Integration of RT-LAMP and Microfluidic Technology for Detection of SARS-CoV-2 in Wastewater as an Advanced Point-of-care Platform

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Abstract

Development of lab-on-a-chip (LOC) system based on integration of reverse transcription loop-mediated isothermal amplification (RT-LAMP) and microfluidic technology is expected to speed up SARS-CoV-2 diagnostics allowing early intervention. In the current work, reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and RT-LAMP assays were performed on extracted RNA of 7 wastewater samples. RT□LAMP assay was also performed on wastewater samples without RNA extraction. Current detection of SARS-CoV-2 is mainly by RT-qPCR of ORF (ORF1ab) and N genes so we targeted both to find the best surrogate marker for SARS-CoV-2 detection. We also performed RT-LAMP with/without RNA extraction inside microfluidic device to target both genes. Positivity rates of RT-qPCR and RT-LAMP performed

on extracted RNA were 100.0% (7/7) and 85.7% (6/7), respectively. RT-qPCR results revealed that all 7 wastewater samples were positive for N gene (Ct range 37-39), and negative for ORF1ab, suggesting that N gene could be used as a surrogate marker for detection of SARS-CoV-2. RT-LAMP of N and ORF (ORF1a) genes performed on wastewater samples without RNA extraction indicated that all 7 samples remains pink (negative). The color remains pink in all microchannels except the one which subjected to RT-LAMP for targeting N region after RNA extraction (yellowish/orange color). This study shows for the first time that SARS-CoV-2 was successfully detected from wastewater samples using RT-LAMP in microfluidic chips.

Keywords: SARS-CoV-2; RT-LAMP; microfluidic device; N gene

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible and pathogenic coronavirus and it is the causative agent of the coronavirus disease 2019 (COVID-19) pandemic [1]. Although there are massive coronavirus vaccination campaigns all over the world, strong public health surveillance and rapid diagnostic testing is considered as the best way to control COVID-19 [2-4]. The gold standard to diagnose COVID-19 is reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) [5]. Droplet digital RT-PCR (RT-ddPCR) offers an attractive platform for quantification of SARS-CoV-2 RNA [6]. Factors such as high sensitivity and specificity, requirement of highly trained personnel, and the need of special facilities and high-cost instrumentation limit its application especially in developing countries [7].

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is an isothermal nucleic acid amplification technique that is being widely used as point-of-care detection of

SARS-CoV-2 in clinical samples [8]. RT-LAMP possesses some fundamental advantages such as sensitivity, speed, exclusion of a thermal cycler, and robustness to sample inhibitor making it a promising alternative to RT-qPCR [9]. LAMP takes less than one hour for amplifying the genetic material of the pathogen, and requires a set of four to six primers, ensuring high specificity [10]. Amplification product of LAMP can be confirmed using different procedures such as changes in fluorescence using intercalating dyes, DNA probes with gold nanoparticles [11, 12], changes in turbidity caused by magnesium pyrophosphate precipitate [13], pH indicators, or gel electrophoresis followed by UV detection [14]. The most frequently used method is based on color change of colorimetric master mix containing a visible pH indicator for rapid and easy detection [4, 14, 15].

Although inhalation of aerosol/droplet and person-to-person contact are the major transmission routes of SARS-CoV-2, current evidence points out that the viral RNA is detected in wastewater, urging the need to better understand wastewater as potential source of epidemiological data and human health risks, which can be applied as an early warning system [16-19]. SARS-CoV-2 may cause asymptomatic or pauci-symptomatic infections [20-22], which could add more limitations to determine the actual degree of SARS-CoV-2 circulation in a community. In the meantime, wastewater surveillance can give an unbiased method of estimating the spread of infection in different places, especially in developing countries, where resources for clinical diagnosis are sparse and limited [23]. Currently, detection of SARS-CoV-2 in wastewater primarily relies on RT-qPCR [24-26], which is laborious, costly, time-consuming, and requires extensive personnel skills [4, 15].

The field of microfluidics provides an alternative to the time-consuming bench assays [27]. Micro-electromechanical systems and microelectronics technologies have an important role in

the emergence of microfluidic devices, which are able to manipulate minute amounts of fluids and extracting information from it, offering the potential to quickly acquire information from the small sample volumes [28]. It has increasingly been used for point of care testing or bedside. There are many available microfluidic devices for early diagnosis of diseases or other health-related conditions such as pneumonia, glucose level, and pregnancy test by the detection of target elements [27, 29]. In recent years, viruses could be also detected using microfluidic devices [30, 31]. Microfluidic devices promise cheaper, faster, sensitive and easy to use methods, so they have a high potential to be an alternative way for the viral RNA detection [32]. Microfluidic devices have previously been applied for detection of RNA viruses such as HIV [33], Hepatitis A virus [34], H1N1 [35], Zika [36], and norovirus [34], with acceptable results.

In the present study, we aim to evaluate the efficacy of RT-LAMP to detect SARS-CoV-2 in wastewater as a point of care method to provide early warning system for COVID-19 transmission in the community. Current detection of SARS-CoV-2 is mainly by RT-qPCR of ORF (ORF1ab) and N genes so we try to find the best surrogate gene marker for SARS-CoV-2 detection. We also aim, for the first time, to assess the application RT-LAMP in microfluidic device as an advanced point of care to detect SARS-CoV-2 in wastewater.

Material and methods

Wastewater sampling

Grab sampling technique was used to collect untreated wastewater samples (sewage samples). During the peak morning flow, 500 ml of wastewater was collected from the midstream into a leak proof, sterile container at a downstream sampling site. Seven wastewater samples were collected in early morning from hot COVID-19 spots in Islamabad, capital of Pakistan on 4 April

2021 and were kept at 4 °C. In the following day (5 April 2021) early morning, samples were transported at 4 °C to the BSL-3 facility at the Institute of Microbiology (IM), University of Veterinary and Animal Sciences (UVAS) Lahore, Pakistan.

Sample processing and RNA extraction

RNA of each wastewater sample was extracted in BSL-3 of IM, UVAS Lahore, Pakistan. Before extraction, each sample was vortexed thoroughly and 1 ml of the sample was transferred to microfuge tube. Samples were centrifuged at 5000 rpm for 15 minutes at 4 ºC. The supernatant was used for RNA extraction [37]. RNA was extracted using Viral Nucleic Acid Extraction Kit II Geneaid (Geneaid Biotech, Taiwan), according to the manufacturer's protocol. The RNA was stored at -80ºC, and used as a template for both RT-qPCR, and RT□LAMP. We performed RNA extraction step on the seven samples directly without virus concentration step to check if virus concentration step is necessary or can be skipped in RT-LAMP of wastewater samples.

RT-qPCR analysis

RT-qPCR analysis of the seven wastewater samples was performed by using the commercially available kit (2019-nCoV Nucleic Acid Diagnostic Kit, Sansure Biotech Inc., China). This kit is used for detection of the ORF (ORF1ab) and N genes of SARS-CoV-2. According to Sansure protocol, we selected FAM (ORF-1ab region) and ROX (N gene) channels. Each reaction mixture contained 26µl of 2019-nCoV-PCR Mix, 4µl of 2019-nCoV-PCR Enzyme Mix, and 20 µl RNA extract so the final volume will be 50 µl. Thermal cycling reactions are shown in Table 1. RT-qPCR analysis was run on CFX96 real-time thermal cycler (Bio-Rad, USA). All RT-qPCR reactions also had positive and negative controls. Interpretation of results is shown in Table 2.

Table 1: Thermal cycling reactions of RT-qPCR of SARS-CoV-2 according to Sansure protocol.

	Steps	Temperature.	Time.	Cycle No.
1	Reverse transcription	50°C	30 min.	1
2	cDNA predenaturation	95°C	1 min.	1
3	Denaturation	95°C	15 sec.	45
	Annealing, extension and fluorescence collection	60°C	30 sec.	
4	Device cooling	25°C	10 sec.	1

Table 2: Explanation of detection result according to Sansure protocol.

Conclusion	Amplification results
SARS-CoV-2 Positive	There is typical S-shape amplification curve detected at FAM and/or ROX channel, and the amplification curve which is detected at CY5 (internal control) channel, Ct≤40.
SARS-CoV-2 Negative	There is no typical S-shape amplification curve (No Ct) or Ct＞40 detected at FAM and ROX channel, and the amplification curve which is detected at CY5 channel (internal control), Ct ≤

	40.

RT□LAMP assays performed with/without RNA extraction

RT□LAMP assays were performed on the seven wastewater samples according to Zhang *et al.* [38] with two sets of LAMP primers targeting ORF (ORF1a) and N genes [38] as shown in Table 3. We selected these two sets, because Zhang *et al.* [38] concluded that these two sets were the best performing among five tested sets targeted the ORF (ORF1a) and N genes [38]. The 5′ frameshift nature of polyproteins (ORF1a/ORF1ab) [39] allows us to use primers for ORF1a, ORF1ab genes in RT-LAMP, RT-qPCR respectively. In other way, ORF1ab, the largest gene, contains overlapping open reading frames that encode polyproteins PP1a and PP1ab [40] so ORF1a is part of ORF1ab.

In brief, the assay was performed in a 20 µl reaction mixture containing 2 µL of 10x primer mix of 16 µM (each) of Forward Inner Primer (FIP) and Backward Inner Primer (BIP), 2 µM (each) of F3 and B3 primers, 4 µM (each) of Forward Loop (LF) and Backward Loop (LB) primers, 10 µL of WarmStart Colorimetric Lamp 2X Master Mix (M1800) (New England Biolabs, USA), 5 µL of DNAse, RNAase free water (Invitrogen, USA), and 3 µl of RNA template. The reaction mixture was set at 65 °C for 30 minutes on a pre-heated dry bath. Yellow color indicates positive reaction, where pink indicates negative one. Orange color indicates positive samples with low viral loads having Ct >30 [41]. For confirmation of RT-LAMP, reactions were run on 2% agarose gel electrophoresis (100 V) for 45 min, stained with ethidium bromide and visualized using a UV transilluminator [41]. We also performed RT□LAMP on the 7 samples directly

without RNA extraction to check if RNA extraction is necessary or can be omitted in RT-LAMP of wastewater samples.

Table 3: Sequences of amplicons and LAMP primers [38]

LAMP primer	Sequence
ORF1a	
ORF1a - F3	CTGCACCTCATGGTCATGTT
ORF1a -B3	AGCTCGTCGCCTAAGTCAA
ORF1a -FIP	GAGGGACAAGGACACCAAGTGTATGGTTGAGCTGGTAGCAGA
ORF1a -BIP	CCAGTGGCTTACCGCAAGGTTTTAGATCGGCGCCGTAAC
ORF1a -LF	CCGTACTGAATGCCTTCGAGT
ORF1a -LB	TTCGTAAGAACGGTAATAAAGGAGC
N	
N-F3	TGGCTACTACCGAAGAGCT
N-B3	TGCAGCATTGTTAGCAGGAT
N-FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG
N-BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT
N-LF	GGACTGAGATCTTTCATTTTACCGT
N-LB	ACTGAGGGAGCCTTGAATACA

RT□LAMP assays in microfluidic device

The microchips were designed using CAD software (SolidWorks, Dassault Systemes) and 8 mm long microchannels were micromachined on a polymethyl methacrylate (PMMA) piece (1.2 mm

thickness) with a cross-section of 0.6 x 0.6 mm (width x depth). A sample container was also micromilled using 5 mm thick PMMA sheets to load 10 μL sample into each well. Before use, microcapillaries were coated with polyvinyl alcohol (PVA) to convert their surfaces from hydrophobic to hydrophilic, allowing liquid to rise in the microchannels [42, 43] with modifications. In brief, 2% PVA (molecular weight 146000–186000) was prepared in deionized water (DI) water and used to fill the microchannels at room temperature. After 30 minutes, excess PVA was removed and the microchannels were dried by injection of compressed air. Then, the microchip was simply dipped into the RT-LAMP reaction solution loaded via capillary action without need of pump.

We performed RT-LAMP inside microfluidic device to target ORF (ORF1a), and N genes of the first sample. We used both RNA extract and direct wastewater sample (without RNA extraction). In brief, the assay was performed in a 20 μl reaction mixture containing 2 μL of 10x primer mix of 16 μM (each) of Forward Inner Primer (FIP) and Backward Inner Primer (BIP), 2 μM (each) of F3 and B3 primers, 4 μM (each) of Forward Loop (LF) and Backward Loop (LB) primers, 10 μL of WarmStart Colorimetric Lamp 2X Master Mix (M1800) (New England Biolabs, USA), 5 μL of DNAse, RNAase free water (Invitrogen, USA), and 3 μl of RNA template (in case of using RNA extracts) or 3 μl of wastewater sample directly (in case of not using RNA extracts). The, we placed this reaction mixture in the wells for successful loading into microchannels. Then, microfluidic device was carefully placed in a pre-heated dry bath at 65 °C for 30 minutes.

Results

Analysis of RT-qPCR and RT-LAMP done on RNA extracts

The results of 7 samples tested by RT-LAMP and RT-qPCR are shown in Table 4. Positivity rates of RT-qPCR and RT-LAMP were 100.0% (7/7) and 85.7% (6/7), respectively. RT-qPCR results revealed that all 7 wastewater samples were positive for N gene (Ct range 37-39), and negative for ORF (ORF1ab). All samples had Ct for internal control (CY5 channel) less than 40. Therefore, according to the guidelines of RT-qPCR kit manufacturer company (Sansure), all 7 samples were positive for SARS-CoV-2. This was also the case for RT-LAMP except for sample ID 3, where it was negative for both N and ORF (ORF1a) as confirmed by visualization in 2% agarose gel stained with ethidium bromide.

Of 7 wastewater samples positive for SARS-CoV-2 by RT-qPCR, 6 were positive by RT-LAMP (sensitivity of 85.7%). As shown in Figure 2, results of RT-LAMP targeted N gene of SARS-CoV-2 revealed that 5 samples (sample id 1, 4, 5, 6, and 7) showed color change to orange color. Sample ID 2, and 3 did not show conclusive change in color. Visualization in 2% agarose gel stained with ethidium bromide revealed that 6 samples (sample id 1, 2, 4, 5, 6, and 7) have characteristic LAMP amplicon profiles. It is worthy to mention that sample id 5 has the strongest intensity when it visualized in 2% agarose gel, which also has the lowest N gene Ct value of 35 among all other samples. As shown in Figure 3, results of RT-LAMP targeted ORF (ORF1a gene) of SARS-CoV-2 revealed are not confirmatory by naked eye. We cannot confirm that these are negative (pink) or positive (yellow/orange) by just naked eye. Visualization in 2% agarose gel stained with ethidium bromide showed that RT-LAMP targeted ORF (ORF1a gene) of these 7 samples are negative with no LAMP amplicon profiles (Figure 1).

RT-LAMP without RNA extraction

We performed RT-LAMP for amplification of N and ORF (ORF1a) genes directly on wastewater samples without RNA extraction, and found that all seven samples remains pink

indicating negative results with naked eyes (Figure 4). Visualization in 2% agarose gel stained with ethidium bromide showed that all 7 samples are negative with no LAMP amplicon profiles.

RT□LAMP assays in microfluidic device

As shown in Figure 5, the color remains pink in all microchannels except the one which subjected to RT-LAMP for targeting N region after RNA extraction (yellowish/orange color). Although there may be slight change in color of microchannel that subjected to RT-LAMP for targeting ORF1a gene after RNA extraction, visualization in 2% agarose gel stained with ethidium bromide showed that it is negative with no LAMP amplicon profiles.

Table 4: Comparison of detection accuracy between RT-LAMP and RT-qPCR

Sample ID	RT-qPCR		RT□LAMP	
	ORF (ORF1ab)	N	ORF (ORF1a)	N
1	41	39	-	+
2	N/A	38	-	+
3	42	37	-	-
4	N/A	39	-	+
5	41	35	-	+
6	N/A	38	-	+
7	44	37	-	+

+ Positive reaction; - Negative reaction; N/A not detected

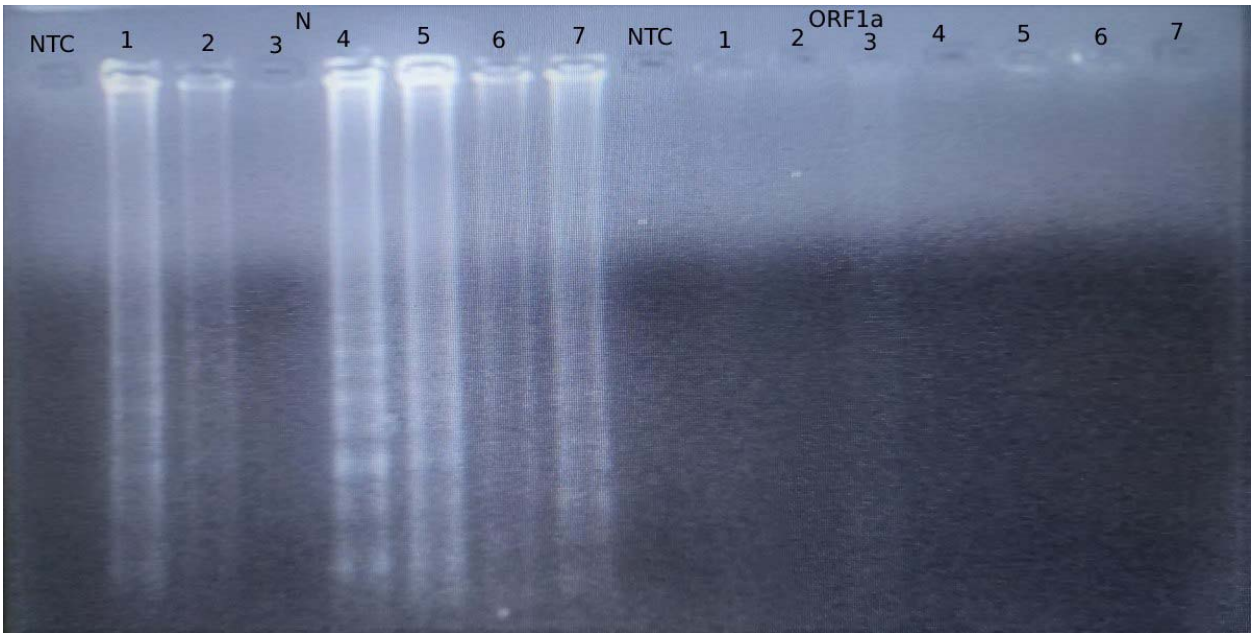

Figure 1: The seven samples were tested for the presence of N gene on the left of the gel, and they were tested again for the presence of ORF1a region on the right of the gel. Amplification visualized in a 2% agarose gel stained with ethidium bromide, showing characteristic LAMP amplicon profiles in positive samples and no amplification in non-template controls (NTC).

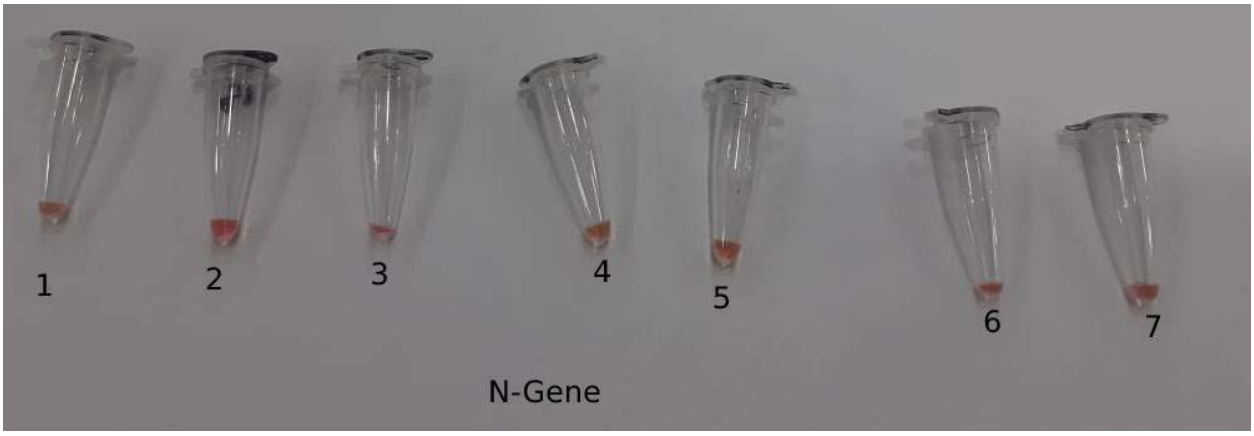

Figure 2: The colorimetric detection of SARS-CoV-2 (N gene) using RT-LAMP of the seven wastewater samples after RNA extraction.

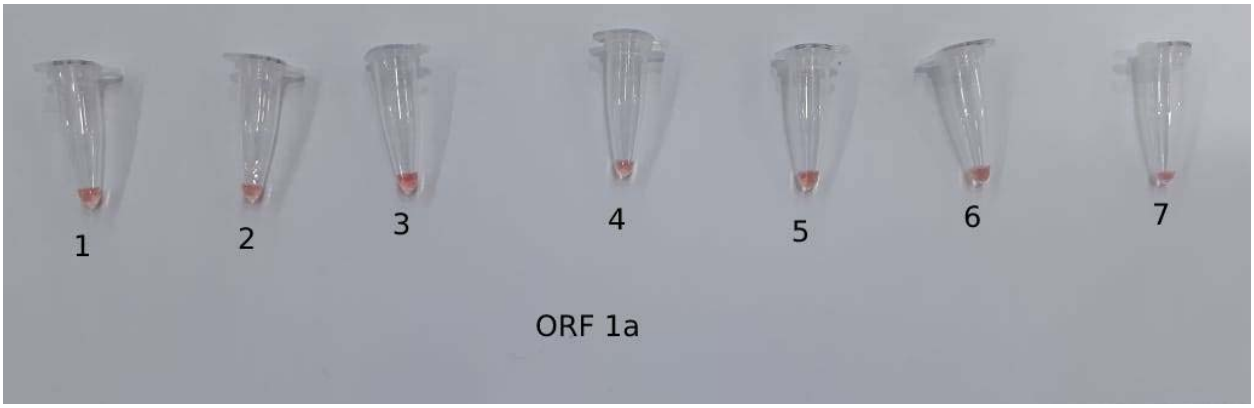

Figure 3: The colorimetric detection of SARS-CoV-2 (ORF1a gene) using RT-LAMP of the seven wastewater samples after RNA extraction.

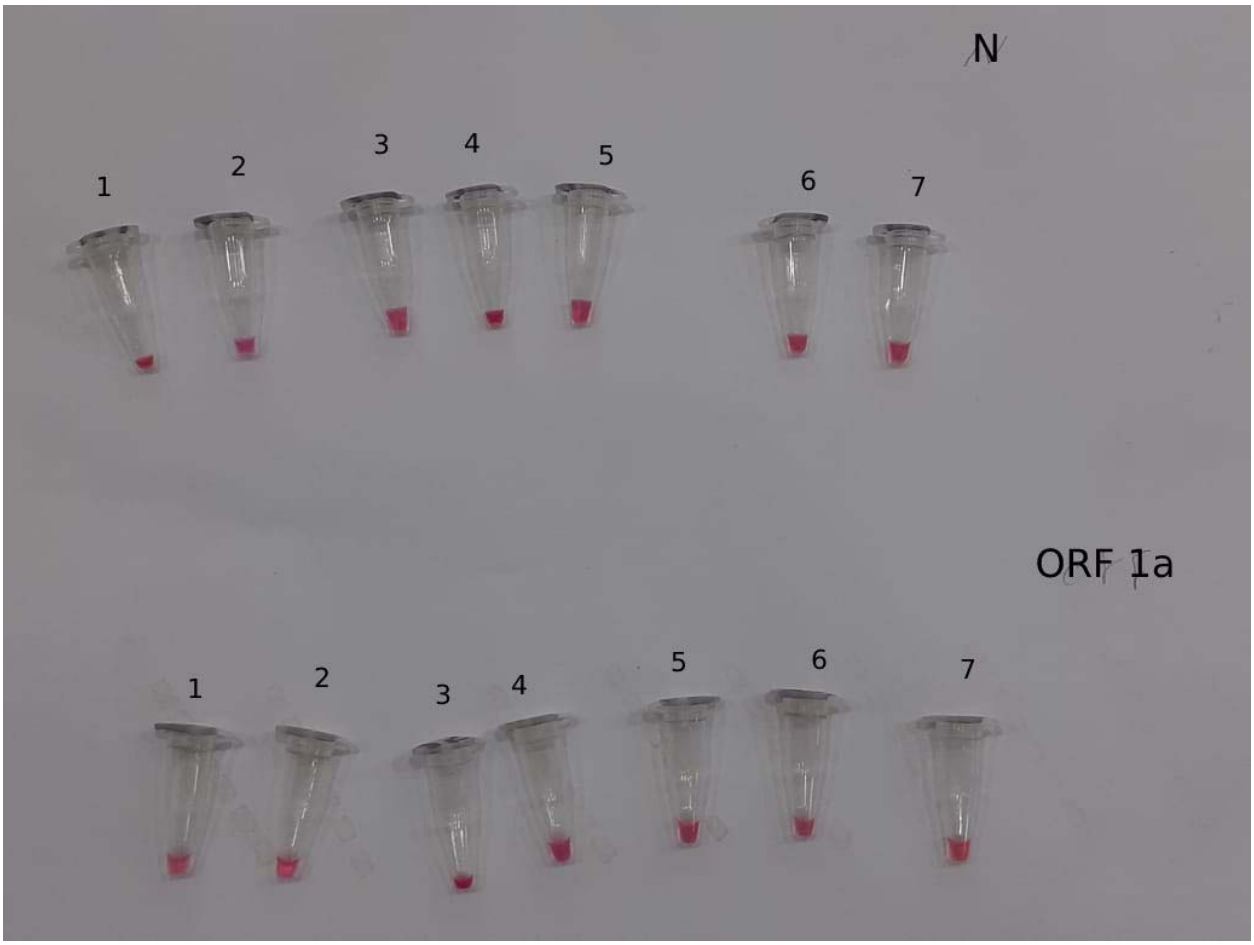

Figure 4: The colorimetric detection of SARS-CoV-2 (N and ORF1a genes) using RT-LAMP of the seven wastewater samples directly without RNA extraction (All seven samples remains pink indicating negative results).

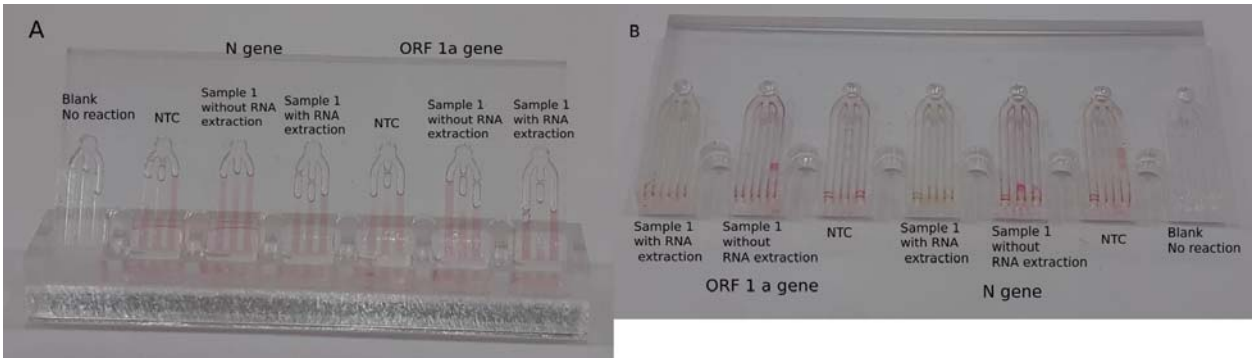

Figure 5: (A) successful loading of RT-LAMP reaction mixture to target N, and ORF1a genes of one sample into microfluidic chip. (B) RT-LAMP reaction results showing change of color into yellowish/orange in samples after RNA extraction in sample 1 targeting N gene**.**

Discussion

RT-LAMP recently emerges as an alternative point-of-care test for detection of SARS-CoV-2, including clinical sample testing, with reaction time varying between 20 and 40 minutes [38, 41, 44]. RT-LAMP has some fundamental advantages such as constant temperature amplification, elimination of a thermal cycler, quick test result, constant temperature amplification, and wide diagnostic capacity, while keeping similar specificity and sensitivity, thus making it more appropriate than the RT-qPCR for monitoring a pandemic such as COVID-19 [45]. To detect SARS-CoV-2 RNA with RT-LAMP, we used the WarmStart Colorimetric LAMP 2X Master Mix (DNA and RNA) from New England Biolabs (USA), which contains two enzymes, an engineered reverse transcriptase (RTx) and a warmStart strand-displacing DNA polymerase (Bst

2.0) in a special low-buffer reaction solution containing a visible pH indicator for easy and rapid detection of LAMP (DNA) and RT-LAMP (RNA) reactions. As a way to avoid nonspecific priming reactions, there are oligonucleotide-based aptamers in the reaction mixture to work as reversible temperature-dependent inhibitors, ensuring that the reaction only starts at high temperature (WarmStart) (https://international.neb.com/products/m1800-warmstart-colorimetric-lamp-2x-master-mix-dna-rna). This aim of this system is to provide a fast, clear visual detection of amplification based on the production of protons, leading to pH drop due to extensive DNA polymerase activity in a LAMP reaction, producing a color change from pink (alkaline) to yellow (red).

Wastewater-based epidemiology is an alternative method to predict virus spread and it considered as an early warning pandemic through detecting pathogens in wastewater [16, 46]. SARS-CoV-2 biomarkers can be detected in the wastewater and/or sewer system, because the SARS-CoV-2 can be isolated from the infected patients' urine and feces [47]. Therefore, wastewater analysis in communities is a potential method to track infected people, and to monitor the epidemiology of the communities [48]. Most of published papers are about using of RT-LAMP for detection of SARS-CoV-2 in patient or clinical samples [14, 41, 49-53], with few of them have focused on detection of SARS-CoV-2 using RT-LAMP in wastewater or sewage samples [54, 55]. We found that results of RT-LAMP for these wastewater samples were largely consistent with those of RT-qPCR, with 6 out of 7 (85.7%) samples were positive for both RT-LAMP and RT-qPCR. Only one sample (sample id 3) tested positive by RT-qPCR was negative by our RT-LAMP. The observed difference in positive predictive value of RT-LAMP and RT-qPCR may be due to more /less RNA input [56]. We also noticed that the final volume of RT-LAMP product of this sample (sample id 3) was very low, which may reflect on the result of RT-

LAMP. This raises concerns about the effect of final volume of RT-LAMP product and interpretation of result. Our RT-LAMP positive samples (40>Ct>35) showed color change to orange color, which is in agreement with previously mentioned [41] that orange color indicates positive samples with low viral loads having Ct >30.

The Nucleocapsid protein can regulate the replication, transcription and packaging, and it is important for viral viability. There is a growing interest in studying the N protein for vaccine development because of its highly immunogenic and its highly conserved amino acid sequence [57]. Currently, the detection of SARS-CoV-2 RNA is mainly performed by RT-qPCR detection of two target genes, including ORF1ab and N [56]. We found that the Ct values of N gene for all of our 7 wastewater samples were lower than the Ct values of ORF1ab gene, suggesting that N gene is the most important gene when monitoring SARS-CoV-2. Our results were similar to what previously reported [58], where the highest proportion of positive results among COVID-19 was the N gene, followed by both ORF1ab and N. They found that the main positive fragment is the N gene, and the proportion of those positive for single ORF1ab was very low [58]. Therefore, we recommended monitoring of the N gene as surrogate marker for detection in clinical and wastewater samples, reducing the time and cost of nucleic acid detection. Amplified products of RT-LAMP targeting N gene of 6 samples visualized in agarose gel showed characteristic LAMP profile, whereas there was negative for ORF1a gene. This again confirms that N gene is the most important gene when monitoring SARS-CoV-2, especially with samples with weakly positive results (Ct>30). Loying and colleagues [59] studied the dynamics of ORF1ab and N genes among hospitalized COVID-19 positive patients, and they found that the persistent of positivity of N gene is significantly for more duration than ORF1ab, indicating that N gene requires longer duration of days to become negative than ORF1ab. This also underscores

our proposition that N gene should be considered as a surrogate marker for detection in clinical and wastewater samples.

One of the most important advantage of RT-LAMP is its ability to detect SARS-CoV-2 directly from clinical samples without the need of RNA extraction [49, 60]. Wei and colleagues [60] developed and tested a highly sensitive and robust method based on RT-LAMP that uses readily available reagents and a simple heat block using contrived spike-in and actual clinical samples. They directly tested clinical nasopharyngeal swab samples in viral transport media without previous time-consuming and laborious RNA extraction with results in just 30 min. Mautner and colleagues [49] developed RT-LAMP assay to directly detect SARS-CoV-2 from pharyngeal swab samples without previous RNA extraction. They found that this method is 10 times cheaper and 12 times faster than RT-qPCR, depending on the assay used. Previous study performed RT-LAMP on wastewater samples after RNA extraction and virus concentration [54]. According to CDC guidelines for wastewater surveillance testing methods (https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/testing-methods.html), small volumes of wastewater (e.g., 1 ml) may be tested without additional concentration processes if levels of SARS-CoV-2 RNA are sufficiently high in wastewater. Therefore, we targeted hotspots in Islamabad with recorded high COVID-19 cases, and took 7 wastewater samples to see the possibility to detect SARS-CoV-2 in wastewater without RNA extraction and virus concentration using direct RT-LAMP alone. Although RNA extraction may be omitted in RT-LAMP performed on clinical samples [49, 60], we found that RNA extraction is necessary in RT-LAMP performed on wastewater samples. However, Ongerth and Danielson detected SARS-CoV-2 in raw sewage samples with no preliminary sample processing for virus concentration and RNA extraction [61].

Microfluidic techniques are emerging as disposable and cost-efficient tools for rapid diagnosis of viral infection [31]. Since microfluidic devices are sensitive, cheaper, faster, and easy to use methods, they have a high potential to be an alternative way for the viral RNA detection [32]. Safavieh *et al.* developed RT LAMP cellulose based paper microchips and amplified the target RNA using the RT LAMP technique and detected the HIV 1 virus via the electrical sensing of LAMP amplicons [33]. Fraisse *et al*. designed RT PCR integrated microfluidic device to detect Hepatitis A and noroviruses in the gut [34]. Song *et al.* developed RT LAMP integrated microfluidic for detection of Zika virus [36]. Recently, Kim *et al.* designed RT PCR integrated microfluidic device for detecting of H1N1 influenza in saliva [35].

For the first time, we used RT-LAMP in microfluidic chip for detection of SARS-CoV-2 in wastewater. After coating of microfluidic chip with PVA, RT-LAMP mixture can be successfully uploaded into microchannels. Then, we observed color change in microfluidic chip after placing it in pre-heated dry bath at 65 °C for 30 minutes. Although we found that detection of SARS-CoV-2 in wastewater using RT-LAMP in microfluidic chip requires RNA extraction, we propose that our workflow (without RNA extraction) could work with clinical samples since there are many reports about successful detection of SARS-CoV-2 in clinical samples using RT-LAMP without RNA extraction [49, 60, 62, 63]. If RNA extraction could be achieved in this microfluidic chip, this could greatly improve the results of this chip. Mauk and colleagues developed simple plastic microfluidic chip for nucleic acid-based testing of blood, other clinical specimens, water, food, and environmental samples [64]. They combines isolation of nucleic acid by solid-phase extraction; isothermal enzymatic amplification such as LAMP, nucleic acid sequence based amplification, and recombinase polymerase amplification; and real-time optical detection of DNA or RNA analytes. Although we could not detect clear color change in sample

not subjected to RNA extraction, we propose that performing RNA extraction inside microchannels could greatly improve results.

Our work is expected to pave the road for designing readymade microfluidic chip with specific chamber for RNA extraction then another amplification chamber for RT-LAMP. This amplification product could be also labeled with a fluorophore reporter that could be excited with a LED light source and monitored in situ in real time with a photodiode or a CCD detector (such as available in a smartphone).

Conclusion

RT-LAMP has been emerging as a great alternative to the RT-qPCR because RT-LAMP is a specific, sensitive, fast, cheap, and easy to use method. We successfully detected SARS-CoV-2 through color change (orange color) in our positive wastewater samples having Ct >30. We also found that the Ct values of N gene for all of our wastewater samples were lower than the Ct values of ORF1ab gene, suggesting that N gene could be used as a surrogate marker for monitoring and surveillance of environmental circulating SARS-CoV-2. This is also confirmed with RT-LAMP, where we detected characteristic RT-LAMP amplicon profile only for N gene. To our knowledge, we, for the first time, successfully detected SARS-CoV-2 from wastewater samples using RT-LAMP in microfluidic chips. This will provide an opportunity for developing more robust and economical approach for using this microchip device along with RT-LAMP as an advanced point of care for detection of SARS-CoV-2 in different samples.

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Conflict of interest

The authors report no conflicts of interest in this work.

Availability of data and material (data transparency)

Data available within the article or its supplementary materials

Code availability (software application or custom code)

Not applicable

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Author contributions

AD and HB conceived and designed the study; AD coordinated, carried out the experiments, drafted the original manuscript, and analyzed the data. AD, MFS, SH, AA, AJ, MN, TY, and HB did necessary editing of the manuscript. All authors read and approved the manuscript.

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