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COMPARATIVE CHARACTERISTICS OF EFFECTIVENESS OF SOME DECONTAMINATION FACILITIES USED FOR CARRYING OUT FORENSIC MOLECULAR GENETIC EXAMINATION

The article is devoted to comparing the performance of disinfectants at different concentrations of a contaminating agent. Contamination of objects can occur at any stage of the study, so effective methods of decontamination should be used to prevent it, which at the same time should be safe for laboratory staff. The purpose of the work is to check the effectiveness of disinfectants while working in biological laboratories and to determine their effects on the genetic information of the cell, the effect on the inhibition of PCR reaction and DNA degradation. The mechanisms of influence on a cell and DNA of chemical solutions are analyzed: Вунсент експресс, Бациллюл АФ, Дезактин 0, 2 %, WIPANIOS Premium, DNAzap™ PCR DNA Degradation Solutions, 70 % и 96 % ethyl alcohols, and physical methods: ultraviolet radiation, alcohol treatment followed by calcination in the flame of the burner and treatment with bidistilled deionized water. While research the contaminating DNA is represented by the synthetic genomic DNA produced by the Promega company. Because of the pilot study, DNAzap™ PCR DNA Degradation Solutions and bidistilled deionized water had the highest decontamination efficiency. Bidistilled deionized water can be used to the fullest in the laboratory if medical waste disposal standards are met. Other methods showed an efficiency of more than 99.98% which also allows them to be used when working in a biological laboratory, following the suggested recommendations. The presented methods of decontamination did not cause inhibition of the PCR reaction. Preserved, after decontamination treatment, DNA for most agents had a low level of degradation. After being treated with ultraviolet radiation, the level of DNA degradation was medium which means that ultraviolet radiation destroys long strands of DNA, leaving strands unbroken up to 200 bp. In addition, increasing the concentration of contaminating DNA did not affect the change in the efficiency of decontamination substances. All the decontaminated substances analyzed can be modified in molecular genetic laboratories.

Keywords: decontamination agents, DNA, inhibition, PCR, efficiency.

Formulation of Research Problem. Polymerase chain reaction (PCR) has been developed to detect pathogens in clinical specimens, which has revolutionized diagnostic approaches in many fields.

The PCR technique allows the detection of deoxyribonucleic acid (DNA) in an amount of less than 100 pg, for example, by low-copy DNA (LCN) amplification or mitochondrial DNA typing.

Sensitivity of this technique implies the perfect sterility of laboratory rooms, equipment, supplies and solutions¹, so methods to avoid contamination are especially important. Typically, contamination of consumables occurs at low levels and will not be detected in a sample with enough DNA. In samples with a small amount of DNA, on the contrary, it can be difficult to distinguish between the profile of the sample and the profile of contaminants. Laboratories utilize negative control for DNA isolation and amplification during screening of reagents for purity of reagents and monitoring of possible contamination.

Each sample, laboratory items, and equipment are potential sources of contaminating DNA, and in addition, many DNA copies exist in the air and on the surfaces of objects in the laboratory during operation. Different sources of contamination require different methods of decontamination.

Contamination of samples and surfaces can occur at any of the stages of operation:

- pre-laboratory (infection of the object of own DNA of the employees who make the selection of objects at the crime scene). Experts working in a DNA laboratory cannot prevent this kind of contamination;
- laboratory (due to violations of the zoning of premises, the transfer of equipment and dispensers between rooms, neglect to use and change of protective clothing; disposable gloves, masks, medical caps and lab clothes).

Laboratory pollution has four main sources:

- contamination of human DNA from the environment: occurs when ignored by experts, biologists, observers or service personnel norms for the use of personal protective equipment in laboratory rooms or the transfer of objects and equipment between areas of the laboratory, without their preliminary cleaning;
- cross-contamination: unintentional contamination of one object with DNA material of another object due to the careless use of equipment, carrying out techniques;
- contamination of PCR reagents and kits for DNA extraction: occurs in the absence of sterility of the reagents, in the preparation of solutions, the use of non-sterile supplies or due to careless handling of research objects;
- contamination by amplification products: transfer of amplified DNA to an object that has not undergone amplification. Amplicons are one of the most common sources of contamination because they can be homologous to target molecules and amplify with higher efficiency than research object.

¹ Tamariz, J. and other. The Application of Ultraviolet irradiation to Exogenous Sources of DNA in Plasticware and Water for the Amplification of Low Copy Number DNA. *Journal of Forensic Sciences*. 2006. Vol 51, № 4. P. 790–794.

The sources of this kind of pollution are dispensers, lab tables and equipment and even the surface of an expert's hands.

Contamination of laboratory surfaces and devices can occur at any stage of the study, which can be avoided by ensuring the cleanliness of the premises and using DNA deactivation techniques.

Scientific problem is to find effective methods of DNA deactivation that could be used in health care settings that were safe for the staff using.

In recent years, various methods of DNA deactivation have been developed to solve this problem, including the use of ultraviolet or γ -radiation, hydroxylamine hydrochloride or ethidium monoazide, treatment with exonuclease III or endonucleases (for example, DNase I or restriction enzymes), as well as autoclaving and deactivation by a gas generator based on radicals. The most affordable and cheapest decontamination methods applicable in PCR laboratories are oxidative reagents and UV radiation¹.

Research on this topic by scientists such as Y. Wu, J. Wu, Z. Zhang, Cheng², Setlow, Parish, P. Zhang², focused on determining the effect of disinfection methods (UV, high temperature, 75% ethanol) and sodium hydrochloride) for certain types of pathogenic microorganisms (*Staphylococcus aureus*, HBV, *Entamoeba histolytica*) and their spores, which in medical laboratories can lead to false positive test results.

Researchers Gefrides, Powell, Donley, Kahn³, determined the effectiveness of autoclaving and ultraviolet irradiation as methods of cleaning supplies from contamination of human DNA. The presented works cover a limited number of disinfection methods that are used in medical and biological laboratories, and do not describe the effect of decontaminants on the contamination of laboratory surfaces of human DNA.

Based on this problem, and based on data on the physicochemical and biological properties of disinfectants used in biological laboratories, the assumption was made to check the effectiveness of these agents when working in biological laboratories and their effect on the genetic information of the cell.

Research Purpose. elucidation, according to the literature, of the mechanisms of action of decontamination means, verification of the

¹ Wu Y. and other. DNA decontamination methods for internal quality management in clinical PCR laboratories. Journal of clinical laboratory analysis. 2017. Vol 32. № 3.

² Setlow, B. and other. Mechanism of killing of spores of *Bacillus anthracis* in a high-temperature gas environment, and analysis of DNA damage generated by various decontamination treatments of spores of *Bacillus anthracis*, *Bacillus subtilis* and *Bacillus thuringiensis*. Journal of Applied Microbiology. 2014. Vol. 116. P. 805–814.

³ Gefrides, L. A. and other. UV irradiation and autoclave treatment for elimination of contaminating DNA from laboratory consumables. Forensic Science international: Genetics. 2010 . Vol 4. № 2. P. 89–94.

effectiveness of disinfection of DNA contamination by selected physical and chemical methods, the effect of disinfectants on the inhibition of PCR reaction and degradation of DNA.

Main content presentation. The analysis of the literature revealed that the mechanisms of decontamination chosen for the study have state registration in Ukraine, methodological instructions for use, approved by the State Sanitary and Epidemiological Service, are used in other laboratories, including international ones.

The presented decontamination agents have different mechanisms of action on the cell and DNA.

We describe them by the mechanism of action and the main active substances:

1. Chemical methods:

A) alcohol-containing drugs:

– 70% and 96% ethyl alcohols have the same mechanism of action on the cell – when interacted with the cell surface, cause its dehydration and denaturation of proteins. Antiseptic effect of an aqueous solution of alcohol has a concentration dependence, so preparations with an ethyl alcohol content of less than 50% do not have a pronounced antiseptic effect, but with an increase in the concentration of alcohol increases its ability to coagulate cell proteins. Antiseptic ability of absolute alcohol drops sharply, as the rapid coagulation of the membrane proteins of the cell prevents the concentrated alcohol from penetrating the cell and destroying its inner contents.

– *Винсент експресс*.

Main active ingredient is 65-70% ethyl alcohol.

The mechanism of action of ethanol is denaturation and coagulation of proteins, dissolution of lipids. The hydroxyl group, which is present in the structure of alcohols, binds to the proteins of the cell wall through a hydrogen bond, disrupts their structure and function, which leads to the deposition of proteins in the precipitate. In addition, hydroxyl ions saponify lipids in the membrane, causing the cell surface structure to break down.

– *Бациллол АФ*. main active substances: 1-propanol 45% and 2-propanol 25%, cause cell dehydration and protein denaturation, which leads to the destruction of the cell wall structure and cell membrane, the release of intracellular components, which ultimately leads to a loss of cell functionality¹.

B) Chlorine-containing preparations:

– Decactin 0, 2%. The main active substances are: sodium chloride, 1, 3-dichloro-5, 5-dimethylhydantoyl. Chlorine compounds have multiple effects on the cell, among them: chlorination of amino acids;

¹ *Sang Su Kim and other*. Comparison of disinfective power according to application order of 70% isopropyl alcohol and 10% povidone-iodine. Korean J Anesthesiol. 2013. Vol 65. P. 519–524.

oxidation of peptide bonds in proteins and their subsequent denaturation¹; reduced absorption of nutrients and oxygen; inhibition of protein synthesis; reduced production of adenosine triphosphate due to K⁺ leakage and malfunctioning of the Na⁺ / K⁺ pump; single and double stranded DNA breaks and inhibition of DNA synthesis; violation of the SOS-DNA repair system².

– *WIP'ANIOS Premium*.

The main active substances are: didecyltrimethylammonium chloride, polyhexamethylenebiguanide hydrochloride. WIP'ANIOS Premium combines the disinfectant properties of chlorides and ammonium compounds. Chlorine compounds cause inactivation of energy-producing enzymes, denaturation of major cellular proteins, and destruction of the cell membrane. Ammonium compounds, for their part, lead to disruption of intermolecular interactions and dissociation of lipid bilayers; reducing the electron transport intensity and reducing the efficiency of the phosphorylation system; protein denaturation; a rapid increase in the permeability of cell membranes and their destruction, followed by leakage of intracellular components. WIP'ANIOS Premium has a long-lasting effect, as it is deposited in the form of a microscopically thin film on objects and surfaces, with prolonged contact with cells, the biocide causes their autolysis³.

C) Drug with copper sulfate

– DNAZap™ PCR DNA Degradation Solutions. Main active substances of this drug are trade secrets. According to the protocol of use, sprays contain metal ions, among which there is copper (II) sulfate, capable of catalyzing the peroxidation of membrane lipids using active forms of oxygen to the cell; oxidize low density lipoproteins; subject the base to oxidation through free oxygen radicals; to induce breaks in DNA strands due to the association of metal ions with various regions of the double helix and binding to individual nucleotides⁴. Copper ions also react with hydrogen peroxide to form free hydroxyl radicals, which initiate a chain reaction of peroxidation that damages the DNA molecule⁵.

¹ *Maris P.* Modes of action of disinfectants. Rev. sci. tech. Off. int. Epiz. 1995. Vol. 14. № 1. P. 47–55.

² Guideline for Disinfection and Sterilization in Healthcare Facilities. Centers for Disease Control and Prevention. 2016. URL : <https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html> (дата обращения: 19.06.2019).

³ *Ioannou, C. J., Hanlon, G. W., Denyer, S. P.* Action of Disinfectant Quaternary Ammonium Compounds against *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy. 2006. Vol. 51. № 1. P. 296–306.

⁴ DNAZap™ PCR DNA Degradation Solutions. Thermo Fisher Scientific. 2017. URL : https://assets.thermofisher.com/TFS-Assets/LSG/manuals/9890M_DNAZapSolutions_Pi.pdf (дата обращения: 20.06.2019).

⁵ *Georgieva S., Попов B., Петров V.* Genotoxic effects of copper sulfate in rabbits. Archives of Biological Sciences. 2013. Vol. 65. № 3. P. 963–967.

2. Physical methods:

A) Ultraviolet radiation inactivates the functional activity of proteins due to the destruction of nitrogenous bases in the active center of the protein; causes photo-oxidation of lipids, with the formation of free radicals and hydroperoxides; leads to the formation of dimers between pyrimidine bases in DNA and disrupts the reaction of transcription and replication of nucleic acids¹.

In addition, UV damage causes suppression of adenosine triphosphate synthesis, increased membrane permeability, loss of activity of biologically active substances.

B) Alcohol treatment followed by calcination in the burner flame.

It combines the benefits of alcohol decontamination, which causes cell dehydration and denaturation of its proteins, and complete cell dehydration by burning the alcohol solution.

Removal of an aqueous component of a cell, when calcined in a flame, leads to an increase in the concentration of salts and minerals, which disrupt the metabolic functions of the cell and cause its death.

In addition, heat denatures proteins, provokes oxidative damage to the cell due to the action of free radicals and leads to the «burning» of all surface biomaterials on objects.

Bidistilled deionized water, so there is a swelling of the cell and then cytolysis, during which the cell's own lysosomal enzymes dissolve the internal contents of the cell.

Research objects

Seven decontamination solutions used in the laboratory were tested during the study (Винсепт экспресс, Бациллол АФ, Дезактин 0, 2%, WIP'ANIOS Premium, DNAZap™ PCR DNA Degradation Solutions, 70% ethyl alcohol, 96% ethyl alcohol), physical decontamination methods (ultraviolet radiation, alcohol treatment followed by calcination in a burner flame) and bidistilled deionized water. The contaminating solution is a synthetic genomic DNA company Promega, a concentration of 50 ng / µl. DNA, which for the study, was reduced to concentrations: 1 ng / µl, 3 ng / µl, and double-distilled deionized water was used to dilute.

Two controls were used in the study:

- negative: a slide on which control DNA was not applied, is an indicator of the purity of the glasses used while research;

- positive: a glass on which the chosen concentration of DNA was applied, but which was not exposed to disinfectants, is an indicator of the efficiency of sampling².

¹ Mark W Le Chevallier, Kwok-Keung Au. Water Treatment and Pathogen Control: Process Efficiency in Achieving Safe Drinking Water. World Health Organization. 2004. P. 41 – 66

² Adé A. and other. Comparison of Decontamination Efficacy of Cleaning Solutions on a Biological Safety Cabinet Workbench Contaminated by

To ensure the purity of the experiment and to avoid cross-contamination, a blind experiment method was conducted, deliberate glass contamination and sampling were separated over time. During all the procedures, the research staff wore appropriate protective equipment (nitrile gloves, a mask, a dressing gown and a hat) and cotton swabs and 1, 5 ml tubes used for washing were previously disinfected in accordance with the technical recommendations for working in the laboratory¹. Nitrile gloves were systematically replaced between successive application and sampling sessions. A sample with control DNA diluted to the required concentration was systematically mixed during operation on a Biosan FV-2400 vortex centrifuge.

Experiment technology

For the research we used microscope slides (hereinafter referred to as glasses), which were pre-immersed for 24 hours in a disinfectant solution, cleaned with detergent and deionized bidistilled water, treated with 70% alcohol solution. After the purification step, the glasses were placed in a laminar box for natural drying.

At the next stage, the glass was contaminated by applying, using dispensers and disposable tips, 3 µl of the solution with the selected DNA concentration to the central region of each glass. Material, mechanically, using a tip, was distributed in a thin layer over the surface of the slide. The contaminated glasses were then allowed to dry completely at room temperature without direct sunlight.

The finished contaminated glasses were divided into groups, namely, three glasses for each disinfectant solution and physical disinfection method. Thus, all disinfectants were tested in three parallel replicates (to evaluate the reproducibility of the results).

The further steps for disinfecting the glass depended on the method of disinfection chosen. A group of glasses selected for research:

- ultraviolet radiation was placed in a laminar box (distance from lamps to objects was 45 cm) and irradiated for 30 minutes;
- chemical disinfecting solutions, 70% and 96% ethyl alcohol and double-distilled deionized water were treated with solutions using the wash method. Rinses were carried out in three «wiping» movements, for each glass, using cotton swabs soaked in disinfectant reagents;
- 70% of the alcohol with subsequent burning was investigated by the method of flushing and burning the entire surface of the glass with an alcohol burner.

Cyclophosphamide. The Canadian Journal of Hospital Pharmacy. 2017. Vol. 70. № 6. P. 407–414.

¹ Derzhavni sanitarni normy i pravyla [State sanitary rules and regulations]: nakaz Ministerstva okhorony zdorovia Ukrainy vid 24.01.2008 № 26. Ofitsiinyi visnyk Ukrainy. 2008. № 11. St. 274 [in Ukrainian].

Degree of cleaning efficiency of the glasses by decontamination agents was determined by the method of washing the samples, using a cotton swab, five times «wiping» movements for each glass tested.

To measure the residual amount of DNA, at the beginning, DNA was extracted from swabs using a Chelex ion exchange resin. 200 µl of 5% Chelex (Bio-Rad Laboratories, Hercules, CA) was added to the test tubes, incubated at 56 ° C for 30 minutes, then the tubes were shaken in a vortex centrifuge and incubated in a water bath for 8 minutes, the vortexing step was repeated, and then centrifuged at a maximum speed of 5 minutes¹. The supernatant with the extracted DNA from each sample was transferred to a new clean tube and the PCR reaction was performed in real time using the Promega PowerQuant TM System kit. The result of real-time PCR is the quantitative determination of total (autosomal) DNA, the degree of degradation and inhibition of DNA on the presented swabs.

Ct values (cycle threshold) and amplification efficiency values were calculated using Real Time PCR System 7500 v2.0 software (Applied Biosystems, Life Technology, ThermoFisher Scientific) provided by the manufacturer.

Statistical analysis and research results

To test the significance of the data obtained, two hypotheses were put forward:

H_0 – there are differences between the mean values of the concentration of the pollutant before and after the use of the decontamination agent.

H_1 – there are differences between the mean values of the concentration of the pollutant before and after the use of the decontamination agent. The decontamination agent works.

The results of the calculation of statistical indicators are shown in the table below. Since we have two related samples that correspond to the normal distribution, we will use paired Student t-test for statistical calculations of the study. The central characteristics of the presented series of results were also calculated: arithmetic mean ($\bar{x}$), confidence interval of arithmetic mean ($\Delta\bar{x}$), standard deviation (SD)². And a separate indicator – decontamination efficiency (DE), is calculated for each washing solution as the measured concentration of the contaminating agent after treatment to the initial concentration of the agent.

The absolute values (modules) of the Student criterion were compared with the table value of the indicator, with the number of degrees

¹ Abbasov R. H., Povkh A. S., Romanchuk S. M. Metodyka provedennia molekuliarno-henetychnykh doslidzhen [Methodology of molecular genetic studies]: metod. rekomend. Kyiv : DNDEKTs MVS Ukrainy, 2018. 75 s. [in Ukrainian].

² Atramentova L. A. Dizajn i statistika biologicheskogo issledovaniya [Design and statistics of biological research]. Har'kov : izdatel'stvo «NTMT», 2015. 269 s. [in Russian].

of freedom equal to $df = N - 1 = 5$ and the significance level equal to 0.05. Two-sided student criterion for a given value of the degree of freedom is 2. 57. Indicators of the criterion Student's study is from 4,422 to 4,473, which exceeds the critical tabular value of the indicator. Thus, for all the presented disinfectant solutions and methods, we can reject the stated null hypothesis and accept the alternative hypothesis, with the statement of a statistically significant difference in the concentration of the contaminant before and after the disinfecting measures.

Table 1

Statistical characteristics of the efficiency of decontamination agents

Methods/Indicators	$x \pm \text{ДИ}$	SD	T	DE, %
Бациллол АФ	0, 00380, 0033	0, 0031	- 4, 470	99, 998
Дезактин 0, 2 %.	0, 0020 $\pm$ 0, 0018	0, 0017	- 4, 472	99, 999
bidistilled water	0, 0003 $\pm$ 0, 0005	0, 0005	- 4, 473	100, 000
70% ethyl alcohol	0, 0025 $\pm$ 0, 0014	0, 0014	- 4, 470	99, 999
96 % ethyl alcohol	0, 0213 $\pm$ 0, 0234	0, 0223	- 4, 422	99, 986
70 % alcohol and calcination	0, 0035 $\pm$ 0, 0036	0, 0034	- 4, 472	99, 998
UV radiation	0, 0198 $\pm$ 0, 0063	0, 0060	- 4, 442	99, 988
Винсепт экспресс	0, 0028 $\pm$ 0, 0019	0, 0018	- 4, 473	99, 998
WIP'ANIOS Premium.	0, 0015 $\pm$ 0, 0018	0, 0018	- 4, 473	99, 999
DNAZap	0, 0003 $\pm$ 0, 0009	0, 0008	- 4, 470	100, 000

Примечание: x – arithmetic mean, ДИ – 95 % confidence interval, SD – standard deviation, T – Student's t-test, DE – decontamination efficiency.

Results and discussion

This work, carried out based on the Department of Biological Research and Accounting of Kharkov Research Institute of the Ministry of Internal Affairs of Ukraine, is a pilot study of the study of the effectiveness of decontamination at different concentrations of the contaminating agent. Results of the quantitative analysis showed that the highest observed concentration after purification was 0, 061 ng (when treated with 96% alcohol), and the lowest observed concentration was the complete absence of DNA (when treated with decactin, bidistilled deionized water, WIP'ANIOS Premium, DNAZapTM PCR DNA Degradation Solutions).

Not detecting a contaminant does not necessarily mean that all the DNA molecules have been completely removed. A small amount could remain on the glass surface but was not measured because the concentration was below the detection limit.

Винсепт экспресс, Бациллол АФ, Дезактин 0, 2 %, WIP'ANIOS Premium, DNAZapTM PCR DNA Degradation Solutions, 70% ethanol,

treatment with alcohol, followed by roasting in a burner flame and bidistilled deionized water removed the control DNA with a concentration of 1 ng / μ l and 3 ng / μ l with equal efficiency, and the effectiveness of these disinfectants was 99.9% after the first surface cleaning session.

The highest 100, 0% decontamination efficiency was demonstrated by DNAZapTM PCR DNA Degradation Solutions and double-distilled deionized water. However, the main effect of bidistilled deionized water is based on the washing of DNA from the glass, and not its complete destruction, as is typical when using DNAZap, therefore, after decontamination, the consumables (wipes, gauze) used to wash them must be disposed of as medical waste. As the study showed, bidistilled deionized water can also be fully used in a biological laboratory as a disinfectant solution, along with other chemical solutions by physical methods.

For 96% ethyl alcohol and ultraviolet radiation, the efficiency index was 99, 98%, these methods work less efficiently than the rest, and after treatment there is a residual concentration of DNA that can contaminate the samples and lead to unreliable genetic analysis results. The property of 96% alcohol to instantly coagulate the proteins of the cell reduces its ability to penetrate the cell and disrupt its operation, which explains the results. In practice, this can be adversely affected, for example, by contamination with a multilayer or multicellular contaminating agent.

Ultraviolet irradiation also has several usage features that, in real conditions, negatively affect its effectiveness: indoor humidity is above 70%, dust (dirt) accumulates on surfaces, long distances from surfaces to an incandescent lamp, the material of which the object is made (porous and opaque materials are not effectively disinfected by radiation)¹.

Taking into account these negative features, the experimental level of disinfection efficiency, the availability and prevalence of these processing methods, it can be said that these methods can be used as independent decontamination agents, but for the complete removal of contaminants it is recommended that, as a first step, treatment with 96% alcohol or ultraviolet radiation, and as a final stage – use one of the other means presented.

Quantitative analysis also evaluated the threshold positive internal control (IPC Ct) cycle and the level of DNA degradation. The performance of the IPC Ct threshold cycle for all samples corresponded to the necessary, according to the manufacturer's protocol, the exit cycle of the internal control, meaning the use of these decontamination substances does not cause inhibition of the PCR reaction.

However, the exception was the samples treated with ultraviolet radiation, the degradation level for them was from 3, 0 to 5,5, which

¹ *Burgener, J.* Position paper on the use of ultraviolet lights in biological safety cabinets. *Applied Biosafety*. 2006. Vol 11. № 4. P. 228–230.

corresponds to the average level of degradation of the sample and means that the irradiation better destroys long strands of DNA, and leaves undamaged DNA fragments less than 200 pn., which, according to literary data, is characteristic of this radiation².

Thus, all disinfection methods significantly reduced the contamination of glass surfaces that were exposed to a certain amount of DNA. In addition, when the concentration of pollutants changed, there was no noticeable difference in the efficiency of the disinfectors, this means that the DNA concentration does not affect the performance of the decontaminants and all the presented processing methods can be fully used in biological laboratories.

Conclusions

1. Results of the quantitative analysis showed that the highest observed concentration after purification was 0,061 ng (when treated with 96% alcohol), and the lowest observed concentration was the complete absence of DNA (when treated with decactin, bidistilled deionized water, WIP'ANIOS Premium, DNAZapTM PCR DNA Degradation Solutions).

2. The highest 100.0% decontamination efficiency was demonstrated by DNAZapTM PCR DNA Degradation Solutions and bidistilled deionized water.

Decontamination efficiency using Vinsept Express, Bacillol AF, Dezactin 0, 2%, WIP'ANIOS Premium, 70% ethyl alcohol, alcohol treatment followed by calcination in the burner flame was 99, 99%.

For 96% of ethyl alcohol and ultraviolet radiation, the decontamination efficiency was 99.98%.

3. The presented decontamination agents do not cause inhibition of the PCR reaction.

4. The DNA degradation index for most decontamination substances was <2, 0, but for the samples treated with ultraviolet radiation, the degradation level was from 3, 0 to 5, 5.

5. High concentration of DNA does not affect the performance of the presented decontamination substances. However, the effect of decontamination agents on trace concentrations and degraded DNA may differ. This problem is a goal for future research.

6. The comparative characteristics of the presented treatment methods showed the difference in the mechanisms of action of decontaminating agents on human DNA and the high efficiency of their work, which allows to apply the selected methods in biological laboratories.

References

- Abbasov, R. H., Povkh, A. S., Romanchuk, S. M. (2018). *Metodyka provedennia molekuliarno-henetychnykh doslidzhen: metodicheskie rekomendacii*. Kyiv: DNDEKTs MVS Ukrainy [in Ukrainian].
- Adé, A. and others (2017). Comparison of Decontamination Efficacy of Cleaning Solutions on a Biological Safety Cabinet Workbench Contaminated by

- Cyclophosphamide' 70 (6) *The Canadian Journal of Hospital Pharmacy*. [in English].
- Atramentova, L.A. (2015). *Dizain i statistika biologicheskogo issledovaniia*. Kharkov: Izdatelstvo «NTMT» [in Russian].
- Burgener, J. (2006). Position paper on the use of ultraviolet lights in biological safety cabinets' 11 (4) *Applied Biosafety*. [in English].
- Deistvie ultrafioletovogo izlucheniia Na mikroorganizmy i bakterii. URL: <http://biofile.ru/bio/4193.html> (data zvernennia 20 June 2019) [in Russian].
- Derzhavni sanitarni Normy i pravyla : Nakaz Ministerstva okhorony zdorovia Ukrainy vid 24.01.2008 № 26 (2008) Ofitsiyni visnyk Ukrainy [in Ukrainian].
- DNAZap™ PCR DNA Degradation Solutions (Thermo Fisher Scientific, 2017). URL: https://assets.thermofisher.com/TFS-Assets/LSG /manuals/9890M_DNAZap_Solutions_PI.pdf (data zvernennia 19 June 2019) [in English].
- Gefrides, L. A. and others (2010). UV irradiation and autoclave treatment for elimination of contaminating DNA from laboratory consumables. 4(2) *Forensic Science International: Genetics* [in English].
- Georgieva, S., Popov, B., Petrov, V. (2013). Genotoxic effects of copper sulfate in rabbits. 65(3) *Archives of Biological Sciences*. DOI: 10.2298/abs1303963g [in English].
- Guideline for Disinfection and Sterilization in Healthcare Facilities (Centers for Disease Control and Prevention, 2016) URL: <https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html> (data zvernennia 19 June 2019) [in English].
- Ioannou, C. J., Hanlon, G. W., Denyer, S. P (2006). Action of Disinfectant Quaternary Ammonium Compounds against *Staphylococcus aureus*. 51(1). *Antimicrobial Agents and Chemotherapy* [in English].
- Maris, P. (1995). Modes of action of disinfectants. 14 (1) *Rev. sci. tech. Off. int. Epiz* [in English].
- Mark, W. LeChevallier, Kwok-Keung, Au Water (2004). *Treatment and Pathogen Control: Process Efficiency in Achieving Safe Drinking Water*. London: IWA Publishing. 41–66 [in English].
- Sang, Su Kim and others (2013). Comparison of disinfective power according to application order of 70% isopropyl alcohol and 10% povidone-iodine 65 *Korean J Anesthesiol*. [in English].
- Setlow, B. and others (2014). Mechanism of killing of spores of *Bacillus anthracis* in a high-temperature gas environment, and analysis of DNA damage generated by various decontamination treatments of spores of *Bacillus anthracis*, *Bacillus subtilis* and *Bacillus thuringiensis* 116. *Journal of Applied Microbiology*. [in English].
- Tamariz, J. and others (2006). The Application of Ultraviolet Irradiation to Exogenous Sources of DNA in Plasticware and Water for the Amplification of Low Copy Number DNA 51 (4) *Journal of Forensic Sciences*. [in English].
- Wu Y. and others (2017). DNA decontamination methods for internal quality management in clinical PCR laboratories' 32 (3) *Journal of clinical laboratory analysis* [in English].

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**СРАВНИТЕЛЬНАЯ ХАРАКТЕРИСТИКА ЭФФЕКТИВНОСТИ
НЕКОТОРЫХ ДЕКОНТАМИНАЦИОННЫХ СРЕДСТВ,
ИСПОЛЬЗУЕМЫХ ПРИ ПРОВЕДЕНИИ СУДЕБНОЙ
МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКОЙ ЭКСПЕРТИЗЫ**

Статья посвящена сравнению эффективности работы дезинфицирующих средств при различной концентрации контаминирующего агента. Контаминация объектов может происходить на любой стадии проведения исследования, поэтому для ее предотвращения необходимо использовать эффективные деконтаминирующие методы, которые в то же время должны быть безопасны для персонала лаборатории. Целью работы является проверка эффективности дезинфицирующих средств при работе в биологических лабораториях и определение их воздействия на генетическую информацию клетки, влияния на ингибицию реакции ПЦР и деградацию ДНК.

Ключевые слова: деконтаминирующие средства; ДНК; ингибция; ПЦР; эффективность.

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