original article

Automated SARS-COV-2 RNA extraction from patient nasopharyngeal samples using a modified DNA extraction kit for high throughput testing

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BACKGROUND: The pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) has prompted a need for mass testing to identify patients with viral infection. The high demand has created a global bottleneck in testing capacity, which prompted us to modify available resources to extract viral RNA and perform reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) to detect SARS-COV-2.

OBJECTIVES: Report on the use of a DNA extraction kit, after modifications, to extract viral RNA that could then be detected using an FDA-approved SARS-COV-2 RT-qPCR assay.

MATERIALS AND METHODS: Initially, automated RNA extraction was performed using a modified DNA kit on samples from control subjects, a bacteriophage, and an RNA virus. We then verified the automated extraction using the modified kit to detect in-lab propagated SARS-COV-2 titrations using an FDA approved commercial kit (S, N, and ORF1b genes) and an in-house primer-probe based assay (E, RdRp2 and RdRp4 genes).

RESULTS: Automated RNA extraction on serial dilutions SARS-COV-2 achieved successful one-step RT-qPCR detection down to 60 copies using the commercial kit assay and less than 30 copies using the in-house primer-probe assay. Moreover, RT-qPCR detection was successful after automated RNA extraction using this modified protocol on 12 patient samples of SARS-COV-2 collected by nasopharyngeal swabs and stored in viral transport media.

CONCLUSIONS: We demonstrated the capacity of a modified DNA extraction kit for automated viral RNA extraction and detection using a platform that is suitable for mass testing.

LIMITATIONS: Small patient sample size.

CONFLICT OF INTEREST: None.

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The human community continually faces the threat of emerging pathogens, some of which spread and infect larger populations and can cause pandemics. In the past two decades, we have faced the spread of viral infections caused by new viruses belonging to the Coronaviridae family of viruses, commonly known as coronaviruses. Coronaviruses are RNA viruses that infect birds, mammals, and humans and affect different bodily systems, including the respiratory system. At least six coronaviruses are known to infect humans and cause diseases of variable severities. Included among these six coronaviruses are the severe acute respiratory syndrome coronavirus (SARS-CoV; 2002–2003 outbreak) and Middle East respiratory syndrome coronavirus (MERS-CoV; 2012 outbreak),[1] and the most recently discovered and cause of the ongoing pandemic, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; 2019 outbreak).[2] The spread of SARS-CoV-2 has prompted the need for widespread rapid diagnostic testing. The method used to test patients for SARS-CoV-2 is based on reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR), which is performed after RNA extraction.[3,4] The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have developed SARS–CoV-2 assays. The assays are based on PCR primer-probe sets for different genes. The CDC and WHO assays differ in their targeted genes and both assays are primer-probe based and have high sensitivity and specificity for SARS–CoV-2. Both assays also have minimal cross reactivity with other circulating strains of coronaviruses, and indicate SARS-COV-2 positivity with a quantification cycle (C_q) of less than 40 for SARS-COV-2 positivity in the PCR test.[5]

There are a number of kits that employ these approaches (such as the cobas SARS-CoV-2 test by Roche Molecular Systems, the RealStar SARS-CoV-2 RT-PCR Kit RUO by Altona-Diagnostic, and the High-throughput Automated Sample Preparation System Nucleic Acid Extraction Kit Genetic Sequencer (DNBSEQ-G50, DNBSEQ-G400 FAST) by Wuhan MGI Tech Co, Ltd, Wuhan, China), but the high demand in the last few months has resulted in a shortage and prompted the use of alternative resources. To counter this shortage, we opted for the use of a bead-based DNA extraction kit after modifications to the buffers used in the extraction of viral RNA followed by a PCR assay based on primers and probes recommended by the WHO for SARS-COV-2 testing. The DNA extraction kit we used is manufactured by Invitrogen and intended for isolation of genomic DNA from forensic specimens, namely the Invitrogen Forensic DNA extraction kit. The modifications of this kit were successful in extracting RNA when performed automatically using a robotic system suitable for mass extraction, which provides an alternative to commercial viral RNA extraction kits and provides a protocol to increase preparedness for future crises. The detection of the extracted RNA was cross validated by kit-based assays and our in-house primer-probe sets, which are based on CDC/WHO recommendations. Our approach provides an alternative to counter the supply chain shortage in SARS-COV-2 diagnostic testing for emergency use.

MATERIALS AND METHODS

Modification of the Invitrogen ChargeSwitch Forensic DNA Purification Kit

The intended use of the ChargeSwitch Forensic DNA Purification Kit (Invitrogen Life Technologies, catalog number CS11200, *https://www.thermofisher.com/*) is to isolate genomic DNA from different types of forensic samples, followed by short tandem repeat analysis. Initially we used the kit according to the manufacturer's instructions without modifications to extract RNA, but with no success. Subsequently, the kit was used according to the manufacturer's instructions with the following modifications to successfully extract human RNA as well as virus RNA (including SARS-COV-2 RNA) by the following steps: deactivation step (intended to deactivate SARS-COV-2 viruses in the sample) for 30 minutes at room temperature for 500 µL of each sample (nasopharyngeal swabs in transport medium) with 800 µL L13 buffer (cell lysis buffer) adjusted to pH 7, 100% ethanol (300 µL, virus deactivation agent), dithiothreitol (DTT) at a final concentration of 2.5 mM (to preserve RNA integrity), β-mercaptoethanol (10 µL/mL, also to preserve RNA integrity) and proteinase K (for complete lysis of cells and virus envelopes) at a final concentration of 20 µg/mL. The total volume was 1.3 mL/each sample preparation in deep well plates (PrepFiler 96-Well Processing Plates, catalog number 4392904). After the viral inactivation step the plate was loaded on the Hamilton Automation system for the automated RNA extraction. The lysis was performed for 30 minutes at 55°C with agitation at 400 rpm speed followed by cooling for 2 minutes and addition of 200 µL purification buffer and 20 µL of ChargeSwitch beads per sample and binding with agitation at 400 rpm at room temperature for 10 minutes. After the binding step, centrifugation at 3000 rpm is performed for 2 minutes to ensure complete settling of the bound beads to the bottom of the deep wells followed by 5 minutes on a magnetic plate, and then the supernatant was discarded followed

by two washes with W12 buffer (wash buffer) for 5 minutes each and elution with 50 µL of the kit elution buffer and transfer of elution buffer containing RNA to a sample plate for subsequent RT-qPCR analysis.

Propagating the SARS-COV-2 virus

Vero CCL-81 cells infected with heat-inactivated SARS-COV-2 isolates were propagated and then diluted in a biosafety level 3 facility (unpublished data).

Reverse transcription real-time PCR

10 µL of extracted RNA (from the final eluted material at the end of the automated viral RNA extraction) was used as input material for the multiplex single-step PCR for three genes using the ThermoFisher Scientific TaqPath One-Step qRT-QPCR kit (TaqPath COVID-19 RT-PCR Kit, Applied Biosystems: A48102) as per manufacturer protocol (annealing at 58°C, TaqPath One-Step RT-QPCR system on the Applied Biosystems 7500 real time cycler for 25 µL per reaction). The in-house primer-probe assay was run using the conditions and primers indicated in **Tables 1, 2 and 3**.[6]

RESULTS

Isolation of single-stranded RNA from human samples, bacteriophage MS2 and encephalomyocarditis virus

To validate the capacity and sensitivity of the modified Invitrogen ChargeSwitch Forensic DNA kit (Invitrogen, catalogue number CS11200) in extracting total RNA from nasopharyngeal swabs, we conducted a series of automated extractions of total RNA experiments from healthy donor nasopharyngeal swabs submerged in transport medium and spiked with MS2 (bacteriophage provided in the Applied Biosystems assay (Applied Biosystems, TaqPath One-Step qRT-QPCR, catalogue number A48102) and an RNA virus (encephalomyocarditis virus, EMCV). A standard two-step RT-qPCR was used to detect human reference genes (rRNA) and EMCV RNA, and single-step RT-qPCR was used to detect bacteriophage MS2 RNA after the automated RNA extraction (spiking experiments). A stepwise dilution of MS2 (from 10 µL to 0.625 µL) and EMCV (10 500 to 325 PFU/µL) were added to the lysis mix and processed using the modified kit on the fully automated extraction. Purified RNA samples were then screened by RT-qPCR using primers specific for human rRNA (forward: ATGGCCGTTCTTAGTTGGTG, reverse: CGCTGAGCCAGTCAGTGTAG), MS2 (Applied Biosystems, TaqPath One-Step qRT-QPCR, catalogue number A48102) and EMCV (FWD: AGA GGC CGG GTA TAA GGT TT, REV: TGC AAT TCC TCA AGC TGT TC).

As shown in **Figure 1 and Tables 4a-4c** the automated extraction using the modified kit isolated sufficient RNA for detection by RT-qPCR to the lowest dilution of MS2 and 325 PFU/µL for EMCV. The quantification cycles at which each RNA product was detected were from C_q of 17.06 to C_t of 20.6 for human rRNA, C_q of 29.7 to 32.3 for MS2 RNA, and C_q of 24.09 to 27.67 for EMCV RNA. Thus, these results indicate that the modified forensic DNA kit is effective for isolation of detectable single-stranded RNA from viruses.

Sensitivity of the Invitrogen ChargeSwitch Forensic DNA Purification Kit for isolation SARS-COV-2 RNA

We validated the modified Invitrogen Forensic DNA Purification kit in extracting in-laboratory propagated SARS-COV-2 RNA by conducting manual and automated extractions on titrations from 15 000 copies to 60 copies of SARS-COV-2 followed by RT-qPCR methods: the commercially available TaqPath One-Step qRT-QPCR kit (using the N, S, and ORF1b genes) and primers and probes from Metabion, Germany to establish an in-house RT-qPCR assay based on E, RdRp2 and RdRp4 gene detection as per recommended SARS-COV-2 testing from CDC and WHO.

The results for the two assays are shown in **Figure 2** and indicate detection of SARS-COV-2 RNA to as few as 60 copies using the commercial kit and 46 copies using the in-house assay. The range of C_q values for SARS-COV-2 detection were N gene: C_q 23.1 (15 000 copies) to C_q 29.6 (60 copies), S gene: C_q 29.6 (15 000 copies) to C_q 36 (60 copies), and ORF1b gene: C_q 22.3 (15000 copies) to C_q 29.4 (60 copies), for TaqPath kit and E gene: C_q 31.0 (3000 copies) to C_q 37.3 (11 copies), RdRp2 gene: C_q 29.3 (3000 copies) to C_q 35.3 (11 copies), and RdRp4 gene: C_q 30.0 (3000 copies) to C_q 38.2 (11 copies), for the in house RT-qPCR assay (**Table 5**).

Modified Invitrogen ChargeSwitch Forensic DNA Purification Kit for isolation of SARS-COV-2 RNA from patient samples

We then validated the capacity of the modified Invitrogen Forensic DNA Purification kit in extracting SARS-COV-2 RNA by conducting manual and automated extractions on SARS-COV-2 nasopharyngeal swabs submerged in transport medium from patient samples (left over from diagnostic materials and shared by our collaborators in the CDC). A standard single-step RT-qPCR was used to detect SARS-COV-2 RNA after the RNA extraction utilizing single-step RT-QPCR assays

Table 1. Primers and probes for the in-house SARS-CoV-2 RT-qPCR diagnostic assay.

Target	Primer and probes	Sequence
E	E_Sarbeco_Forward	ACA GGT ACG TTA ATA GTT AAT AGC GT
	E_Sarbeco_Reverse	ATA TTG CAG CAG TAC GCA CAC A
	E_Sarbeco_Probe	ACA CTA GCC ATC CTT ACT GCG CTT CG [5′] FAM [3′] BHQ-1
RdRp 2	RdRp/nCoV_IP2-Forward	ATG AGC TTA GTC CTG TTG
	RdRp/nCoV_IP2-Reverse	CTC CCT TTG TTG TGT TGT
	RdRp/nCoV_IP2-Probe	AGA TGT CTT GTG CTG CCG GTA [5′] HEX [3′] BHQ-1
RdRp 4	RdRp/nCoV_IP4-Forward	GGT AAC TGG TAT GAT TTC G
	RdRp/nCoV_IP4-Reverse	CTG GTC AAG GTT AAT ATA GG
	RdRp/nCoV_IP4-Probe	TCA TAC AAA CCA CGC CAG G [5′] FAM [3′] BHQ-1

Adapted from Charité Institute of Virology, Universitätsmedizin Berlin, Germany.

Table 2. PCR master mix component and concentration.

Multiplex mix	Volume (μL)	Final concentration
H_2O PPI	1.3	
Reaction mix 2X	12.50	3.0 mM Mg
$MgSO_4$ (50mM)	0.40	0.8 mM Mg
Forward Primer (10μM)	1.00	0.4 μM
Reverse Primer (10μM)	1.00	0.4 μM
Forward Primer (10μM)	1.00	0.4 μM
Reverse Primer (10μM)	1.00	0.4 μM
Probe (10μM)	0.40	0.16 μM
Probe (10μM)	0.40	0.16 μM
SupersriptIII RT/ Platinum Taq Mix	1.00	
Final volume	25.00	

Table 3. RT-qPCR amplification cycles.

Cycle	Temperature (°C)	Time	Number of cycles
Reverse transcription	55°C	20 min	X1
Denaturation	95°C	3 min	X1
Amplification	58°C	30 sec	X40 Acquisition
Cooling	40°C	30 sec	X1

(Applied Biosystems brand, ThermoFisher Scientific). A number of nine samples were processed using the fully automated extraction. Purified RNA samples were then screened by RT-QPCR using the assay primers-probe sets for 3 genes (ORF 1b, S, and N genes).

Figure 3 and Table 6 show the amplification curve and quantification cycle values using the Applied Biosystems assay. The automated extraction using the modified kit isolated sufficient RNA for detection by RT-qPCR. The quantification cycle at which each RNA product was detected ranged from C_q of 15.9 to C_q of 34.8 for the Applied Biosystems assay. These results indicated that the automated extraction of SARS-COV-2 RNA using the modified forensic DNA kit is effective for isolation of detectable SARS-COV-2 RNA from patient samples.

DISCUSSION

On the 11th of March, 2020, the World Health Organization declared the SARS-CoV-2 virus as a pandemic threat with global reports of more than 2 878 196 confirmed (85 530 deaths) (*https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200427-sitrep-98-covid-19.pdf?sfvrsn=90323472_4*). There are multiple reasons for this high rate of virus transmission, for instance; most of the cases were asymptomatic and considered carriers[7] and responses to the epidemic were delayed.[8] Accordingly, it is crucial to screen everyone to confirm and clear positive suspected cases, and this approach will be the key factor to counter global outbreak situations. Hence, there has been an unprecedented global effort to increase the mass testing capacity for SARS-COV-2 for the sake of both clinical practice and pub-

lic health. This worked successfully in two countries: In Germany and South Korea the case-fatality rates dropped to less than 0.5% probably because of the mass testing that they implemented.[9] In addition, the mass testing in China played an important role in identifying SARS-CoV-2.[10] Yet, very few countries have used the mass testing approach, the most important reason probably being the limited resources for testing.

Although SARS-COV-2 kits are available commercially, the huge world-wide demand over the last few months created a shortage and delay in obtaining them, particularly in the large amounts needed for mass testing. Thus, we aimed to use our current available resources, reagents and facility to overcome this shortage. We have adapted the reverse transcription real-time polymerase chain reaction RT-qPCR–based assays, which are the gold standard for SARS-COV-2 diagnostics for upper and lower respiratory specimens. This assay utilizes oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan) to increase sensitivity levels. The adapted set of primers and probes are among the lists that have been evaluated and recommended by CDC/WHO for any in-house assay development for SARS-COV-2 testing. The assay also requires an additional step, the use of an RNA extraction kit for viral SARS-COV-2 extraction prior to the RT-qPCR assay. Despite this nucleic acid test several studies have reported false-negative results,[2,11,12] with a reported sensitivity of almost 80%.

In order to start our mass testing platform, we first addressed the shortage of RNA extraction kits, which is a bottleneck in the testing capacity, at a global level. We opted for the use of a bead-based DNA extraction kit after modifications to the buffers used in the extraction, to extract viral RNA. The DNA extraction kit we used is produced by Invitrogen and intended for isolation of genomic DNA from forensic specimens. This strategy requires investigating whether these kits after modifications are indeed sufficient in extracting RNA in general and viral RNA in particular. The Invitrogen ChargeSwitch Forensic DNA kit was modified to perform automated extraction of RNA from nasopharyngeal swab samples, an approach that is suitable for mass extraction. The first step in extracting DNA or RNA is the cell lysis step in order to release DNA and RNA. We modified the lysis step by adding agents that protect RNA integrity (from RNAses) and to lyse virus envelopes more efficiently. We also added ethanol for the purpose of deactivating the highly infectious SARS-COV-2. The ultimate method to validate a successful extraction of viral RNA is detecting it by RT-qPCR as this is the final output. In our study we successfully vali-

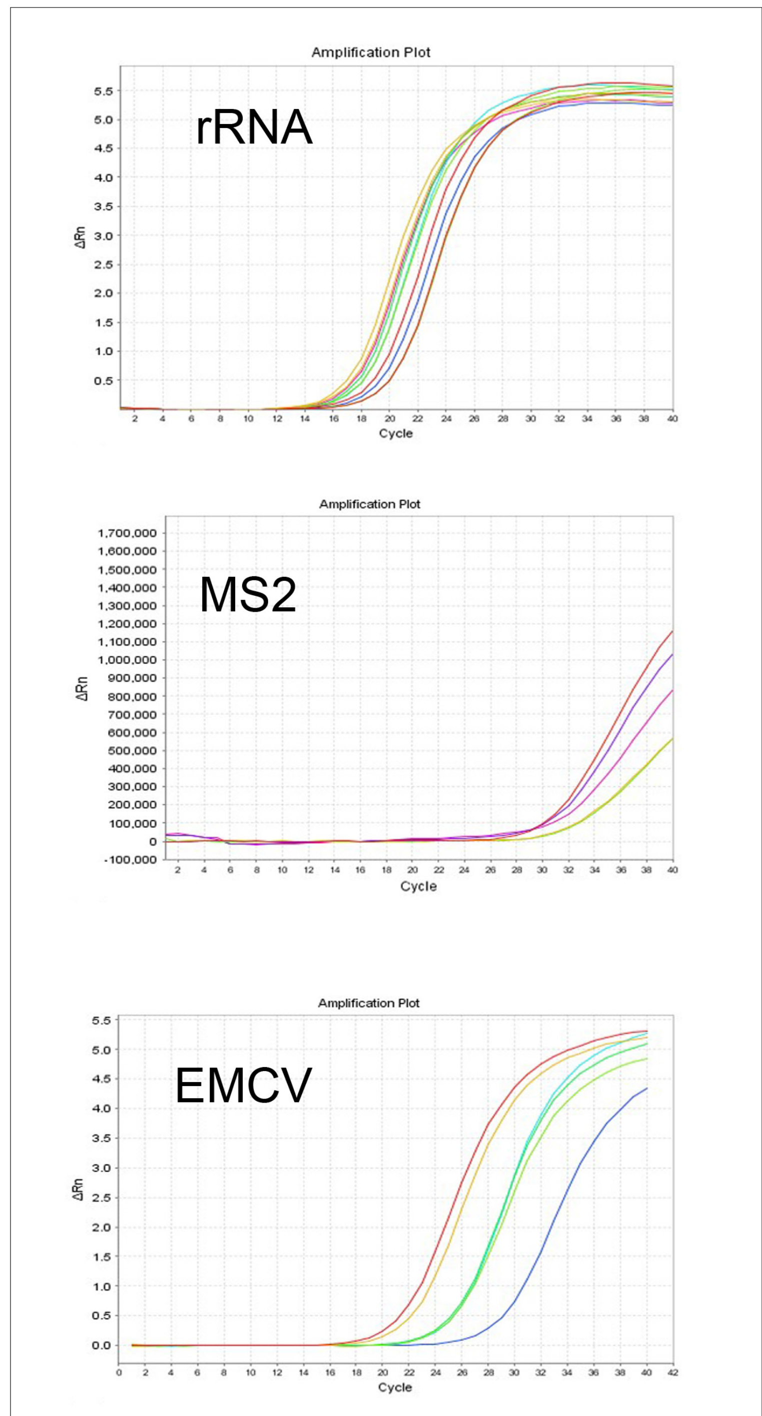

Figure 1. Amplification curves produced by RT-qPCR for healthy donor samples prepared from nasopharyngeal swabs submerged in transport medium and spiked with MS2 and ECMV with step-down titrations. The y axis is ΔRn (the normalized reporter value, also called "Rn value", of an experimental reaction minus the "Rn value" of the baseline signal generated by the instrument) and the x axis are reference cycles. rRNA is the human housekeeping gene, MS2 is a positive-sense single-stranded RNA bacteriophage and EMCV is the encephalomyocarditis virus, a small non-enveloped single-stranded RNA virus.

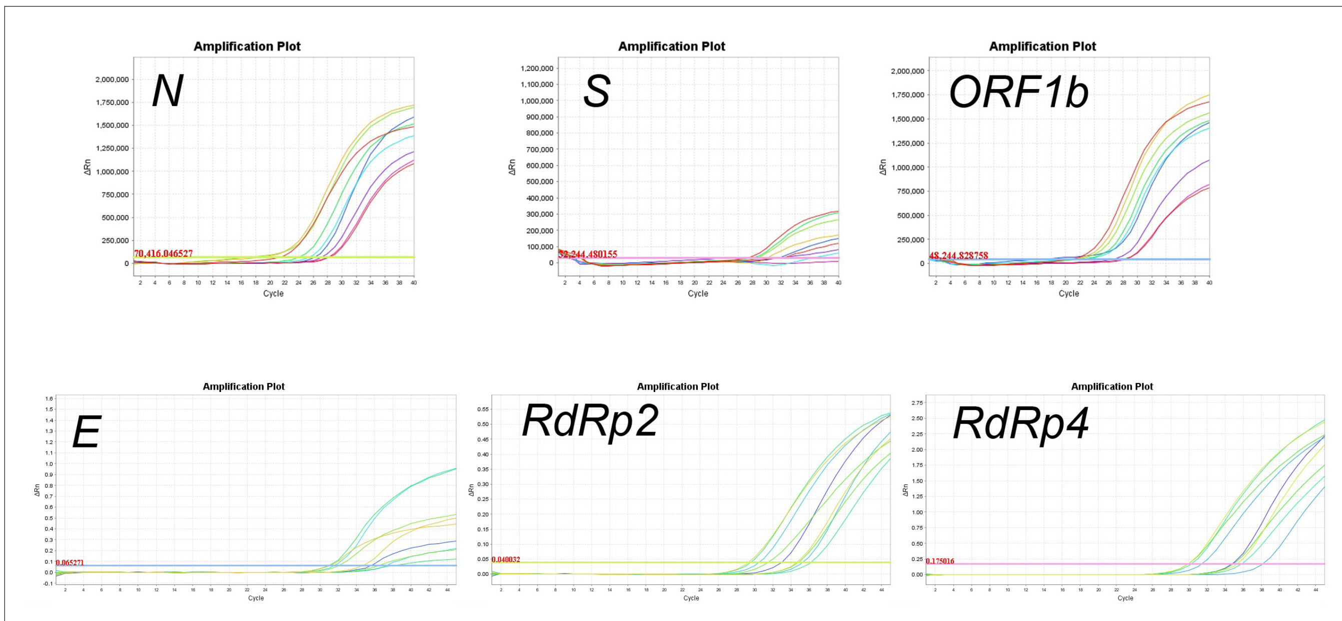

Figure 2. Amplification curves produced by RT-qPCR for healthy donor samples prepared from nasopharyngeal swabs submerged in transport medium and spiked with heat inactivated lab-propagated SARS2-COV-2 viral particles. The S, N, and ORF1b gene amplification curves were produced after automated RNA extraction of decreasing titrations of SARS-COV-2 (from 15000 copies/test to ~60 copies/test followed by one-step RT-qPCR using the TaqPath assay by Applied Biosystems. The E, RdRp2, and RdRp4 gene amplification curves were produced after automated RNA extraction of decreasing titrations of SARS-COV-2 (from 3000 copies/test to 8 copies/test followed by one-step RT-qPCR using the in-house assay. The y axis is ΔRn and x axis are quantification cycles.

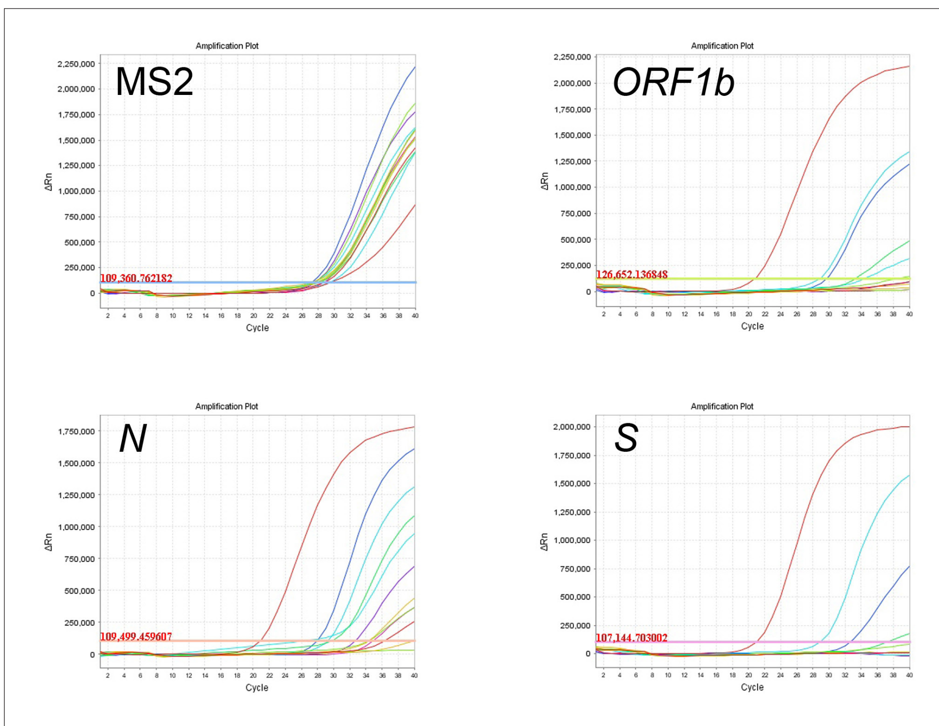

Figure 3. Amplification curves produced by RT-qPCR for SARS-COV-2 samples obtained after diagnostic tests were performed (using leftover specimens), and prepared from nasopharyngeal swabs submerged in transport medium. The S, N, and ORF1b gene amplification curves were produced after automated RNA extraction SARS-COV-2 by one-step RT-qPCR using the TaqPath assay by Applied Biosystems. The samples were spiked by MS2, the internal control for RNA extraction. The y axis is ΔRn and x axis are quantification cycles.

dated the detectability of extracted viral RNA using an FDA-approved assay as well as an in-house developed assay. Our initial experiments in which we validated the RNA extraction using human reference gene showed efficient detection of RNA at low quantification cycle values (high nucleic acid/RNA extraction efficiency). Moreover, automated extraction using the modified kit on MS2 (bacteriophage provided in the Applied Biosystems kit for RNA extraction control) and ECMV (RNA virus) also showed efficient detection of RNA at low quantification cycle values (high nucleic acid/RNA extraction efficiency). The sensitivity of the detection of MS2 was achieved at as low a titration as 0.625 μL and 325 PFU/μL for ECMV. We compared the sensitivity and specificity of the modified kit using the commercially available TaqPath One-Step qRT-QPCR kit (uses N, S, and ORF1b genes) and an in-house primer-probe based assay (E, RdRp2 and RdRp4 genes) in which the

Table 4a. RT-qPCR for rRNA after automated RNA extraction.

Sample	Quantification cycle (C_q)
1	19.4
2	17.9
3	18.7
4	18.3
5	18.7
6	19.9
7	18.1
8	20.6
9	17.6
10	20.6

Table 4b. RT-qPCR for MS2 titrations after automated RNA extraction followed by ThermoFisher PCR assay.

Titration (μL)	Quantification cycle (C_q)
10	29.7
5	30.0
2.5	29.7
1.25	32.1
0.625	32.3

Table 4c. RT-qPCR for EMCV titrations after automated RNA extraction followed by ThermoFisher PCR assay.

Titration (PFU/μL)	Quantification cycle (C_q)
10500	19.9
5250	20.8
2625	24.0
1312	23.8
656	24.1
328	27.6

Table 5. Limit of SARS-COV-2 detection after automated RNA extraction using the modified DNA kit.

	TaqPath™ Assay		In-house Assay		
Titration (copies)	Target	C_q	Titration (copies)	Target	C_q
15000	*N*	23.1	3000	*E*	31.08
	S	29.64		*RdRp2*	29.35
	ORF1b	22.58		*RdRp4*	29.99
7500	*N*	22.35	1500	*E*	32.09
	S	32.36		*RdRp2*	31.53
	ORF1b	23.19		*RdRp4*	29.98
3750	*N*	22.46	750	*E*	31.04
	S	30.73		*RdRp2*	29.58
	ORF1b	23.75		*RdRp4*	30.31
1875	*N*	25.44	375	*E*	31.63
	S	30.35		*RdRp2*	30.51
	ORF1b	24.76		*RdRp4*	31.43
937.5	*N*	26.73	187	*E*	35.61
	S	0.00		*RdRp2*	32.96
	ORF1b	25.22		*RdRp4*	34.82
468.75	*N*	27.38	93	*E*	34.86
	S	34.56		*RdRp2*	34.70
	ORF1b	23.58		*RdRp4*	35.35
234.37	*N*	28.45	46	*E*	36.55
	S	39.92		*RdRp2*	34.86
	ORF1b	28.07		*RdRp4*	34.97
117.18	*N*	29.39	23	*E*	38.23
	S	0.00		*RdRp2*	36.21
	ORF1b	29.39		*RdRp4*	35.84
58.59	N Gene	29.58	11	*E*	37.30
	S Gene	36.11		*RdRp2*	35.35
	ORF 1b	29.40		*RdRp4*	38.17

Table 6. RT-qPCR for 9 SARS-COV-2 patients after automated RNA extraction followed by ThermoFisher PCR assay.

Sample	Gene (positive control)	Quantification cycle (C_q)
CDC1	*Orf1b*	33.5
	S	0.00
	N	28.7
	(MS2)	31.5
CDC8	*Orf1b*	0.00
	S	0.00
	N	32.5
	(MS2)	30.8
CDC10	*Orf1b*	0.00
	S	0.00
	N	27.9
	MS2	30.9
CDC13	*Orf1b*	0.00
	S	0.00
	N	0.00
	(MS2)	31.3
CDC67	*Orf1b*	27.8
	S	30.6
	N	25.8
	(MS2)	28.2
CDC59	*Orf1b*	34.8
	S	0.00
	N	30.1
	(MS2)	29.8
CDC63	*Orf1b*	0.00
	S	0.00
	N	31.9
	(MS2)	30.9
CDC15	*Orf1b*	0.00
	S	0.00
	N	27.5
	(MS2)	31.1
CDC73	*Orf1b*	29.3
	S	33.5
	N	15.9
	(MS2)	31

TaqPath method detected down to 60 copies and the in-house assay down to 46 copies of SARS-COV-2 RNA. This prompted us to test the automated extraction on nine SARS-COV-2 samples that we obtained after clinical testing was conducted at the Centers for Disease Control and Prevention in Riyadh, Saudi Arabia. The RNA extracted from these samples was validated by running the PCR using the FDA-approved assay from ThermoFisher Scientific TaqPath One-Step qRT-QPCR (Applied Biosystems, A48102), in which SARS-COV-2 RNA was detected in 8 out of the 9 samples. These results indicated the capacity of detecting SARS-COV-2 RNA after automated extraction with the modified DNA extraction kit.

The analytical sensitivity and specificity of SARS-COV-2 RT-qPCR primer-probe sets varies across different assays. At viral load of 500 copies and higher, the primer-probe sets have comparable sensitivities with quantification cycle values ranging between 30 and 40 cycles, however at a lower copy number some probes have lower sensitivity than others.[6] Other factors may also affect detection of SARS-COV-2 in samples including sample collection and transport; hence most kit based assays include two or three genes for SARS-COV-2 testing. WHO, for instance, recommends a cocktail of E and RdRp2 and RdRp4 genes for SARS-COV-2. Two recent studies have evaluated the different sets of primers and probes recommended by CDC/WHO. One reported that the RdRp and E are among the best to be tested with a high specificity for SARS-CoV-2, no cross-reactivity with other respiratory viruses with a limit of detection of about 790 viral copies,[6] while another report indicates that the lower sensitivities failed to reach 500 copies.[13] In this study we tested the limit of detection of SARS-COV-2 after automated RNA extraction by two assays, in which the commercially available assay from Applied Biosystems had a SARS-COV-2 limit of detection of ~60 copies and the in-house assay had a SARS-COV-2 limit of detection of 46 copies. However, these different reports on viral load measurement may indicate that no standardized process exists yet. In addition, there is no established threshold for interpretation of viral loads, which may vary in different labs, by assay used and by different targeted genes. In addition, the detection rates in each sample type may vary from patient to patient and may change over the course of individual patients' illnesses.[13]

In conclusion, due to the urgent need for high volume SARS-COV-2 screening, we report the use of the modified DNA extraction kit on an automated platform for SARS-COV-2 RNA extraction with a cost of 3USD

per sample. Our findings suggest that use of this protocol will be satisfactory for emergency use after further validation on patient samples.

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Ethical consideration

The study is approved by the institutional research committee and the de-identified samples were left over after completion of diagnostic tests; hence this study requires no consenting as per institutional ethics committee regulations.

Disclaimer

All authors have given final approval of this manuscript to be published. In addition they have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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