treatment with the Savinap-R disinfectant (0.2%) was most effective in reducing the viral RNA concentration in swabs from nonporous surfaces.

Swabs from cardboard have a number of features that distinguish this material from the others analyzed in this work. It was found that the viral concentration on cardboard was greatly reduced, which could affect viral RNA detection by RT-PCR. It was observed that UV irradiation and irrigation with disinfectants did not significantly reduce the SARS-CoV-2 RNA concentration. This can be associated with the high hygroscopicity of the material and/or its chemical composition.

Taking the requirements for Russian exporters about the presence of SARS-CoV-2 genetic material on the surface of foods and packaging into account, special attention should be paid to compliance with sanitary and epidemiological regulations that establish hygiene standards and food safety norms, as well as recommendations of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing aimed at preventing SARS-CoV-2 spreading.

Table 2. Reducing the SARS-CoV-2 RNA concentration in swabs from the cardboard surface after disinfection

Disinfectant	ΔC_t[a]		Decrease in RNA concentration, log(cop/mL)[b]
	SARS-CoV-2 gene *E* fragment	SARS-CoV-2 gene *N* fragment	
Trilox-Spray	3.93 ± 0.75	3.7 ± 1.14	1.27
BabyDez Ultra	5.38 ± 0.43	4.45 ± 0.81	1.63
Dezital	2.80 ± 0.50	2.72 ± 1.02	0.92
Sanivap-R	6.34 ± 0.24	4.47 ± 0.32 (in 2 of 3 swabs)	1.80
UV light	5.11 ± 2.09	3.65 ± 0.84	1.46

[a] The amplification parameters were: $R^2_{\text{gene }E} = 0.99$, $R^2_{\text{gene }N} = 0.98$, $E > 0.94$ for each of the detected genes. [b] The average decrease in RNA concentration for two genes in swabs from surfaces treated with a disinfectant is shown.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest.

This article does not contain any studies involving animals performed by any of the authors.

This article does not contain any studies involving human participants performed by any of the authors outside the scope of people's normal professional activities.

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