Egypt. J. Vet. Sci., pp. 1-16

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Application and Optimization of Reverse Transcription Loop-Mediated Isothermal Amplification Assay for Rapid SARS-CoV-2 Detection in Humans and Animals

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Abstract

SEVERE Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) requires quick and dependable diagnostic solutions. Our study aimed to optimize the rapid detection of SARS-CoV-2 using the reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay and to compare its performance with the reverse transcription real-time TaqMan quantitative polymerase chain reaction (RT-qPCR) of RNA extraction protocols. Nasopharyngeal swabs (NPS), oropharyngeal swabs (OPS), mixed nasopharyngeal and saliva samples, and sputum samples from individuals with or without COVID-19 symptoms (outpatient n = 180, inpatient n = 50) were collected and stored at -80 °C. These samples were tested using RT-qPCR, RT-LAMP, and RT-PCR for comparison. Additionally, animal samples were tested, including cattle (n = 20), buffaloes (n = 10), sheep (n = 25), goats (n = 30), horses (n = 5), donkeys (n = 5), mules (n = 3), camels (n = 5), dogs (n = 10), cats (n = 5), and rabbits (n = 6). The detection rates of RT-qPCR were 7.3 ± 0.56, 8.0 ± 0.98, and 5.0 ± 0.56 for nasopharyngeal swabs (NPS), oropharyngeal swabs (OPS), and mixed nasopharyngeal and saliva samples mixed with sputum samples from outpatients (n = 180), respectively. For inpatients (n = 50), the means and SD were 43 ± 0.264, 43 ± 0.245, and 26 ± 0.376, respectively. The sensitivity of RT-LAMP was comparable to RT-qPCR, with 88.9% for outpatients and 99.4% for inpatients. Both assays demonstrated 100% specificity in both groups. The findings demonstrated the direct RT-qPCR assay, both with and without RNA extraction, produced positive results

Keywords: Coronavirus, COVID-19, Loop-mediated isothermal amplification, Real-Time quantitative Polymerase Chain Reaction, Reverse Transcriptase Polymerase Chain Reaction.

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(Received 27 July 2024, accepted 21 October 2024)
DOI: 10.21608/EJVS.2024.307529.2280

Introduction

COVID-19 is caused by SARS-CoV-2, a pandemic with an unknown an unknown animal reservoir [1]. A SARS-CoV-2 strain genetically similar to the human isolates was isolated from pangolins and bats [2]. SARS-CoV-2 belongs the Betacoronavirus subgenus Sarbecovirus and has RNA genome that encodes four structural proteins; envelope (E), spike (S), nucleocapsid (N) and membrane (M) proteins [2, 3]. The remaining viral polyproteins are cleaved into various non-structural proteins essential for viral replication [3, 4]. Like other coronaviruses, SARS-CoV-2 binds to host cells through the angiotensin-converting enzyme 2 (ACE2) receptor using the receptor-binding domain (RBD) on its spike protein [5, 6]. This binding facilitates viral entry and subsequent airborne transmission from person to person [7, 8]. SARS-CoV-2 infection can manifest with various pathological signs, including influenza-like respiratory illnesses, dry cough, and fever [9, 10].

While RT-qPCR remains the gold standard for SARS-CoV-2 diagnosis, its high cost, time-intensive nature, and need for specialized equipment present challenges [11, 12]. RT-qPCR provides flexibility, enabling precise control over reaction parameters and the ability to perform multiplex analysis [13]. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) has emerged as a promising alternative for rapid SARS-CoV-2 detection. It provides advantages in terms of simplicity, speed, and cost-effectiveness compared to traditional RT-qPCR methods [13]. The RT-LAMP assay begins by converting viral RNA into complementary DNA (cDNA) using reverse transcriptase [14]. Extensive research has supported the development of effective RT-LAMP assays targeting different regions of the SARS-CoV-2 genome, including the nucleocapsid (N) gene, envelope (E) gene, and RNA-dependent RNA polymerase (RdRp) gene [15]. The use of saliva samples offers significant advantages in their non-invasive nature and the potential for self-collection [16, 17]. This minimizes transmission risk for both the individual and healthcare workers [18, 19]. Furthermore, the RT-LAMP test's suitability for point-of-care (POC) diagnostics plays a vital role in effective disease management and outbreak control [20, 21]. The study aimed to compare the performance of a one-step, single-tube RT-LAMP assay with RT-qPCR and RT-PCR assays for detecting SARS-CoV-2 in different human and animal samples [22].

Material and Methods

Sample collection

In this study, we included participants from various governorates in Egypt to examine COVID-19 for travel purposes (outpatients) and collected samples from hospitalized patients (inpatients). All participants provided written consent. Nasal, oropharyngeal, and nasopharyngeal swab samples were collected from 180 random outpatients of various ages and genders, and 50 samples were collected from inpatients. These samples were stored at -80°C and included individuals with or without fever, respiratory symptoms, or known contact with COVID-19 cases, all of whom were attended at the Central Laboratory of the Ministry of Health (CLMH), Egypt. The healthcare professional collected an oropharyngeal swab by tilting the patient's head back slightly and asking them to open their mouth wide. This allowed access to the pharyngeal tonsils on both sides for swabbing. The swab was used to gently wipe both sides of the tonsils at least three times, followed by wiping the posterior pharyngeal wall up and down at least three times. Nasal swabs were collected by instructing patients to blow their noses before the procedure Nasopharyngeal swab collection, a swab was carefully inserted with a gentle rotating motion. Insertion continued until resistance was felt at the level of the turbinate, approximately 1 centimetre or less from the nostril opening. The swab was then rotated against the nasal wall several times before being carefully removed. Additionally, nasopharyngeal swabs were collected from various animals: cattle (n = 20), buffaloes (n = 10), sheep (n = 25), goats (n = 30), horses (n = 5), donkeys (n = 5), mules (n = 3), camels (n = 5), dogs (n = 10), cats (n = 5), and rabbits (n = 6). Whole uncoagulated blood samples were also collected from these animals. The seal was removed from the sample treatment tube, and the swab was placed in the tube, submerged in a viral transport medium (VTM) solution. This solution consisted of phosphate-buffered saline (PBS) with phenol red (Gibco), supplemented with 2% fetal bovine serum (FBS; Gibco), 0.5 µg/ml amphotericin B, and 100 µg/ml gentamicin. Each swab was rotated and pressed ten times against the side and bottom of the tube. Then, it was squeezed along the inner tube wall to release any collected material into the tip. The processed samples were then frozen at -80°C for storage. Nasopharyngeal (NP) and oropharyngeal (OP) swabs were placed together in a 3-ml tube

containing VTM, whereas saliva samples were placed in a separate tube. Complete coagulated blood samples (3 mL) were collected from the same person. Specimens were collected by physicians or professionally trained individuals at a specific location (Respiratory Emergency Room at CLMH). A total of 180 outpatient samples were examined and classified as positive or negative using molecular tests, specifically RT-PCR and RT-LAMP, following the guidelines outlined [23].

RNA extraction

RNA was isolated from clinical samples using a QIAamp viral RNA mini kit (QIAGEN, Germany). The protocol followed the manufacturer's instructions with modifications as described by Toptan et al., [23]. All specimen handling procedures strictly adhered to the WHO's laboratory biosafety guidelines for COVID-19 prevention. Following manufacturer instructions, extracted RNA was reconstituted to a final concentration of 50 ng in a 40 µL elution volume. Control reactions for reverse transcription real-time TaqMan quantitative polymerase chain reaction and reverse transcription loop-mediated isothermal amplification assays Positive control reactions were conducted using commercially available SARS-CoV-2 RNA (e.g., Viro Gene company, Egypt) to validate the specificity and efficiency of reagents, the reaction itself, and the performance of the RT-qPCR thermal cycler.

Negative control allows for the exclusion of contaminations

The RT-qPCR and RT-LAMP runs rely on the successful inclusion and performance of both positive and negative control reactions.

Internal control is based on the detection of artificial RNA

An internal control target, co-amplified in the HEX channel in each reaction, acts as a quality check. It helps evaluate factors such as proper sampling, sample storage and transport, preparation, and the overall performance of RT-qPCR and RT-LAMP assays.

One-step endpoint reverse transcription polymerase chain reaction for SARS-COV2 *detection*

The reaction was performed with 5 µL of RNA, 12.5 µL of 2x reaction buffer, 1 µL of 25x enzyme mix, 1 µL each of forward and reverse primers (10 pmol/µL), and 0.5 µL each of probes (10 pmol/µL) as detailed in Table 1. The process included an initial denaturation at 95°C for 3 minutes, followed by incubation at 50°C for 2 minutes, then 120 minutes at 37°C, and a final step at 85°C for 5 minutes. Amplification involved 38 cycles: denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension was done at 72°C for 5 minutes. Results were verified by observing amplicons on 2% agarose gels, according to CDC guidelines, according to CDC guidelines for RT-PCR SARS-CoV-2 detection.

Reverse transcription real-time TaqMan quantitative polymerase chain reaction (RT-qPCR)

A 20-µL reaction mixture was prepared using 5 µL of RNA, 12.5 µL of 2x reaction buffer (from the one-step RT-qPCR kit), 1 µL of 25x enzyme mixture, 1 µL each of forward and reverse primers (10 pM concentration), and 0.5 µL each of probes (10 pM concentration) as specified in Table 1. Reverse transcription was performed at 50°C for 30 minutes, followed by inactivation of the reverse transcriptase at 95°C for 10 minutes. PCR amplification then proceeded with 40 cycles, each consisting of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 hour.

Reverse transcription loop-mediated isothermal amplification assay primer design

Four sets of LAMP primers targeting the SARS-CoV-2 Orf1ab and RNase genes were designed using Primer Explorer (http://primerexplorer.jp/e/), a dedicated LAMP primer design software, and deposited in NCBI GenBank. The LAMP assay utilized six primers to accelerate the reaction, forward primer (F3), reverse primer (B3), forward inner primer (FIP), reverse inner primer (BIP), forward loop primer (LF), and reverse loop primer (LB). Details on the designed primers and DNA fragments alongside a 2019-nCoV reference sequence are provided in (Table 2).

Reverse transcription loop-mediated isothermal amplification assay

The specific primer set "Primer 1" (P1) was selected for the RT-LAMP reaction, which was performed in triplicate. The 25 µL reaction mixture included 1 µL each of the outer primers (F3 and B3; final concentration 2 µM), inner primers (FIP and BIP; final concentration 16 µM), and loop primers (LF and LB; final concentration 4 µM), as well as 1 µL of the diluted RNA sample. For RT-LAMP, either WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix with UDG (one-step) or modified WarmStart (DNA and RNA) (two-step) from New England Biolabs was used.

A direct patient swabs without RNA extraction were used for real-time quantitative polymerase chain reaction, and Reverse transcription loop-mediated isothermal amplification assay.

To advance rapid and accessible diagnostic tools, we evaluated using nasopharyngeal and oropharyngeal swabs directly in RT-PCR, RT-qPCR, and RT-LAMP assays, eliminating the need for prior RNA extraction. We tested two methods with swab specimens collected in VTM: one involved heating the samples on a heating block at 95°C for 5 or 10 minutes, and the other used the samples directly without heating for RT-PCR, RT-qPCR, and RT-LAMP assays.

Statistical analysis

Descriptive statistics were calculated, including mean and standard deviation (SD). Student's t-test (significance level of 0.05) was used to assess differences within the dataset. Data analysis was performed using SPSS (version 15, Windows 7). Sensitivity, specificity, and percentage agreement (assessed via the kappa coefficient) were determined utilizing the MedCalc Diagnostic Test Evaluation Calculator available online (MedCalc's Diagnostic test evaluation calculator) and a trial version of MedCalc software. Real-time qPCR, LAMP, and PCR were carried out under reaction using the same primers set and evaluated equation as follows

$$\text{Sensitivity} = \frac{True\ Postive}{True\ Positive + False\ Negative} X\ 100 \ ,$$

$$\text{Specificity} = \frac{True\ Negative}{True\ Negative + False\ Postive} X\ 100 \ .$$

The strength of the kappa coefficients was assessed according to the following categories: 0.01 - 0.20: Indicates slight agreement; 0.21 - 0.40: Indicates fair agreement; 0.41 - 0.60: Indicates moderate agreement; 0.61 - 0.80: Indicates substantial agreement, and 0.81 - 1.00: Indicates almost perfect agreement.

Results

RT-qPCR with or without RNA extraction may identify the SARS-CoV-2 from NP swabs. The different samples were extracted with an extraction Kit or used directly after heating at 95 °C for 5, and 10 minutes. RT-qPCR detected SARS-CoV-2 RNA in NPS samples as shown in Figure 1 amplification plot of RT-qPCR revealed positive results 1 to 5 while 6 gave negative results. Figure 4. Detection of SARS-Co2 RNA extracts from patients' sample specimens using different molecular methods (RT-PCR and rRT-qPCR. RT-qPCR assay had a higher detection rate than did the RT-PCR of the SARS-CoV-2 RNA from all sample specimens (NPS, NOPS, and SA mixed with sputum). The rates of SARS-CoV-2 in outpatient samples were 4.44, 3.88, 4.44, and 8.33 NPS, NOPs, and SPs, respectively. While, inpatient samples of whole blood, NP, and OP were 8, 80 and 84 respectively. The molecular identification performed via gel-based RT-PCR used the N, E, and Orf1a primer sets to detect SARS-CoV-2. Table 3. The SARS-CoV-2 RNA can be detected from NP swabs by RT-qPCR with or without RNA extraction. SARS-CoV-2 detection is shown in a real-time PCR amplification plot. RT-qPCR was used to test NP swabs using an *N* primer/probe pair. The mixture was either subjected to RNA extraction using the QiagenQIAamp Viral RNA Mini Kit or directly added to the RT-qPCR reaction, with or without a preheating step (0, 5, and 10 min at 95 °C for "NP, OP, NOP, and sputum samples treated with heat at 95 °C for "NP, Op, and Nop, and sputum sample treated with heat at 95 °C for "NP, Op, and NO) As a control, the Ministry of Health generously provided the required quantities of positive-control SARS-CoV-2 RNA in transport medium, which was purified using the QIAamp Viral RNA Mini Kit and screened using RT-qPCR. Direct RT-qPCR was used to test NP swabs from 180 sample specimens for SARS.

Discussion

Diagnostics have been essential to the COVID-19 pandemic response. There are three primary methods for detecting SARS-CoV-2 infection, and their roles have evolved throughout the pandemic. Molecular tests, as real-time reverse transcriptase-polymerase chain reaction, reverse transcription-PCR and reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assays are highly sensitive and specific in detecting viral RNA. The WHO recommends these tests for confirming diagnoses in symptomatic individuals and for initiating public health measures [13, 24]. The PCR technique is considered the gold-standard approach for viral detection because of its rapidity and high sensitivity and specificity [24, 25]. Table 3 demonstrated that RT-qPCR had a higher detection rate of SARS-CoV-2 RNA from all sample specimens compared to RT-PCR (NPS,

OP, NOPS, and SA mixed with sputum). The detection rates for NPS were 4.44%, 3.88%, 4.44%, and 8.33% for NPS, NOPS, and SPs, respectively. In 180 persons without confirmed COVID-19, nasopharyngeal and oropharyngeal swabs, sputum, and whole uncoagulated blood samples were collected for SARS-CoV-2 identification using real-time RT-qPCR. We found that NPS was more sensitive than OPS for detecting SARS-CoV-2, with a higher viral load in NPS specimens. Despite the viral load in the upper respiratory tract decreasing as the patient's health improved, NPS tests detected this variation. NPS, NOPS, and SP showed significantly higher sensitivity than OPS samples from 180 specimens: 9/180 (5%), 9/180 (5%), 6/160 (10%), and 7/180 (3.88%), respectively. The sensitivity difference was not influenced by the patient's condition, indicating that NPS is more diagnostically accurate than OPS. Currently, rapid and effective nasopharyngeal and oropharyngeal swabs are recommended for sample collection and examination using the current WHO diagnostic test techniques. After RNA extraction from patient sample swabs, RT-qPCR amplification of the collected RNA is necessary to identify the viral RNA in RT-qPCR tests [25]. The RT-PCR experiment is also a time-consuming process that requires the use of specialized equipment. Therefore, a quicker, more convenient, and less complicated test is required to address these issues. The RT-PCR test might be considered the primary approach for detecting the COVID-19 causal agent, SARS-CoV-2 [26]. However, RT-PCR has a disadvantage in that it can produce false-negative and false-positive results [26, 27]. RT-PCR tests are expensive to execute, although the fact that they are designed to be as precise as possible based on the conserved sections of the viral genome [28]. The RT-qPCR method had a higher detection rate than RT-PCR for SARS-CoV-2 RNA across all sample specimens (NPS, NOPS, and SA mixed with sputum). The detection rates for NPS were 4.44%, 3.88%, 4.44%, and 8.33% for NPS, NOPS, and SPs, respectively, as shown in Table 3 and Figures 1 and 2. These findings are consistent with several previous studies [28]. RT-PCR detected eight positive samples of SARS-CoV-2 using a specific primer. RT-qPCR is an extremely accurate and rapid procedure that enhances the laboratory diagnosis of SARS-CoV-2. In real-time RT-qPCR, 9 out of 180 suspected samples tested positive for SARS-CoV-2, which is similar to the 8 positive samples detected by RT-PCR. These results suggest that RT-qPCR has higher sensitivity and specificity than the RT-PCR method. As shown in Table 4 and Figure 2, negative results were more likely to occur in samples with a low viral load. A common SARS-CoV-2 test relies on RNA extraction kits, which are currently sold out, and reagents for both manual and automated equipment remain scarce, with uncertain supply chains [29]. As demonstrated in Figure 2, the direct approach produced the best findings and was highly consistent with the SARS-CoV-2 infection diagnosis based on conventional RNA extraction. Due to limited funding compared to foreign projects, the study included only a small number of samples [30]. Interestingly, SARS-CoV-2 has shown varied environmental stability [31]. The findings in Table 4 demonstrated that SARS-CoV-2 RNA could be detected by RT-qPCR without the need for an RNA extraction step. This result is supported by other studies indicating that RT-qPCR can effectively identify the virus even in the absence of a conventional RNA extraction process [32]. Because of oligonucleotide cross-contamination during synthesis or processing, certain oligonucleotide batches may contain trace quantities of target areas from the SARS-CoV-2 genome [33, 34]. The findings indicate that the combined use of RT-PCR and RT-qPCR identified the highest percentage of SARS-CoV-2 in both nasopharyngeal (NP) and nasopharyngeal with oropharyngeal (NOP) samples. While RT-LAMP offers potential benefits, it faces challenges such as false positives due to aerosol contamination [35] and the need to open reaction tubes for end-point detection, which hinders large-scale screening and on-site use [36]. This study evaluated a novel RT-LAMP assay for SARS-CoV-2 detection, achieving a sensitivity of 88.9% and a specificity of 99.4% (Figures 3 and 4; Table 4). Compared to RT-PCR, the RT-LAMP assay presents several advantages: (The RT-LAMP assay is easy to use; it requires less specialized equipment and expertise, making it more accessible in various settings; the test completes in 60 minutes, significantly faster than the 120 minutes required for RT-PCR; and results are visually apparent, facilitating interpretation without the need for specialized equipment.) These features highlight the potential of RT-LAMP as a valuable point-of-care (POC) testing tool, owing to its simplicity, speed, and ease of result interpretation (Figures 1-3; Table 4). Furthermore, the positive results of the assay were

evident, making them easily recognizable [37]. The RT-LAMP assay proves to be a significant point-of-care (POC) detection tool, highlighting one of the key strengths of this study. Additionally, the results of this test are straightforward and easy to interpret [38]. The well-established RT-LAMP assay has significant clinical implications, especially considering that COVID-19 is present in 203 nations, regions, or territories, with increasing numbers of confirmed cases and deaths [39]. No detectable SARS-CoV-2 RNA was found in nasal swabs or blood samples from cattle, buffalo, sheep, goats, horses, donkeys, mules, camels, dogs, cats, or rabbits. This result contrasts with positive findings obtained from previously tested human samples using both RT-qPCR and RT-LAMP. Several factors may explain these negative results, including missed viral shedding windows or changes in infection dynamics. Importantly, many of the species tested, such as cats, ferrets, mink, white-tailed deer, and non-human primates, have shown moderate to high susceptibility to SARS-CoV-2 in both natural and experimental settings [39]. This suggests potential interspecies transmission. However, several limitations of our study warrant consideration. The detection window for SARS-CoV-2 using RT-qPCR in infected animals is relatively short. Viral shedding periods vary: skunks shed for about 5 days, cattle for 0-3 days, while cats and white-tailed deer can shed for up to 21 days. Deer may shed an infectious virus for around 7 days. Negative RT-qPCR results might indicate that testing occurred outside the viral shedding window. Additionally, without antibody testing, it is possible that animals with prior SARS-CoV-2 exposure were not actively shedding at the time of testing. Although high-risk animals were targeted, there remains a possibility of missing susceptible or previously infected animals [39]. Finally, the novel RT-LAMP assay shows promise for rapid and cost-effective SARS-CoV-2 detection. However, it requires further validation and standardization before it can be widely adopted in clinical settings. While it should not replace existing diagnostic tests, its potential benefits make it a valuable option, especially in resource-limited environments.

Conclusions

RT-LAMP has shown promising results for detecting SARS-CoV-2, it is not yet a widely accepted diagnostic method and should not be used as a standalone test for COVID-19 diagnosis; it should be used in conjunction with other diagnostic tests, such as RT-qPCR and/or serological testing, to ensure accurate diagnosis of COVID-19. Also, it is dependent on the quality of the sample collected, and improper collection, storage, or transportation of samples can lead to false-negative results. Therefore, it is essential to follow proper sample collection and handling procedures to ensure accurate test results. Our results did not, detected SARS-CoV-2 in any animal sampling does not rule out susceptibility or interspecies transmission; ongoing surveillance is still necessary for future monitoring. Overall, while the RT-LAMP assay shows great potential as a diagnostic tool for SARS-CoV-2, further validation and standardization of the assay are needed before it can be widely implemented in clinical settings.

Acknowledgments

We would like to acknowledge National Research Centre, Dokki, Egypt, for facilities and funds Project No # MP-121201 # during this work

Funding statement

This work funded by Project No# MP-121201 # National Research Centre fund (NRC)

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

Ethical statement the study was approved ethically by the Medical Research Ethical Committee, National Research Centre, and Egypt under registration number # 6-1-3-1 #. We confirmed that all experiments were performed according to the relevant guidelines and regulations of the World Medical Association (WMA) for the declaration of Helsinki. We confirm that our physician team obtained an informed consent letter from all participants and/or their legal guardians in the Virology Department, Central Public Health Laboratories, the Ministry of Health and Population, and the National Research Center Cairo, Egypt. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay design and optimization

Based on the operational criteria for developing a rapid diagnostic test for COVID-19, the following criteria were included for evaluating the newly developed test, rapidity (test results were obtained in less than 30 minutes); simplicity (test was completed in one or two steps and required no special equipment, as well as minimal training and equipment); and easy interpretation

TABLE 1. Sequence of primers for real-time quantitative polymerase chain reaction.

Target genome	Type of PCR	Target gene	Primer name	Forward primer (5′–3′)	Reverse primer (5′–3′)	Size (bp)
SARS-CoV-2	Real-time PCR (rRT-qPCR) ,Multiplex PCR	RdRP	RdRP2<u>9</u>	GTGARATGGTCATGTGT GGCGG	CARATGTTAAASACACTATTA GCATA	CAGGTGGAACCTCATC AGGAGATGC (FAM probe) 101
		S	S2<u>9</u>	GCTGGTGCTGCAGCTTATTA	AGGGTCAAGTGCACAGTCTA	107
		N	N1<u>9</u>	CAATGCTGCAATCGTGCTAC	GTTGCGACTACGTGATGAGG	117
		E	E2<u>9</u>	ACAGGTACGTTAATAGTTAAT AGCGT	ATATTGCAGCAGTACGCACACA	ACACTAGCCATCCTTACT GCGCTTCG- ′ (FAM probe) 116
	RT- PCR	RdRP	RdRP 1	GCTCGCAAACATACAACGTG	CATTAACATTGGCCGTGACA	202
		RdRP	RdRP 2	TGAAATCAATAGCCGCCACT	TGTTTGCGAGCAAGAACAAG	199
		S	S 1	CAGATGCTGGCTTCATCAAA	GGTTGGCAATCAATTTTTGG	291
		S	S 2	ACTGTTTTGCCACCTTTGCT	AGCTTGTGCATTTTGGTTGA	300
		N	N 1	AAGGAAATTTTGGGGACCAG	GAGTCAGCACTGCTCATGGA	399
		N	N 2	AAAGGCCAACAACAACAAGG	GCTCTGTTGGTGGGAATGTT	393

FAM: 6-carboxyfluorescein, BBQ: Blackberry quencher, ROX: Carboxyrodamine

The final concentration represents the concentration of each oligonucleotide primers 1 (µM) while each probe concentration 0. 2 (µM) in reaction mixture (20 µL).

TABLE 2. Sequences of the RT-LAMP primers used for the detection of SARS-CoV-2

Target gene	Primer	Sequence (5′–3′)
N	F3	TGGCTACTACCGAAGAGCT
	B3	TGCAGCATTGTTAGCAGGAT
	FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG
	BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT
	LF	GGACTGAGATCTTTCATTTTACCGT
	LB	ACTGAGGGAGCCTTGAATACA
ORF1a	F3	ACTTGTCACGCCTAAACG
	B3	CTACCCAATTTAGGTTCCTGG
	FIP	AGGACACGGGTCATCAACTACAAGCTGCATTTCACCAAGAA
	BIP	AGGAGCTAGAAAATCAGCACCTATGGGTGATTTAGAACCAGC
	LF	TGGTTGATGTTGAGTACATGAC
	LB	AATTGAATTGTGCGTGGATGAG
RNase P POP7	F3	TTGATGAGCTGGAGCCA
	B3	CACCCTCAATGCAGAGTC
	FIP	GTGTGACCCTGAAGACTCGGTTTTAGCCACTGACTCGGATC
	BIP	CCTCCGTGATATGGCTCTTCGTTTTTTTCTTACATGGCTCTGGTC
	LF	ATGTGGATGGCTGAGTTGTT
	LB	CATGCTGAGTACTGGACCTC

The in-house Viroselect assay was considered positive if ORF1a (nsp3) and human RNase P (internal control) were detected; negative if ORF1a (nsp3) was not detected but RNase P was detected; and invalid if RNase P was not detected.

TABLE 3. Direct detection of SARS-CoV-2 RNA from patient swab samples with /or without an RNA extraction step

Patients	Samples No	RT- PCR				RT-qPCR				RT- LAMP			
		95°C at 5 min	95°C at 10 min	Non	Extraction kits	95°C at 5 min	95°C at 10 min	Non heat	Extraction Kits	95°C at 5min	95°C at 10 min	Non heat	Extraction Kits
Outpatients samples	N P (n=180)	7.6 ± 0.345	7.66± 0.45	4.0 ±.816	7.66± 0.87	8.17± .87	8.6±0.87	4.0 ± .81	8.6±0.87	8.17± 0.87	8.6± .87	4.0 ± 0.81	8.6±0.87
	OP (n=180)	7.33± 0.546	7.3 ± 0.67	3.7± .957	7.3± 0.65	73 ± 0.76	7.3±0.76	3.7± .957	7.3 ± 0.56	7.3 ± 0.76	7.3±0.43	3.7± 0.95	7.3 ± 0.56
	N O P (n=180)	7.0 ± 0.675	8.0± 0.87	4.0± .81	8.0±0.65	8.00± 0.45	8.0 ±0.9	4.0 ± .816	8.0± 0.98	8.00± 0.45	8.0 ±0.56	4.0 ± .816	8.0± 0.98
	SA+SP (n=60)	5.0 ± 0.04	5.0 ± 0.34	4.2± 0.95	5.0± 0.45	5.0± 0.56	5.0 0.87	4.25 ±.957	5.0 ±0.56	5.0± 0.56	5.0 ±0.87	4.25±.957	5.0 ±0.56
	B l o o d (n=30)	00.± 0.00	00± 0.00	00± .0.00	00± 0.00	0.0 ±٠0.	0.0 ±٠0.	0.0± 0 .0	0.0 ±٠0.	0.0 ±٠0.	0.0 ±٠0.	00± 0 .0	0.0 ±٠0.
Inpatients samples	NP (n = 50)	40 ± 0.868	41 ± 0.764	35 ± 0.268	41 ± 0.264	41± 0.868	42 ± 0.764	36 ± 0.268	43 ± 0.264	41 ± 0.868	41 ± 0.764	35 ± 0.268	42± 0.264
	OP (n = 50)	40 ± 0.556	40 ± 0.645	35 ± 0.256	41 ± 0.245	41 ± 0.556	41 ± 0.645	36 ± 0.256	43 ± 0.245	40 ± 0.556	40 ± 0.645	35 ± 0.256	42± 0.245
	B l o o d (n = 50)	22± 0.366	22± 0.416	14± 0.356	22± 0.376	22± 0.366	22± 0.416	16± 0.356	26± 0.376	22± 0.366	22± 0.416	14± 0.356	24± 0.376

*Mean values of logarithmic count for different products with different superscript letters in the same rows are significantly different at (P<0.05).

TABLE 4. Evaluation of the detection of the SARS-CoV-2 RNA from direct sample specimens by RT-PCR and RT-LAMP.

Patients	Samples No	Samples No	RT- PCR				RT-qPCR				RT- LAMP			
			95 °C at 5 min	95 °C at 10 min	Non	Extraction kits	95 °C at 5 min	95 °C at 10 min	Non heat	Extraction Kits	95 °C at 5min	95 °C at 10 min	Non heat	Extraction Kits
Outpatients samples	NP (n=180)	Sensitivity %	77.7	88.9	44.4	44.4	77.7	88.9	44.4	88.9	44.4	77.7	77.7	88.9
		Specificity %	98.8	99.4	97.2	97.2	98.8	99.4	97.2	98.8	97.2	98.8	98.8	98.8
	OP (n=180)	Sensitivity %	77.7	88.9	44.4	44.4	77.7	88.9	44.4	77.7	44.4	77.7	77.7	77.7
		Sensitivity %	98.8	99.4	97.2	97.2	98.8	99.4	97.2	98.8	97.2	98.8	98.8	98.8
	SA+SP (n=60)	Sensitivity %	66.6	83.6	44.4	44.4	66.6	83.6	44.4	66.6	44.4	66.6	66.6	66.6
		Specificity %	96.6	98.2	97.2	97.2	96.6	98.2	97.2	96.6	97.2	96.6	96.6	96.6
Inpatients samples	NP (n = 50)	Sensitivity %	93.2	93.2	81.33	93.18	95.2	95.2	83.33	100	95.2	95.2	83.33	100
		Specificity %	76.9	76.9	65.2	81.1	83.33	83.33	68.18	100	83.33	83.33	68.18	100
	OP (n = 50)	Sensitivity %	93	93	65.2	93.18	95.2	95.2	83.33	100	95.2	93	83.33	97.7
		Sensitivity %	76.9	76.9	65.2	81.1	83.33	83.33	68.18	100	83.33	76.9	68.18	88.8
	Blood (n = 50)	Sensitivity %	84.6	84.6	58.33	84.6	84.6	84,6	66.6	100	84.6	84,6	58.33	97.7
		Specificity %	87.5	87.5	78.2	87.5	87.5	87.5	81.8	100	87.5	87.5	78.2	88.8

Nasopharyngeal (NP), oropharyngeal (OP), and nasal and oropharyngeal (NOP) swabs, as well as saliva (SA), sputum (SP), and uncoagulated whole-blood samples were used h ere. The antigen found by various diagnostic procedures did not differ substantially ($p \leq 0.005$).

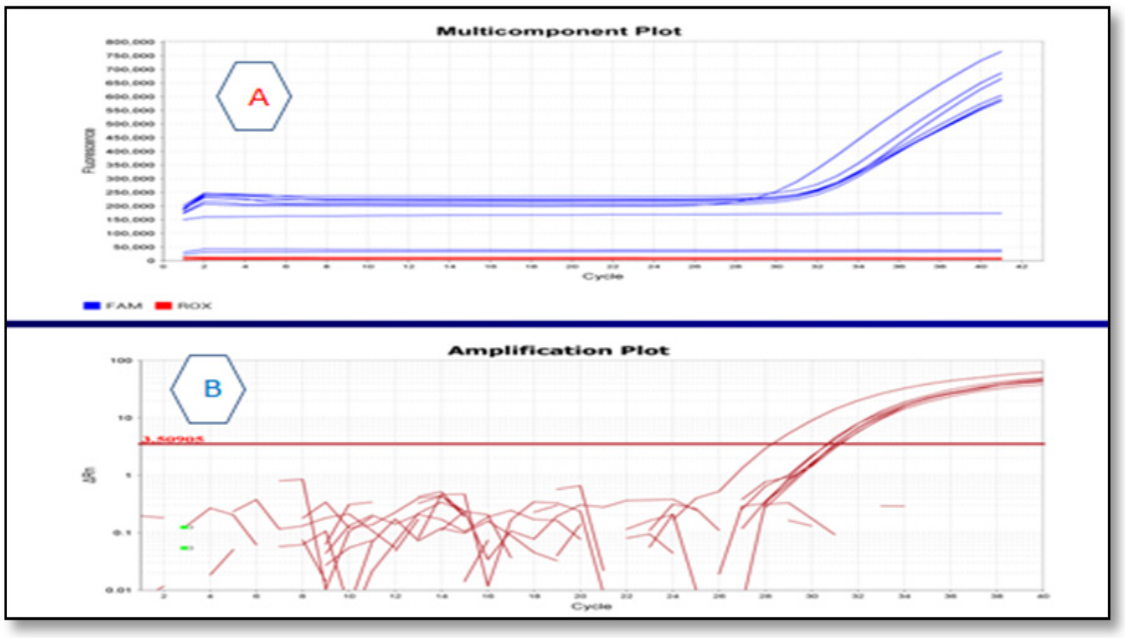

Fig. 1. (A and B). Showed amplification plot of real-time PCR. Fig.1 A and B: revealed that RT-qPCR with or without RNA extraction identified the SARS-CoV-2 RNA from NP swabs, amplification plot of real-time PCR revealed positive results.

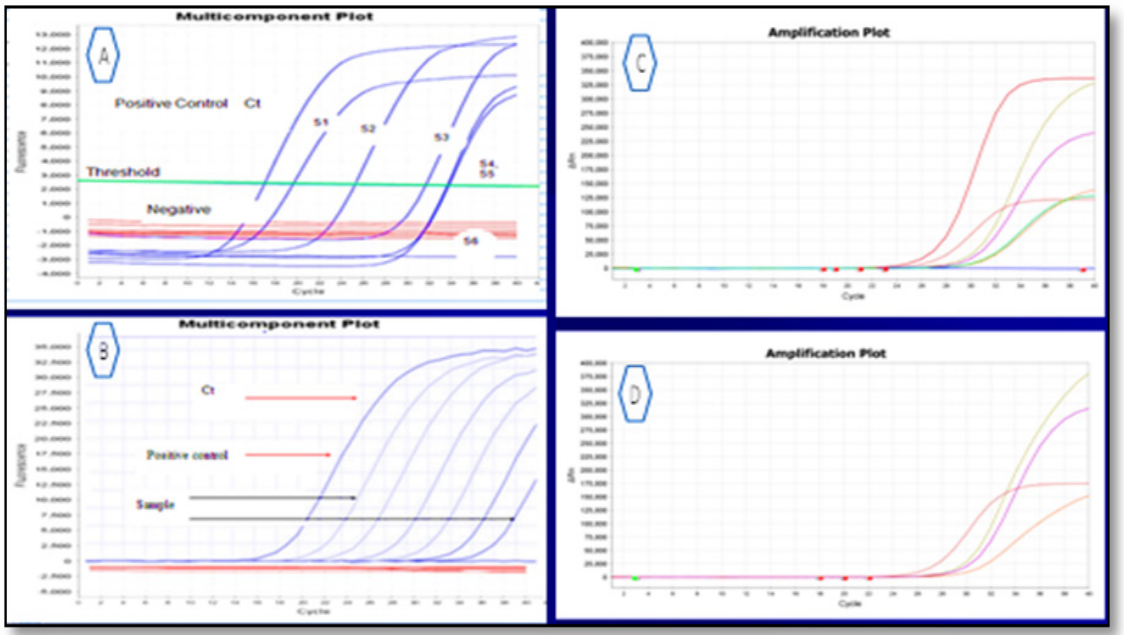

Fig. 2. (A- D). Showed amplification plot of real-time PCR that showing RT-qPCR with or without RNA extraction may identify the SARS-CoV-2 RNA.

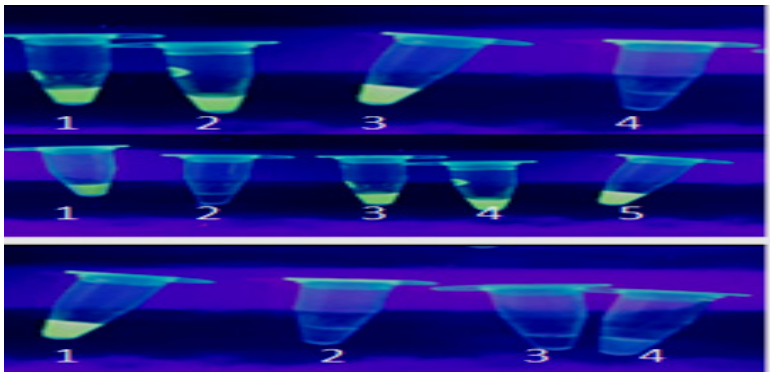

Fig. 3. LAMP based rapid detection of SARS-CoV-2 in saliva by photographed Smartphone camera. Successive green-colored LAMP amplification tubes indicate successful LAMP amplification. Color green positive and colorless negative tubes.

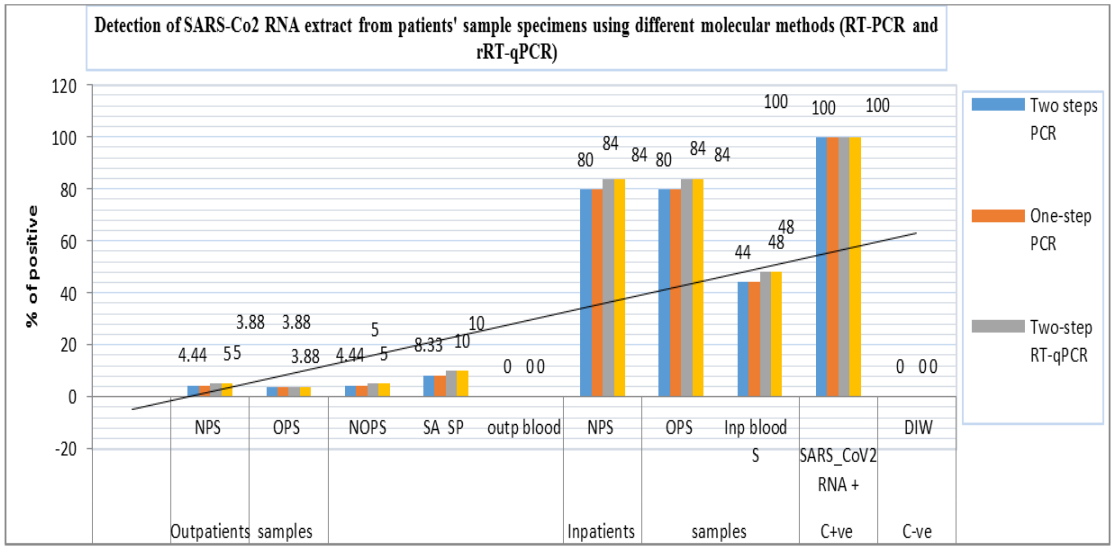

Fig. 4. Detection of SARS-Co2 RNA extract from patients' sample specimens using different molecular methods (RT-PCR and rRT-qPCR).

References

1. Alekseenko, A., Barrett, D., Pareja-Sanchez, Y., Howard, R. J., Strandback, E., Ampah-Korsah, H. Rovšnik, U., Zuniga-Veliz, S., Klenov, A., Malloo, J. and Ye, S . Direct detection of SARS-CoV-2 using non-commercial RT-LAMP reagents on heat-inactivated samples. *Scientific Reports*, **11**(1), 1820-1838. (2020). DOI: https://www.doi.org/10.1038/s41598-021-81554-1

2. do Vale, B., Lopes, A. P., Fontes, M. D. C., Silvestre, M., Cardoso, L., and Coelho, A. C. Bats, pangolins, minks and other animals - villains or victims of SARS-CoV-2?. *Veterinary Research Communications*, **45**(1), 1-19. (2021). DOI: https://www.doi.org/10.1007/s11259-021-09787-2

3. Biscayart, C., Angeleri, P., Lloveras, S., Chaves, T. D. S. S., Schlagenhauf, P., and Rodríguez-Morales, A. J. The next big threat to global health? 2019 novel coronavirus (2019-nCoV): What advice can we give to travellers? - Interim recommendations January 2020, from the Latin-American society for Travel Medicine (SLAMVI). *Travel Medicine and Infectious Disease*, **33**, 101567 (2020). DOI: https://www.doi.org/10.1016/j.tmaid.2020.101567

4. Sharifkashani, S., Bafrani, M. A., Khaboushan, A. S., Pirzadeh, M., Kheirandish, A., Yavarpour Bali, H. and Rezaei, N.. Angiotensin-converting enzyme 2 (ACE2) receptor and SARS-CoV-2: Potential therapeutic targeting. *European Journal of Pharmacology*, **884**, 1-6 (2020). Available at: https://www.journals.elsevier.com/european-journal-of-pharmacology

5. Clementini, M., Raspini, M., Barbato, L., Bernardelli, F., Braga, G., Di Gioia, C. Littarru C, Oreglia F, Brambilla, E., Iavicoli, I., Pinchi, V., Landi, L., Marco Sforza, N., Cavalcanti, R., Crea, A., and Cairo F., Aerosol transmission for SARS-CoV-2 in the dental practice. A review by SIdP Covid-19 task-force. *Oral Diseases*, **28**(1), 852-857 (2022). Available at: https://onlinelibrary.wiley.com/journal/16010825

6. Young, B. E., Ong, S. W. X., Kalimuddin, S., Low, J. G., Tan, S. Y., Loh, J. Ng ,O.T., Marimuthu, K., Ang, L.W., Mak, T.M., Lau, S.K., Anderson, D.E., Chan KS, Tan, T.Y., Ng, T.Y., Cui, L., Said ,Z., Kurupatham L, Chen, M.I,, Chan, M., Vasoo, S., Wang, L.F., Tan ,B.H., Lin, R.T.P., Lee, V.J.M,, Leo, Y.S., and Lye, D.C., Singapore 2019 Novel Coronavirus Outbreak Research Team. Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. *Journal of the American Medical Association (JAMA)*, **323**(15), 1488-1494 (2020). DOI: https://www.doi.org/10.1001/jama.2020.3204

7. Abdalhamed, A. M., Naser, S. M., Mohamed, A. H., and Zeedan, G. S. G. Development of gold nanoparticles-lateral flow test as a novel field diagnostic assay for detecting foot-and-mouth disease and lumpy skin disease viruses. *Iranian Journal of Microbiology*, **14**(4), 574-586 (2022). Available at: https://ijm.tums.ac.ir

8. Zeedan, G. S. G., Mahmoud, A. H., Abdalhamed, A. M., El-Razik, K. A. E. A., Khafagi, M. H., and Abou Zeina, H. A. Detection of lumpy skin disease virus in cattle using real-time polymerase chain reaction and serological diagnostic assays in different governorates in Egypt in 2017. *Veterinary World*, **12**(8), 1093-1100 (2019). DOI: https://www.doi.org/10.14202/vetworld.2019.1093-1100

9. Artika, I. M. and Ma'roef, C. N. Laboratory biosafety for handling emerging viruses. *Asian Pacific Journal of Tropical Biomedicine*, **7**(6), 483-491 (2017). Available at: https://www.journals.elsevier.com/asian-pacific-journal-of-tropical-biomedicine

10. Alcoba-Florez, J., González-Montelongo, R., Inigo-Campos, A., de Artola, D. G. and Gil-Campesino, H., The Microbiology Technical Support Team, Ciuffreda L, Valenzuela-Fernández A, Flores C. Fast SARS-CoV-2 detection by RT-qPCR in preheated nasopharyngeal swab samples. *International Journal of Infectious Diseases*, **97**, 66-68 (2021). DOI: https://www.doi.org/10.1016/j.ijid.2020.05.053

11. Behrmann, O., Bachmann, I., Spiegel, M., Schramm, M., Abd El Wahed, A., Dobler, G. Dame,., G., and Hufert FT. Rapid Detection of SARS-CoV-2 by Low Volume Real-Time Single Tube Reverse Transcription Recombinase Polymerase Amplification Using an Exo Probe with an Internally Linked Quencher (Exo-IQ). *Clinical Chemistry*, **66**(9), 1047-1054 (2020). DOI: https://www.doi.org/10.1093/clinchem/hvaa153

12. Białobrzeska, W., Ficek, M., Dec, B., Osella, S., Trzaskowski. B., Jaramillo-Botero, A., Pierpaoli M., Rycewicz M., Dashkevich, Y., Łęga T, Malinowska N, Cebula Z, Bigus D, Firganek D, Bięga E, Dziąbowska, K., Brodowski, M., Kowalski, M., Panasiuk, M., Gromadzka, B., Żołędowska, S., Nidzworski, D., Pyrć K., Goddard, W.A., and Bogdanowicz R. Performance of electrochemical immunoassays for clinical diagnostics of SARS-CoV-2 based on selective nucleocapsid N protein detection: Boron-doped diamond, gold and glassy carbon evaluation. *Biosensors and Bioelectronics*, **22**, 209-219 (2022). DOI: https://www.doi.org/10.1016/j.bios.2021.114212

13. Nagura-Ikeda, M., Imai, K., Tabata, S., Miyoshi, K., Murahara, N., Mizuno, T., Horiuchi, M. Kato K, Imoto Y, Iwata, M, Mimura ,S, Ito T., Tamura, K., and Kato Y. Clinical Evaluation of Self-Collected Saliva by Quantitative Reverse Transcription-PCR (RT-qPCR), Direct RT-qPCR, Reverse Transcription-Loop-Mediated Isothermal Amplification, and a Rapid Antigen Test to Diagnose COVID-19. *Journal of Clinical Microbiology*, **58**(12), 438-448 (2020). DOI: https://www.doi.org/10.1128/JCM.01892-20

14. Riccò, M., Ferraro, P., Gualerzi, G., Ranzieri, S., Bragazzi, N. L., Balzarini, F., and Signorelli C. Point-of-Care diagnostic of SARS-CoV-2: knowledge, attitudes, and perceptions (KAP) of medical workforce in Italy. *Acta Biomedica*, **91**(2), 57-67 (2020). Available at: https://www.actabiomedica.it

15. Brattelid, T. and Levy, F. O. Quantification of GPCR mRNA using real-time RT-PCR. *Methods in Molecular Biology*, **746**, 165-193 (2011). Available at: https://link.springer.com/protocol/10.1007/978-1-61779-160-6_9

16. Ehrlich, M., Madden, C., McBride, D. S., Nolting, J. M., Huey, D., Kenney, S. Wang, Q., Saif ,L.J., Vlasova, A., Dennis, P., Lombardi, D., Gibson S, McLaine A, Lauterbach S, Yaxley P, Winston, J.A., Diaz-Campos, D., Pesapane ,R., Flint, M., Flint, J., Junge, R., Faith, S.A., Bowman A.S., and Hale, V.L.. Lack of SARS-CoV-2 Viral RNA Detection) among a Convenience Sampling of Ohio Wildlife, Companion, and Agricultural. *Animals*, **13**(1), 25-54 (2023). DOI: https://www.doi.org/10.3390/animals13010025

17. Fernandes, Q., Inchakalody, V. P., Merhi, M., Mestiri, S., Taib, N., Moustafa Abo El-Ella, D. Bedhiafi T, Raza A, Al-Zaidan L, Mohsen MO, Yousuf Al-Nesf, M.A,, Hssain, A.A,, Yassine, H.M., Bachmann, M.F., Uddin, S,, and Dermime, S. Emerging COVID-19 variants and their impact on SARS-CoV-2 diagnosis, therapeutics and vaccines. *Annals of Medicine*, **54**(6), 524-540 (2022). DOI: https://www.doi.org/10.1080/07853890.2022.2104138

18. Graham, T. G. W., Dugast-Darzacq, C., Dailey, G. M., Nguyenla, X. H., Van Dis, E., Esbin, M. N., Abidi A, Stanley, S.A., Darzacq, X., and Tjian, R.. Open-source RNA extraction and RT-qPCR methods for SARS-CoV-2 detection. *PLoS ONE*, **16**(6), e0252175 (2021). DOI: https://www.doi.org/10.1371/journal.pone.0252175

19. Hammer, A. S., Quaade, M. L., Rasmussen, T. B., Fonager, J., Rasmussen, M., Mundbjerg, K., Lohse, L., Strandbygaard, B., Jørgensen, C.S., Alfaro-Núñez, A, Rosenstierne, M.W., Boklund, A., Halasa, T., Fomsgaard, A., Belsham, G.J., and Bøtner, A.. SARS-CoV-2 Transmission between Mink (Neovison vison) and Humans, Denmark. *Emerging Infectious Diseases*, **27**(2), 547-551 (2021). DOI: https://www.doi.org/10.3201/eid2702.203159

20. Huang, W. E., Lim, B., Hsu, C. C., Xiong, D., Wu, W., Yu, Y., Jia, H., Wang, Y., Zeng, Y., Ji, M., Chang, H., Zhang. X., Wang, H., and Cui, Z. RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microbial Biotechnology*, **13**(5), 950-961 (2020). DOI: https://www.doi.org/10.1111/1751-7915.13536

21. Huang, X., Tang, G., Ismail, N. and Wang, X. Developing RT-LAMP Assays for Detection of SARS-CoV-2 in Saliva. *MedRxiv* [Preprint]. (2021) DOI: https://www.doi.org/10.1101/2021.04.25.21256085

22. Mohon, A. N., Oberding, L, Hundt, J, van Marle, G, Pabbaraju, K, Berenger, B.. M., Lisboa, L, Griener, T., Czub ,M., Doolan, C., Servellita, V., Chiu, C.Y., Greninger, A.L., Jerome, K.R., and Pillai ,D.R., Optimization and clinical validation of dual-target RT-LAMP for SARS-CoV-2. *Journal of Virological Methods*, **286**, 113972 (2020). DOI: https://www.doi.org/10.1016/j.jviromet.2020.113972

23. Toptan, T., Eckermann, L., Pfeiffer, A. E., Hoehl, S., Ciesek, S., Drosten, C., and Corman, V.M. Evaluation of a SARS-CoV-2 rapid antigen test: Potential to help reduce community spread. *Journal of Clinical Virology*, **135**, 145 (2021). DOI: https://www.doi.org/10.1016/j.jcv.2021.104717

24. Kasibhatla, S. M., Kinikar, M , Limaye, S., Kale, M. M., and Kulkarni-Kale, U. Understanding evolution of SARS-CoV-2: A perspective from analysis of genetic diversity of RdRp gene. *Journal of Medical Virology*, **92**(10), 1932-1937 (2020). DOI: https://www.doi.org/10.1002/jmv.25909

25. Li, L., Xie, F., Li, J., Gong, K., Xie, X., Qin, Y., Qin, M. and Hu, J. Diagnostic analysis of regional ozone pollution in Yangtze River Delta, China: A case study in summer 2020. *Science of The Total Environment*, **812**, 151-171 (2020). DOI: https://www.doi.org/10.1016/j.scitotenv.2021.151227

26. Lv, Y., Ma, Y., Si, Y., Zhu, X., Zhang, L., Feng, H. Tian D, Liao Y, Liu T, Lu H, and Ling Y. Rapid SARS-CoV-2 antigen detection potentiates early diagnosis of COVID-19 disease. *Bioscience Trends*, **15**(2), 93-99 (2021). DOI: https://www.doi.org/10.5582/bst.2021.01201

27. Wozniak, A., Cerda, A., Ibarra-Henríquez, C., Sebastian, V., Armijo, G.., Lamig, L.. Miranda, C., Lagos, M., Solari, S., Guzmán, A.M., Quiroga, T., Hitschfeld, S., Riveras, E., Ferrés, M., Gutiérrez, R.A., and García, P. A simple RNA preparation method for SARS-CoV-2 detection by RT-qPCR. *Scientific Reports*, **10**, 16608 (2020). DOI: https://www.doi.org/10.1038/s41598-020-73608-y

28. Oba, J., Taniguchi, H., Sato, M., Takamatsu, R., Morikawa, S., Nakagawa, T. Takaishi, H., Saya, H., Matsuo, K., and Nishihara, H, RT-PCR Screening Tests for SARS-CoV-2 with Saliva Samples in Asymptomatic People: Strategy to Maintain Social and Economic Activities while Reducing the Risk of Spreading the Virus. *Keio Journal of Medicine*, **70**(1), 35-43 (2021). DOI: https://www.doi.org/10.2302/kjm.70.35

29. Premraj, A., Aleyas, A. G., Nautiyal, B., and Rasool, T. J. Nucleic Acid and Immunological Diagnostics for SARS-CoV-2: Processes, Platforms and Pitfalls. *Diagnostics (Basel)*, **10**(11), 866-882 (2020). DOI: https://www.doi.org/10.3390/diagnostics10110866

30. Renuse, S., Vanderboom, P. M., Maus, A. D., Kemp, J. V., Gurtner, K. M., Madugundu. A. K., Chavan S, Peterson, J.A., Madden BJ, Mangalaparthi, K.K., Mun D.G., Singh, S., Kipp , B.R., Dasari, S., Singh, R.J., Grebe, S.K., and Pandey, A., A mass spectrometry-based targeted assay for detection of SARS-CoV-2 antigen from clinical specimens. *EBioMedicine*, **69**, 103465. (2021) DOI: https://www.doi.org/10.1016/j.ebiom.2021.103465

31. Safiabadi Tali, S. H., LeBlanc, J. J., Sadiq, Z., Oyewunmi, O. D., Camargo, C., Nikpour, B., Armanfard, N., Sagan, S. M. and Jahanshahi-Anbuhi, S. Tools and Techniques for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)/COVID-19 Detection. *Clinical Microbiology Reviews*, **34**(1), 12-48 (2021). DOI: https://www.doi.org/10.1128/CMR.00043-20

32. Shen, M., Zeng, Z., Song, B., Yi, H., Hu, T., Zhang, Y., Zeng, G. and Xiao, R. Neglected microplastics pollution in global COVID-19: Disposable surgical masks. *Science of The Total Environment*, **790**, 148-159 (2021). DOI: https://www.doi.org/10.1016/j.scitotenv.2021.148159

33. Silva Júnior, J. V. J., Merchioratto, I., de Oliveira, P. S. B., Rocha Lopes, T. R., Brites, P.C., de Oliveira, E.M., Weiblen, R., and Flores, E.F.. End-point RT-PCR: A potential alternative for diagnosing coronavirus disease 2019 (COVID-19). *Journal of Virological Methods*, **288**, 114007 (2021). DOI: https://www.doi.org/10.1016/j.jviromet.2021.114007

34. Villota, S. D, Nipaz, V. E., Carrazco-Montalvo, A. and Hernandez, S. Alternative RNA extraction-free techniques for the real-time RT-PCR detection of SARS-CoV-2 in nasopharyngeal swab and sputum samples. *Journal of Virological Methods*, **298**, 114302 (2021). DOI: https://www.doi.org/10.1016/j.jviromet.2021.114302

35. Vogels, C. B. F., Brito, A. F., Wyllie, A. L., Fauver, J. R., Ott, I. M., Kalinich, C. C. Petrone , M.E., Casanovas-Massana, A., Catherine Muenker, M., Moore, A.J., Klein, J., Lu, P., Lu-Culligan, A., Jiang, X., Kim, D.J., Kudo, E., Mao T., Moriyama, M., Oh, J.E., Park, A., Silva, J., Song, E., Takahashi, T., Taura, M., Tokuyama , M., Venkataraman, A., Weizman, O.E., Wong, P., Yang, Y., Cheemarla, N.R., White, E.B., Lapidus, S., Earnest, R., Geng, B., Vijayakumar P., Odio, C.., Fournier, J.., Bermejo, S., Farhadian S., Dela Cruz, C.S., Iwasaki, A., Ko ,A.I., Landry, M.L., Foxman, E.F., and Grubaugh, N.D. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets. *Nature Microbiology*, **5**(12), 1299-1305 (2020). DOI: https://www.doi.org/10.1038/s41564-020-0765-5

36. Weber, J. N., Kalbe, M., Shim, K. C., Erin, N. I., Steinel, N. C., Ma, L. and Bolnick, D. I. Resist globally, infect locally: a transcontinental test of adaptation by stickleback and their tapeworm parasite. *The American Naturalist*, **189**(1), 43-57 (2017). DOI: https://www.doi.org/10.1086/688760

37. Yan, C., Cui, J., Huang, L., Du, B., Chen, L., Xue, G. Li, S., Zhang, W., Zhao, L., Sun, Y., Yao, H., Li, N., Zhao, H., Feng, Y., Liu, S., Zhang, Q., Liu, ,D., and Yuan, J. Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. *Clinical Microbiology and Infection*, **26**(6), 773-779 (2020). DOI: https://www.doi.org/10.1016/j.cmi.2020.03.031

38. Zeedan, G. S., Abdalhamed, A. M., Shaapan, R. M. and El-Namaky, A. H. Rapid diagnosis of Toxoplasma gondii using loop-mediated isothermal amplification assay in camels and small ruminants. *Beni-Suef University Journal of Basic and Applied Sciences*, **11**(1), 23 (2022). DOI: https://www.doi.org/10.1186/s43088-022-00293-1

39. Zou, J., Xia, H., Xie, X., Kurhade, C., Machado, R. R. G., Weaver, S. C. Ren, P., and Shi P.Y. Neutralization against Omicron SARS-CoV-2 from previous non-Omicron infection. *Nature Communications*, **13**, 852 (2022). DOI: https://www.doi.org/10.1038/s41467-022-28316-5

دراسة ميكروبيولوجية تفاضلية على تطبيق وتحسين تقنية التضخيم الحراري الثابت القائمة على النسخ العكسي للكشف السريع عن فيروس كورونا الجديد (سارس-كوف-٢) لدى البشر والحيوانات

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[٥] قسم الفيروسات، معهد بحوث صحة الحيوان، شارع النادي، الدقي، الجيزة، مصر

الملخص

تتطلب جائحة كوفيد19-، الناجمة عن فيروس كورونا الجديد الشديد الحدة (سارس-كوف2-)، حلول تشخيصية سريعة وموثوقة. هدفت هذه الدراسة إلى تحسين الكشف السريع عن سارس-كوف2- باستخدام تقنية التضخيم اللوبي الحرارة الثابتة القائمة على النسخ العكسي (RT-LAMP) ومقارنة أدائها مع تقنية تفاعل البوليميراز التسلسلي الكمي في الوقت الحقيقي القائم على النسخ العكسي (RT-qPCR) لبروتوكولات استخلاص الحمض النووي الريبوزي. تم جمع مسحات الأنف البلعومية، والمسحات البلعومية الفموية، وعينات الأنف البلعومية واللعاب المختلطة، وعينات البلغم من الأفراد الذين يعانون أو لا يعانون من أعراض كوفيد19- (مرضى خارجيين n = 180، مرضى داخليين n = 50) وخزنوها عند درجة حرارة 80- درجة مئوية. تم اختبار هذه العينات باستخدام RT-qPCR وRT-LAMP و RT-PCR للمقارنة. بالإضافة إلى ذلك، تم اختبار عينات حيوانية، بما في ذلك الأبقار (n = 20)، الجاموس (n = 10)، الأغنام (n = 25)، الماعز (n = 30)، الخيول (n = 5)، الحمير (n = 5)، البغال (n = 3)، الإبل (n = 5)، الكلاب (n = 10)، القطط (n = 5)، والأرانب (n = 6). كانت معدلات الكشف عن RT-qPCR 7.3 ± 0.56 و8.0 ± 0.98 و 5.0 ± 0.56 لمسحات الأنف البلعومية ومسحات البلعوم الفموي وعينات الأنف البلعومية واللعاب المختلطة مع عينات البلغم من المرضى الخارجيين (n = 180) على التوالي. بالنسبة للمرضى الداخليين (n = 50)، كانت المتوسطات والانحراف المعياري 43 ± 0.264 و43 ± 0.245 و26 ± 0.376 على التوالي. كانت حساسية RT-LAMP قابلة للمقارنة مع RT-qPCR، حيث بلغت ٪88.9 للمرضى الخارجيين و٪99.4 للمرضى الداخليين. أظهر كلا الفحصين خصوصية بنسبة ٪100 في المجموعتين. أعطى RT-PCR المباشر وRT-qPCR بدون استخلاص الحمض النووي الريبوزي نتائج مماثلة لتلك التي تم الحصول عليها باستخدام أطقم استخلاص الحمض النووي الريبوزي. بالإضافة إلى ذلك، لم يتم الكشف عن أي حمض نووي ريبوزي لفيروس سارس-كوف2- في عينات مسحات الأنف من مختلف الحيوانات باستخدام RT-qPCR و RT-LAMP. أظهرت النتائج أن اختبار RT-qPCR المباشر، مع أو بدون استخلاص الحمض النووي الريبوزي، أنتج نتائج إيجابية قابلة للمقارنة مع تلك التي تم الحصول عليها باستخدام RT-LAMP. وهذا يدل على إمكاناته كاختبار سريع وبسيط وخاص وحساس للكشف عن سارس-كوف2-.

الكلمات الدالة: الفيروس التاجي, مرض فيروس كورونا ٢٠١٩, تفاعل البلمرة المتسلسل الكمي في الزمن الحقيقي , تفاعل البلمرة المتسلسل القائم على النسخ العكسي.