DOI: https://doi.org/10.17816/brmma107133

DEVELOPMENT OF A TEST SYSTEM AND A METHOD FOR DETECTING RIBONUCLEIC ACID OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 USING REAL-TIME POLYMERASE CHAIN REACTION

O.A. Miteva, A.V. Smirnova, I.A. Myasnikova, K.A. Bykova, B.A. Kanevsky, V.A. Myasnikov

State Research and Testing Institute of Military Medicine, Saint Petersburg, Russia

ABSTRACT. Given the rapid spread of coronavirus disease 2019 (COVID-19) globally, test systems are needed for its diagnosis, timely treatment, and introduction of quarantine measures. In the shortest possible time, a diagnostic system based on real-time reverse-transcription polymerase chain reaction to detect the ribonucleic acid of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal and oropharyngeal smears was developed and registered. The method determines the nucleocapsid and small-membrane protein genes and the human PGK1 gene, acting as internal control reactions. The nucleotide sequences of SARS-CoV-2 were analyzed, and primers were selected. The conditions for carrying out real-time reverse-transcription polymerase chain reaction and the composition of a set of reagents were set. The diagnostic sensitivity and specificity of the kit were tested on biological samples, with the addition of inactivated SARS-CoV-2. The high analytical characteristics of the developed set of reagents were demonstrated, with a sensitivity of at least 103 GE/mL and a specificity of 100%, and no false-positive or false-negative results were recorded. The high specificity of the test system was shown on a representative sample of genetic materials of respiratory viral pathogens. Clinical and laboratory tests of the diagnostic “SARS-CoV-2 test” were conducted in the N.F. Gamalei National Research Center for Epidemiology and Microbiology. A set of reagents for the detection of ribonucleic acid of SARS-CoV-2 through on real-time reverse-transcription polymerase chain reaction for in vitro diagnostics “SARS-CoV-2 test” was registered in the Russian Federation as a medical device (Registration certificate no. RZN 2020/10632, dated 06/03/2020).

Keywords: diagnostics of infectious diseases; molecular diagnostics; new coronavirus infection; reverse-transcription and polymerase chain reaction in real-time; coronavirus of severe acute respiratory syndrome-2; genetic material; nucleocapsid; pathogens of respiratory viral infections.

To cite this article:
Miteva OA, Smirnova AV, Myasnikova IA, Bykova KA, Kanevsky BA, Myasnikov VA. Development of a test system and a method for detecting ribonucleic acid of severe acute respiratory syndrome coronavirus 2 using real-time polymerase chain reaction. *Bulletin of the Russian Military Medical Academy*. 2022;24(4):675–682. DOI: https://doi.org/10.17816/brmma107133

Received: 05.05.2022 **Accepted:** 27.10.2022 **Published:** 25.12.2022

УДК 616.98:578.2

DOI: https://doi.org/10.17816/brmma107133

РАЗРАБОТКА ТЕСТ-СИСТЕМЫ И СПОСОБА ВЫЯВЛЕНИЯ РИБОНУКЛЕИНОВОЙ КИСЛОТЫ КОРОНАВИРУСА ТЯЖЕЛОГО ОСТРОГО РЕСПИРАТОРНОГО СИНДРОМА-2, ВОЗБУДИТЕЛЯ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ, ИСПОЛЬЗУЯ ПОЛИМЕРАЗНУЮ ЦЕПНУЮ РЕАКЦИЮ В РЕЖИМЕ РЕАЛЬНОГО ВРЕМЕНИ

О.А. Митева, А.В. Смирнова, И.А. Мясникова, К.А. Быкова, Б.А. Каневский, В.А. Мясников

Государственный научно-исследовательский испытательный институт военной медицины, Санкт-Петербург, Россия

Резюме. В связи с быстрым распространением новой коронавирусной инфекции по всему земному шару появилась необходимость в большом количестве тест-систем для ее диагностики с целью назначения своевременного лечения и введения карантинных мероприятий. В кратчайшие сроки была разработана и зарегистрирована диагностическая система в формате обратной транскрипции и полимеразной цепной реакции в реальном времени для выявления рибонуклеиновой кислоты коронавируса тяжелого острого респираторного синдрома-2 в мазках со слизистой оболочки носоглотки и ротоглотки, основанная на определении генов нуклеокапсида и малого белка мембранной оболочки, а также гена PGK1 человека, выступающего в качестве внутреннего контроля реакции. Проведен анализ нуклеотидных последовательностей коронавируса тяжелого острого респираторного синдрома-2 и осуществлен подбор праймеров. Подобраны условия для проведения обратной транскрипции и полимеразной цепной реакции в реальном времени и состав набора реагентов. Исследование диагностической чувствительности и специфичности набора проводили на образцах биологического материала, с добавлением инактивированного вируса тяжелого острого респираторного синдрома-2. Продемонстрированы высокие аналитические характеристики разработанного набора реагентов: чувствительность не менее 10^3 ГЭ/мл, специфичность — 100%, не было зарегистрировано ложноположительных или ложноотрицательных результатов. Высокая специфичность тест-системы показана на репрезентативной выборке генетического материала возбудителей респираторно-вирусных инфекций. Клинико-лабораторные испытания диагностикума «SARS-CoV-2-тест» были проведены на базе Национального исследовательского центра эпидемиологии и микробиологии имени Н.Ф. Гамалеи. Набор реагентов для выявления рибонуклеиновой кислоты коронавируса тяжелого острого респираторного синдрома-2 путем полимеразной цепной реакции в режиме реального времени для диагностики *in vitro* «SARS-CoV-2-тест» зарегистрирован в Российской Федерации как медицинское изделие, регистрационное удостоверение № РЗН 2020/10632 от 03.06.2020.

Ключевые слова: диагностика инфекционных заболеваний; молекулярная диагностика; новая коронавирусная инфекция; обратная транскрипция и полимеразная цепная реакция в реальном времени; коронавирус тяжелого острого респираторного синдрома-2; генетический материал; нуклеокапсид; возбудители респираторно-вирусных инфекций.

Как цитировать:
Митева О.А., Смирнова А.В., Мясникова И.А., Быкова К.А., Каневский Б.А., Мясников В.А. Разработка тест-системы и способа выявления рибонуклеиновой кислоты коронавируса тяжелого острого респираторного синдрома-2, возбудителя новой коронавирусной инфекции, используя полимеразную цепную реакцию в режиме реального времени // Вестник Российской военно-медицинской академии. 2022. Т. 24, № 4. С. 675–682. DOI: https://doi.org/10.17816/brmma107133

Рукопись получена: 05.05.2022 **Рукопись одобрена:** 27.10.2022 **Опубликована:** 25.12.2022

ЭКО • ВЕКТОР

INTRODUCTION

Viral infections account for more than 60% of all known human infectious diseases and are one of the main mortality causes in both developed and developing countries [1]. In the process of evolution, more and more new types of pathogens occur, so new, so-called emerging or emergent, infections with extremely high epidemic potential are regularly recorded. One of critical situations instantly developed following a local outbreak of new respiratory infection caused by Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2), the causative agent of a new coronavirus infection COVID-19 [2].

In December 2019, a previously undetectable coronavirus, originally named 2019 novel coronavirus (2019-nCoV), caused an outbreak of acute respiratory infection in Wuhan, China [3]. On February 11, 2020, the World Health Organization (WHO) assigned the official names to the virus and the corresponding disease, Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) and Coronavirus infection disease 2019 (COVID-19), respectively[1]. In January 2020, in Russia, COVID-19 was added to the list of diseases posing a danger to others, along with other highly infectious diseases such as plague, cholera, and smallpox[2]. In March 2020, the WHO classified the spread pattern of the new infection as a pandemic[3].

Such a rapid spread of SARS-CoV-2 raised key issues related to the urgent development of early diagnostic tools necessary for timely and correct diagnostics, selection of treatment strategy, and implementation of disease control measures. According to generally accepted recommendations, most tests designed to detect COVID-19 are based on molecular biological methods and are implemented in the real-time polymerase chain reaction (RT-PCR) format[4,5].

Due to favorable analytical and technical characteristics and simple and rapid reaction, this methos was recognized as successful and promising for development of PCR-based test systems to be used in clinical laboratory practice and epidemiological monitoring studies. Developing diagnostic tools of such a type is facilitated by a variety of high-tech platforms for selecting and optimizing primers [4]. Selection criteria for best PCR conditions were well known and standardized [5]. This allowed to quickly develop and scale up the production of diagnostic PCR kits for the public health system [6, 7].

The first data on the genetic sequence of SARS-CoV-2 was published in the GenBank database and the Global Initiative on Sharing Avian Influenza Data (GISAID) portal on January 10 and 12, 2020, respectively. This allowed rapid development of diagnostic PCR tests to detect a new infection[6]. Just two weeks after deciphering the SARS-CoV-2 genome, BGI & Co-Diagnostics (China) offered the first commercial diagnostic kits for quantitative reverse transcription combined with real-time polymerase chain reaction (RT-PCR). To date, the technical manual posted on the WHO website[7] presents a wide list of test systems developed by different countries and includes hundreds of molecular genetic and immunoanalytical diagnostic kits which are developed or under development.

The aim of the study was to develop a set of reagents for laboratory COVID-19 diagnostics using real-time PCR as well as to evaluate its analytical characteristics and diagnostic significance.

MATERIALS AND METHODS

Samples for nucleic acid (NA) isolation were prepared according to Guidelines 1.3.2569-09 “Workflow Management in Laboratories using Nucleic Acid Amplification Techniques When Working with Materials Containing Group I–IV Pathogens”[8]. Ribonucleic acid (RNA) was isolated from biomaterial samples by manual isolation of NA using a medical device Reagent kit for RNA/DNA extraction from clinical material "RIBO-prep" according to TU 9398-071-01897593-2008 of Central Scientific Research Institute of Epidemiology of Rospotrebnadzor. The recommended elution volume is 50 µL.

The development of the reagent kit was started in January 2020 when there was no clinical material in the Russian Federation. As a result, a region of the SARS-CoV-2 genome was synthesized *de novo*, and its fragment was cloned into a plasmid construct with isolating a recombinant RNA containing a portion of the SARS-CoV-2 genome. It was

[1] WHO. The name of the disease caused by coronavirus (COVID-19), and the viral pathogen. [Epub] URL: https://www.who.int/ru/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it. (Accessed on September 29, 2022).

[2] On Amending the List of Diseases Posing a Danger to Others: Decree of the Government of the Russian Federation of January 31, 2020 No. 66. In: Collection of Laws of the Russian Federation. 2020. No. 6. Art. 674.

[3] WHO. Director General’s opening remarks at the media briefing on COVID-19 — 11 March 2020 [Epub] URL: https://www.who.int/dg/speeches/detail/who-director-general-sopening-remarks-at-the-media-briefing-on-covid-19--11-march-2020 (Accessed on March 23, 2020).

[4] S. N. Avdeyev et al. Prevention, Diagnosis and Treatment of Novel Coronavirus Infection (COVID-19): Interim Guidelines. Version 15. The Ministry of Health of the Russian Federation. Moscow. 2022. 245 pp.

[5] WHO. Technical Guidance–coronavirus disease (COVID-19) [Epub]. URL: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance-publications (Accessed on September 29, 2022).

[6] GISAID. [Epub] URL: https://www.gisaid.org/epiflu-applications/next-hcov-19-app/ (Accessed on March 23, 2020).

[7] WHO. Novel Coronavirus (2019-nCoV) technical guidance: laboratory guidance. [Epub] URL: https://www.who.int/emergencies/ diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance (Accessed on March 23, 2020).

[8] Workflow Management in Laboratories using Nucleic Acid Amplification Techniques When Working with Materials Containing Group I–IV Pathogens: Guidelines 1.3.2569-09. Effective date: April 05, 2010. [Epub].URL: http://docs.cntd.ru/document/1200077791 (Accessed on Septmeber 29, 2022).

necessary for further studies and used as a positive control sample. By the time the kit was developed, the nucleotide sequences of 183 full-length virus genomes with minor genetic differences had been included in the database of the National Center for Biotechnology Information (NCBI) and GISAID. The sequences were aligned using the Mega X program and the Clustal W algorithm. Primers and probes for the selected target genes were designed using the Primer Express software from Applied Biosystems (USA). Preliminary specificity of primers and characteristics of fluorescent probes were assessed using the MEGA software package and online NCBI database resources. Each probe was labelled with a fluorescent reporter dye (FAM, HEX, or Cy5). Amplification products were detected using TaqMan Assay probes. Primers and probes were synthesized by a closed joint stock company (CJSC) Evrogen (Russia).

To obtain more accurate and reliable data, control samples were included in the developed set of reagents. A positive control sample was obtained by cloning the synthesized deoxyribonucleic acid (DNA) fragments carrying a virus-specific insert (a conservative fragment of the SARS-CoV-2 E-gene) into the pUC57 plasmid from Evrogen CJSC (Russia). A positive control sample should be included into the analysis during the NA extraction step as a separate sample. PGK1, the human housekeeping gene, was used as an internal endogenous control of the reaction. Plasmid DNA concentration was determined using a commercial Quant-IT DNA HS reagent kit and a QUBIT fluorometer from Invitrogen and ThermoFisher Scientific (USA) in accordance with the manufacturer's instructions.

The developed set of reagents is suitable for conducting reverse transcription and real-time PCR in one step. The analysis is carried out according to the developed instructions. The total volume of the reaction mixture is 25 μL, including 5 μL of the RNA sample. The reaction mixture is composed of the following components: 17 μL of SARS-CoV-2 test RT-PCR mixture, 3 μL of SARS-CoV-2 test FS mixture, 5 μL of RNA sample. The amplification program consists of the following steps: reverse transcription for 15 min at 50°C; further denaturation at 95°C for 15 min; 45 cycles, each included DNA denaturation at 94°C for 15 s, primer annealing at 55°C for 45 s, and chain elongation at 72°C for 15 s. Curves of fluorescent signal accumulation are analyzed by three channels: the channel for the FAM fluorophore registers a signal indicating the accumulation of the amplification product of the SARS-CoV-2 N-gene fragment; the VIC fluorophore channel registers a signal indicating the accumulation of an amplification product of the SARS-CoV-2 E-gene fragment; the Cy5 fluorophore channel registers a signal indicating the accumulation of the amplification product of the internal endogenous control gene fragment.

The result was evaluated by threshold method, determining Ct by the intersection of the fluorescence curve with a threshold line set at the middle of the exponential section of the fluorescence increase graph on a logarithmic scale. The amplification results were interpreted as positive if the intersection of the fluorescence curve with the threshold line set at the appropriate level was observed. The SARS-CoV-2-test reagent kit was optimized using CFX96 PCR diagnostic devices registered in the Russian Federation as medical devices from Bio-Rad Laboratories (USA) and DT-light from DNA Technology (Russia) .

Clinical and laboratory tests of "SARS-CoV-2-test" were conducted at the N.F. Gamaleya National Research Center for Epidemiology and Microbiology. To determine the analytical sensitivity, 52 nasal swabs were tested with the addition of an inactivated GK2020/1 strain of the SARS-CoV-2 in serial dilutions. The following virus concentrations were used to prepare model samples: 10^5 genomic equivalents per mL (GE/mL) with 10 replicates, 10^4 GE/mL with 10 replicates, 10^3 GE/mL with 10 replicates, 10^2 GE/mL with 10 replicates, 10^1 GE/mL with 6 replicates, 0 GE/mL with 6 replicates. Total number was 52 samples. The sensitivity threshold was set according to the minimum dilution detected in all replicates. To determine the analytical specificity, 9 samples with pathogens of other respiratory infections (*Influenza A virus* (H1N1, H3N2, H7N9)), *Influenza B virus*, human adenovirus A-5 (strain Adeno-5), human metapneumovirus (HM-1 virulent strain), parainfluenza virus type 1 (Sendai Biomed strain), *Haemophilus influenzae* (ATCC 49619), *Streptococcus pneumoniae* (CCBH-101/14). The presence or absence of Influenza A (H1N1 and H3N2) and Influenza B virus markers (nucleic acids) was confirmed by testing with AmpliSens® *Influenza virus A/B-FL* reagent kits (TU 9398-080-01897593-2012 LOT 02.03.20) and AmpliSens® *Influenza virus A/H1-swine-FL* (TU 9398-101-01897593-2009 LOT 05.03.20) reagent kits. For other infections, we used characterized museum strains from the State Virus Collection of the National Research Center for Epidemiology and Microbiology based on data presented in strain passports provided (with a biological activity of at least 1.0×10^5 PFU/mL). Samples with respective pathogens were tested in quadruplicate each.

Diagnostic sensitivity and specificity were assessed using samples of human biomaterial taken during the diagnostic and treatment process, with the addition of an inactivated GK2020/1 strain of SARS-CoV-2. A total of 48 nasal model swabs containing SARS-CoV-2 RNA and 35 nasal swabs without SARS-CoV-2 RNA were tested. To obtain samples with the final concentration (5×10^4 GE/mL), 10 μL of GK2020/1 archival strain of SARS-CoV-2 was added to 1000 μL of each biological sample. The assessment of diagnostic characteristics included comparative studies of the same biomaterial samples using the developed and reference set of reagents. The validated Vector-PCRv-2019-nCoV-RG test system from Vector (Russia) was used as a reference set.

Diagnostic sensitivity (D Se) was calculated using the formula:

$$D\ Se = TP / (TP + FN) \times 100\%,$$

where: TP is the number of true positive results, FN is the number of false negative results.

Diagnostic specificity (D Sp) was calculated using the formula:

$$D\ Sp = TN / (TN + FP) \times 100\%,$$

where: TN is the number of true negative results, FP is the number of false positive results.

RESULTS AND DISCUSSION

The developed technique is based on RNA reverse transcription and subsequent cDNA amplification consisting of repeated cycles of thermal DNA denaturation, annealing of primers with complementary sequences, and subsequent completion of polynucleotide chains from these primers with Taq polymerase. Reverse RNA transcription and PCR amplification of cDNA are performed using one tube.

When developing PCR techniques for indicating and identifying dangerous pathogens, genetic determinants are most often used as markers. They are highly specific and conservative for this type of pathogens and are absent in closely related organisms. For a new coronavirus infection, *N*, *E*, *S* genes (peplomer spike protein gene) and *RdRP* (RNA-dependent RNA polymerase gene) are recommended as target genes[9]. Most international and national health organizations recommend using the *N* gene and one of additional genes as a target when developing diagnostic kits. This approach prevents future diagnostics errors due to false negative results, which are possible in case of new mutations in the SARS-CoV-2 genome. In accordance with these recommendations, when developing our own original set of reagents for COVID-19 diagnostics, target genes were sequences consisting of sections of the *N* and *E* genes of SARS-CoV-2 and the human *PGK1* gene, acting as an internal endogenous control of the reaction. Control samples were used to evaluate the effectiveness of all stages of analysis.

The reaction mixture contains fluorescently labeled oligonucleotide probes, which hybridize with complementary regions of the target cDNA being amplified, resulting in an increase in fluorescence intensity. This allows to register accumulation of a specific amplification product by measuring the intensity of the fluorescent signal in real time. Primers were designed using sequences that served as targets for selection of specific oligonucleotides to effectively detect currently circulating isolates (including variants α-, δ-, o-).

At the first stage, monoplex PCRs were used for each detection channel to determine the optimal range of fluorescent signal values, sufficient to detect specific fluorescence and non-overlapping with the signals of other channels, as well as to select the amplification program and the ratio of primer and probe concentrations in each reaction mixture without increase in background fluorescence. When choosing the optimal conditions for the real-time PCR, the following reaction parameters were determined: composition of the reaction mixture including concentrations of primers, probes, Taq polymerase, and magnesium ions; amplification program with primer annealing temperature, duration of each step of the amplification cycle, number of cycles, duration of preliminary denaturation. Using the data obtained during monoplex reactions, the composition of the reaction mixture for multiplex real-time RT-PCR was selected to ensure a stable increase in the fluorescence level in the detection channels (FAM, VIC, and Cy5) without distorting the signal in other channels. When developing optimal conditions for one-stage PCR with the RT stage, the optimal concentrations of enzymes (Taq-polymerase and MMLV reversetase), magnesium ions, deoxynucleotide triphosphates were selected, and the temperature protocol for one-stage PCR were established. Based on above findings, the maximum value of the threshold cycle (Ct) was determined, at which the reaction result is considered positive. In this case, the fluorescence curve of the sample should cross the threshold line in the area of the characteristic exponential rise in fluorescence. An appropriate algorithm was proposed to interpret amplification results (Table 1). In case of discrepancy between the results obtained and tabular data, the analysis shall be repeated, starting with RNA extraction.

The optimized composition of the reagent kit is shown in Table 2.

According to studies, the analytical sensitivity of the SARS-CoV-2 test kit for qualitative detection of SARS-CoV-2 RNA by real-time PCR for *in vitro* diagnostics was at least 10^3 GE/mL, which meets the criteria provided in technical specification (TU). Specificity assessment did not reveal any cross-reactions with closely related and heterologous microbial species. Fluorescence in these samples did not exceed the background level on all detection channels.

Findings obtained when assessing diagnostic characteristics of the developed reagent set are presented in Table 3.

The diagnostic sensitivity of the kit was 100% (95% confidence interval (CI): 94.4%, 100%). The diagnostic specificity was 100% (95% CI: 92.3%, 100%). Studies showed that the detection limit for SARS-CoV-2 RNA (analytical sensitivity of the reagent kit) in nasal swabs is 1×10^3 GE/mL.

[9] WHO. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance. 2 March 2020. [Epub]. URL: https://apps.who.int/iris/bitstream/handle/10665/331329/WHO-COVID-19-laboratory-2020.4. (Accessed on September 29, 2022).

Table 1. Detection of SARS-CoV-2 RNA by a set of reagents for SARS-CoV-2 test
Таблица 1. Учет результатов выявления РНК SARS-CoV-2 набором реагентов «SARS-CoV-2-тест»

Name of sample	Detection by channel			Interpretation of results
	FAM (*N*-gene)	VIC (*E*-gene)	Cy5 (*PGK1*-gene)	
	Ct			
Test sample	⩽ 40	⩽ 40	⩽ 38	To be considered. Viral RNA detected
	Absent	Absent	⩽ 38	To be considered. Viral RNA not detected
Negative control sample	Absent	Absent	Absent	To be considered.
Positive control sample	⩽ 32	⩽ 32	⩽ 38	To be considered.
Internal control sample	Absent	Absent	⩽ 38	To be considered.

Table 2. Composition of a set of reagents for qualitative detection of SARS-CoV-2 RNA by real-time polymerase chain reaction for *in vitro* diagnostics “SARS-CoV-2 test” according to TU 21.20.23-001-07669108-2020
Таблица 2. Состав набора реагентов для качественного выявления РНК вируса SARS-CoV-2 путем полимеразной цепной реакции в режиме реального времени для диагностики *in vitro* «SARS-CoV-2-тест» по ТУ 21.20.23-001-07669108-2020

Name of set components	Description	Number per pack
FS reaction mixture	Clear, colorless liquid without sediment	1 test tube (0.310 mL)
RT-PCRm reaction mixture	Pink liquid without sediment	1 test tube (0.744 mL)
Positive control sample	Clear, colorless liquid without sediment	1 test tube (0.280 mL)
Negative control sample	Clear, colorless liquid without sediment	1 test tube (0.280 mL)

Table 3. Diagnostic characteristics of a set of reagents for the detection of SARS-CoV-2 virus RNA by real-time polymerase chain reaction
Таблица 3. Результат оценки диагностических характеристик набора реагентов для детекции РНК вируса SARS-CoV-2 путем полимеразной цепной реакции в режиме реального времени

Result	Quantity
True positive	48
False positive	0
True negative	35
False negative	0
Total	83

CONCLUSION

The State Scientific Research Institute of Military Medicine has developed a diagnostic test system for detecting SARS-CoV-2 RNA by real-time RT-PCR. These studies allowed to develop an experimental set of reagents and after laboratory testing, instructions for use, technical specification, pilot-scale production regulations, and a quality certificate were prepared. Clinical and laboratory testing confirmed the favorable analytical and diagnostic characteristics of the developed test system. According to clinical findings, this set of reagents meets technical and operational requirements for functional characteristics, effectiveness, quality, and safety of a medical device. Clinical trials confirmed quality of this medical device as well as its effectiveness and safety. As a result, a marketing authorization was issued by the Federal Service for Surveillance in Healthcare (Roszdravnadzor) dated June 03, 2020 No. RZN 2020/10632 for a medical device Reagent kit for the qualitative detection of SARS-CoV-2 RNA by real-time polymerase chain reaction for *in vitro* diagnostics "SARS-CoV-2-test" according to TU 21.20.23-001-07669108-2020. To obtain complete and reliable data on the developed medical device when used by Russian Military Medical Service in 2020, the test system was tested in various Centers for Disease Control and Prevention of the Russian Ministry of Defense. The system was recognized as promising for use in disease control and prevention units of the Russian Military Medical Service (including Centers for Disease Control and Prevention of the Russian Ministry of Defense, sanitary platoon of a separate medical company / brigade, sanitary laboratory of the division).

REFERENCES

1. Medunitsyn NV, Katlinsky AV, Vorslov LO. Editors. *Vaccinology.* Moscow: Practical Medicine; 2022. 480 p. (In Russ.)

2. Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med.* 2020;382(13):1199–1207. DOI: 10.1056/NEJMoa2001316

3. Wang H, Wang W, Tan W. Summary of the detection kits for SARS-CoV-2 approved by the National Medical Products Administration of China and their application for diagnosis of COVID-19. *Virol Sin.* 2020;35(6):699–712. DOI: 10.1007/s12250-020-00331-1

4. Park S, Zhang Y, Lin S, et al. Advances in microfluidic PCR for point-of-care infectious disease diagnostics. *Biotechnol Adv.* 2011;29(6):830–839. DOI: 10.1016/j.biotechadv.2011.06.017

5. Esbin MN, Whitney ON, Chong S, et al. Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection. *RNA.* 2020;26(7):771–783. DOI: 10.1261/rna.076232.120

6. Tan W, Zhao X, Ma X. et al. A novel coronavirus genome Identified in a cluster of pneumonia cases. Wuhan, China 2019–2020. *China CDC Weekly.* 2020;2(4):61–62. DOI: 10.46234/ccdcw2020.017

7. Dong L, Zhou J, Niu C, et al. Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR. *Talanta.* 2021;224:121726. DOI: 10.1016/j.talanta.2020.121726

СПИСОК ЛИТЕРАТУРЫ

1. Вакцинология / под ред. Н.В. Медуницына, А.В. Катлинского, Л.О Ворслова. Москва: Практическая медицина, 2022. 480 с.

2. Li Q., Guan X., Wu P., et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia // N Engl J Med. 2020. Vol. 382, No. 13. P. 1199–1207. DOI: 10.1056/NEJMoa2001316

3. Wang H., Wang W., Tan W. Summary of the detection kits for SARS-CoV-2 approved by the National Medical Products Administration of China and their application for diagnosis of COVID-19 // Virol Sin. 2020. Vol. 35, No. 6. P. 699–712. DOI: 10.1007/s12250-020-00331-1

4. Park S., Zhang Y., Lin S., et al. Advances in microfluidic PCR for point-of-care infectious disease diagnostics // Biotechnol Adv. 2011. Vol. 29, No. 6. P. 830–839. DOI: 10.1016/j.biotechadv.2011.06.017

5. Esbin M.N., Whitney O.N., Chong S., et al. Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection // RNA. 2020. Vol. 26, No. 7. P. 771–783. DOI: 10.1261/rna.076232.120

6. Tan W., Zhao X., Ma X., et al. A novel coronavirus genome Identified in a cluster of pneumonia cases. Wuhan, China 2019–2020 // China CDC Weekly. 2020. Vol. 2, No. 4. P. 61–62. DOI: 10.46234/ccdcw2020.017

7. Dong L., Zhou J., Niu C., et al. Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR // Talanta. 2021. Vol. 224. P. 121726. DOI: 10.1016/j.talanta.2020.121726

AUTHORS INFO

***Olga A. Miteva,** applicant for an academic degree; e-mail: letto2004@inbox.ru; ORCID: 0000-0002-3874-6954; SCOPUS: 55195685300; eLibrary SPIN: 2070-7250

Anna V. Smirnova, applicant for an academic degree; e-mail: gniii_7@mil.ru; eLibrary SPIN: 4897-0219

Irina A. Myasnikova, candidate of medical sciences; e-mail: gniii_7@mil.ru; eLibrary SPIN: 8883-1534

Ksenia A. Bykova, junior research assistant; e-mail: gniii_7@mil.ru; eLibrary SPIN: 3310-3572

Boris A. Kanevsky, deputy chief; e-mail: gniii_7@mil.ru; eLibrary SPIN: 2549-9294

Vadim A. Myasnikov, candidate of medical sciences; e-mail: gniii_7@mil.ru; eLibrary SPIN: 5084-2723

ОБ АВТОРАХ

***Ольга Анатольевна Митева,** соискатель ученой степени; e-mail: letto2004@inbox.ru; ORCID: 0000-0002-3874-6954; SCOPUS: 55195685300; eLibrary SPIN: 2070-7250

Анна Владимировна Смирнова, соискатель ученой степени; e-mail: gniii_7@mil.ru; eLibrary SPIN: 4897-0219

Ирина Анатольевна Мясникова, кандидат медицинских наук; e-mail: gniii_7@mil.ru; eLibrary SPIN: 8883-1534

Ксения Александровна Быкова, младший научный сотрудник e-mail: gniii_7@mil.ru; eLibrary SPIN: 3310-3572

Борис Александрович Каневский, заместитель начальника; e-mail: gniii_7@mil.ru; eLibrary SPIN: 2549-9294

Вадим Александрович Мясников, кандидат медицинских наук; e-mail: gniii_7@mil.ru; eLibrary SPIN: 5084-2723

* Corresponding author / Автор, ответственный за переписку