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The development of primers and the installation of PCR for the detection of coronavirus SARS-CoV-2

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Abstract. The relevance of this study stems from the need to improve the accuracy and sensitivity of laboratory diagnostics for COVID-19, particularly in the early stages of infection and with low viral loads. The aim of the study was to validate and experimentally evaluate the performance of a pair of primers targeting the SARS-CoV-2 nucleocapsid gene using a plasmid positive control. The study utilised computer-aided primer design using standard criteria, testing their specificity against nucleotide sequence databases, modelling a plasmid construct with the target fragment, and performing one-step reverse transcription followed by polymerase chain reaction and agarose gel analysis of the products. Primers N9171F and N9368R, 20-22 nucleotides in length, with optimal GC content and consistent melting temperatures, were developed and characterised. Bioinformatics analysis revealed a lack of significant tendency to form stable hairpins and dimers, and confirmed minimal homology with off-target sequences, which increases amplification specificity. Recombinant plasmid DNA pJET1.2, containing a 197-bp insert of the target fragment, was used as a positive control. Visualisation of the construct map confirmed the presence of the diagnostic insert and its ease of use for reaction control. Experimental results demonstrated the production of

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a specific product of the expected size (197 bp) during SARS-CoV-2 RNA analysis, while the negative amplification control did not produce any, confirming the accuracy of the reaction and reducing the risk of false negative results with a valid control. The practical value of this work lies in the potential for using the proposed primer pair and plasmid control in laboratories for RT-PCR testing in laboratory diagnostics, epidemiological monitoring, and the development of diagnostic test systems for detecting the coronavirus pathogen

Keywords: plasmid; nucleotide; diagnostics; amplification; BLAST

Introduction

The need to develop and optimise primers for the SARS-CoV-2 nucleocapsid (N) gene stems from the virus's continued circulation, the emergence of new genetic variants, and the need to ensure reliable laboratory diagnostics at the national level. Despite a decline in global incidence compared to the peak levels of 2020-2022, COVID-19 remains a significant respiratory infection causing severe complications in vulnerable population groups. Given the genetic variability of the virus, the effectiveness of existing test systems may decline, requiring regular revision of primer and probe designs, particularly for conserved regions of the genome such as the N gene. D. Dutta *et al.* (2022) analysed the diagnostic performance of RT-PCR in clinical samples from 312 patients and demonstrated that test systems targeting the N gene provided a detection limit of up to 10 RNA copies/reaction at a specificity of 99.3%, which exceeded the performance of tests targeting the S gene exclusively. The authors emphasised that the high transcriptional activity of the N gene results in a greater quantity of subgenomic RNA, increasing the likelihood of virus detection in the early stages of infection.

In their study, A. Mentes *et al.* (2022) proposed an *in silico* pipeline for identifying genomic variants that overlap with the target regions of widely used SARS-CoV-2 PCR primer sets, based on large datasets including sequences from GISAID. They found that mutations affecting primer target sites – particularly in Omicron lineages – impair detection by some assays, increasing the proportion of samples classified as misclassified. These findings underscore the need for regular *in silico* assessment of primer-template complementarity as circulating variants evolve. D. Negrón *et al.* (2022) conducted a large-scale *in silico* analysis of over 1.6 million SARS-CoV-2 genomes and demonstrated that the accumulation of mutations in primer and probe binding regions can lead to a reduction in the sensitivity of PCR assays. This indicates a potential decline in assay sensitivity over time and highlights the need for continuous monitoring and redesign of primers. H. Li *et al.* (2020) developed a modified set of primers targeting a conserved region of the N2 region and tested it on 186 clinical samples. The sensitivity of the method was 98.9%, and the limit of detection was 5 RNA copies per reaction. Compared with the reference protocol, sensitivity increased by 6.3%, particularly in samples with a viral load of less than 10^3 copies/mL.

In their review of COVID-19, M. Cascella *et al.* (2025) provided a comprehensive overview of the clinical features, epidemiology and diagnostic methods for SARS-CoV-2, including a discussion of molecular diagnostics using PCR. The authors noted that SARS-CoV-2, like other RNA viruses, rapidly accumulates mutations in its genome, which influences the virus's evolution and requires careful consideration when selecting targets for diagnostic assays, as changes in target sequences can potentially reduce the sensitivity of RT-qPCR tests. This highlights the importance of monitoring circulating variants to maintain the effectiveness of existing diagnostic primers. Research by R. Wang *et al.* (2020) demonstrates that the N gene remains one of the most stable and diagnostically significant targets of SARS-CoV-2. The constant evolution of the virus necessitates regular analysis of genomic data, optimisation of primer sequences, and experimental validation of their efficacy. Furthermore, the one-step RT-PCR format is more cost-effective and technologically convenient for routine use in clinical diagnostic laboratories. Combining the reverse transcription and amplification steps in a single reaction tube reduces the duration of the assay by an average of 30-40% compared to two-step protocols, reduces the consumption of enzymes and plasticware, and minimises the risk of contamination. This is particularly important during mass testing and when handling a high throughput of clinical samples. Reducing the number of steps decreases staff workload and improves the reproducibility of results, making one-step RT-PCR the optimal tool for rapid and reliable laboratory diagnosis of SARS-CoV-2.

According to the WHO (2022), equitable distribution of and access to COVID-19 diagnostic tools, including molecular tests such as PCR kits, remain inadequate in many low- and middle-income countries. The report highlights persistent gaps in regulation, supply and domestic delivery mechanisms that hinder timely and reliable access to diagnostic tools, underscoring the ongoing challenges countries face in maintaining a stable diagnostic capacity for SARS-CoV-2. For example, not all laboratories in the Republic of Kazakhstan and the Kyrgyz Republic have stable and timely access to commercial diagnostic kits for detecting SARS-CoV-2. Constraints related to supply logistics, currency dependence and periodic disruptions in the global reagent market may reduce the efficiency of laboratory

surveillance of infectious diseases, particularly in remote regions. Given the ongoing circulation of the virus and the need to monitor new variants, such reliance on imported test kits poses a potential epidemiological risk. In this regard, the development in such countries of test systems based on their own molecular genetic solutions, including the design of primers and probes targeting conserved regions of the SARS-CoV-2 N gene, takes on particular significance. The creation of local diagnostic methods makes laboratory diagnostics more accessible, ensures flexibility in adapting to new variants of the virus, and reduces the cost of testing. Furthermore, the implementation of nationally developed technologies helps to strengthen the region's scientific and technological capacity and establish a sustainable biosafety system.

The optimisation of primers for the SARS-CoV-2 N gene in a one-step RT-PCR format is of not only scientific and methodological but also strategic importance, ensuring laboratories' independence from external suppliers and enhancing the preparedness of the healthcare systems in Kazakhstan and Kyrgyzstan for current and future epidemiological challenges. The aim of this study was to develop and experimentally optimise primers for conserved regions of the SARS-CoV-2 N gene, taking into account current genomic data on circulating variants of the virus, and to evaluate their analytical sensitivity and specificity in a one-step RT-PCR assay.

Materials and Methods

The research was conducted at the Research Institute for Biological Safety Issues of the QazBioPharm National Holding (Republic of Kazakhstan) in 2025. The SARS-CoV-2 strain SARS-CoV-2/human/KAZ/Delta-020/2021 was used in the study. Viral RNA was isolated under BSL-2 biosafety conditions using the innuPREP Virus DNA/RNA Kit (IST Innuscreen GmbH, Berlin, Germany) in accordance with the manufacturer's instructions. The concentration and quality of the isolated RNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The A260/A280 and A260/A230 ratios were used to assess the purity of the preparation, with values in the range of 1.8-2.0 and > 1.8, respectively, being considered acceptable.

A constructed plasmid DNA containing a fragment of the SARS-CoV-2 N gene was used as a positive control. A 197-base-pair (bp) nucleotide sequence corresponding to the SARS-CoV-2 N-gene fragment was cloned into the pJET1.2 plasmid vector using the CloneJET PCR Cloning Kit (Thermo Fisher Scientific Baltics, Lithuania). Transformation was carried out in competent E. coli DH5α cells, and recombinant clones were selected on LB agar supplemented with ampicillin (100 μg/mL). Plasmid DNA was isolated using a plasmid extraction kit and verified by Sanger sequencing. The resulting recombinant plasmid was used as a positive control in the OT-PCR assay. A model of the pJET1.2 plasmid construct with the N-gene fragment inserted was generated using PlasMapper 3.0 software.

Primers for the detection of SARS-CoV-2 were selected based on multiple alignment of the nucleotide sequences of the relevant viral genes. Sequences of SARS-CoV-2 isolates from various geographical regions, available in the GenBank (n.d.) database, were used for the analysis. The N-gene sequences of the SARS-CoV-2 virus available in GenBank were used for alignment (Table 1).

Table 1. GenBank accession numbers for the N gene of the SARS-CoV-2 virus

Name of the gene and virus	Identification numbers of SARS-CoV-2 virus sequences in the GenBank database
The N gene of the SARS-CoV-2 virus	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/KAZ/Omicron-XBB.1.9.1-399/2023 [Genbank: OR485702.1, Kazakhstan], Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/KAZ/Omicron-XBB.1.9-400/2023 [Genbank: OR485705.1, Kazakhstan], Isolate SARS-CoV-2/human/KAZ/AST-S396/2023 [Genbank: PP905483.1, Kazakhstan], isolate SARS-CoV-2/HUMAN/CHN/Delta CS/2021[Genbank: PX952996.1, China], isolate SARS-CoV-2/Vero E6/CHN/Delta CAS1\2/2024 1 [Genbank: PX952990.1, China], isolate SARS-CoV-2/human/CHN/CCS12-BA.2/2022 [Genbank: PX587945.1, China], isolate SARS-CoV-2/human/RUS/P693/2024 [Genbank: PQ510397.1, Russia]

Source: compiled by the authors based on GenBank (n.d.)

Nucleotide sequences in FASTA format were imported into MEGA version 11.0 (Molecular Evolutionary Genetics Analysis) via the Align → Edit/Build Alignment module. Multiple alignment was performed using the ClustalW algorithm. Alignment parameters: gap opening penalty – 15, gap extension penalty – 6.66. Conservative regions selected for primer design were defined as areas with a sequence identity of at least 95% among the isolates analysed.

Primer pairs were designed using the Vector NTI Advance™ software (Thermo Fisher Scientific, USA) in the "Primer Design" section. The target nucleotide sequence was imported in FASTA format into the Vector NTI → Tools → Primer Design module for the automatic selection of optimal primers. The criteria for primer selection were: length 18-24 nucleotides, guanine-cytosine (GC) content 40-60%, annealing temperature 55-65°C, absence of significant secondary structures (ΔG > -3 kcal/mol for hairpins and dimers), and an amplicon size of 150-300 bp. Primer specificity was verified using the BLAST (Basic Local Alignment Search Tool) algorithm against the NCBI (n.d.) nucleotide sequence

database to exclude cross-reactivity with the genomes of other microorganisms and humans.

For the detection of viral RNA by RT-PCR, primers N9171F and N9368R, targeting the SARS-CoV-2 N gene, were used. The primers were synthesised by Metabion International AG (Germany) to HPLC-grade purity. The total reaction volume was 25 µL and comprised: 12.5 µL of 2× buffer, 1 µL of enzyme mix, 1 µL each of forward and reverse primers (10 nmol), 2 µL of RNA template (5-50 ng) and deionised water to the final volume. The composition of the 2× buffer included Tris-HCl (pH 8.3), KCl, $MgCl_2$ (concentration 3 mM in the final reaction) and dNTPs (0.4 mM of each). The enzyme mix comprised reverse transcriptase, which facilitates the synthesis of complementary DNA (cDNA) from the RNA template, and Taq DNA polymerase, which carries out the amplification of the cDNA.

The RT-PCR temperature-time profile comprised a reverse transcription step at 58°C for 30 seconds, an initial denaturation step at 94°C for 1 minute, followed by 40 cycles: denaturation at 94°C for 15 s, annealing/extension at 58°C for 30 s. Amplification was performed on a SimpliAmp Thermal Cycler (Thermo Fisher Scientific, USA). The amplification products were analysed by electrophoresis in a 2% agarose gel (SeaKem LE Agarose, Lonza) with the addition of ethidium bromide (0.5 µg/mL) in 1× TAE buffer. Electrophoresis was performed at 120 V for 40 minutes. The GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific) was used as a molecular weight marker. Visualisation and documentation of the results were carried out using a gel documentation system with a UV transilluminator.

The specificity of the primers developed was analysed using the NCBI BLAST online tool. All experiments were conducted in triplicate. No statistical analysis was performed due to the qualitative nature of the RT-PCR method.

Results and Discussion

The primers were designed in accordance with standard PCR design criteria: primer length ranged from 18 to 24 nucleotides, GC content was between 40% and 60%, and the melting temperatures (Tm) of the forward and reverse primers differed by no more than 5°C. To enhance specificity, a GC-clamp was introduced at the 3′ end and the propensity for secondary structure formation was minimised. Homology with off-target sequences was checked *in silico* using nucleotide sequence databases. Table 2 lists the characteristics of the selected primers.

Table 2. Characteristics of the selected primers N9171F and N9368R for the N gene of SARS-CoV-2

Name of the primer	Nucleotide sequence (5′ → 3′)	Length, nt	GC, %	Tm, °C	Amplicon size, bp
N9171F	CATTGGCCGCAAATTGCACA	20	50.0	59	197
N9368R	TGGTGGGAATGTTTTGTATGCG Complementary: CGCATACAAAACATTCCCACCA	22	45.5	60	

Note: nt – nucleotides; GC – percentage of guanine (G) and cytosine (C) nucleotides; Tm – primer melting temperature; bp – base pairs

Source: compiled by the authors

The N9171F and N9368R primers have an optimal length (20-22 nt) and GC content (45-50%), ensuring stable and specific amplification. Melting temperature calculations showed good agreement in Tm values between the primers (difference < 2°C). Analysis of secondary structures revealed no stable hairpins or significant dimers capable of inhibiting PCR, allowing these primers to be recommended for use in PCR. Figure 1 shows the localisation of the primers on the nucleotide sequence of the coronavirus genome.

The pJET1.2 plasmid DNA is used as a positive control in PCR, as it contains the target nucleotide sequence of the SARS-CoV-2 virus's N gene. The presence of amplification when using this control confirms the correctness of the reaction conditions, the functionality of the primers and the enzyme system, and also allows false-negative test results to be ruled out (Fig. 2).

Modelling has yielded a circular map of the recombinant pJET1.2 plasmid containing an insert of the SARS-CoV-2 N gene, with a total length of 3,205 bp. The 197-bp insert, cloned into plasmid DNA, contains the target fragment of the SARS-CoV-2 N gene and is used as a positive control in PCR. The results obtained confirm that RT-PCR targeting the N gene allows for the effective detection of viral RNA, including at low viral loads, which is particularly important for the early diagnosis of COVID-19 and screening studies. The results of RT-PCR using the N9171F and N9368R primers for the detection of the SARS-CoV-2 N gene are presented in Figure 3.

The RT-PCR results for the identification of the SARS-CoV-2 virus showed that a specific PCR product of 197 bp in size was amplified using SARS-CoV-2 viral RNA. The selection of the N gene as a target for SARS-CoV-2 diagnosis is scientifically justified. As noted by P. Zhou *et al.* (2020), SARS-CoV-2 exhibits 96% identity in its whole-genome sequence with the bat coronavirus RaTG13 and 79.6% with SARS-CoV, indicating the phylogenetic proximity of this virus to coronaviruses of animal origin. Furthermore, as shown in the study by F. He *et al.* (2020), coronaviruses are a highly diversified group of RNA-containing viruses that can cause respiratory, intestinal, hepatic and neurological diseases of varying severity in humans and animals. In this regard, the selection of conserved genomic regions for

diagnostic purposes becomes of critical importance. The SARS-CoV-2 coronavirus nucleocapsid protein N plays a key role in the packaging of viral RNA and viral replication, which further confirms the stability and diagnostic significance of the gene encoding it (Chang *et al.*, 2006; Dangi *et al.*, 2021; Chen *et al.*, 2022). W. Song *et al.* (2023) emphasise that the N gene is highly conserved across different viral strains and is expressed in large quantities in infected cells, which increases the sensitivity of the detection method. Using the N gene as a target reduces the likelihood of false-negative results, particularly when analysing samples with a low viral load, which is of fundamental importance for the early diagnosis of infection.

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CDS             28206..29456
                /gene="N"
                /codon_start=1
                /product="nucleocapsid phosphoprotein"
                /protein_id="UUS91996.1"
                /translation="MSDNGPQNQRNALRITFGGPSDSTGSNQNGGARSKQRRPQGLPN
                NTASWFTALTQHGKEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLS
                PRWYFYYLGTGPEAGLPYGANKDGIIWVATEGALNTPKDHIGTRNPANNAAIVLQLPQ
                GTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSKRTSPARMAGNGGDAALAL
                LLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAYNVTQAFGRRGP
                EQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAI
                KLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLL
                PAADLDDFSKQLQQSMSRADSTQA"
28321 aactggcagt aaccagaatg gtggggcgcg atcaaaacaa cgtcggcccc aaggtttacc
28381 caataatact gcgtcttggt tcaccgctct cactcagcat ggcaaggaag accttaaatt
28441 ccctcgagga caaggcgttc caattaacac caatagcagt ccagatgacc aaattggcta
28501 ctaccgaaga gctaccagac gaattcgtgg tggtgacggt aaaatgaaag atctcagtcc
28561 aagatggtat ttctactacc taggaactgg gccagaagct ggacttccct atggtgctaa
28621 caaagacggc atcatatggg ttgcaactga gggagccttg aatacaccaa aagatcacat
28681 tggcacccgc aatcctgcta acaatgctgc aatcgtgcta caacttcctc aaggaacaac
28741 attgccaaaa ggcttctacg cagaagggag cagaggcggc agtcaagcct cttctcgttc
28801 ctcatcacgt agtcgcaaca gttcaagaaa ttcaactcca ggcagcagta aacgaacttc
28861 tcctgctaga atggctggca atggcggtga tgctgctctt gctttgctgc tgcttgacag
28921 attgaaccag cttgagagca aaatgtctgg taaaggccaa caacaacaag gccaaactgt
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29101 tggggaccag gaactaatca gacaaggaac tgattacaaa cattggccgc aaattgcaca
29161 atttgccccc agcgcttcag cgttcttcgg aatgtcgcgc attggcatgg aagtcacacc
29221 ttcgggaacg tggttgacct acacaggtgc catcaaattg gatgacaaag atccaaattt
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29341 agagcctaaa aaggatcaaa agaagaaggc tgatgaaact caagccttac cgcagagaca
29401 gaagaaacag caaactgtga ctcttcttcc tgctgcagat ttggatgatt tctccaaaca
29461 attgcaacaa tccatgagcc gtgctgactc aactcaggcc taaactcatg cagaccacac
29521 aaggcagatg ggctatataa acgttttcgc ttttccgttt acgatatata gtctactctt
29581 gtgcagaatg aattctcgta actacatagc acaagtagat gtagttaact ttaatctcac
29641 atagcaatct ttaatcagtg tgtaacatta gggaggactt gaaagagcca ccacattttc
```

Figure 1. Localisation of primers on the nucleotide sequence of the SARS-CoV-2 coronavirus genome
Source: compiled by the authors based on NCBI (n.d.)

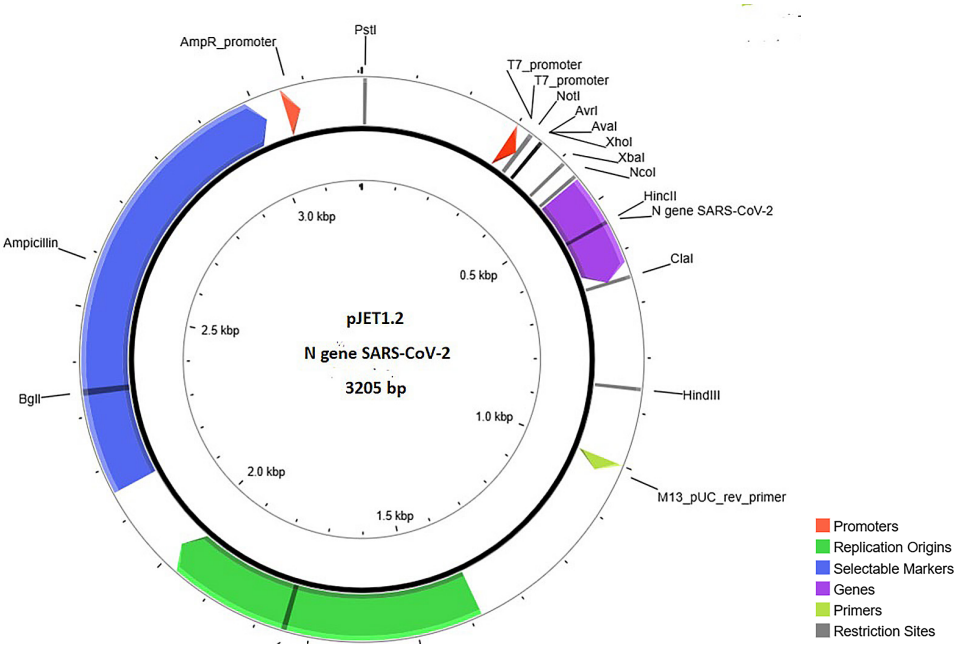

Figure 2. Circular map of the recombinant plasmid pJET1.2 containing the N gene fragment of the SARS-CoV-2 virus
Source: compiled by the authors using PlasMapper 3.0

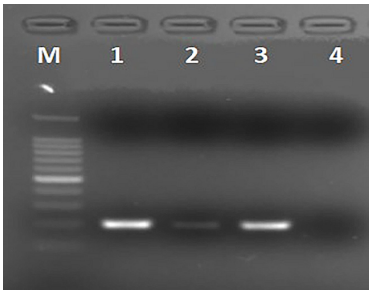

Figure 3. Results of PCR testing using the N9171F and N9368R primers for the diagnosis of SARS-CoV-2
Note: M – DNA molecular weight marker; 1 – positive control; 2, 3 – SARS-CoV-2 viral RNA; 4 – negative control
Source: compiled by the authors

The use of MEGA 11.0 software for multiple alignment of nucleotide sequences is consistent with modern approaches to bioinformatics analysis (Tamura *et al.*, 2021). This software package is widely used for phylogenetic analysis and enables the identification of conserved genomic regions that are optimal for the design of diagnostic primers. When developing PCR systems for the detection of SARS-CoV-2, the envelope protein (E) and RNA-dependent RNA polymerase (RdRp) genes are also widely used as diagnostic targets (Chu *et al.*, 2020; Pabbaraju *et al.*, 2021). The E gene is highly conserved among members of the Sarbecovirus subgenus and is frequently used in screening PCR tests due to its stability (Rangaiah *et al.*, 2021). I. Barjaktarović *et al.* (2023) highlight the diagnostic importance of detecting the E gene of the SARS-CoV-2 genome in the comprehensive diagnosis of COVID-19. The RdRp gene, which encodes a key enzyme in viral genome replication, exhibits high specificity for SARS-CoV-2 and is frequently used in confirmatory diagnostic tests (Abbasi *et al.*, 2022). Compared to the E and RdRp genes, the N gene is characterised by a combination of high conservation, specificity and expression levels. The N gene is widely used as the primary or one of the key targets in commercial and research diagnostic kits for the detection of SARS-CoV-2 (Corman *et al.*, 2020; Hu *et al.*, 2022).

A. Nalla *et al.* (2020) conducted a comparative evaluation of the effectiveness of SARS-CoV-2 detection using seven different pairs of primers and probes, including kits targeting the N gene. The results demonstrated that N-gene-based systems provide high sensitivity and specificity in the detection of viral RNA in clinical samples. The importance of accurate laboratory diagnosis of COVID-19 is also emphasised in the broader context of the pandemic's development. S. Perlman (2020) noted in his editorial that, a decade after the SARS outbreak and seven years after the emergence of MERS, humanity is facing yet another coronavirus, which highlights the need to develop reliable and accessible diagnostic systems. B. Udugama *et al.* (2020) emphasised in their review the central role of molecular diagnostics, in particular RT-PCR, as the gold standard for detecting SARS-CoV-2, whilst noting the need for validated diagnostic tools. T. Dong *et al.* (2023), in a comprehensive review of SARS-CoV-2 diagnostic and analytical methods, noted that PCR-based molecular detection methods remain the most reliable for the early detection of infection, particularly when combined with the use of multiple targets, including the N gene.

Overall, the results of this study confirm that the developed one-step PCR system, utilising the N9171F and N9368R primers targeting the conserved region of the N gene, is an effective tool for the detection of SARS-CoV-2. The use of a one-step RT-PCR format reduces analysis time, lowers the risk of contamination and simplifies the protocol compared to two-step methods, making this approach optimal for routine laboratory diagnostics. The use of the recombinant plasmid pJET1.2 containing a cloned fragment of the N-gene as a positive control allows the reaction setup procedure to be standardised and ensures the validity of the results obtained. The combination of bioinformatic analysis for primer selection and experimental verification of their efficacy demonstrates a comprehensive approach to the development of diagnostic systems. The data obtained can be used in the development of domestic diagnostic kits for the detection of SARS-CoV-2, which will help to increase the availability of laboratory diagnostics for COVID-19 in the region and reduce dependence on imported test systems.

Conclusions

As a result of the study, specific primers for the detection of SARS-CoV-2 using a one-step reverse transcription polymerase chain reaction (RT-PCR) method were developed and experimentally validated, which is of practical significance for the laboratory diagnosis of COVID-19 in the Republic of Kazakhstan and the Kyrgyz Republic. Based on bioinformatic analysis of multiple alignment of N-gene nucleotide sequences of SARS-CoV-2 isolates from various geographical regions, as presented in the GenBank database, conserved regions of the genome with a sequence identity of at least 95% were identified. Using Vector NTI Advance software, primers N9171F (20 nucleotides) and N9368R (22 nucleotides) were developed, characterised by an optimal GC content of 45-50%, melting temperatures differing by less than 2°C, and no tendency to form stable secondary structures. Verification of primer specificity using the BLAST method confirmed minimal homology with off-target sequences, which reduces the risk of non-specific amplification.

A recombinant pJET1.2 plasmid containing a 197-base-pair target fragment of the SARS-CoV-2 N gene was constructed and used as a positive control for the validation of the RT-PCR assay. The use of a plasmid control ensures standardisation of the reaction and helps to eliminate false-negative results, which is

critical for the reliability of laboratory diagnostics. The performance of the developed primers has been demonstrated experimentally: when analysing RNA from the SARS-CoV-2/human/KAZ/Delta-020/2021 strain using a one-step RT-PCR method, a specific amplification product of the expected size of 197 base pairs was obtained, visualised by agarose gel electrophoresis. The absence of amplification in the negative control confirms the specificity of the reaction and the correctness of the selected conditions.

Future research plans include validating the developed method on an expanded panel of clinical samples from patients with confirmed COVID-19, determining the analytical sensitivity and specificity of the method, assessing its effectiveness in detecting new genetic variants of SARS-CoV-2, adapting the protocol for real-time PCR with fluorescent detection, and developing a multiplex test system with simultaneous detection of several target genes (N, E, RdRp) to improve the reliability of diagnosis and differentiation of SARS-CoV-2 from other respiratory pathogens.

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Conflict of Interest

None.

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SARS-CoV-2 коронавирусун аныктоо үчүн Праймерлерди иштеп чыгуу жана Птрди коюу

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Аннотация. Бул изилдөөнүн актуалдуулугу COVID-19 үчүн лабораториялык диагностиканын тактыгын жана сезгичтигин жогорулатуу зарылдыгынан келип чыгат, айрыкча инфекциянын алгачкы этаптарында жана вирустук жүктөмү төмөн болгондо. Изилдөөнүн максаты SARS-CoV-2 нуклеокапсид генин плазмиддик позитивдүү башкарууну колдонуу менен бутага алган бир жуп праймердин иштешин текшерүү жана эксперименталдык түрдө баалоо болгон. Изилдөөдө стандарттуу критерийлерди колдонуу менен компьютердик жардам менен праймердин дизайны колдонулган, алардын нуклеотиддик ырааттуулук маалымат базаларына карата спецификалуулугун текшерген, максаттуу фрагмент менен плазмиданын конструкциясын моделдеген жана бир баскычтуу тескери транскрипцияны, андан кийин полимераз чынжыр реакциясын жана продуктулардын агароз гелинин анализин жүргүзгөн. Узундугу 20-22 нуклеотидден турган, оптималдуу GC курамы жана туруктуу эрүү температурасы бар N9171F жана N9368R праймерлери иштелип чыккан жана мүнөздөлгөн. Биоинформатикалык анализ туруктуу чач тырмактарын жана димерлерди түзүү тенденциясынын жоктугун көрсөттү жана максаттан тышкаркы ырааттуулуктар менен минималдуу гомологияны тастыктады, бул амплификациянын спецификалуулугун жогорулатат. Максаттуу фрагменттин 197-bp кошумчасын камтыган рекомбинанттык плазмидалык ДНК pJET1.2 оң контроль катары колдонулган. Конструкция картасын визуалдаштыруу диагностикалык кошумчанын бар экендигин жана аны реакцияны башкаруу үчүн колдонуунун оңойлугун тастыктады. Эксперименталдык жыйынтыктар SARS-CoV-2 РНК анализи учурунда күтүлгөн өлчөмдөгү (197 bp) белгилүү бир продуктунун өндүрүлгөнүн көрсөттү, ал эми терс күчөтүү контролу эч кандай натыйжа берген жок, бул реакциянын тактыгын тастыктап, жаракту контроль менен жалган терс натыйжалардын коркунучун азайтты. Бул иштин практикалык баалуулугу сунушталган праймер жубун жана плазмидалык контролду лабораториялык диагностикада, эпидемиологиялык мониторингде жана коронавирустун козгогучун аныктоо үчүн диагностикалык тест системаларын иштеп чыгууда RT-ПЦР тестирлөө үчүн лабораторияларда колдонуу мүмкүнчүлүгүндө жатат

Негизги сөздөр: плазмид; нуклеотид; диагностика; күчөтүү; BLAST

Разработка праймеров и постановка ПЦР для выявления коронавируса SARS-CoV-2 на основе N-гена

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Аннотация. Актуальность работы обусловлена необходимостью повышения точности и чувствительности лабораторной диагностики COVID-19, особенно на ранних стадиях инфекции и при низкой вирусной нагрузке. Целью исследования было обосновать и экспериментально оценить работоспособность пары праймеров, нацеленных на нуклеокапсидный ген SARS-CoV-2, с использованием плазмидного положительного контроля. В работе применяли компьютерный дизайн праймеров по стандартным критериям, проверку их специфичности по базам нуклеотидных последовательностей, моделирование плазмидной конструкции с целевым фрагментом, а также проведение одношаговой обратной транскрипции с последующей полимеразной цепной реакцией и анализ продуктов в агарозном геле. Были разработаны и охарактеризованы праймеры N9171F и N9368R длиной 20-22 нуклеотида с оптимальным содержанием GC и согласованными температурами плавления. При биоинформатической оценке установлено отсутствие выраженной склонности к образованию устойчивых шпилек и димеров, а также подтверждена минимальная гомология с внецелевыми последовательностями, что повышает специфичность амплификации. В качестве положительного контроля использована рекомбинантная плазмидная ДНК pJET1.2, содержащая вставку целевого фрагмента длиной 197 пар оснований; визуализация карты конструкции подтверждала наличие диагностической вставки и удобство ее применения для контроля постановки реакции. Экспериментально показано получение специфического продукта ожидаемого размера 197 пар оснований при анализе РНК SARS-CoV-2, тогда как отрицательный контроль амплификации не давал, что свидетельствует о корректности реакции и снижает риск ложноотрицательных результатов при валидном контроле. Практическая ценность работы заключается в возможности использования предложенной пары праймеров и плазмидного контроля в лабораториях для проведения ОТ-ПЦР-метода в лабораторной диагностике, эпидемиологическом мониторинге и при создании диагностических тест-систем для выявления возбудителя коронавирусной инфекции

Ключевые слова: плазмида; нуклеотид; диагностика; амплификация; BLAST