



Malachite Green-Based Detection of SARS-CoV-2 by One-Step Reverse Transcription Loop-Mediated Isothermal Amplification

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Abstract

The pandemic of severe acute respiratory syndrome 2 (SARS-CoV-2) revealed the necessity of diagnosis of the infected people to prevent the prevalence infection cycle. Many commercial pathogen diagnosis methods are based on the detection of genomic materials. Isothermal amplification methods such as loop-mediated-isothermal amplification (LAMP) are the method of choice in these cases. Reverse transcription steps are efficiently coupled to LAMP for the detection of pathogens with genomic RNAs such as SARS-CoV-2. Many detection systems for LAMP include fluorescent readout systems. Although such systems result in desirable limits of detection, the need for special instrumentation is the main dispute of such systems to become real point of care assays. In contrast, colorimetric detection methods would reduce costs and improve the applicability of the system. In this study one-step reverse transcription-LAMP reaction was established that enables visual detection of the SARS-CoV-2 genome. Nasopharyngeal RNA samples were first validated by reverse transcription quantitative polymerase chain reaction and then subjected to RT-LAMP. To lower the cost associated with the readout system equipment, malachite green (MG) was used. The color change of MG to blue allowed visual detection of the virus. Firstly, experiments were set up as two-step RT-LAMP reaction to identify the best primer sets. In addition, MG concentration was optimized with the significant colorimetric signal for the positive samples. Next, a one-step colorimetric method was developed for the detection of SARS-CoV-2 based on MG color shift in 2 h.

Keywords RT-LAMP · SARS-CoV-2 · Malachite green · Isothermal amplification

1 Introduction

Severe acute respiratory syndrome 2 (SARS-CoV-2) is the viral agent that causes coronavirus disease 2019 (COVID-19). In recent years, the importance of rapid detection of genetic materials of pathogens and finding therapeutic approaches against viruses got more pronounced (Rosenthal 2020; Vandenberg et al. 2021; Zaman et al. 2020; Dastjerdi-Khorzoghi et al. 2019). In the fight against viral pathogens, many techniques have been developed for the

detection of the genomic materials of the pathogens to overcome the uncontrolled infections. In this respect, commercial tests for COVID-19 diagnosis comprise three main categories: molecular detection of genetic materials, antigen tests, and serological methods (Machado et al. 2021) from which the former is considered the gold standard method by the World Health Organization (WHO) for detection of SARS-CoV-2. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) is the most applied method; however, this conventional method has limitations such as the long time to results (TTR), up to six hours, as well as its dependence on high-tech instrumentations, e.g., fluorescently equipped thermal cyclers. The development of methods that provide detection of the genetic materials of the virus in less equipped laboratories is necessary for the rapid isolation of asymptomatic and symptomatic patients, especially in low-budget countries. The method would be more beneficial if it could reduce TTR (Song et al. 2021; Afzal 2020).

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Isothermal amplification techniques are the method of choice to provide molecular diagnostic tests for the detection of genetic materials that accommodate low TTR with the least instrumentation. These methods have been validated as a point of care (POC) test as well (Bodulev and Sakharov 2020). Methods such as loop-mediated isothermal amplification (LAMP) (Nzulu et al. 2019), helicase-dependent amplification (HDA) (Ma et al. 2017), rolling circle amplification (RCA) (Shen et al. 2019), strand displacement amplification (SDA) (Becker et al. 2019), recombinase polymerase amplification (RPA) (Rostron et al. 2019), and nucleic acid sequence-based amplification (NASBA) (Zhai et al. 2019), unified amplifier-based primer exchange reaction (UniAmPER) (Tavakoli-Koopaei et al. 2022) are examples of isothermal amplification methods that have been developed in recent years for the detection of genetic materials of viruses. Among these methods, LAMP was able to achieve governmental approvals as IVD (Zhao et al. 2016; Zhang et al. 2015; Etchebarne et al. 2017).

LAMP starts with four primers (forward primer, backward primer, forward internal primer, and backward internal primer). After the first round of synthesis, loop primers are attached to the newly synthesized products and continue a highly specific and effective amplification (Nagamine et al. 2002). The reaction products are long double-stranded DNAs.

The LAMP reaction products are produced with high efficiency and can provide colorimetric signals for visual detection with bare eyes. Visual detection methods are the

method of choice in many detection systems (Sadeghi et al. 2017; Javani et al. 2017; Anantharaj et al. 2020). The signal could be generated with either pH indicators or magnesium pyrophosphate indicators (Scott et al. 2019; Gachugia et al. 2020; Goto et al. 2009). Phenol red is a pH indicator and malachite green (MG) and hydroxyl naphthol blue (HNB) are magnesium pyrophosphate indicators (Lucchi et al. 2016; Kudyba et al. 2019; Choopara et al. 2017). Real-time monitoring of the reactions is another beneficial aspect that has been addressed in many studies (Dehghanian et al. 2020; Higuchi et al. 1993). Real-time detection of the LAMP reaction is possible through the interaction of the double-stranded long products of LAMP with intercalating dyes (Aglietti et al. 2019; Ristaino et al. 2020).

A clear advantage of the method is that it may get coupled with reverse transcription reactions to target RNA genomes as reverse transcription loop-mediated isothermal amplification (RT-LAMP) in one step (da Silva et al. 2019). RT-LAMP is one of the useful methods for rapid diagnosis of SARS-CoV-2 (Alekseenko et al. 2021; Fowler et al. 2021; Lim et al. 2021).

Primer design is a crucial step in LAMP-based assays. Software such as Primer Explorer (Park et al. 2020; Zhang et al. 2020) and LAMP designer (da Silva Gonçalves et al. 2014; Abdulmawjood et al. 2016; Elvira-González et al. 2017) has been developed to design LAMP primer sets for candidate genomic regions (Jia et al. 2019; Shirshikov et al. 2019). Such software usually results in tens of primer sets that their sensitivity and specificity need to be investigated.

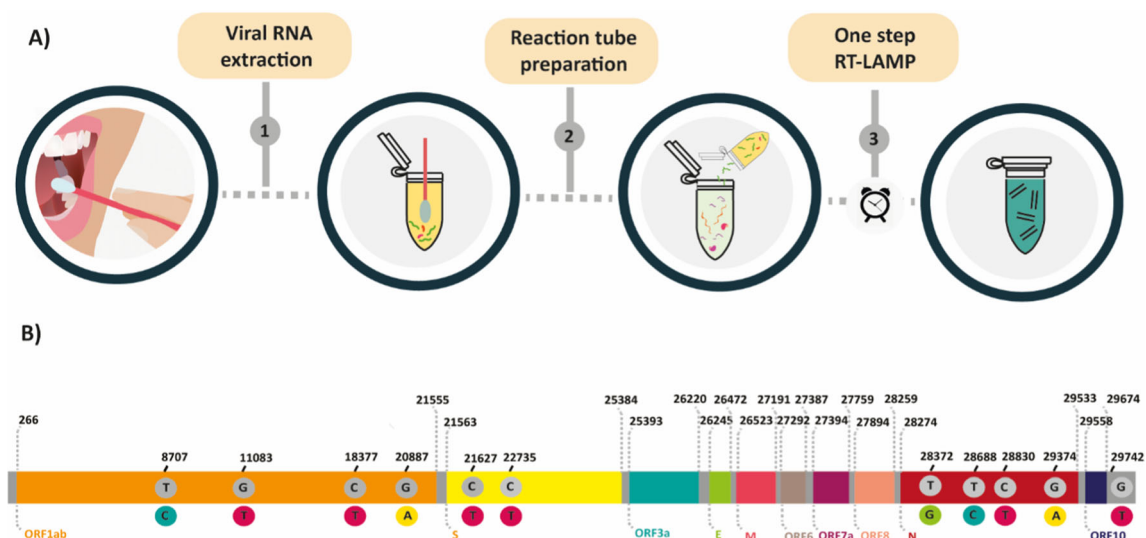


Fig. 1 **A** The steps of reverse transcription loop-mediated isothermal amplification (RT-LAMP). The sample was collected by nasopharyngeal swab at the first step after the RNA was extracted and applied as a template for RT-LAMP reaction in presence of malachite green. The colorimetric RT-LAMP product signals were visible with the naked eye after incubation at 37 °C for 2 h. **B** Hot spots region of

SARS-CoV-2 genomes that were sequenced from samples of Iranian patients. The gray circles specify the parent nucleotide at the reference genome (MN908947). The colored circle is the mutated nucleotide. The numbers above each circle indicate the position of the mutation at the reference genome

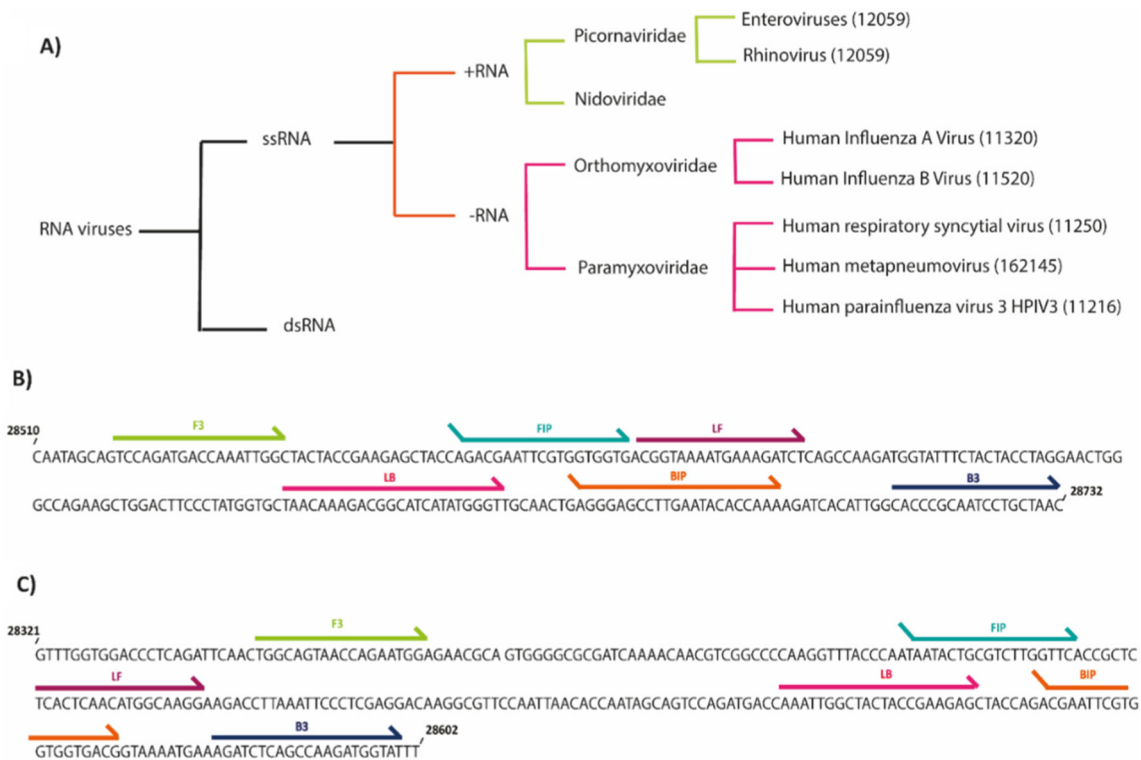


Fig. 2 Details for specific binding sites and sequences of primers. **A** Organisms that were analyzed for cross-reactivity check with primer blast. Taxids are mentioned in parentheses. **B** and **C** The

primers binding sites of Primer Explorer and LAMP designer on the N gene of SARS-CoV-2, respectively

The objective of the study was to develop a validated RT-LAMP primer set that enables accurate and fast visual detection of the SARS-CoV-2 by malachite green. In the first step, the RT-LAMP primer sets were designed for conserved regions of the SARS-CoV-2 genome using both Primer Explorer and LAMP designer software. The top scored designed primer sets of both software were experimentally validated with RT-LAMP assay, and then, the best primer set was chosen for the development of the malachite green-based one-step SARS-CoV-2 diagnostic test (Fig. 1A).

2 Experimental Details

2.1 Materials

All primers were supplied by Tag Copenhagen. Co. Ltd. (Denmark). RNA isolation kit and one-step RT-qPCR kit were supplied by Behgene, Biotech, Iran, and Sansure Biotech, China, respectively. AddScript cDNA Synthesis Kit was from AddBio, Korea. Bst DNA polymerase large fragment (M0275L) was purchased from New England Biolabs, USA. Mineral oil was from Sinaclone, Iran. Malachite green, agarose, tris (hydroxymethyl)-

aminomethane (Tris), boric acid, ethylenediaminetetraacetic acid (EDTA), ethidium bromide solution, and deoxyribonucleotides were purchased from Sigma-Aldrich. The reaction tubes were incubated in LightCycler[®] 96 instrument (Roche, Germany).

2.2 Clinical Samples and Materials

This study included samples of hospitalized patients with a respiratory infection or suspected patients who were in contact with affected individuals or indicated some clinical signs of COVID-19 and routinely referred to healthcare centers affiliated with Isfahan University of Medical Sciences, Isfahan, Iran. The nasopharyngeal/oropharyngeal swab samples were collected and kept in collection tubes containing 2-ml virus transport medium, delivered to the laboratory, worked with biosafety level 2, and inactivated by mixing to lysis buffer and heating at 65 °C.

2.3 RNA Extraction and RT-qPCR Validation Tests

The genomic RNAs were extracted by RNA isolation kit. A 10- μ l aliquot of extracted SARS-CoV-2 RNA was used for RT-qPCR tests by a specific probe-based one-step RT-qPCR kit, targeting ORF1ab, and N genes according to the

Table 1 Designed primer sets with both primer explorer and LAMP designer software for N genes of SARS-CoV-2

Primer explorer best result for the conserved region of N gene			
Primer	Sequence	Length (nt)	T _m (°C)
F3	TCCAGATGACCAAATTGGCT	20	59.0
B3	GTTAGCAGGATTGCGGGTG	19	60.4
FIP	TCTGGCCCAGTTCCTAGGTAGTGACGAATTCGTGGTGGTGA	41	65.0
BIP	AGCTGGACTTCCCTATGGTGCTTTTGGTGTATTACAGGGCTCC	42	65.0
LF	TGGACTGAGATCTTTCATTTTACCG	25	60.8
LB	AACAAAGACGGCATCATATGGG	22	60.7
LAMP designer best result for conserving region of N gene			
Primer	Sequence	Length (nt)	T _m
F3	GGCAGTAACCAGAATGGAG	19	59.9
B3	AATACCATCTTGGACTGAGATC	22	59.4
FIP	TTGGAACGCCTTGCTCTCGATACTGCGTCTTGGTTTAC	38	62.0
BIP	ACCAATAGCAGTCCAGATGACCCGTCACCACCACGAATTC	40	64.0
LF	TTCCTTGCCATGTTGAGTGA	20	62.0
LB	AATTGGCTACTACCGAAGAGC	21	62.2

manufacturer's protocol. The amplification process was carried out in 25 µl. According to the manufacturer's protocol, the reverse transcription step was done with incubation for 30 min at 50 °C. Cyclic amplification of synthesized cDNA was carried out as follows: 1 min 95 °C for primary denaturation, followed by 45 cycles at 95 °C (15 s) and 60 °C (30 s). The calculated SARS-CoV-2 cycle threshold (Ct) values were analyzed and applied for quantification of the input RNA sample in the RT-LAMP reaction. Purified RNA samples with the matching number of Ct values were maintained at - 80 °C for further analysis.

2.4 LAMP Primer Design

To define conserved regions of the SARS-CoV-2 genome, Iranian partial and complete genomes which were deposited in both the GISAID and NCBI databases were aligned and compared with the reference genome (MN908947) by Clustal Omega software. The hot spots of nucleotides in Iranian genomes are shown in Fig. 1B. Primer Explorer V5 and LAMP designer were applied to design primers for non-mutated regions of gene N of SARS-CoV-2. The top score primer sets of each software were subjected to cross-reactivity analysis by the NCBI Primer Blast tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Primers were blasted with similar organism genomes as mentioned in Fig. 2A. Primer details are listed in Table 1 and Fig. 2B and C.

2.5 Two-Step RT-LAMP

A reverse transcription reaction was performed with a reverse transcription kit containing 6 µl of extracted RNA, 10 µl of 2X reaction buffer, 1 µM B3 primer, 0.2 mM dNTPs. The reaction volume was 20 µl. LAMP reaction was carried out with a final volume of 25 µl reaction mixture containing 0.2 µM B3 and F3, 1.6 µM of BIP and FIP, 0.8 µM of LF and LB, 1X isothermal amplification buffer [20 mM of Tris-HCl (pH 8.8), 10 mM of KCl, 10 mM of (NH₄)₂SO₄, 2 mM of MgSO₄, 0.1% of Triton-X100], 1.2 mM of dNTP mix, supplementary 8 mM of MgSO₄, 8 U of Bst DNA polymerase large fragment and 2 µl of cDNA as template. Finally, 25 µl of mineral oil was added on top. The LAMP reactions were carried out at 65 °C for 60 min, and the products were subjected to agarose gel electrophoresis and post-staining with ethidium bromide (0.5 µg/ml) to validate the LAMP reaction. For the colorimetric RT-LAMP assay, the reaction tubes were prepared with the addition of malachite green prior to the reaction.

2.6 One-Step, MG-Assisted RT-LAMP

One-step LAMP reaction was carried out in 25 µl in presence of 10 µl of 2X AddScript reaction buffer, 0.2 µM B3 and F3 each, 1.6 µM BIP and FIP each, 0.8 µM LF and LB each, 1X isothermal amplification buffer, 1.2 mM the dNTPs mix, supplementary 8 mM MgSO₄, 8 U Bst DNA polymerase large fragment, 6 µL extracted RNA, and 0.02% malachite green, except mentioned otherwise. Finally, 25 µl mineral oil was added on top of the reaction. Tubes were incubated in a thermal cycler with two-step

program: 50 °C for reverse transcription reaction within the first 1 h and 65 °C LAMP amplification within the second 1 h. To confirm the result 5 µl of the reactions was loaded on 2% agarose gel and stained by ethidium bromide.

3 Results

3.1 RT-qPCR for Quantification of RNA Samples

RNA-extracted clinical samples were analyzed by RT-qPCR. Figure 3 depicts the Ct values for the same samples that were applied for RT-LAMP reactions. From this list, two samples were proven to be negative in SARS-CoV-2 RNA and the rest had Ct values from 10 to 33 according to RT-qPCR tests.

3.2 Validation of RT-LAMP Primers Set

Initially, the efficiency and accuracy of the two primer sets were investigated in two-step RT-LAMP reactions with samples 1 and 2, with respected Ct values of 18 and 19. Since the number of primers for amplification via LAMP is higher than other isothermal methods, there are higher chances for false-positive results upon primer dimer extensions and self-amplification (Meagher et al. 2018); hence, control reactions that contained all 6 primers and reaction components except the template were necessarily applied here to indicate if false positives are present (Fig. 4A). Both primer sets were efficient in the detection of SARS-CoV-2 genomic RNA from positive clinical

samples. Notably, the primer set which was designed by the Primer Explorer did not show any extension in the no-template control. However, the primer set that was designed by LAMP designer software showed extended bands, apparently due to the presence of primer dimers. Therefore, the first primer set was selected for further experiments in this study.

LAMP primer sets must not show any cross-reactivity with the human genome and transcriptome. Usually, nasopharyngeal samples contain human cells as well as viral particles. Therefore, it is necessary to make sure that the primer set does not bind any RNA from the human transcriptome. To this endeavor, two-step RT-LAMP reactions were performed in presence of negative controls that contained RNA templates, but the RNA samples were from individuals that were negative in SARS-CoV-2 according to RT-qPCR, i.e., samples 3 and 4. Figure 4B shows the results of the RT-LAMP reaction for RNA-negative samples 3 and 4 and RNA-positive samples 5 and 6.

3.3 Two-Step Colorimetric RT-LAMP

RT-LAMP products may be read upon fluorometric or colorimetric signals. Fluorometric signals are usually generated by SYBR green (Naji et al. 2019) or CYTO9 (Tangkanchanapas et al. 2018) which require equipped instruments for detection. On the other hand, colorimetric RT-LAMP products are detectable by the naked eye. As an example, some commercial colorimetric RT-LAMP kits are based on the pH change during RT-LAMP reaction with pH indicators such as phenol red.

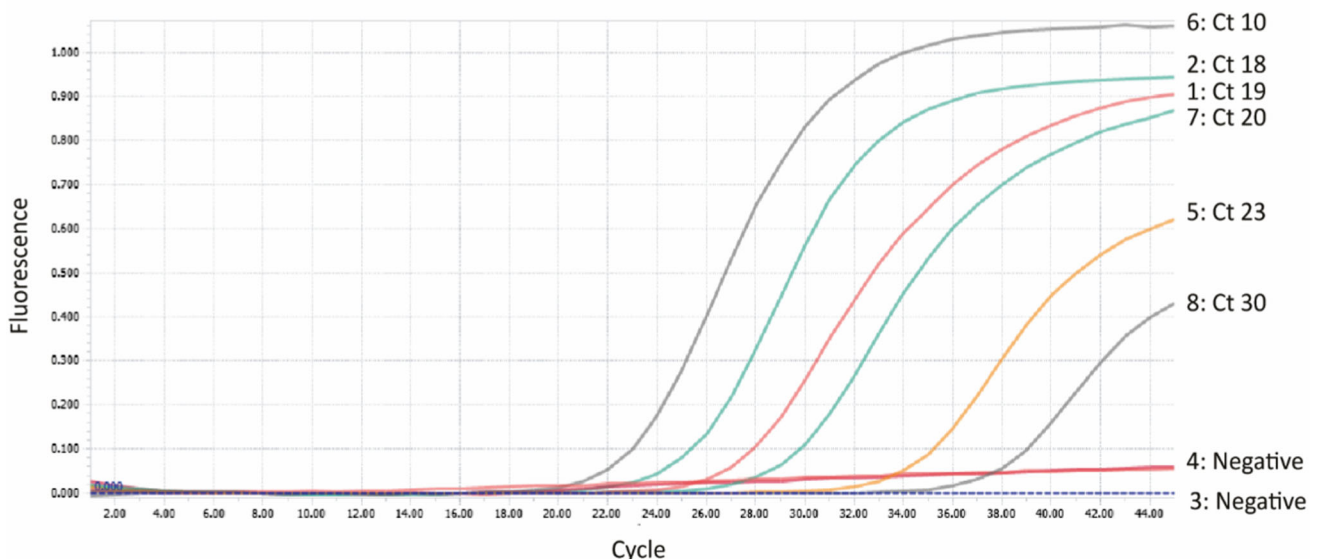


Fig. 3 Quantification of extracted RNA samples based on one-step RT-qPCR. The corresponding Ct values of each sample are depicted on the side

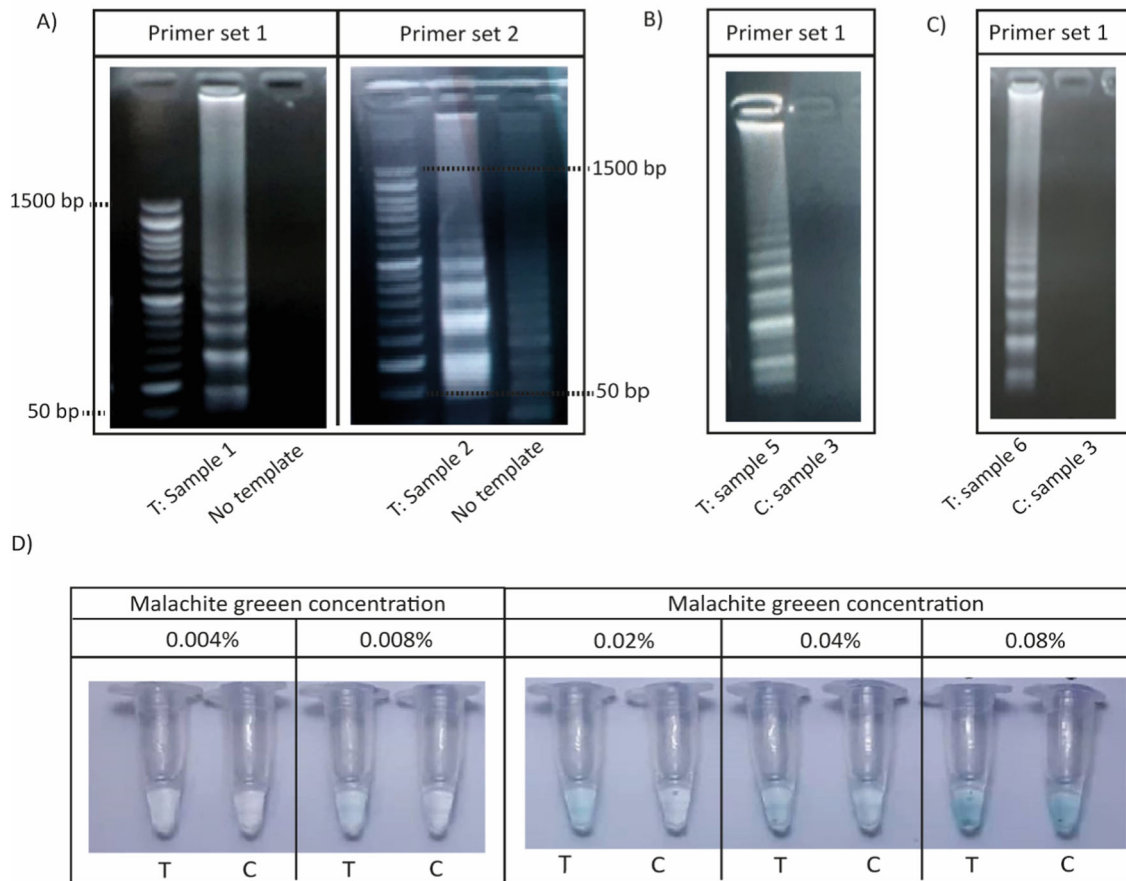


Fig. 4 **A** LAMP primer sets validation and RT-LAMP reaction in presence of a different concentration of malachite green. **A**: Two-step RT-LAMP reactions were performed with no-template controls to investigate of primer dimer formation. Primer set 1 which was designed by Primer Explorer software was shown any primer dimer formation neither in the presence nor absent of a template. The top score primer sets of the LAMP designer (primer set 2) were shown primer dimer formation, and it was not suitable for further RT-LAMP

reaction. **B** Primer set 1 was subjected to RT-LAMP reaction in presence of negative control which contains human's RNA. **C** RT-LAMP reaction in presence of 0.02% MG (the optimized concentration) with control reaction was loaded into 2% agarose gel. **D** The two-step RT-LAMP reaction was performed with 0.004, 0.008% MG as mentioned in the previous study. The RT-LAMP assay with MG concentrations of 0.02%, 0.4%, and 0.08% was also done to find an optimized MG concentration

Hydroxy naphthol blue (Goto et al. 2009) and malachite green (Nzulu et al. 2016) are other reagents that produce colorimetric signals in RT-LAMP assays. Both reagents are the indicator of magnesium pyrophosphate that is synthesized during the polymerization reaction as a by-product. Here, malachite green-based two-step RT-LAMP (MG-RT-LAMP) assays were performed with positive clinical samples in the presence of 0.004% MG (Lucchi et al. 2016). In addition, the concentration of MG was varied up to 20 fold. Reaction tubes are shown in Fig. 4C. MG in concentrations 0.004% and 0.008 could not pose any obvious difference between positive and negative controls. Here the positive control was sample number 6, and the negative control was sample 3. The color generated in the control tube was detectable from the test when the MG concentration raised above 0.02%. However, the background was also increased, while the concentration of MG went higher. The color produced in the test and control

tubes was the same when the malachite green concentration reached 0.04% and above. Increasing the MG concentration enhanced the background signal and prevented the test and control tubes to be distinguished.

Therefore, the best colorimetric signal was produced with 0.02% of the MG concentration. The samples were loaded into an agarose gel to validate the presence of the RT-LAMP reaction products. The result showed that the reaction was efficient in the presence of the optimized concentration of malachite green (Fig. 4D).

3.4 One-Step Colorimetric RT-LAMP

Finally, the reactions were performed with different RNA concentrations as quantified with RT-qPCR. Three tubes of MG-RT-LAMP assays were analyzed with RNA samples with Ct values of 20 (sample 7) and 33 (sample 8) and also a negative RNA (sample 4). The reaction tubes were

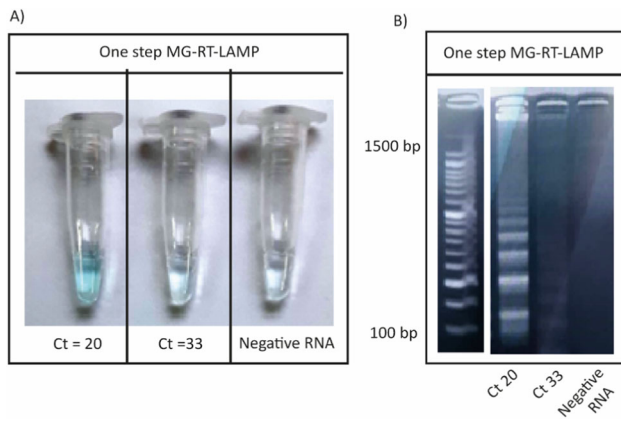


Fig. 5 One-step MG-RT-LAMP reactions with different RNA concentrations. **A:** RT-LAMP reaction was performed in presence of 0.02% malachite green. The test tubes contained extracted RNA with corresponding Ct 20 and 33, respectively. Negative virus samples were also present as the control. **B:** The same samples of A were loaded at 2% agarose gel as shown above

incubated at 65 °C for 2 h and loaded into agarose gel (Fig. 5). The colorimetric signal of RNA samples with Ct value 20 significantly was above the signal that has been generated in negative RNA samples. However, the RNA samples with Ct value 33 did not show any significant difference to the negative RNA samples. Further experiments validated that SARS-CoV-2 RNA genomes with Ct values below 33 could be detected using one-step MG-RT-LAMP assay with the primer set that was designed by Primer Explorer software, but the system had limitations for detection of lower concentrations of genomic RNAs.

4 Discussion and Future Prospect

Rapid diagnosis of SARS-CoV-2 is necessary to prevent the transmission cycle of the disease among people. In response to this urgency, fast and non-expensive methods are required that support detection of the people with various financial and work situations. Isothermal amplification strategies are hence the method of choice in this respect, and many of them have been developed for rapid detection of COVID-19 during pandemic (Huang et al. 2020; Dao Thi et al. 2020; Aoki et al. 2021). LAMP was the most attractive method among other isothermal amplification methods since it applies non-modified primers and allows detection by simple readout systems (Amaral et al. 2021; Alhamid et al. 2022). However, in general, isothermal amplification methods, including LAMP, are not as sensitive as the gold standard detection system (i.e., RT-qPCR) for SARS-CoV-2, which means the limit of detection of the system (LOD) is higher than that of RT-qPCR. Such limitation still allows diagnosis of people

with high viral load, which is sufficient to block the infection cycle, since the virus is mostly transmitted by patients with high viral load (Wyllie et al. 2020; Gupta et al. 2020; Wölfel et al. 2020). With an acknowledge to being fast and non-expensive, RT-LAMP would be beneficial for application in low-income countries to avoid the complications of health challenges. RT-LAMP has a simple protocol, and its reaction is performed at an instant temperature. Optimization of the colorimetric RT-LAMP method was investigated in this study by using malachite green, during the amplification process. The results of this study are applicable to further development of visual RT-LAMP systems and being prepared for future pandemics.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

- Abdulmawjood A, Wickhorst J, Hashim O, Sammra O, Hassan AA, Alsahen M, Lämmle C, Prenger-Berninghoff E, Klein G (2016) Application of a loop-mediated isothermal amplification (LAMP) assay for molecular identification of *Trueperella pyogenes* isolated from various origins. *Mol Cell Probes* 30:205–210. <https://doi.org/10.1016/j.mcp.2016.05.003>
- Afzal A (2020) Molecular diagnostic technologies for COVID-19: limitations and challenges. *J Adv Res* 26:149–159. <https://doi.org/10.1016/j.jare.2020.08.002>
- Aglietti C, Luchi N, Pepori AL, Bartolini P, Pecori F, Raio A, Capretti P, Santini A (2019) Real-time loop-mediated isothermal amplification: an early-warning tool for quarantine plant pathogen detection. *AMB Express* 9:1–14. <https://doi.org/10.1186/s13568-019-0774-9>
- Alekseenko A, Barrett D, Pareja-Sanchez Y, Howard RJ, Strandback E, Ampah-Korsah H, Rovšnik U, Zuniga-Veliz S, Klenov A, Malloo J (2021) Direct detection of SARS-CoV-2 using non-commercial RT-LAMP reagents on heat-inactivated samples. *Sci Rep* 11:1–10. <https://doi.org/10.1038/s41598-020-80352-8>
- Amaral C, Antunes W, Moe E, Duarte AG, Lima LMP, Santos C, Gomes IL, Afonso GS, Vieira R, Helena Sofia Teles S (2021) A molecular test based on RT-LAMP for rapid, sensitive and inexpensive colorimetric detection of SARS-CoV-2 in clinical samples. *Sci Rep* 11:1–12. <https://doi.org/10.1038/s41598-021-95799-6>
- Anantharaj A, Das SJ, Sharanabasava P, Lodha R, Kabra SK, Sharma TK, Medigeshi GR (2020) Visual detection of SARS-CoV-2 RNA by conventional PCR-induced generation of DNazyme Sensor. *Front Mol Biosci*. <https://doi.org/10.3389/fmolb.2020.586254>
- Aoki MN, de Oliveira B, Coelho LG, Góes B, Minoprio P, Durigon EL, Morello LG, Marchini FK, do RiedigerCarmo Debur INM, Nakaya HI (2021) Colorimetric RT-LAMP SARS-CoV-2

- diagnostic sensitivity relies on color interpretation and viral load. *Sci Rep* 11:1–10. <https://doi.org/10.1038/s41598-021-88506-y>
- Becker WR, Ober-Reynolds B, Jouravleva K, Jolly SM, Zamore PD, Greenleaf WJ (2019) High-throughput analysis reveals rules for target RNA binding and cleavage by AGO2. *Mol Cell* 75(741–55):e11. <https://doi.org/10.1016/j.molcel.2019.06.012>
- Bodulev OL, Yu I, Sakharov (2020) Isothermal nucleic acid amplification techniques and their use in bioanalysis. *Biochem Mosc* 85:147–166. <https://doi.org/10.1134/s0006297920020030>
- Choopara I, Arunrut N, Kiatpathomchai W, Dean D, Somboonna N (2017) Rapid and visual chlamydia trachomatis detection using loop-mediated isothermal amplification and hydroxynaphthol blue. *Lett Appl Microbiol* 64:51–56. <https://doi.org/10.1111/lam.12675>
- da Silva S, Ribeiro J, Paiva MHS, Guedes DRD, Krokovsky L, Lopes F, de Melo M, Lopes A, da Silva A, da Silva C, Ayres FJ, Pena LJ (2019) Development and validation of reverse transcription loop-mediated isothermal amplification (RT-LAMP) for rapid detection of ZIKV in mosquito samples from Brazil. *Sci Rep* 9:1–12. <https://doi.org/10.1038/s41598-019-40960-5>
- da Gonçalves SD, Cassimiro APA, de Oliveira CD, Rodrigues NB, Moreira LA (2014) Wolbachia detection in rodents through LAMP: loop mediated isothermal amplification. *Parasit Vectors* 7:1–5. <https://doi.org/10.1186/1756-3305-7-228>
- Dastjerdi-Khorzoghi P, Javadi-Zarnaghi F, Hojati Z (2019) Targeting a viral DNA sequence with a deoxyribozyme in a preparative scale. *Biochimie* 165:161–169. <https://doi.org/10.1016/j.biochi.2019.07.022>
- Dehghanian F, Najafi ZB, Hojati Z, Javadi-Zarnaghi F (2020) DMLR: a toolkit for investigation of deoxyribozyme-mediated ligation based on real time PCR. *Biochem Biophys Res Commun* 524:405–410. <https://doi.org/10.1016/j.bbrc.2020.01.075>
- Elvira-González L, Puchades AV, Carpino C, Ana Alfaro-Fernández MI, Font-San-Ambrosio LR, Galipienso L (2017) Fast detection of southern tomato virus by one-step transcription loop-mediated isothermal amplification (RT-LAMP). *J Virol Methods* 241:11–14. <https://doi.org/10.1016/j.jviromet.2016.12.004>
- Etchebarne BE, Li Z, Stedtfeld RD, Nicholas MC, Williams MR, Johnson TA, Stedtfeld TM, Kostic T, Khalife WT, Tiedje JM (2017) Evaluation of nucleic acid isothermal amplification methods for human clinical microbial infection detection. *Front Microbiol* 8:2211. <https://doi.org/10.3389/fmicb.2017.02211>
- Fowler VL, Armson B, Gonzales JL, Wise EL, Howson ELA, Vincent-Mistiaen Z, Fouch S, Maltby CJ, Gripton S, Munro S (2021) A highly effective reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the rapid detection of SARS-CoV-2 infection. *J Infect* 82:117–125. <https://doi.org/10.1016/j.jinf.2020.10.039>
- Galyah A, Tombuloglu H, Motabagani D, Dana Motabagani AA, Rabaan KU, Dorado G, Al-Suhaimi E, Unver T (2022) Colorimetric and fluorometric reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for diagnosis of SARS-COV-2. *Funct Integr Genomics*. <https://doi.org/10.1007/s10142-022-00900-5>
- Gachugia J, Chebore W, Otieno K, Ngugi CW, Godana A, Kariuki S (2020) Evaluation of the colorimetric malachite green loop-mediated isothermal amplification (MG-LAMP) assay for the detection of malaria species at two different health facilities in a malaria endemic area of western Kenya. *Malar J* 19:1–10. <https://doi.org/10.1186/s12936-020-03397-0>
- Goto M, Honda E, Ogura A, Nomoto A, Hanaki K-I (2009) Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxy naphthol blue. *Biotechniques* 46:167–172. <https://doi.org/10.2144/000113072>
- Gupta S, Parker J, Smits S, Underwood J, Dolwani S (2020) Persistent viral shedding of SARS-CoV-2 in faeces—a rapid review. *Colorectal Dis* 22:611–620. <https://doi.org/10.1111/codi.15138>
- Higuchi R, Fockler C, Dollinger G, Watson R (1993) Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Bio/technology* 11:1026–1030. <https://doi.org/10.1038/nbt0993-1026>
- Huang WE, Lim B, Hsu C-C, Xiong D, Wei Wu, Yejiang Yu, Jia H, Wang Y, Zeng Y, Ji M (2020) RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microbial Biotechnol* 13:950–961. <https://doi.org/10.1111/1751-7915.13586>
- Javani A, Javadi-Zarnaghi F, Rasaei MJ (2017) Development of a colorimetric nucleic acid-based lateral flow assay with non-biotinylated capture DNA. *Appl Biol Chem* 60:637–645. <https://doi.org/10.1007/s13765-017-0321-9>
- Jia B, Li X, Liu W, Changde Lu, Xiaoting Lu, Ma L, Li Y-Y, Wei C (2019) GLAPD: whole genome based LAMP primer design for a set of target genomes. *Front Microbiol* 10:2860. <https://doi.org/10.3389/fmicb.2019.02860>
- Kudyba Heather M, Louzada J, Ljolje D, Kudyba KA, Muralidharan V, Oliveira-Ferreira J, Lucchi NW (2019) Field evaluation of malaria malachite green loop-mediated isothermal amplification in health posts in Roraima state Brazil. *Malar J* 18:1–7. <https://doi.org/10.1186/s12936-019-2722-1>
- Lim B, Ratcliff J, Nawrot DA, Yejiang Yu, Sanghani HR, Hsu C-C, Peto L, Evans S, Hodgson SH, Skeva A (2021) Clinical validation of optimised RT-LAMP for the diagnosis of SARS-CoV-2 infection. *Sci Rep* 11:1–11
- Lucchi NW, Ljolje D, Silva-Flannery L, Udhayakumar V (2016) Use of malachite green-loop mediated isothermal amplification for detection of plasmodium spp. parasites. *PLoS One* 11(3):e0151437. <https://doi.org/10.1371/journal.pone.0151437>
- Ma F, Liu M, Tang Bo, Zhang C-Y (2017) Sensitive quantification of microRNAs by isothermal helicase-dependent amplification. *Anal Chem* 89:6182–6187. <https://doi.org/10.1021/acs.analchem.7b01113>
- Machado BA, Souza KV, Hodel S, Barbosa-Júnior VG, Soares MBP, Badaró R (2021) The main molecular and serological methods for diagnosing COVID-19: an overview based on the literature. *Viruses* 13:40. <https://doi.org/10.3390/v13010040>
- Meagher RJ, Priye A, Light YK, Huang C, Wang E (2018) Impact of primer dimers and self-amplifying hairpins on reverse transcription loop-mediated isothermal amplification detection of viral RNA. *Analyst* 143:1924–1933. <https://doi.org/10.1039/c7an01897e>
- Nagamine K, Hase T, Notomi TJMCP (2002) Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Mol Cell Probes* 16:223–229. <https://doi.org/10.1006/mcpr.2002.0415>
- Naji E, Fadaian Z, Afshar D, Fazeli M (2019) Comparison of reverse transcription loop-mediated isothermal amplification method with SYBR green real-time RT-PCR and direct fluorescent antibody test for diagnosis of rabies. *Jpn J Infect Dis JJID* 2019:009. <https://doi.org/10.7883/yoken.jjid.2019.009>
- Nzulu CO, Cáceres AG, Guerrero-Quincho S, Tineo-Villafuerte E, Rodriguez-Delfin L, Mimori T, Uezato H, Katakura K, Gomez EA, Guevara AG (2016) A rapid molecular diagnosis of cutaneous leishmaniasis by colorimetric malachite green-loop-mediated isothermal amplification (LAMP) combined with an FTA card as a direct sampling tool. *Acta Trop* 153:116–119. <https://doi.org/10.1016/j.actatropica.2015.10.013>
- Nzulu CO, Kato H, Peters NC (2019) Loop-mediated isothermal amplification (LAMP): An advanced molecular point-of-care technique for the detection of Leishmania infection. *PLoS Negl Trop Dis* 13:e0007698. <https://doi.org/10.1371/journal.pntd.0007698>

- Park G-S, Keunbon Ku, Baek S-H, Kim S-J, Kim SI, Kim B-T, Maeng J-S (2020) Development of reverse transcription loop-mediated isothermal amplification assays targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *J Mol Diagn* 22:729–735. <https://doi.org/10.1016/j.jmoldx.2020.03.006>
- Ristaino JB, Saville AC, Paul R, Cooper DC, Wei Q (2020) Detection of *Phytophthora infestans* by loop-mediated isothermal amplification, real-time LAMP, and droplet digital PCR. *Plant Dis* 104:708–716. <https://doi.org/10.1094/pdis-06-19-1186-re>
- Rosenthal PJ (2020) The importance of diagnostic testing during a viral pandemic: early lessons from novel coronavirus disease (CoVID-19). *Am J Trop Med Hyg* 102:915. <https://doi.org/10.4269/ajtmh.20-0216>
- Rostron P, Pennance T, Bakar F, Rollinson D, Knopp S, Allan F, Kabole F, Ali SM, Ame SM, Webster BL (2019) Development of a recombinase polymerase amplification (RPA) fluorescence assay for the detection of *Schistosoma haematobium*. *Parasit Vectors* 12:1–7. <https://doi.org/10.1186/s13071-019-3755-6>
- Sadeghi S, Ahmadi N, Esmaili A, Javadi-Zarnaghi F (2017) Blue-white screening as a new readout for deoxyribozyme activity in bacterial cells. *RSC Adv* 7:54835–54843. <https://doi.org/10.1039/C7RA09679H>
- Scott A, Jackson K, Carter M, Comeau R, Layne T, Landers J (2019) Rapid sperm lysis and novel screening approach for human male DNA via colorimetric loop-mediated isothermal amplification. *Forensic Sci Int Genet* 43:102139. <https://doi.org/10.1016/j.fsigen.2019.102139>
- Shen C, Liu S, Li X, Yang M (2019) Electrochemical detection of circulating tumor cells based on DNA generated electrochemical current and rolling circle amplification. *Anal Chem* 91:11614–11619. <https://doi.org/10.1021/acs.analchem.9b01897>
- Shirshikov FV, Pekov YA, Miroshnikov KA (2019) MorphoCatcher: a multiple-alignment based web tool for target selection and designing taxon-specific primers in the loop-mediated isothermal amplification method. *PeerJ* 7:e6801. <https://doi.org/10.7717/peerj.6801>
- Song Qi, Sun X, Dai Z, Gao Y, Gong X, Zhou B, Jinbo Wu, Wen W (2021) Point-of-care testing detection methods for COVID-19. *Lab Chip* 21:1634–1660. <https://doi.org/10.1039/D0LC01156H>
- Tangkanchanapas P, Höfte M, De Jonghe K (2018) Reverse transcription loop-mediated isothermal amplification (RT-LAMP) designed for fast and sensitive on-site detection of Pepper chat fruit viroid (PCFVd). *J Virol Methods* 259:81–91. <https://doi.org/10.1016/j.jviromet.2018.06.003>
- Tavakoli-Koopaei R, Javadi-Zarnaghi F, Mirhendi H (2022) Unified-amplifier based primer exchange reaction (UniAmPER) enabled detection of SARS-CoV-2 from clinical samples. *Sens Actuators B Chem*. <https://doi.org/10.1016/j.snb.2022.131409>
- Thi D, Loan V, Herbst K, Boerner K, Meurer M, Kremer LPM, Kirmmaier D, Freistaedter A, Papagiannidis D, Galmozzi C, Stanifer ML (2020) A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. *Sci Transl Med*. <https://doi.org/10.1126/scitranslmed.abc7075>
- Vandenberg O, Martiny D, Rochas O, van Belkum A, Kozlakidis Z (2021) Considerations for diagnostic COVID-19 tests. *Nat Rev Microbiol* 19:171–183. <https://doi.org/10.1038/s41579-020-00461-z>
- Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA et al (2020) Virological assessment of hospitalized patients with COVID-2019. *Nature* 581(7809):465–469
- Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, Warren JL, Bertie Geng M, Muenker C, Moore AJ (2020) Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N Engl J Med* 383:1283–1286. <https://doi.org/10.1056/nejmc2016359>
- Zaman W, Saqib S, Ullah F, Ayaz A, Ye J (2020) COVID-19: phylogenetic approaches may help in finding resources for natural cure. *Phytother Res*. <https://doi.org/10.1002/ptr.6787>
- Zhai L, Liu H, Chen Q, Zhaoxin Lu, Zhang C, Lv F, Bie X (2019) Development of a real-time nucleic acid sequence-based amplification assay for the rapid detection of salmonella spp. from food. *Braz J Microbiol* 50:255–261. <https://doi.org/10.1007/s42770-018-0002-9>
- Zhang W, Chen C, Cui J, Bai W, Zhou J (2015) Application of loop-mediated isothermal amplification (LAMP) assay for the rapid diagnosis of pathogenic bacteria in clinical sputum specimens of acute exacerbation of COPD (AECOPD). *Int J Clin Exp Med* 8:7881
- Zhang Y, Odiwuor N, Xiong J, Sun L, Nyaruaba RO, Wei H, Tanner NA (2020) Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric LAMP. *MedRxiv*. <https://doi.org/10.1101/2020.02.26.20028373>
- Zhao N, Liu JX, Li D, Sun DX (2016) Loop-mediated isothermal amplification for visual detection of hepatitis B virus. *Zhonghua gan zang bing za zhi = Zhonghua ganzangbing zazhi = Chin J Hepatol* 24:406–411. <https://doi.org/10.3760/cma.j.issn.1007-3418.2016.06.003>

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