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Using aerosols to decontaminate surfaces from nucleic acids

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ABSTRACT

The occurrence of cross-contamination, which leads to false-positive results when analyzing samples, is an important problem in the PCR laboratory. There are many ways to solve this problem, but none of them is universal. Treatment with aerosol is preferable for decontamination of large areas of complex surfaces. The goal of this study was to determine the efficiency of different modes of aerosol treatment for disinfection and nucleic acids decontamination. The solutions of compounds evolving active chlorine and active oxygen were used for decontamination of surfaces artificially contaminated with exogenous nucleic acids and bacteria. The potency of active ingredients of these solutions was assessed based on results of disinfection and nuclear acids decontamination. We have demonstrated effective modes of aerosol decontamination. The differences in conditions necessary for the nucleic acid decontamination and bacterial disinfection of laboratory surfaces are discussed.

Keywords: PCR, DNA, amplicons, decontamination, disinfectants, aerosol

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INTRODUCTION

The polymerase chain reaction (PCR) is one of the most common DNA amplification methods used in laboratory diagnostics. It finds routine application in clinical laboratories for detecting microorganisms that are difficult to culture. However, PCR poses a significant challenge due to its high sensitivity which makes it susceptible to cross-contamination.

The contamination of the working area, instruments, or reagents with DNA amplicons can affect the outcome of PCR analysis, resulting in false positives [1]. The presence of amplicons on working surfaces and equipment may lead to DNA re-amplification and subsequent contamination of the test samples, thereby complicating research and diagnostics [1]. Amplicon contamination is relevant for all laboratories utilizing nucleic acid amplification techniques (NATs). The sources of amplicons include the air within the working premises, equipment, and clothing of employees. Additionally, emergencies can cause bacterial contamination of working surfaces. Sample contamination can lead to false-positive test results [2]. This issue is particularly critical for clinical and forensic laboratories where accuracy is paramount. Identifying the source of contamination is a costly and time-consuming process [3].

Several methods are currently used to decontaminate a NAT laboratory from nucleic acids, including

ultraviolet irradiation [4], treatment with enzymes that digest nucleic acid molecules [5], or application of disinfectants (DS) [3]. The following enzymes can be used for DNA destruction: exonuclease III, DNase I, and DNA restriction enzymes [6, 7]. The most common disinfectants that ensure DNA decontamination are based on active chlorine and active oxygen compounds [3].

None of the existing methods of DNA decontamination is universal [1, 8]. The laboratory equipment can be large and complex in design or contain metal and plastic parts that are sensitive to acids and oxidizing agents. UV radiation is only effective on exposed surfaces, which makes it unsuitable for devices with complex constructions due to incomplete removal of DNA fragments; UV can also degrade plastic materials when used repeatedly [9, 10]. In addition, most common decontamination methods fail to remove short DNA fragments with low molecular weight (less than 200 bp) [11].

Thus, establishing reliable standardized methods for surface decontamination in laboratories using NATs is a pressing issue [5]. Among the available decontamination methods, treatment with disinfectants remains the most cost-effective, efficient, and convenient approach for DNA decontamination in laboratory practice. However, the existing list of disinfecting agents and application procedures needs to be updated.

Classical methods, such as wiping work surfaces and equipment or immersing consumables and tools in disinfectants, are suitable for partial (local) decontamination. When it comes to decontaminating extensive areas with intricate surfaces, the aerosol method employing aerosol generators is considered preferable [12]. This study aimed to determine effective modes of applying disinfectant solutions for both disinfection and DNA decontamination using the aerosol method.

MATERIALS AND METHODS

Experiment design

Surfaces were decontaminated from DNA by the aerosol method using disinfectant solutions of hydrogen peroxide (HP), chlorine dioxide (CD), peracetic acid (PAA), dichloroisocyanuric acid (DICA), or sodium hypochlorite (SHC). The list of substances active against nucleic acids was published earlier [13]. The surfaces of BSL-2 type A microbiological safety cabinets, or PCR cabinets (Lamsystems, Miass, Russia), were used for the decontamination experiments in this study. The surfaces were pre-treated following National Sanitary and Epidemiological Requirements for the Prevention of Infectious Diseases (3.3686-21). Work surfaces of the PCR cabinets were contaminated with genomic DNA or *Escherichia coli* 1257 bacterial culture, treated with aerosols of the disinfectant solutions and then the degree of DNA degradation was analyzed.

Bacterial strains and culturing conditions

The *E. coli* 1257 strain was provided by the State Collection of Pathogenic Microorganisms, Obolensk (B-8556). *E. coli* 1257 was cultivated in the hydrolyzed fish meal (HFM) broth as a liquid nutrient medium (State Research Center for Applied Microbiology and Biotechnology (SRC), Obolensk, Russia) under aeration conditions and stirring at 150 rpm at 37°C for 18 h, and on the HFM-agar dense nutrient medium (SRC, Obolensk, Russia) at 37°C for 24 h.

Isolation of genomic DNA

Genomic DNA was isolated from the *E. coli* 1257 bacterial culture following the phenol-chloroform extraction protocol [14]. Cells were lysed by adding 30 µl of 10% SDS buffer and proteinase K. After adding 0.5 ml of the mixture containing phenol, chloroform, and isoamyl alcohol in a ratio of 25:24:1, cell lysates were mixed on a Vortex shaker (Biosan, Riga, Latvia) and centrifuged at 12,000 g and 4°C for 10 min. Next, the aqueous fraction of the supernatant containing DNA was aspirated. Isopropanol (80% of the sample volume) and a saturated NaCl solution (1/9 of the sample volume) were added to the supernatant. Samples were incubated at 20°C for 20 min followed by centrifugation at 12,000 g for 10 min. After discarding the supernatant, the precipitate was washed with 400 µl of 70% ethanol and centrifuged at 12,000 g for 10 min. The supernatant was removed, and the samples were air-dried until complete evaporation of ethanol. The DNA pellet was resuspended in 20 µl of 1x TE buffer (10 mM Tris, 1 mM EDTA; pH 8.0). The DNA concentration in the samples was determined using a NanoDrop spectrophotometer (Thermo Scientific, USA). The samples were used for DNA detection by PCR. The remaining DNA was stored at -20°C.

Evaluation of DNA decontaminating activity of different disinfectants

E. coli 1257 cultured in the HFM-broth liquid nutrient medium was precipitated by centrifugation at 5,000 g at room temperature for 20 min. The cells were then washed three times in phosphate-buffered saline and DNA was isolated. The amplification product of PCR with specific 16S rRNA primers was used as a DNA sample. The sample, at a concentration of 1×10^5 copies/µl, was applied to the work surface of the PCR cabinet in ten different spots and

allowed to dry at room temperature for 20-30 min. In order to control the level of initial contamination, DNA was sampled from five points on the working surface. Next, the interior surface of the cabinet was treated with aerosols of different disinfectant solutions following various decontamination modes. Treatment with the same volume of water was used as a negative control. Swabs were taken from the remaining five points on the work surface after the aerosol treatment, transferred to a neutralizing solution (1.0% sodium thiosulfate) for 10 min, and then placed in separate microtubes with TE buffer (pH 8.0; Evrogen, Moscow, Russia). DNA was isolated from the obtained samples as described above and then used as a template for quantitative real-time PCR (RT-qPCR) using primers for the 16S rRNA gene. The absence of any PCR inhibition effect caused by disinfectant agents was confirmed by including an internal control as described by Fischer et al. [11].

Decontamination from bacterial cultures and DNA

Disinfection of the work surfaces of the PCR cabinets contaminated with a daily culture of *E. coli* 1257 was carried out together with intracellular DNA decontamination. A daily culture grown on an HFM-agar dense nutrient medium was suspended in saline to a turbidity equivalent to 3.0 according to the McFarland standard (1.0×10^9-2.0×10^9 CFU/ml). The resulting bacterial suspension was applied to the work surface of the PCR cabinet at ten points and dried at room temperature for 20-30 min. In order to control the level of initial contamination, swabs were taken from five points on the work surface. Aerosol treatment was then carried out as described below. Subsequently, 0.1 ml of each collected sample was inoculated on an HFM-agar dense nutrient medium, followed by DNA extraction and RT-qPCR.

Treatment of PCR cabinet work surfaces with aerosol

The interior space of the cabinet (0.5 m^3) was filled with aerosols of the studied disinfectant solutions using a Mobile Hygiene Center device (LLC ASKM, St. Petersburg, Russia). The aerosol particle size varied from 20 to 50 μm (mist) at a flow rate of the working solution of 60-200 ml/m^3. The exposure time after the treatment was 15 or 30 min. The distribution of aerosol on the PCR cabinet surfaces was determined empirically. Pre-weighed test objects – 10×10 cm pieces of filter paper – were placed on horizontal and vertical surfaces of the cabinet and the interior space of the cabinet was sprayed with 3% HP solution. Changes in the weight of the test objects were then used to calculate the volume of the precipitated solution resulting from the sedimentation of the aerosol on the paper; the density of the studied disinfectant solutions was close to one.

Quantitative RT-PCR

The amount of residual DNA in swabs was quantified by RT-qPCR on a CFX96-touch instrument (BioRad, Hercules, CA, USA) using qPCRmixHS SYBR reagents (Evrogen, Moscow, Russia) and specific 16S rRNA primers. The primers (forward: 5'-CGGAAACGGGCGCTAAT-3'; reverse: 5'-CCCCACTTTCTCCCTCAGG-3') [14] used in this study were manufactured by Sintol Ltd. (Moscow, Russia). RT-qPCR was carried out in triplicates according to the following program: 95°C – 5 min (95°C – 20 sec, 61°C – 20 sec, 72°C – 30 sec) × 40 cycles.

Statistical data analysis

The obtained PCR results were normalized according to the Pfaffl method [16]. The relative expression coefficient (R) was calculated by the formula $R=\frac{E_{GOI}^{\Delta c_{q;GOI}}}{E_{REF}^{\Delta c_{q;REF}}}$, where $\Delta c_{q;GOI}$ is the change in the threshold cycle (Ct) of the gene of interest (GOI); $\Delta c_{q;REF}$ is the change in the threshold cycle of the reference gene (REF); *E* is the reaction efficiency. The efficiency of the reaction was calculated by calculating the average PCR efficiency across all wells for each gene (E=1.8). The change of the Ct value for the reference gene was taken as zero. The efficiency of surface decontamination from microorganisms or DNA was calculated as a percentage. The degree of DNA degradation was determined according to Champlot et al. using the formula $E_{deg}=\frac{100}{(1+E)^{\Delta Ct}}$ [8]. The effectiveness of disinfection was calculated by the formula $E_{dec}=1-\frac{N_d}{N_c}$, where N_d is the CFU on a Petri dish after treatment with DS; N_c is the CFU on a control Petri dish (treatment with water).

Data processing and interpretation were carried out using the GraphPad Prism software version 8.0.1 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Statistical analysis was performed using an unpaired Student's t-test. The results were considered statistically significant at a p-value <0.05.

RESULTS

Determination of the working solution flow rate

An important parameter to consider when treating surfaces with DS aerosols is the flow rate of a working solution, as the volume of the released liquid affects the number of deposited particles. We determined the amount of the precipitated solution after aerosol sedimentation by

measuring the change in weight of the test objects placed on horizontal and vertical surfaces. The test objects were weighed before and after aerosol treatment.

No change in the test objects' weight was observed at the flow rate of 40 ml/m^3. Increasing the flow rate of the working solution up to 80 ml/m^3 led to a small amount of solution precipitating on the test objects (2-4 ml/m^2). At the flow rate of 100 ml/m^3, the volume of the precipitated solution reached 20 ml/m^2 on the horizontal surface and 10-13 ml/m^2 on vertical surfaces.

At the flow rate of 200 ml/m^3, a twofold increase in the volume of the precipitated solution was observed both on horizontal and vertical surfaces (Fig. 1). Based on these data, the flow rate of the working solution in the range from 60 to 200 ml/m^3 was chosen for further experiments.

Decontamination of surfaces from DNA

The activity of different disinfectants in decontamination from short DNA fragments (94 bp) was studied using the aerosol method under conditions simulating contamination of the inner surfaces of a biosafety cabinet with a PCR product. The decontamination efficiency was assessed by measuring DNA in swabs from the work surfaces of microbiological cabinets before and after the treatment with the aerosols under study. Active chlorine and active oxygen compounds were chosen as active ingredients for the working solutions that were tested in this project. The list and modes of application of these compounds have been published earlier [13].

Active chlorine compounds

No DNA degradation was found after treating the DNA-contaminated cabinets with aerosols of working solutions containing CD, DICA, and SHC compounds at the flow rate of 60 ml/m^3. At this flow rate, no significant differences were found between the results of experiments with any studied disinfectants and control experiments, regardless of aerosol application modes. With an increase in the flow rate to 80 ml/m^3, only a 30-minute exposure to the SHC working solution resulted in at least a tenfold decrease in the number of amplicons found on the cabinet surfaces compared to the results of the control experiment (Fig. 2).

Target DNA fragments were not detected when the cabinet was treated with 0.03% CD working solution at a flow rate of 100 ml/m^3 and a 30-minute exposure. Our experiments showed that it was possible to achieve the same result by increasing the flow rate to 200 ml/m^3 while keeping the exposure time at 30 min and reducing the CD concentration to 0.02%. Alternatively, increasing the concentration of the working solution to 0.03% allowed for reducing the exposure time to 15 min at the same flow rate (Fig. 2A).

The minimum concentration of DICA leading to DNA destruction within 30 min was 0.02% at a 100 ml/m^3 flow rate of the working solution. An increase in the flow rate to 200 ml/m^3 allowed to shorten the treatment time to 15 min (Fig. 2B).

SHC solution showed decontaminating activity against DNA at a concentration of 0.1%, a flow rate of 100 ml/m^3, and an exposure time of 15 min (Fig. 2C).

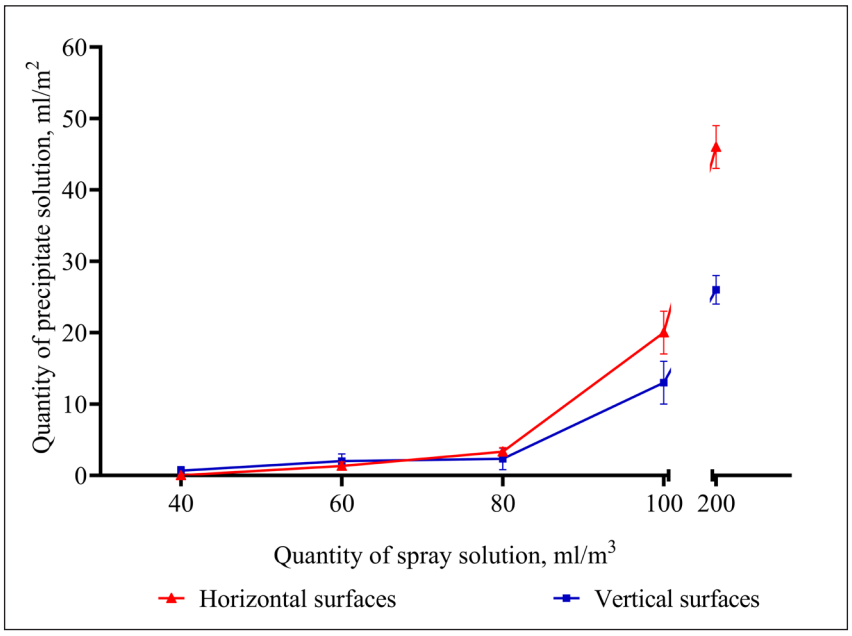

Fig. 1. Aerosol precipitation on the surfaces of a microbiological safety cabinet.

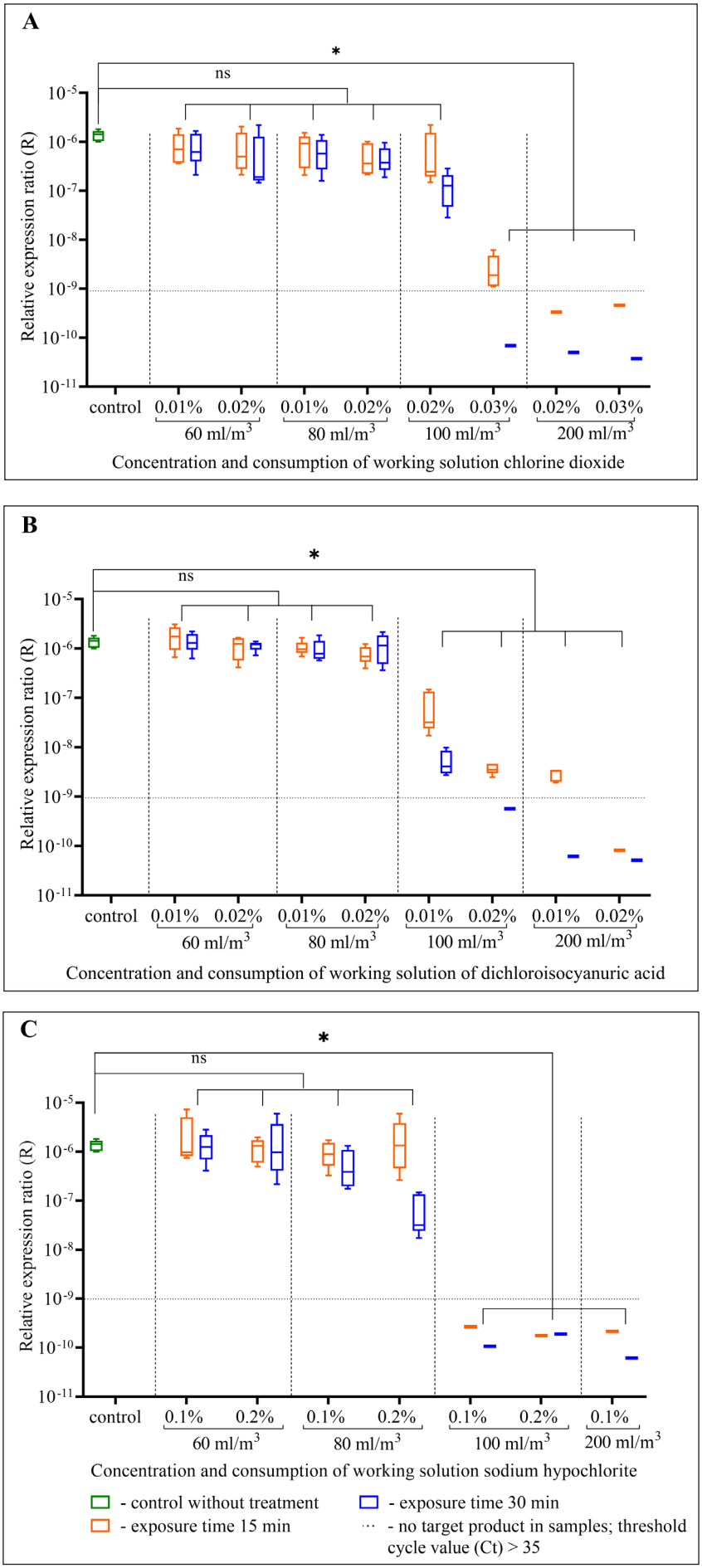

Fig. 2. Decontaminating activity of disinfectant solutions containing active chlorine compounds; **A** – chlorine dioxide (CD), **B** – dichloroisocyanuric acid (DICA), **C** – sodium hypochlorite (SHC), ns – no significant differences, (*) indicates $p \leqslant 0.05$ according to Student's t-test.

Active oxygen compounds

HP and PAA were used as active oxygen compounds. At working solution flow rates of 60-80 ml/m^3, only a slight decrease in the number of amplicons was observed when compared to the control samples. Aerosol treatment of the inside space of the cabinet with a 2.0% HP solution or a 0.24% PAA solution at a flow rate of 100 ml/m^3 destroyed DNA within 15 min (Fig. 3).

Decontamination of surfaces from bacteria and intracellular DNA

Using working disinfectant solutions of various concentrations at a flow rate of 100 ml/m^3, PCR cabinets were treated with disinfectant aerosols to determine which modes of their application allowed for the decontamination of surfaces from bacteria and the simultaneous destruction of intracellular DNA. The effectiveness of different working solutions was determined by the degree of decontamination of surfaces from bacterial cells and the degree of degradation of their DNA.

Active chlorine compounds

Aerosol treatment of cabinets with a 0.05% CD solution at a 30-minute exposure time led to the destruction of intracellular DNA, while the minimum bactericidal concentration was 0.02% at the same flow rate and exposure time (Fig. 4A). Aerosol of DICA at a concentration

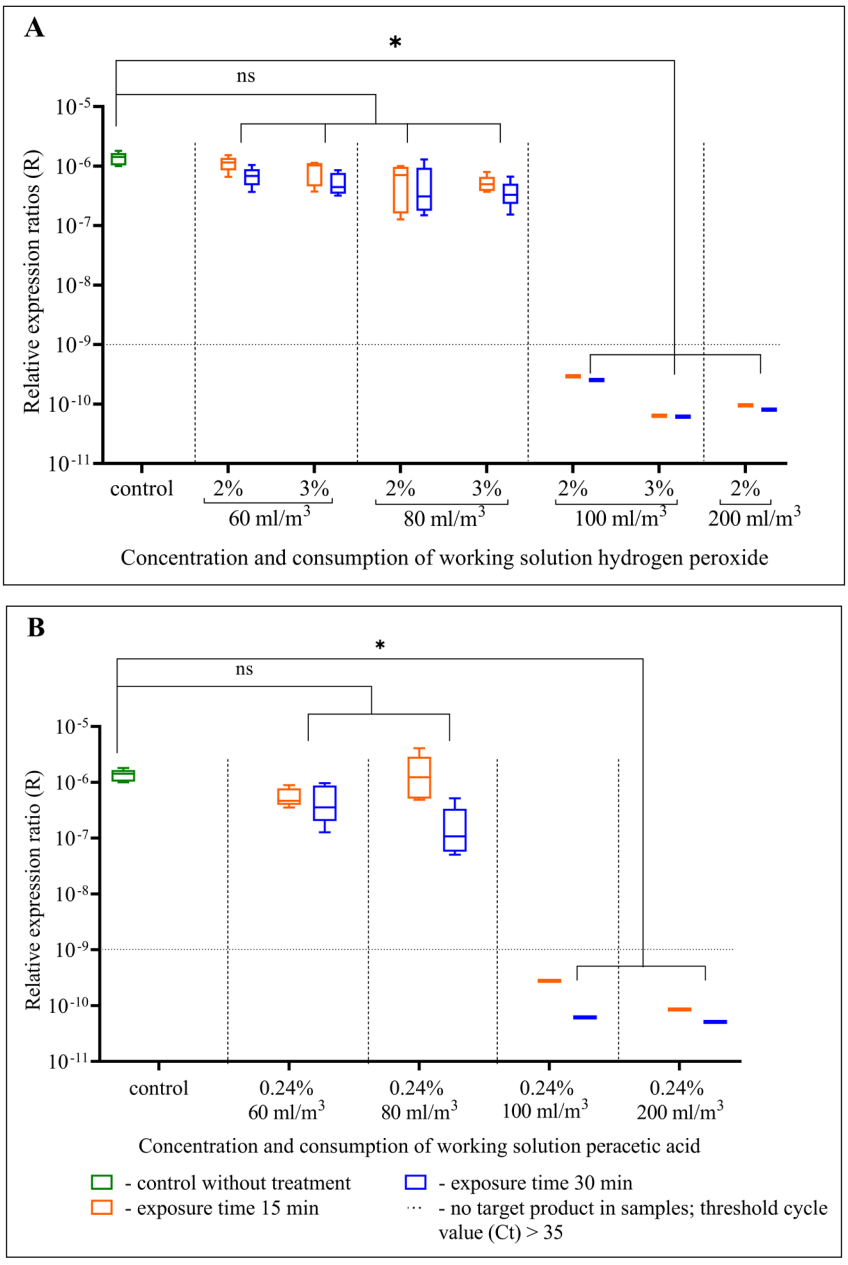

Fig. 3. Decontaminating activity of disinfectant solutions containing active oxygen compounds; **A** – hydrogen peroxide (HP), **B** – peracetic acid (PAA), ns – no significant differences, (*) indicates p≤0.05.

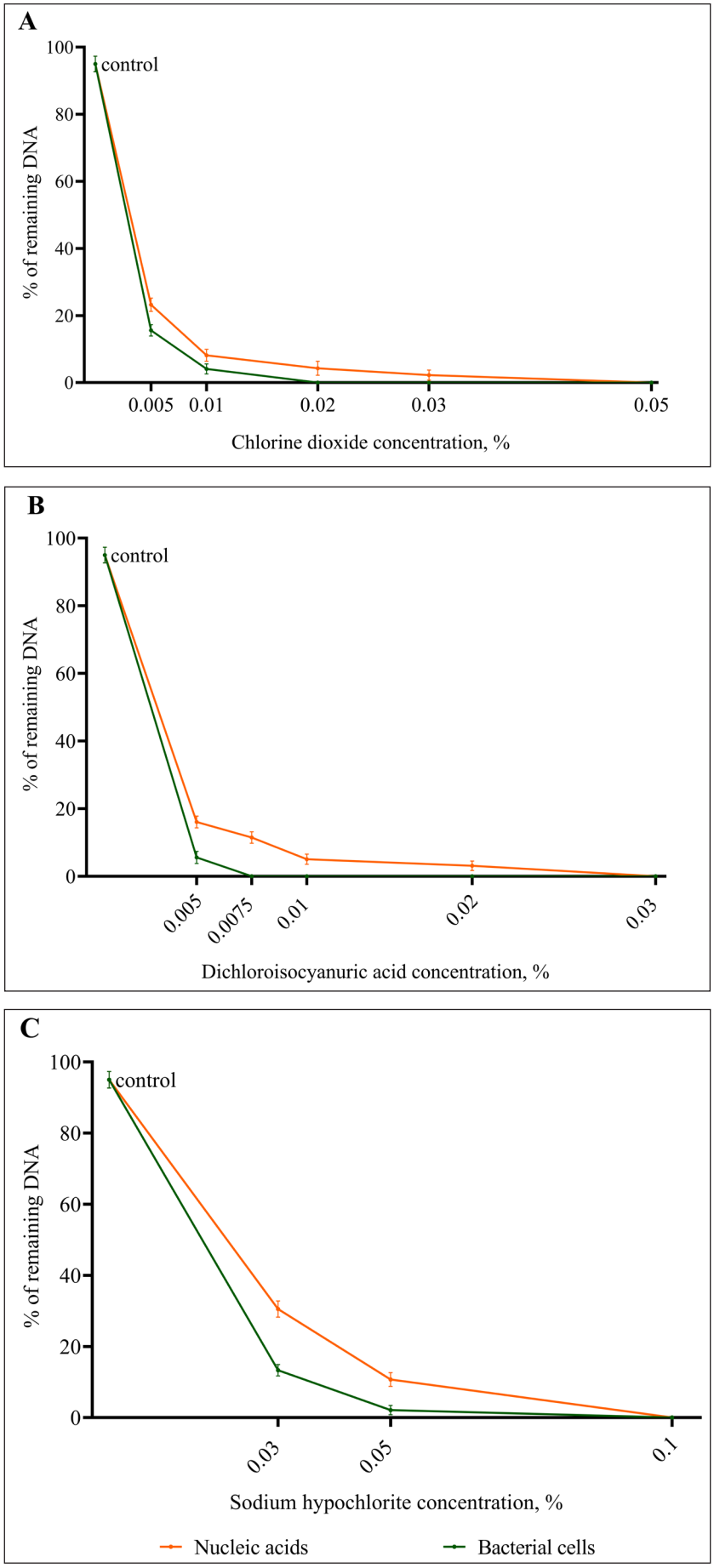

Fig. 4. Decontaminating activity of disinfectant solutions containing active chlorine compounds against bacteria and DNA; **A** – chlorine dioxide (CD), **B** – dichloroisocyanuric acid (DICA), **C** – sodium hypochlorite (SHC).

of 0.0075% ensured the complete elimination of bacteria that contaminated the inner surfaces of the cabinet after 30 min of exposure. Increasing the concentration of DICA to 0.03% resulted in DNA degradation (Fig. 4B). Unlike other active chlorine compounds, SHC solutions provided surface decontamination from both bacteria and DNA at the same concentration. The application of SHC was effective under the following conditions: 0.1% solution at a flow rate of 100 ml/m^3 and a 15-minute exposure time (Fig. 4C).

Active oxygen compounds

Intracellular DNA in the samples was fully destroyed after the treatment of the cabinet with a 3.0% HP solution at a 15-minute exposure time. The minimum bactericidal concentration of HP in the same application mode was 2.0% (Fig. 5A). A 0.06% PAA solution destroyed bacterial cells but did not result in DNA decontamination. Increasing the concentration to 0.24% allowed us to achieve complete decontamination of the tested surfaces from both bacteria and DNA (Fig. 5B).

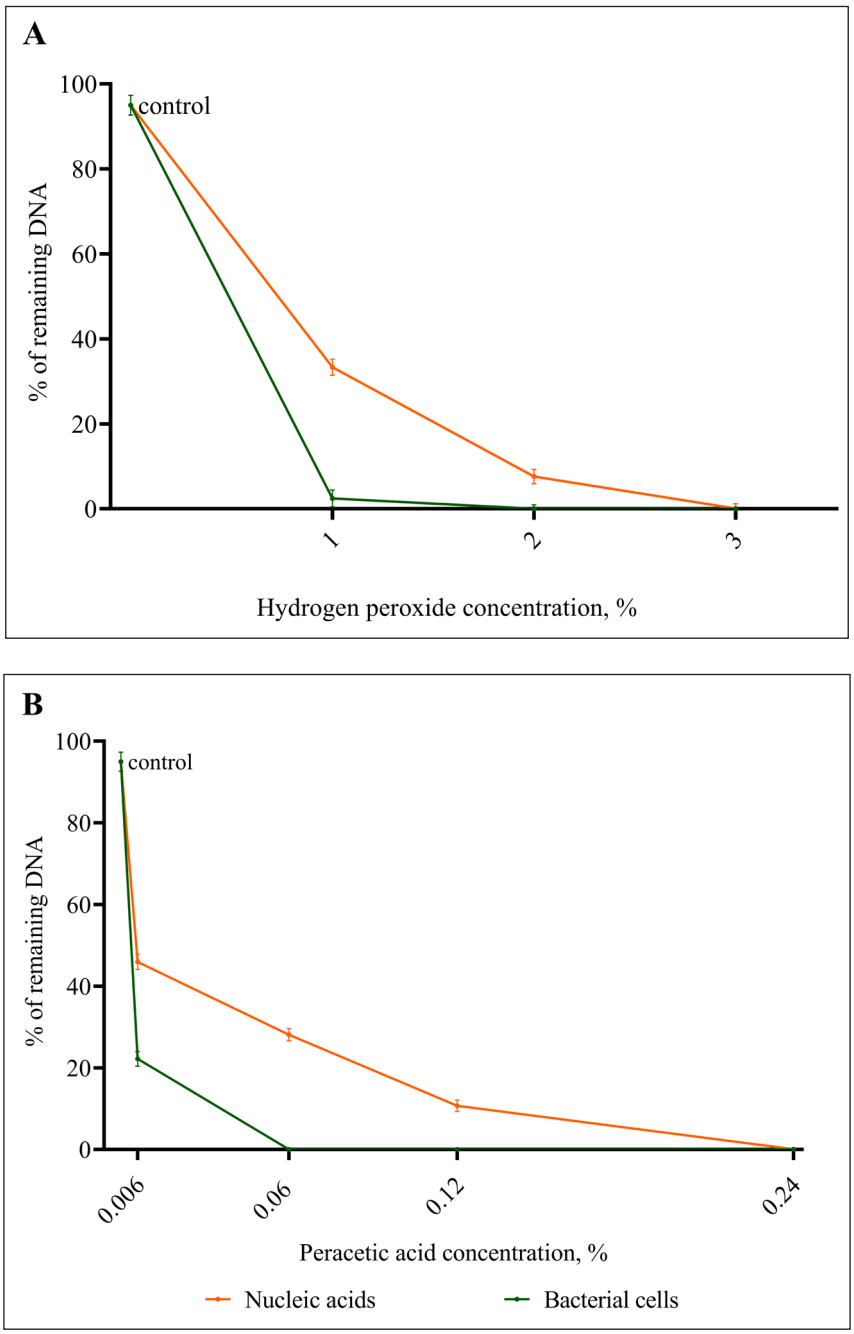

Fig. 5. Decontaminating activity of disinfectant solutions containing active oxygen compounds against bacteria and DNA; **A** – hydrogen peroxide (HP), **B** – peracetic acid (PAA).

Comparison of aerosol decontamination modes

By comparing various modes of aerosol treatment at the same flow rate of working solutions (100 ml/m^3), we assessed differences in effective concentrations of several disinfectant solutions that are utilized for the decontamination of laboratory surfaces from bacteria and DNA.

The effective CD concentration was found to be dependent on the type of surface contamination (bacteria or DNA amplicons): the destruction of intracellular DNA required a higher concentration of the working solution. Similar results were observed when surfaces were treated with HP solutions of various concentrations. It was shown that the bactericidal concentration of the PAA solution was four times lower than the concentration required for DNA decomposition. A dependence of the effective working solution concentration on the contamination type was observed in experiments with DICA as well. However, when we used SHC as the active component of the working solution, we did not observe such a dependence (Table 1).

DISCUSSION

Traditional methods of decontamination, such as wiping or spraying, do not provide complete removal of DNA from hard-to-reach surfaces. When decontaminating large areas of complex surfaces, it is preferable to use the aerosol method. In this paper, we describe a method for decontaminating surfaces from bacteria and DNA in laboratories using NATs. The method employs disinfectant aerosols containing active chlorine and active oxygen compounds.

Experiments evaluating the distribution of aerosol on the test surfaces revealed that the volume of deposited aerosol depends on the flow rate of a working solution. When a disinfectant solution is distributed at the flow rate of 60 ml/m^3, the volume of the precipitated solution is 1-2 ml/m^2. Increasing the flow rate to 100 ml/m^3 leads to a tenfold increase in the volume of the precipitated solution. At this flow rate, the air space of the cabinets is likely to be oversaturated with aerosol particles, which can result in their coagulation and subsequent precipitation on the surface [17].

In the previous studies [13], 150 ml/m^2 of a 0.01% CD solution was used for the decontamination of surfaces from DNA by the wiping method. The results of our experiments show that a 0.03% CD solution at a flow rate of 100 ml/m^3 is effective for aerosol decontamination of surfaces from amplicons, that corresponds to precipitation of 10-20 ml of working solution per 1 m^2 of the treated surface. The destruction of intracellular DNA occurs at a higher concentration of CD (0.05%) than the inactivation of bacterial cells (0.02%). The results obtained in our study are consistent with the data published by Xue et al. [18].

Similar results were observed in experiments with DICA. For DNA amplicon decontamination, the concentrations of solutions differed by a factor of two between wiping and aerosol application. However, in the case of bacterial disinfection and decontamination from intracellular DNA, the concentration difference between the two methods increased to a factor of four. The difference between wiping and aerosol application was presumably due to the fact that part of the active disinfectant is lost as chlorine gas during spraying [19]. According to recommended laboratory practices MU 1.3.2569-09, decontamination of surfaces from DNA must be carried out by wiping with a 0.2% solution of DP-2T that contains trichloroisocyanuric acid. Our studies demonstrated that when using the aerosol decontamination method, the minimum effective concentration of DICA can be ten times lower, reaching 0.02%.

SHC is a strong oxidizing agent that is effective against a wide range of bacteria and viruses [20-24]. Ballantyne et al. [25] showed that spraying a 1% SHC solution on a surface contaminated with DNA leads to DNA destruction within 5 min. Nilsson et al. [3] demonstrated that wiping surfaces with a 0.4% SHC solution could be used for the efficient removal of DNA. Our studies indicate that efficient decontamination of surfaces from DNA amplicons, bacteria, and their intracellular DNA could be achieved with a 0.1%. SHC solution aerosol.

Table 1. Concentrations of disinfectant working solutions that are effective at aerosol disinfection and decontamination of surfaces from bacteria and DNA

Active substance	Concentration of the active substance in solution, %			Exposure time, min
	Bacteria	Amplicons	Intracellular DNA	
Chlorine dioxide (CD)	0.03	0.03	0.05	30
Dichloroisocyanuric acid (DICA)	0.0075	0.02	0.03	30
Sodium hypochlorite (SHC)	0.1	0.1	0.1	15
Hydrogen peroxide (HP)	2.0	2.0	3.0	15
Peracetic acid (PAA)	0.06	0.24	0.24	30

HP is widely used both in the form of liquid and aerosol for the disinfection of various objects [26–28]. It is known that free peroxide radicals cause oxidative damage to DNA [29]. Our experiments showed that exposure of contaminated surfaces to a 2% HP solution leads to the complete destruction of amplicons and partial degradation of intracellular DNA. If bacterial decontamination together with the destruction of intracellular DNA is necessary, the concentration of the HP solution should be increased to 3%. The results obtained in our experiments are consistent with the data published by Afonyushkin et al. [30], who showed that treatment with a 2% solution of HP inactivates plasmids and reduces the concentration of chromosomal DNA of *Cl. perfringens* by 28-49 times.

PAA demonstrates disinfectant activity against bacteria, fungi, viruses, and spores [31, 32]. It destroys sulfhydryl (–SH) groups and disulfide (S–S) bonds in proteins as well as some important components of cell membranes. Hydroxyl radicals formed during PAA decomposition destroy bacterial cell walls and lead to DNA denaturation [32]. Nevertheless, Zhang et al. [33] showed that treatment with PAA solutions leads to only partial degradation of bacterial plasmids and other mobile genetic elements. These data are consistent with the results of our experiments, which demonstrate that the destruction of DNA requires four times higher concentrations of working solutions than antibacterial decontamination.

CONCLUSION

In this study we identified efficient modes of applying disinfectant solutions containing active chlorine and active oxygen compounds for aerosol decontamination of laboratory surfaces from DNA. The optimal flow rate of working solutions for decontaminating PCR cabinets was found to be 100 ml/m^3, equivalent to the deposition of 10-20 ml of solution per m^2 of cabinet surfaces. CD and DICA in the aerosol form required a threefold and twofold increase in the working solution concentration, respectively, compared to decontamination by wiping. In contrast, the effective working concentrations of SHC, HP, and PAA remained consistent regardless of the treatment method. We showed that different effective concentrations of disinfectants are necessary for aerosol disinfection and for decontamination from DNA. Aerosol decontamination with HP and SHC solutions required the shortest exposure time, and in addition SHC based disinfectants are less corrosive, which is an undoubted advantage. In conclusion, the developed modes of aerosol treatment using different disinfectant solutions allow for effective decontamination of various complex surfaces in laboratories while providing a less toxic working environment for humans and less corrosive conditions for equipment.

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