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RESEARCH ARTICLE

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Comparison of COVID-19 laboratory diagnosis by commercial kits: Effectivity of RT-PCR to the RT-LAMP

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Abstract

Coronavirus disease 2019 or COVID-19 caused by novel coronavirus/severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or 2019-nCoV) is an ongoing pandemic that has emerging global effects and requires rapid and reliable diagnostic testing. Quantitative reverse transcription-polymerase chain reaction (q-RT-PCR) is the gold standard method for SARS-CoV-2 detections. On the other hand, new approaches remedy the diagnosis difficulties gradually. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) as one of these novel approaches may also contribute to faster and cheaper field-based testing. The present study was designed to evaluate this rapid screening diagnostic test that can give results in 30–45 min and to compare the effectiveness of LAMP to the q-RT-PCR. The 30 randomly chosen patient samples were generated by nasopharyngeal swabs with a portion of the SARS-CoV-2 nucleic sequence. The sample of quantification cycle (Cq) values was tested using RT-LAMP as well as by conventional q-RT-PCR. The patient samples were tested with four different kits (SENSObiz COVID-19 [SARS-CoV-2] LAMP Assay, the QIAseq DIRECT SARS-CoV-2 kit, Biospeedy SARS-CoV-2 Variant Plus kit, and CoVirion-CV19-2 SARS-CoV-2 OneStep RT-PCR kit) and two different PCR devices (GDS Rotor-Gene Q Thermocycler and Inovia Technologies GenX series). Based on 30 patient samples, the positive/negative ratio (P/N) was 30/0 as Biospeedy and Covirion (positivity 100%), 28/2 as Qiagen kit (positivity 93.3%) for the samples studied on the Inovia device while the same samples on the Rotor-Gene device were 30/0 as Biospeedy and Covirion (positivity 100%), 29/1 as Qiagen kit at the first day (96.7%). On the fifth day, the samples were studied in the Inovia device and the respective results were obtained: 27/3 as Biospeedy (positivity 90%), 16/14 as Qiagen (positivity 53.3%), 28/2 as Covirion kit (positivity 93.3%). When these samples were studied in the Rotor-Gene device, it was 29/1 in Biospeedy and Covirion (positivity 96.7%), 19/11 in the Qiagen kit (positivity 63.3%). When these samples were compared with the LAMP method it was found to be 19/11 (positivity 63.3%) on the first day and 18/12 (positivity 60%) on the fifth day. SARS-CoV-2 test studies will contribute to a proactive approach to the development of rapid diagnosis systems. The LAMP approach presents promising results to monitor exposed individuals and also improves screening efforts in potential ports of entry.

KEYWORDS
Coronavirus, Covid-19, LAMP-PCR, q-RT-PCR, SARS-CoV-2

1 | INTRODUCTION

Throughout history, three types of coronavirus families have affected humans. Severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in Guangdong, China, while the Middle East respiratory syndrome coronavirus (MERS-CoV/SARS-CoV-1) emerged in Saudi Arabia. In late December 2019, an unknown disease reported as pneumonia appeared in Wuhan city, Hubei Province, China, which was called SARS-CoV-2 (genus Betacoronavirus, subgenus Sarbecoronavirus). Globally, there have been 262.178.403 confirmed cases of COVID-19 including 5 215 745 deaths, reported to WHO (December 1, 2021). As of November 30, 2021, a total of 7 880 127 721 vaccine doses have been administered.[1] Generally, droplets from person to person, contaminated materials, and direct contacts of the virus are also the main reasons for the transmission of the virus. Fever, back pain, taste and smell loss, cough, and diarrhea are the main symptoms of the disease. Moreover, multiorgan system failure because of cytokine storm and respiratory failures are described in severe cases.[2] Asymptomatic cases have been also detected with positive polymerase chain reaction (PCR) results depending on their viral loads that represent the ratio range from 8% to 80%.[3] Incubation time is significant to detect the disease clinically and it was updated as 6.4 days with recent studies.[4]

The coronavirus family members are positive-sense enveloped single-stranded RNA viruses that are categorized into four genera as beta, alpha, gamma, and delta coronavirus. The SARS-CoV-2 is a beta type and only affects mammals. Mainly, it is composed of a 30 kb genome with 14 open reading frames (ORFs) encoded to the spike protein (S), nucleocapsid protein (N), a small membrane protein (SM), and membrane glycoprotein (M) with an additional membrane glycoprotein (HE).[5] The spike protein is the actual part for binding the specific host receptor, angiotensin-converting enzyme 2 (ACE2). When the spike protein binds to the receptor, it divides two main subunits; first, an amino-terminal subunit (S1) and a carboxyl-terminal subunit (S2) by host furin-like proteases, as shown in Figure 1.[6]

The genetic sequence of SARS-CoV-2 shares 79.5% with SARS-CoV and 96.2% identical genome with RaTG13 which is a short RNA-dependent RNA polymerase (RdRp) region that originated from bats of CoV.[7] A new version of the coronavirus family, SARS-CoV-2 was first reported in the Wuhan city of China in December 2019 and spread throughout the whole world rapidly[8] The first case in our country (Tukey) was reported on March 11, 2020, and the Ministry of Health established COVID-19 diagnostic laboratories in public hospitals and private diagnostic centers to detect the SARS-CoV-2 virus.

The major aspect to control pandemics throughout the whole world is the understanding of SARS-CoV-2 genome mutations. Recently, numerous types of variants of SARS-CoV-2 have been described. Thus, rapid and reliable detection of severe acute respiratory syndrome SARS-CoV-2 is crucial to control the spread rate of the virus.[9,10] The creation of sufficient awareness and preparedness of the virus is the main effective aspect so as to prevent the transmission of the virus. In particular, many patients show asymptomatic infection and are the most frequent carriers of the disease. These patients contribute to the spreading of the infection. Thus, to control the spreading of the virus infection, the first step is the diagnosis of the disease. According to this perspective, four different strategies are q-RT-PCR, serological tests, LAMP, and point of care (POC), respectively.

The RT-PCR is a quantitative and gold standard method that is commonly preferred. In this technique, three main genes are targeted for SARS-CoV-2 virus detection including the N-gene (N protein), Orf1b gene (human RNA polymerase protein), and the E-gene (E protein). In the RT-PCR technique, the specificity of the confirmatory test relies on the probe-target sequence.[11] Clinical specimens are resourced from nasopharyngeal or oropharyngeal samples of

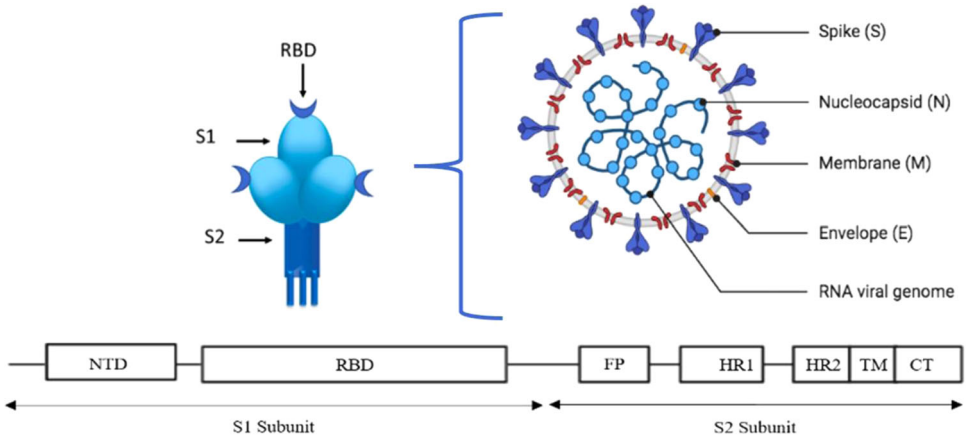

FIGURE 1 The structure of SARS-CoV-2. CT, C-terminal domain; FP, fusion peptide; HR1, and HR2, Heptad repeat 1 and 2; NTD, N terminal domain; RBD, receptor-binding domain; S, spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TM, transmembrane

patients. On the other hand, the immunoassays are alternatively utilized test types used the enzyme-linked immunosorbent assay (ELISA) that are cheaper but also less sensitive compared to PCR. The immunofluorescence assay (IFA) is utilized for the diagnosis of SARS-CoV-2 with the presence of immunoglobulin G (IgG).[12] The blood-based tests are serological tests that are used to identify whether the person had an infection. The antibodies are utilized to detect the disease. Antibodies (IgM and IgG) are specific to an antigen within the blood. Principally, the immune system recognizes these antigens of an infected person as a foreign element and specific antibodies can be created to fight the infection within the body. Thus, these antibodies can act as labels for the disease that are generally produced after the second week of the virus infection. Although IgM antibodies can be detected after 10–20 days, IgG is determined after 20 days of SARS-CoV-2 infection.[13] Moreover, the POC tests are rapid diagnostic tests for immunodiagnostic detection of SARS-CoV-2. However, tests are available only in research settings recommended by WHO. Although these tests give the result within several minutes, they can only detect actively replicating viruses.[14] Although there are many techniques to detect the SARS-CoV-2, nowadays the scientific world has changed direction to the loop-mediated isothermal amplification with simultaneous reverse-transcription LAMP (RT-LAMP) technique.

RT-LAMP technique is a recently preferred technique which is a rapid and sensitive method used in SARS-CoV-2 detection. Nucleic acid detections occur over 1 h and this easily interpretable colorimetric assay requires only a heat source.[15] LAMP technique is the fast and low-cost simple colorimetric technique that makes RT-LAMP an effective solution for ramping up global testing capacity. Moreover, it is single tube technology to detect the target nucleic acid sequences.[16] On the basis of the LAMP technique, six primers are utilized, including four primers selected by combining parts of the target DNA and two additional loop primers that are used to amplify a specific gene region. Recently, RT-LAMP has been applied for POC for many RNA virus infections.[17] According to this perspective, LAMP has taken an important place for the diagnosis of virus infections, such as SARS-CoV-2. Thus, in this study, it is aimed to compare the COVID-19 diagnosis effectivity of RT-PCR to the RT-LAMP. In addition, our results contribute to the proactive approach for the development of a rapid diagnosis system.

2 | MATERIAL AND METHOD

2.1 | Sample collection and transportation

Nasopharyngeal swab samples of SARS-CoV-2 patients were collected by trained staff and transferred to Private VIROMED Istanbul Central Laboratory and Imaging Center, Istanbul, in a viral transport medium (VTM) solution tube. Thirty randomly selected patient samples were tested with four different kits as SENSObiz COVID-19 (SARS-CoV-2) Reverse Transcriptase PCR (isothermal) Assay (LAMP) (NANOBIZ Technologies A.Ş.), the QIAseq DIRECT SARS-CoV-2 kit (QIAGEN), Biospeedy SARS-CoV-2 Variant Plus kit (BIOEKSEN R&D Technologies), and CoVirion-CV19-2 SARS-CoV-2 OneStep RT-PCR kit (MEDAMET Medical ITH. IHR. SAN. TIC. LTD, Turkey). Additionally, three of these kits (QIAseq DIRECT SARS-CoV-2 kit, Biospeedy SARS-CoV-2 Variant Plus kit, and CoVirion-CV19-2 SARS-CoV-2 OneStep RT-PCR kit) were also tested in GDS Rotor-Gene Q Thermocycler (QIAGEN) and Inovia Technologies GenX series (INOVIA) RT-PCR systems. The study was conducted based on positivity and negativity of results on the first and fifth days and the Cq results of the samples that were positively changed daily.

2.2 | RT-PCR tests

Throughout the RT-PCR, the kits did not require any extra RNA extraction step because of the VTM solution usage with nucleic acid extraction property. The vortex was enough for RNA extraction of swap samples with VTM solution. For the RT-PCR, three different kit systems were utilized which were the QIAseq DIRECT SARS-CoV-2 kit, Biospeedy SARS-CoV-2 Variant Plus kit, and CoVirion-CV19-2 SARS-CoV-2 OneStep RT-PCR kit.

2.3 | Biospeedy SARS-CoV-2 Variant Plus kit

In this kit, 6-carboxy- fluorescein (FAM), phosphoramidite (Hex), 6-carbocyl-X-Rhoddamine (ROX) and, carboxylic acid (Cy5) channels were utilized for ORF1ab, RNaseP, Spike (S) gene and, Nucleocapsid (N), respectively. Based on the kit protocol, 2.5 µl patient samples with VTM were added to a 7.5 µl ready kit mixture to achieve a 10 µl PCR mixture in total. Thermal cycle parameters of RT-PCR amplification were as follows: 52°C for 3 min for reverse transcription, 95°C for 10 s for holding, then 35 cycles of 85°C for 1 s and 60°C for 1 s for denaturation, annealing, and extension, respectively.

2.4 | CoVirion-CV19-2 SARS-CoV-2 OneStep RT-PCR kit

In this kit, FAM, HEX, and Texas Red were used for S, Orf1ab, and RNaseP gene, respectively. According to the kit protocol, 5 µl patient samples with VTM were added to a 15 µl ready kit mixture to achieve 20 µl PCR mixture in total. Thermal cycle parameters of RT-PCR amplification were as follows; 55°C for 10 min for reverse transcription, 95°C for 10 s for holding, then 40 cycles, and 60°C for 30 s for denaturation, annealing, and extension, respectively.

2.5 | QIAseq DIRECT SARS-CoV-2 kit

In this kit, FAM, HEX, and Cy5 were used for S, Orf1ab, and RNaseP gene, respectively. According to the kit protocol, 5 µl patient samples with VTM were added to a 15 µl ready kit mixture to achieve a 20 µl

PCR mixture in total. Thermal cycle parameters of RT-PCR amplification were as follows; 50°C for 10 min for reverse transcription, 95°C for 2 min for holding, then 40 cycles, and 58°C for 30 s for denaturation, annealing, and extension, respectively.

2.6 | LAMP-PCR tests—SENSObiz COVID-19 (SARS-CoV-2) RT PCR kit

In the LAMP procedure, the kit does not require any extra RNA extraction step due to the use of VTM solution, and another advantage is that swap samples can be used just after being taken from the patient. Thus, SENSObiz COVID-19 (SARS-CoV-2) Reverse Transcriptase PCR (isothermal) Assay was utilized using LAMP4U device (NANOBIZ Technologies A.Ş.). The mix should be completely thawed on ice and mixed thoroughly by inverting several times. The RNA isolate of the samples (minimum 0.5 ng/µl) was added to the reaction mix (23 µl). The prepared tubes were mixed gently by finger tapping, briefly centrifuged (spin), and placed in the thermal cycler. It gave a positive result at the end of 45 min. Amplification and extension temperatures were 63°C and 4°C, respectively. Moreover, each patient sample was studied as duplicate and can be observed with bare eyes, as negative control tubes were purple and positive control tubes as blue, as summarized in Figure 2.

2.7 | Test interpretation

The special rotary design of the Rotor-Gene Q makes it the most precise and versatile real-time PCR cycler currently available. Each tube spins in a chamber and keeps all samples at precisely the same temperature during rapid thermal cycling. Cq value was arranged automatically in Rotor-Gene Q as 200. Cq values below 37 for FAM channel irrespective of HEX values. Nonsigmoidal signals or sigmoidal signals with Cq values above 37 in the FAM channel and sigmoidal signals with Cq values below 37 in the HEX channel were interpreted as negative. Nonsigmoidal signals and sigmoids were below 37 Cq on both FAM and HEX channels.

In the Inovia Technologies GenX series, positive values were interpreted as sigmoids with Cq values below 37 for the FAM channel, irrespective of HEX values. Nonsigmoidal signals or sigmoidal signals with Cq values above 37 in the FAM channel and sigmoidal signals with Cq values below 37 in the HEX channel were interpreted as negative. Nonsigmoidal signals and sigmoids below 37 Cq on both FAM and HEX channels were interpreted as invalid results according to the kit protocol. On the other hand, the threshold value was set as 200 according to kit protocol and on the Inovia Technologies GenX series, it was set automatically.

The LAMP4U device was used to perform the RT-LAMP technique. RT-LAMP kit primer sets for SARS-CoV-2 were designed from conserved regions of the RdRP, E, and N genes. Throughout LAMP primers, including two outer primers (forward primer F3 and backward primer B3), two inner primers (forward inner primer FIP and backward inner primer BIP), and two loop primers (forward loop primer LF and backward loop primer LB). The kit contains fully ready-to-use COVID-19 Reaction Mix tubes and gives qualitative (yes/no) results visible to the bare eye. The bare eye is sufficient for test results and negative control tubes were observed as purple and positive control tubes as blue. Although each patient sample was studied as duplicate, purple in both sample tubes means SARS-CoV-2 negative result, and blue in both sample tubes means SARS-CoV-2 positive result. If one of the sample tubes was purple and the other is blue, the test is repeated as summarized in Table 1.

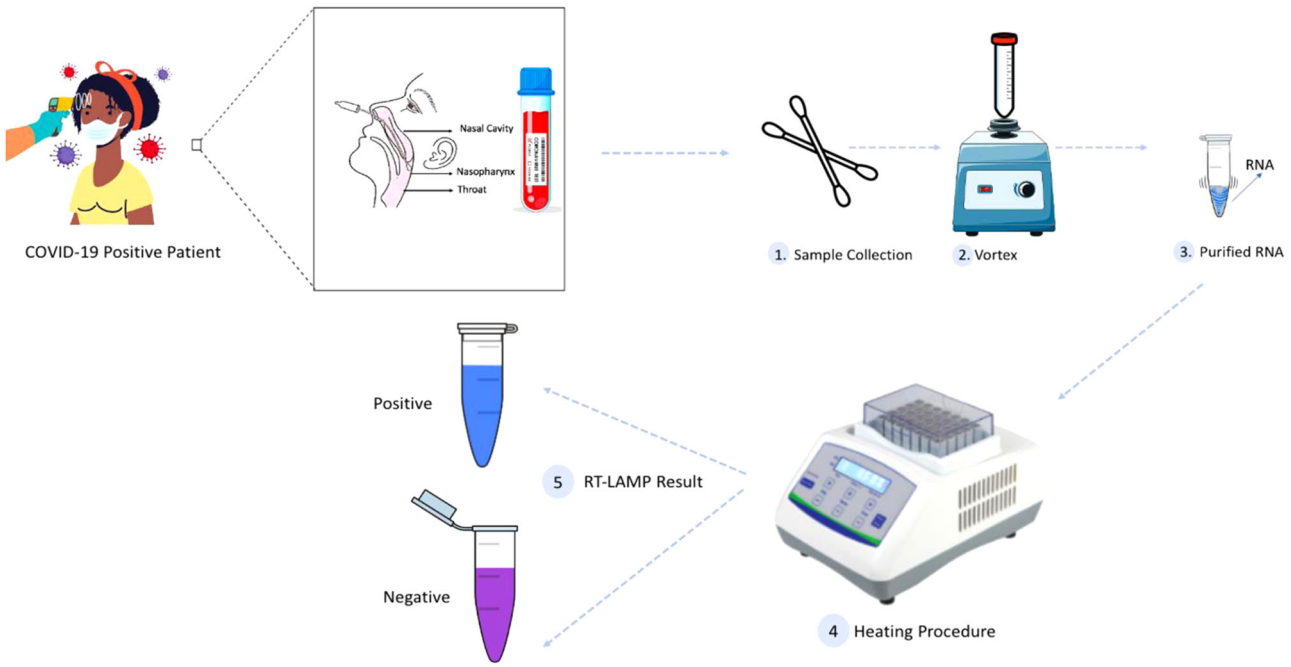

FIGURE 2 Reverse transcription loop-mediated isothermal amplification (RT-LAMP) study protocol

TABLE 1 LAMP result evaluation

Before evaluation				After evaluation	
Negative control	Positive control	Both sample tubes are the same color	Result evaluation	Color of both sample tubes	Result
Purple	Blue	Yes	Yes	Purple	SARS-CoV-2 negative
				Blue	SARS-CoV-2 positive
Purple	Blue	No	No	Retest is recommended	

Abbreviations: LAMP, loop-mediated isothermal amplification; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 2 The Cq values of the kits studied on the Rotor-Gene and the Inovia device on the first and fifth days

PCR devices and days	Kits	*n*	Mean	Std. deviation	95% confidence interval for mean		*p*
					Lower bound	Upper bound	
Inovia Cq (1st day)	Biospeedy	30	18.4333	3.87462	16.9865	19.8801	0.000
	Qiagen	30	25.6667	5.07416	23.7719	27.5614	
	Covirion	30	22.4667	3.44146	21.1816	23.7517	
	Total	90	22.1889	5.09879	21.1210	23.2568	
Inovia Cq (5th day)	Biospeedy	30	20.6667	5.77947	18.5086	22.8248	0.000
	Qiagen	30	33.0000	7.21588	30.3055	35.6945	
	Covirion	30	26.7333	5.01675	24.8600	28.6066	
	Total	90	26.8000	7.85429	25.1550	28.4450	
Rotor-Gene Cq (1st day)	Biospeedy	30	17.5333	3.39100	16.2671	18.7996	0.000
	Qiagen	30	26.0667	4.18481	24.5040	27.6293	
	Covirion	30	25.0000	4.09373	23.4714	26.5286	
	Total	90	22.8667	5.43025	21.7293	24.0040	
Rotor-Gene Cq (5th day)	Biospeedy	30	19.7667	4.81150	17.9700	21.5633	0.000
	Qiagen	30	33.3667	6.38146	30.9838	35.7495	
	Covirion	30	26.5667	4.24819	24.9804	28.1530	
	Total	90	26.5667	7.60699	24.9734	28.1599	

Note: One-way analysis of variance statistical analysis.

Abbreviations: Cq, quantification cycle; LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction.

2.8 | Statistical analyses

The data were analyzed using the SPSS 25.0 package program. The distribution of the data was examined with the Kolmogorov–Smirnov test. Student's *t* test, one-way analysis of variance (ANOVA), χ^2, and Fisher Exact test were used for parametric data as well as descriptive statistical methods (mean, standard deviation, frequency) while evaluating the study data. It was calculated in the 95% confidence interval when evaluating the study.

3 | RESULTS

By taking samples of 30 different COVID-19 positive patients, on the first and fifth days of our experiment, three different kits were studied on two different q-RT-PCR devices. The same samples were studied in the LAMP-PCR device on Days 1 and 5. The threshold value of the LAMP device was determined automatically for each study and gave negative and positive results. According to the first observation result, the positive and negative results of the first and fifth days of the samples and the Cq results of the samples that were positive were compared on a day-by-day basis in Bioeksen, Qiagen, and Covirion kits to measure the kit sensitivity. This comparison was made on two different devices and a common finding and result were obtained. The comparison of the findings obtained in the first observation with the results obtained in the LAMP-PCR device was evaluated in terms of kit, device, and day, and the confidence interval was calculated according to q-RT-PCR for the results of the first and fifth days as shown in Table 2.

When the kit sensitivity was considered in terms of Cq values, in the first day Rotor-Gene group, no significant difference was found between the Qiagen and Covirion pairs. There was a significant

difference between all the other pairs. The Biospeedy kit worked better on the first and fifth days in both the Inovia and the Rotor-Gene device than Covirion and Qiagen. On the other hand, it was concluded that the Covirion kit worked better than the Qiagen kit in both the first and fifth days in the Inovia and Rotor-Gene devices.

When the kit was considered in terms of the positive and negative values of the samples in the Inovia device, there was no difference between the Biospedy and Covirion kit on the first day. The Qiagen kit was also in the 93.3% confidence interval compared to the Biospedy and Covirion. This confidence interval was insignificant in terms of kit sensitivity and does not reveal any significance, as shown in Table 3.

When the kit was considered in terms of the positive and negative values of the samples in the Inovia device; the rates of positive and negative groups showed significant differences according to kits in subgroups. There was no difference between the Biospeedy and Covirion kit, and it is observed that the sensitivity of the Qiagen kit was decreased compared to the Biospeedy and Covirion in terms of significance, as summarized in Tables 4 and 5.

When the Rotor-Gene device was considered the use of the kit in terms of the positivity and negativity values of the samples, there was no difference between the Biospedy and Covrion kit on the first day. Moreover, the Qiagen kit was also in the 63.3% confidence interval compared to the Biospedy and Covirion in terms of significance. This confidence interval was the kit sensitivity, as summarized in Table 6.

The positivity and negativity values of the samples in the Rotor-Gene device exhibit significant differences according to kits in subgroups. There was no difference between the Biospedy and the Covrion kit. It was observed that the sensitivity of the Qiagen kit was decreased compared to the Biospedy and Covirion kit in terms of significance, as summarized in Table 7.

When the LAMP group was compared with the inovia and the Rotor-Gene device on the first day, a significance of 63.3% was detected, and when the cost, speed, and laboratory requirement used in rapid diagnosis systems were considered, these results are found to be in an ideal confidence interval as summarized in Tables 7 and 8.

On the fifth day, when the LAMP group was compared with the Rotor-Gene device and the Inovia device, a 60% significance was detected. The positivity values of the samples were approximately the same after 5 days compared to the experiment performed on the first day. They were statistically insignificant, even during long-term sample scans of the LAMP system and when the samples were stored as summarized in Tables 9 and 10.

TABLE 3 Positive and negative (P/N) results of the kits on the Inovia device on the first day

			Groups kits			
			Biospeedy	Qiagen	Covirion	*p*
P/N 1-day Inovia	Positive	*n*	30	28	30	0.326
		%	100.0%	93.3%	100.0%	
	Negative	*n*	0	2	0	
		%	0.0%	6.7%	0.0%	

Note: Fisher's exact test.

TABLE 4 Positive and negative results of the kits on the inovia device on the fifth day

			Groups kits			
			Biospeedy	Qiagen	Covirion	*p*
P/N 5th day Inovia	Positive	*n*	27	16	28	0.000
		%	90.0%	53.3%	93.3%	
	Negative	*n*	3	14	2	
		%	10.0%	46.7%	6.7%	

TABLE 5 Positive and negative results of the kits on the Rotor-Gene device on first day

			Groups kits			
			Biospeedy	Qiagen	Covirion	*p*
P/N 1st day Rotor-Gene	Positive	*n*	30	29	30	1.000
		%	100.0%	96.7%	100.0%	
	Negative	*n*	0	1	0	
		%	0.0%	3.3%	0.0%	

TABLE 6 Positive and negative results of the kits on the Rotor-Gene device on the fifth day

			Groups kits			
Device and day			Biospeedy	Qiagen	Covirion	*p*
P/N 5th day Rotor-Gene	Positive	*n*	29	19	29	0.000
		%	96.7%	63.3%	96.7%	
	Negative	*n*	1	11	1	
		%	3.3%	36.7%	3.3%	

TABLE 7 Evaluation between inovia and LAMP on the first day

			LAMP_Group_1st day		
Device and day			Positive	Negative	Total
P/N 1st day Inovia	Positive	*n*	19	11	30
		%	63.3%	36.7%	100.0%

Abbreviation: LAMP, loop-mediated isothermal amplification.

TABLE 8 Evaluation between the Rotor-Gene and the LAMP group on the first day

			LAMP_Group_1st day		
Device and day			Positive	Negative	Total
P/N 1st day Rotor-Gene	Positive	*n*	19	11	30
		%	63.3%	36.7%	100.0%

Abbreviation: LAMP, loop-mediated isothermal amplification.

TABLE 9 Evaluation between the Inovia group on the first day and the LAMP group on the fifth day

Device and day			LAMP_Group_5th day Positive	Negative	Total
P/N 1st day Inovia	Positive	*n*	18	12	30
		%	60.0%	40.0%	100.0%

Abbreviation: LAMP, loop-mediated isothermal amplification.

TABLE 10 Evaluation between the Rotor-Gene on first day and the LAMP group on fifth day

Device and day			LAMP_Group_5th day Positive	Negative	Total
P/N 1st day Rotor-Gene	Positive	*n*	18	12	30
		%	60.0%	40.0%	100.0%

Abbreviation: LAMP, loop-mediated isothermal amplification.

TABLE 11 Evaluation between the Inovia group on the fifth day and the LAMP group on the first day

Device and day			LAMP_Group_1 day Positive	Negative	*p*
P/N 5th day Inovia	Positive	*n*	18	9	0.266
		%	94.7%	81.8%	
	Negative	*n*	1	2	
		%	5.3%	18.2%	

Abbreviation: LAMP, loop-mediated isothermal amplification.

TABLE 12 Evaluation between the Rotor-Gene group on the fifth day and the LAMP group on the first day

Device and day			LAMP_Group_1 day Positive	Negative	*p*
P/N 5th day Rotor-Gene	Positive	*n*	18	11	1.000
		%	94.7%	100.0%	
	Negative	*n*	1	0	
		%	5.3%	0.0%	

Abbreviation: LAMP, loop-mediated isothermal amplification.

When Tables 11 (p= 0.266) and 12 (p= 1.00) were examined, no significant difference was observed in terms of days of statistically studied samples compared to the findings of the kit group used in the Inovia device of the LAMP system. This result confirms the confidence interval between the LAMP system and the q-RT-PCR system.

When Tables 13 (p = 0.325) and 14 (p= 0.170) were examined, no significant difference was observed in terms of days of statistically studied samples compared to the findings of the kit group used in the Inovia device of the LAMP system. When these results were evaluated together with the results in Tables 10 and 12, it confirms the confidence interval between the LAMP system and the q-RT-PCR system.

TABLE 13 Evaluation between the inovia group on the fifth day and the LAMP group on the fifth day

Device and day			LAMP_Group_5th day Positive	Negative	*p*
P/N 5th day Inovia	Positive	*n*	17	10	1.000
		%	94.4%	83.3%	
	Negative	*n*	1	2	
		%	5.6%	16.7%	

Abbreviation: LAMP, loop-mediated isothermal amplification.

TABLE 14 Evaluation between the Rotor-Gene group on the fifth day and the LAMP group on the fifth day

Device and day			LAMP_Group_5th day Positive	Negative	*p*
P/N 5th day Rotor-Gene	Positive	*n*	18	11	0.170
		%	100.0%	91.7%	
	Negative	*n*	0	1	
		%	0.0%	8.3%	

Abbreviation: LAMP, loop-mediated isothermal amplification.

Threshold values of the first day were found to be significantly different between the positive and negative groups, as shown in Table 15, but there was no significant difference between these groups in the Cq value of the fifth day as shown in Table 16.

4 | DISCUSSION

Nowadays, SARS-CoV-2 is also defined as a chemical, biological, radiological, and nuclear defense (CBRN defense or CBRNE defense) member as a disaster or biological disaster. Disaster is defined as the holistic state of natural or human-induced events that develop suddenly, controllability requires a systematic approach, interrupts or stops social life that causes loss of life, property and often cannot be overcome with local capacity.[18] Biological disasters can be human-induced as well as naturally infectious diseases and epidemiological emergence. It has become significant to prepare a plan, which includes information and practical actions, including all situations that require urgency and actions to be taken in emergencies that may occur in workplaces.[19,20] In particular, it is important to understand how the COVID-19 disease spreads. As patients are divided into two as symptomatic and nonsymptomatic. Although patients with symptoms can be detected by various diagnostic methods, patients who do not show symptoms, which are called asymptomatic, are the main reason for the spreading of the disease. For this reason, developed countries have turned direction to more rapid tests. In this way, it is

TABLE 15 LAMP group Cq value on the first day

Group statistics					
	LAMP_Group_1st day	*n*	Mean	Standard deviation (SD)	*p*[a]
Cq value_1st day	Positive	19	0.5463	0.33679	0.009
	Negative	11	0.9000	0.33045	

Abbreviation: Cq, quantification cycle; LAMP, loop-mediated isothermal amplification.
[a]Student's *t* test.

TABLE 16 LAMP group Cq value on the fifth day

Group statistics					
	LAMP_Group_5th day	*n*	Mean	Standard deviation (SD)	*p*[a]
Cq value_5th day	Positive	18	1.3222	0.56460	0.979
	Negative	12	1.3167	0.58002	

Abbreviation: Cq, quantification cycle; LAMP, loop-mediated isothermal amplification.
[a]Student's *t* test.

thought that they will perform a continuous screening. In the early stages of the infection, false-negative results are the actual problem in RT-PCR. The insufficient and improper extraction of nucleic acids for RT-PCR causes false-negative results. Thus, as a complementary tool, the computerized tomography scan of the chest is suggested.[21] The problem of RT-PCR with inaccurate results was increasingly exposed.[22] Moreover, RT-PCR requires trained medical staff, specialized instrumentation, technical labor, and special chemicals or reagents.[23] The accuracy of the serological tests is also not enough to detect the SARS-CoV-2 infection. Generally, these tests can be coupled with the RT-PCR based on the presence of viral RNA.[24] The LAMP technique is an alternative to the conventional quantitative RT-PCR methods that do not require expensive instruments to perform the reaction or interpret the results and LAMP may achieve higher sensitivity on crude clinical samples than RT-PCR.[25] RT-LAMP is a nucleic acid amplification assay like RT-PCR, which is a simple, low cost and fast method.[26] Catarina Amaral et al. created a one-tube test based on RT-LAMP which allows visual detection of less than 100 viral genome copies of SARS-CoV-2 within 30 min. In that study, 177 nasopharyngeal RNA samples for COVID-19 were compared with RT-PCR. For viral loads greater than 100 copies, the sensitivity of the RT-LAMP assay was 100% and the specificity was 96.1%.[27] In our study, we present the development of a LAMP-based method to detect SARS-CoV-2 genes that are ORF8 and N directly from pharyngeal swab samples. The test was sensitive and highly specific for SARS-CoV-2 detection under 45 min. Moreover, the RT-LAMP technique detects SARS-CoV-2 directly from pharyngeal swab samples without a time-consuming and laborious RNA extraction step. The presented study shows that the LAMP technique is sensitive enough to be used in the field. RT-PCR is a gold standard method, and it exhibits a value close to 100% even when working with various kits and devices. In addition, we have shown that the LAMP technique can be used for rapid tests in the field with the positivity detection rate of LAMP on the first day being 63%. Mautner et al. stated that this method is very ideal in rapid diagnosis systems since it does not show cross-reactivity when tested on other 20 respiratory tract pathogens, and it is 12 times faster and 10 times cheaper than routine RT-PCR depending on the test used.[28] As the study consisted of random patients with routine positive results, the high confidence interval of the results makes it ideal for this method in which the LAMP system is applied. Unlike the studies in the literature, in the study, various kits and devices used in RT-PCR studies were compared. Based on 30 patient samples, the positivity was caught 100% as Biospeedy and Covirion, 93.3% as Qiagen kit for the samples studied on the Inovia device while the same samples on the Rotor-Gene device are 100% as Biospeedy and Covirion, 96.7% as Qiagen kit at the first day. On the fifth day, the samples studied in the Inovia device were recorded as 90% for Biospeedy, 53.3% for Qiagen, 93.3% for the Covirion kit. When these samples were studied in the Rotor-Gene device, positivity was 96.7% in Biospeedy and Covirion, 63.3% in the Qiagen kit. The Qiagen kit showed a significant decrease in positivity on the fifth day. The Covirion and Biospeedy kit did not show as much decrease as the Qiagen kit in both the Inovia and the lamp device. This may be due to susceptibility to reduced viral load in expected samples. Kits produced in Turkey can also be considered as more specific effects than genomic sequencing in samples collected in Turkey. It is not possible to evaluate this situation accurately on 30 samples. These evaluations can be better clarified as a result of expanding the population in a more comprehensive study. In addition, another reason why the Qiagen kit did not catch a positivity on the fifth day may be the damage to the kit in the freeze-thaw process and it can be considered as a kit problem or the kit used (QIAprep & amp Viral RNA UM kit, GTIN 04053228039679, LOT-166044095, Date: 2021-03-21 REF 221415). The protocol was produced according to the first SARS-CoV-2 structure, recreating the kit with new variants or over new samples can solve this problem. In such a case, the study can be repeated with the new version kits of QIAGEN. When these samples were compared with the LAMP method, positivity was found at 63.3% on the first day and 60% on the fifth day. In addition, all results were compared with the LAMP method, which reveals that

the sensitivity of the confidence interval of LAMP to RT-PCR. Additionally, the results obtained with this technique can be seen with the bare eye without the requirement for any reading device. Therefore, this is the most important advantage of the RT-LAMP method that distinguishes it from other methods.

The incubation period of the disease is thought to extend to 14 days, with a median time of 4–5 days from exposure to symptoms.[29] A study reported that 97.5% of people with COVID-19 who have symptoms will do so within 11.5 days of SARS-CoV-2 infection.[30] Experiments were carried out on the first and fifth days since the incubation period was 4–5 days for the first occurrence. According to the results (as shown in Table 1) positivity was caught ideally by inovia and Rotor-Gene devices for all kits on the first day. However, on the fifth day, the Qiagen kit showed a significant decrease in positivity rate based on the first day, which is 53.3% for the inovia device and 63.3% for Rotor-Gene. On the other side, when the LAMP is examined, the first-day positivity rate was 63.3% and the fifth day was 60%. All experiments were performed on 30 different patients with known positivity. This study focused on the sensitivity of the LAMP-PCR system, whose confidence interval has proven to be above acceptability in urgent needs for pandemic guidelines. It works without the need for any complicated device, especially with its portable device form. At the same time, the denaturation, annealing, and extension steps applied in RT-PCR are not found in the LAMP technique, and the most striking feature is that the whole experiment is performed at constant temperature (54°C). In this way, it is possible to perform these steps even by using only a water bath. As the use of LAMP PCR in this field is very new, comprehensive studies are needed.[31] Also Chaouch et al. revealed that the RT-LAMP method has reliable application for SARS-CoV-2 diagnosis due to its simple application and low technical requirements, thus presenting a potentially effective test to help us to fight the ongoing COVID-19 pandemic.[32] The hope is that this strategy could be applied rapidly, and confirmed for viability with clinical samples, before being rolled out for mass-diagnostic testing in these current times.[33] This approach could be used for monitoring exposed individuals or potentially aid with screening efforts in the field and potential ports of entry.[20,34] During the event, the time for the development of the kits and the devices is further shortened. Ultimately, it is an analysis system that can read both quantitatively and qualitatively.

With this study, we foresee it will be used frequently in the future in cases of epidemics or pandemics in many CBRN agents, as well as in the triage stages of the emergency services of hospitals. Furthermore, the study showed that there may be differences between the kits used in the diagnosis and their results. The results of this study will shed light on further studies to be conducted in larger samples.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This study protocol was reviewed and approved by the Ethics Committee of Uskudar University—Date: 31.05.2021 Subject No: 61351342/May-2020-61 and Republic of Turkey, Ministry of Health, Covid-19 Scientific Research Studies Approval No: YakupArtik-2021-05-22T01_36_00.

AUTHOR CONTRIBUTIONS

Yakup Artik developed the protocol, summarized and analyzed the data, experiment in the laboratory. Wrote the article and vouched forit. Alp B. Coşğun developed the protocol, summarized and analyzed the data, experiment in the laboratory. Nevra P. Cesur development of review and regulation. Nedret Hizel offers on academic consultancy and administrative process management throughout the entire research process. Yavuz Uyar interpreted the results and provided support on academic consultancy. Haydar Sur calculated the statistics. Alp Ayan provided support on academic consultancy.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article [and/or] its supplementary material files. Further inquiries can be directed to the corresponding author.

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REFERENCES

1. Taştan C, Yurtsever B, SirKarakuŞ G, et al. SARS-CoV-2 isolation and propagation from turkish COVID-19 patients. *Turkish J Biol* 2020; 44(3):192-202. doi:10.3906/biy-2004-113
2. Woloshin S, Schwartz LM. US food and drug administration approval of flibanserin. *JAMA Intern. Med.* 2016;176(4):439-442. doi:10.1001/jamainternmed.2016.0073
3. Zheng J. SARS-CoV-2: an emerging coronavirus that causes a global threat. *Int J Biol Sci*. 2020;16(no. 10):1678-1685. doi:10.7150/ijbs.45053
4. Zhai P, Ding Y, Wu X, Long J, Zhong Y, Li Y. The epidemiology, diagnosis and treatment of COVID-19. *Int J Antimicrob Agents*. 2020; 55:(5):105955. doi:10.1016/j.ijantimicag.2020.105955
5. M. S.-D, P. J. S., Koehorst JJ, et al. Vitor AP martins dos santos MS-D and PJS. Global initiative on sharing all influenza data (GISAID).

Phylogeny of SARS-like betacoronaviruses including novel coronavirus (nCoV). Oxford [Internet], Vol. 34, Oxford; 2017;1401-1403.
6. Taylor S, Landry CA, Paluszek MM, Fergus TA, McKay D, Asmundson GJG. COVID stress syndrome: concept, structure, and correlates. *Depress Anxiety*. 2020;37(8):706-714.
7. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. A origem proximal do SARS-CoV-2. *Nat Med*. 2020;26(4):450-452.
8. World Health Organization. COVID-19: Weekly Epidemiological Update (3 November 2020). Accessed November 13, 2020. https://www.who.int/publications/m/item/weekly-epidemiological-update—3-november-2020
9. Yilmaz Gulec E, Cesur NP, Yesilyurt Fazlioğlu G, Kazezoğlu C. Effect of different storage conditions on COVID-19 RT-PCR results. *J Med Virol*. 2021;93:(12):6575. –6581. doi:10.1002/jmv.27204
10. Artik Y, Cesur NP, Kurtulmus MS, Mart Komurcu SZ, Kazezoglu C, Kocatas A. Clinic evaluation of the destrovir spray effectiveness in SARS-CoV-2 disease. *Electron J Gen Med*. 2022;19(1):emXXX.
11. Hatcher EL, Zhdanov SA, Bao Y, et al. Virus variation resource–improved response to emergent viral outbreaks. *Nucleic Acids Res*. 2017;45(D1):D482-D490.
12. Ehrhart J-C, Bennetau B, Renaud L, et al. A new immunosensor for breast cancer cell detection using antibody-coated long alkylsilane self-assembled monolayers in a parallel plate flow chamber. *Biosens Bioelectron*. 2008;24(3):467-474.
13. Hou H, Wang T, Zhang B, et al. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. *Clin Transl Immunol*. 2020; 9(5):e1136.
14. Erdem Ö, Derin E, Sagdic K, Yilmaz EG, Inci F. Smart materials-integrated sensor technologies for COVID-19 diagnosis. *Emergent Mater*. 2021;4:(1):169. –185. doi:10.1007/s42247-020-00150-w
15. Tasrip NA, Mohd Desa MN, Khairil Mokhtar NF, et al. Rapid porcine detection in gelatin-based highly processed products using loop mediated isothermal amplification. *J Food Sci Technol*. 2021;58:1-10.
16. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020; 25(3):2000045.
17. Singh R, Singh DP, Savargaonkar D, Singh OP, Bhatt RM, Valecha N. Evaluation of SYBR green I based visual loop-mediated isothermal amplification (LAMP) assay for genus and species-specific diagnosis of malaria in *P. vivax* and P. falciparum endemic regions. *J Vector Borne Dis*. 2017;54(1):54.
18. Varol N, Bulus Kirikkaya E. Afetler Karşısında Toplum Dirençliliği. *Resilience*. 2017;1:(1):1. –9. doi:10.32569/resilience.344784
19. Artik Y, Cesur N, Kenar L, Ortatatli M. Biological Disasters: In The First Quarter Of 2021 Covid-19 Overview. *Afet ve Risk Dergisi*. 2021;doi:10.35341/afet.977488
20. Komurcu SZM, Artik Y, Cesur NP, et al. The evaluation of potential global impact of the N501Y mutation in SARS-COV-2 positive patients. *J Med Virol*. 2021. doi:org/10.1002/jmv.27413
21. Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for typical 2019-nCoV pneumonia: relationship to negative RT-PCR testing. *Radiology*. 2020;200343.
22. Liu X, Feng J, Zhang Q, et al. Analytical comparisons of SARS-COV-2 detection by qRT-PCR and ddPCR with multiple primer/probe sets. *Emerg Microbes Infect*. 2020;9(1):1175-1179.
23. Wu S, Liu X, Ye S, et al. Colorimetric isothermal nucleic acid detection of SARS-CoV-2 with dye combination. *Heliyon*. 2021;7(4): e06886.
24. Gonzalez JM, Shelton WJ, Díaz-Vallejo M, et al. Immunological assays for SARS-CoV-2: an analysis of available commercial tests to measure antigen and antibodies. *MedRxiv*. 2020.
25. Song J, El-Tholoth M, Li Y, Single-and Two-Stage, Closed-Tube, Point-of-Care, Molecular detection of SARS-CoV-2. *Anal chem*. 2021;93(38):13063-13071. http://doi.org/10.1021/acs.analchem.1c03016
26. Kitajima H, Tamura Y, Yoshida H, et al. Clinical COVID-19 diagnostic methods: comparison of reverse transcription loop-mediated isothermal amplification (RT-LAMP) and quantitative RT-PCR (qRT-PCR). *J Clin Virol*. 2021;139:104813.
27. Amaral C, Antunes W, Moe E. A molecular test based on RT-LAMP for rapid, sensitive and inexpensive colorimetric detection of SARS-CoV-2 in clinical samples. *Sci Rep*. 2021;11:16430.
28. Mautner L, Baillie CK, Herold HM, et al. Rapid point-of-care detection of SARS-CoV-2 using reverse transcription loop-mediated isothermal amplification (RT-LAMP). *Virol J*. 2020;17(1):1-14.
29. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720.
30. Lauer SA, Grantz KH, Bi Q, et al. The incubation period of coronavirus disease 2019 (CoVID-19) from publicly reported confirmed cases: estimation and application. *Ann Intern Med*. 2020;172(9): 577-582.
31. Altındiş M, Elmas B, Kılıç Ü, Aslan FG, Küçükkara G, Köroğlu M. Grup A Streptokokların hızlı moleküler tanısında loop-mediated isothermal amplifi cation PCR (LAMP-PCR). *J Biotechnol Strateg Heal Res*. 2017; 1(1):11-16.
32. Chaouch M. Loop-mediated isothermal amplification (LAMP): an effective molecular point-of-care technique for the rapid diagnosis of coronavirus SARS-CoV-2. *Rev Med Virol*. 2021;31:e2215.
33. Kashir J, Yaqinuddin A. Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19. *Med Hypotheses*. 2020;141:109786.
34. Lamb LE, Bartolone SN, Ward E, Chancellor MB. Rapid detection of novel coronavirus (COVID19) by reverse transcription-loop-mediated isothermal amplification. *PLOS One*. 2020;15: e0234682.

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