Brief Communication *Highlight article*

High-quality RNA improves sensitivity of SARS-CoV-2 detection by colorimetric RT-LAMP

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Impact statement

Given the global nature of the coronavirus disease 2019 (COVID-19) pandemic, there is a critical need for rapid and reliable disease detection. In our laboratory, we developed a protocol for SARS-CoV-2 detection based on RT-LAMP technology. We demonstrate the importance of RNA quality for rapid detection of SARS-CoV-2 in suspected cases, including those with a low viral load. The RT-LAMP technique can detect samples with Ct values between 8 and 35, similar to those detected by the gold standard method, RT-qPCR.

Abstract

The global SARS-CoV-2 pandemic requires a rapid, reliable, and user-friendly diagnostic test to help control the spread of the virus. Reverse transcription and quantitative PCR (RT-qPCR) is currently the gold standard method for SARS-CoV-2 detection. Here, we develop a protocol based on reverse transcription loop-mediated isothermal amplification (RT-LAMP) and demonstrate increased sensitivity of this technique using fresh RNA extracts compared to RNA samples subjected to freezing/thawing cycles. We further compare RT-LAMP to RT-qPCR and demonstrate that the RT-LAMP approach has high sensitivity in fresh RNA extracts and can detect positive samples with Ct values between 8 and 35.

Keywords: SARS-CoV-2, RT-LAMP, high-quality RNA

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Introduction

The ongoing pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first detected in the city of Wuhan (China), and spread thereafter in multiple countries, infecting over 130 million people worldwide. To slow this spread, large-scale diagnostics of infected individuals were implemented in many countries.

Current diagnostic methods combine clinical symptoms and molecular techniques. The most common clinical symptoms include fever, shortness of breath, cough, fatigue/malaise, and confusion (ISARIC platform: https://isaric.org/). The gold standard method to detect SARS-CoV-2 infection is based on reverse transcription and quantitative PCR (RT-qPCR) assays which require two steps: (i) RNA extraction from a nasopharyngeal (NP) swab and (ii) RT-qPCR amplification to detect viral RNA.

RT-qPCR is the most commonly used method for detection of SARS-CoV-2,[1] but it requires costly equipment and trained personnel. Therefore, many efforts have been dedicated to developing a simple and more broadly applicable testing method. An advocated alternative diagnostic method to RT-qPCR is reverse transcription loop-mediated isothermal amplification (RT-LAMP).[2–5] Only a thermoblock is needed for the RT-LAMP protocol, enabling transfer of this technique outside of specialized molecular

biology laboratories. RT-LAMP is a one-step nucleic acid amplification method based on PCR technology. RT-LAMP is performed at a constant temperature, with reverse transcription and genomic material amplification in a single step. RT-LAMP results can be visualized by a change in the color of the reaction, based on the presence of pH-sensitive dye phenol red. Amplification increases the acidification of the reaction and the phenol red changes to a yellow color. Positive samples are detected by the naked-eye after a 40-min incubation at 65°C.[6,7] This method can be used to detect Zaire Ebola virus RNA and Salmonella in ready-to-eat fruits and vegetables.[8,9]

The main limitation of the RT-LAMP technique is its low sensitivity. Detection of SARS-CoV-2 directly from NP swabs, without an RNA extraction step, is affected by RNA degradation and the presence of enzymatic inhibitors in the media. In addition, low viral load is observed in some patients with COVID-19. This may cause inconsistent results during serial testing.[4,10,11]

Recent publications present a series of improvements in the RNA isolation step that may help overcome the challenges encountered in RT-LAMP.[3,4,11,12] In our study, we evaluate the sensitivity of RT-LAMP by comparing fresh RNA extracts with RNA samples that have been subjected to freeze/thaw cycles. We further compare RT-LAMP results with those obtained using RT-qPCR.

Materials and methods

Clinical sample collection

Specimens were collected with nasopharyngeal swabs in the province of Girona (Spain). Samples were transported in sterile containers within a few hours of collection and delivered to the ICS-IAS Girona Clinical Laboratory of Hospital Santa Caterina, located in Girona. Nasopharyngeal swabs were processed in a biosafety level 2 cabinet. The virus was inactivated with a lysis buffer. We divided the cases into two different groups:

- Group 0 = A total of 40 samples in which the RNA was extracted with a Biosprin 96 DNA blood kit (Qiagen, Germany). This RNA was fully analyzed by RT-qPCR and frozen at –80°C. These samples were subjected to different freezing/thawing cycles before RT-LAMP analysis.
- Group 1 = A total of 19 samples in which the RNA was isolated from NP swabs using Chemagen MSM I (PerkinElmer, Germany) with the Chemagic viral DNA/RNA kit special H96 following the manufacturer's instructions (PerkinElmer). This kit uses magnetic particles with high affinity to nucleic acid and low protein binding. We assessed the concentration of the samples by spectrophotometry (Nanodrop, ThermoScientific, Massachusetts, USA). The RT-qPCR and RT-LAMP tests were performed immediately following RNA isolation.

RT-qPCR

RT-qPCR was performed using the 2019-nCoV CDC EUA commercial kit (Integrated DNA Technologies, IDT, Iowa, USA) and TaqPath™ 1-step RT-qPCR MM (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. First, RT-qPCR was validated using the 2019-nCoV_N_positive control (Integrated DNA Technologies, IDT) and analyzing three different regions of the viral N-gene: N1, N2, and N3. To correlate the Ct value to the number of viral copies, a serial dilution of the 2019-nCoV_N_positive control (IDT) was performed (1, 1:10, 1.20, 1:40, 1:100, 1:1000) (1 µL of positive control is equivalent to 200,000 copies of virus).

Afterward, 3 µL of isolated RNA in a final volume of 10 µL were amplified using the QuantStudio 7 Flex PCR System (Thermo Fisher Scientific) under the following conditions: 15 min at 55°C, 2 min at 95°C and 45 cycles at 95°C for 30 s and 55°C for 30 s. RNAseP POP7 was used as a control for human RNA isolation. Results were analyzed using QuantStudio™ Real-time PCR software v 1.2 (Thermo Fisher Scientific). Samples were considered positive when one or more viral N-genes amplified at a cycle threshold (Ct) < 40. Detection of the human RNaseP POP7 gene is essential to validate the RT-qPCR results, and results without amplification of this gene were considered invalid.

RT-LAMP primer design

The RT-LAMP primer sets used in this study were designed against the N, E, and ORF1 genes of SARS-CoV-2 described by Zhang *et al.*,[2] and the primers against the RNAseP POP7 human gene were from the SARS-CoV-2 LAMP Diagnostic Assay (version 1.2) (New England Biolabs). Primers were synthesized by Conda laboratories (Madrid, Spain). For the test, we used a 10× primer mix containing 2 µM each of the F3 and B3 primers, 4 µM each of the forward loop (FL) and backward loop (BL) primers, and 16 µM each of the forward inner primer (FIP) and backward inner primer (BIP). The FIP and BIP were HPLC purified (Table 1).

Colorimetric RT-LAMP primer assay

Colorimetric reactions were performed at room temperature in a total volume of 20 µL per reaction using 10 µL of the WarmStart Colorimetric RT-LAMP 2X Master Mix (M1800, New England Biolabs), 2 µL of 10X primer mix (Table 1), 1 µL guanidine hydrochloride at a final concentration of 40 mM (Sigma, Misuri, USA), and 4 µL of nuclease-free water (ThermoFisher). Finally, 3 µL of RNA were added. Reaction mixes were then incubated in a thermocycler at 65°C for 40 min with the lid heated to 75°C. Reactions were stopped by placing tubes on ice for 2 min, and photographs were taken using a conventional camera.

RNA 2019-nCoV_N_positive control (IDT) was used in each experiment. RNAseP POP7 was amplified as a control of human RNA isolation. Samples were considered negative if the original pink color of the phenol red was maintained and positive if the pink color turned to yellow-orange for the SARS-CoV-2 and RNAse POP7

Table 1. Sequences of RT-LAMP primers sets used in the colorimetric RT-LAMP assay.

Gene N-A	
GeneN-A-F3	TGGCTACTACCGAAGAGCT
GeneN-A-B3	TGCAGCATTGTTAGCAGGAT
GeneN-A-LF	GGACTGAGATCTTTCATTTTACCGT
GeneN-A-LB	ACTGAGGGAGCCTTGAATACA
GeneN-A-FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG
GeneN-A-BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT
ORF1a-A	
ORF1a-A-F3	CTGCACCTCATGGTCATGTT
ORF1a-A-B3	AGCTCGTCGCCTAAGTCAA
ORF1a-A-LF	CCGTACTGAATGCCTTCGAGT
ORF1a-A-LB	TTCGTAAGAACGGTAATAAAGGAGC
ORF1a-A-FIP	GAGGGACAAGGACACCAAGTGTATGGTTGAGCTGGTAGCAGA
ORF1a-A-BIP	CCAGTGGCTTACCGCAAGGTTTTAGATCGGCGCCGTAAC
Gene N2	
GeneN2-F3	ACCAGGAACTAATCAGACAAG
GeneN2-B3	GACTTGATCTTTGAAATTTGGATCT
GeneN2-LF	GGGGGCAAATTGTGCAATTTG
GeneN2-LB	CTTCGGGAACGTGGTTGACC
GeneN2-FIP	TTCCGAAGAACGCTGAAGCGGAACTGATTACAAACATTGGCC
GeneN2-BIP	CGCATTGGCATGGAAGTCACAATTTGATGGCACCTGTGTA
Gene E1	
GeneE1-F3	TGAGTACGAACTTATGTACTCAT
GeneE1-B3	TTCAGATTTTTAACACGAGAGT
GeneE1-LF	CGCTATTAACTATTAACG
GeneE1-LB	GCGCTTCGATTGTGTGCGT
GeneE1-FIP	ACCACGAAAGCAAGAAAAAGAAGTTCGTTTCGGAAGAGACAG
GeneE1-BIP	TTGCTAGTTACACTAGCCATCCTTAGGTTTTACAAGACTCACGT
RNaseP-POP7	
RNaseP POP7-F3	TTGATGAGCTGGAGCCA
RNaseP POP7-B3	CACCCTCAATGCAGAGTC
RNaseP POP7-LF	ATGTGGATGGCTGAGTTGTT
RNaseP POP7-LB	CATGCTGAGTACTGGACCTC
RNaseP POP7-FIP	GTGTGACCCTGAAGACTCGGTTTTAGCCACTGACTCGGATC
RNaseP POP7-BIP	CCTCCGTGATATGGCTCTTCGTTTTTTTCTTACATGGCTCTGGTC

genes, with the exception of the positive control, which does not contain the RNase POP7 gene.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed on 14 samples to verify the RT-LAMP reaction. One half of each RT-LAMP reaction was electrophoresed on a 2% agarose gel in 1× TAE buffer (40 nM Tris, 20 mM acetic acid, 1 mM EDTA) at 90 V for 90 min. Agarose gels were imaged under UV light using a Quantum transilluminator (Biotech, Canada). Lanes containing a laddering pattern indicated RT-LAMP-positive samples.

Statistical analysis

To evaluate the performance of the RT-LAMP assay in SARS-CoV-2 positive and negative patients, we estimated the sensitivity and specificity values. The 95% confidence interval (95% CI) was reported using the Wilson score method. RT-qPCR is considered the gold standard for this evaluation. Statistical analyses were performed using NCSS 2020 Statistical Software (NCSS, LLC. Kaysville, UT, USA, ncss.com/software/ncss).

Results

Our study was designed to investigate the effect of RNA quality on the sensitivity and specificity of the colorimetric RT-LAMP assay and evaluate its suitability as an alternative to RT-qPCR testing for detecting SARS-CoV-2 in RNA isolated from nasopharyngeal swabs. First, to determine the optimal incubation time, we incubated the RT-LAMP reactions for 20, 30, and 40 min at 65°C. Optimal results were obtained after 40 min of incubation at 65°C. Then, we tested different primer sets (Table 1) and found that primers targeting the N-A gene worked best with these incubation conditions (data not shown).

Finally, we established the correlation between the number of viral copies and Ct value using the N-A gene primer set with the 2019-nCoV_N_positive control. The minimum number of copies of virus that could be detected by the RT-LAMP technique was 2×10^2 copies, which corresponds to a qPCR Ct value of 35 (Figure 1).

RNA samples subjected to different freeze/thaw cycles (group 0) and fresh RNA extracts (group 1) were tested using the RT-LAMP protocol. In group 0, 11 samples were detected as positive and 29 samples as negative (Figure 2(a)). In group 1, 16 positive and 2 negative samples were detected (Figure 2(b)). In all groups, the positive control was amplified and no amplification occurred in the NTC.

(a)

	Dilution	Ct N1	N2	N3
2019-nCoV_N_ positive control	1	22.3	23.8	22.6
	1:10	25.4	26.4	25.5
	1:20	25.6	26.5	25.9
	1:40	28.5	28.5	27.2
	1:100	29.2	30.0	29.3
	1:1000	34.1	35.7	35.3

(b)

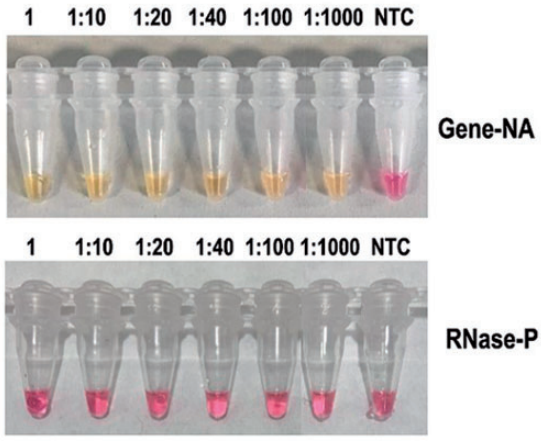

Figure 1. Determination of the correlation between the number of viral copies and Ct value. (a) Serial dilutions of 2019-nCoV_N_positive control were tested by RT-qPCR (b) RT-LAMP results from different dilutions and the non-control-template (NTC) after 40 min of incubation at 65°C are shown. (A color version of this figure is available in the online journal.)

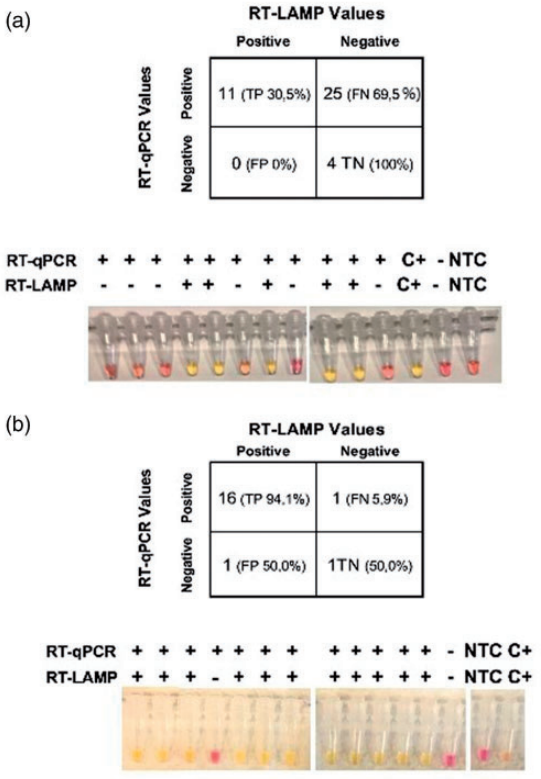

Figure 2. Classification of true positive (TP), true negative (TN), false positive (FP), and false negative (FN) numbers and RT-LAMP test results compared to standard RT-qPCR results in samples with some cycles of freezing/thawing (a) and fresh samples (b). End point color change photographs of RT-LAMP using RNA samples with some cycles of freezing/thawing (a) and fresh RNA samples (b). (A color version of this figure is available in the online journal.)

Therefore, the results were considered valid (Figure 2(c) and (d)).

To determine the sensitivity and specificity of the RT-LAMP approach, we compared it to RT-qPCR, which is considered the gold-standard. Among 36 total RT-qPCR-positive group 0 samples, only 11 were detected as positive by the RT-LAMP assay (30.56%, 95% CI: 18.0%–46.86%) (Figure 2(a), Table 2). However, the sensitivity reached 100% among samples with a Ct value $\leq$25 (Figure 3(a)). Moreover, the RT-LAMP technique allowed identification of all RT-qPCR-negative samples (true negatives), resulting in a specificity of 100% (95% CI: 51.01%–100.0%) (Figure 2(a)).

To evaluate whether the low sensitivity was caused by low RNA quality due to freezing/thawing, we performed the same experiment using fresh RNA samples (group 1). The sensitivity in this group of samples increased to 94.14% (95% CI: 73.02%–98.95%) (Figure 2(b), Table 2) and reached 100% sensitivity when the Ct threshold was increased to $\leq$35 (Figure 3(b)). However, the specificity was only 50.0% (95% CI: 9.4%–90.55%) (Figure 2(b)). This reduced specificity could be due to the low number of negative samples in this group.

The RT-LAMP results were further verified by 2% agarose gel electrophoresis, which revealed different band patterns between positive and negative RT-LAMP samples. Positive RT-LAMP samples had a common band pattern, as recently reported by Dao Thi *et al.*, whereas RT-LAMP-negative samples produced a smear pattern (Figure 4).

Table 2. Sensitivity values of RT-LAMP technique compared to the gold standard RT-qPCR method.

	% of sensitivity (95% C.I.)	
	Group 0 ($N_T = 40$)	Group 1 ($N_T = 19$)
$C_T \leq 35$	30.5% (18–46.9)	94.1% (73–98.8)
$C_T \leq 30$	80.0% (49–94.3)	100% (78.4–100)
$C_T \leq 25$	100% (56.6–100)	100% (74.1–100)

In summary, using fresh RNA extracts resulted in increases in true positive results and decreases in false negative results. Moreover, all positive group 0 samples detected by RT-LAMP had Ct values $\leq$25. We were not able to detect group 0 samples with Ct $\geq$ 30. However, the RT-LAMP technique was able to detect group 1 samples with Ct values between 8 and 35.

Discussion

RT-LAMP is a simple and rapid assay to detect SARS-CoV-2 in RNA samples. RNA isolation before detection is a crucial

(a)

			RT-LAMP		
		Ct	Positive	Negative	Total
RT-qPCR	Positive	0-25	5	0	5
		25-30	3	2	5
		30-35	2	7	9
		35-40	1	16	17
	Negative	Negative	0	4	4
Total			11	29	40

(b)

			RT-LAMP		
		Ct	Positive	Negative	Total
RT-qPCR	Positive	0-25	11	0	11
		25-30	3	0	3
		30-35	2	0	2
		35-40	0	1	1
	Negative	Negative	1	1	2
Total			17	2	19

Figure 3. RT-qPCR and RT-LAMP results using RNA subjected to different freeze/thaw cycles (a) and fresh RNA samples (b), stratified by Ct values. Specificity and sensitivity values are calculated from these numbers.

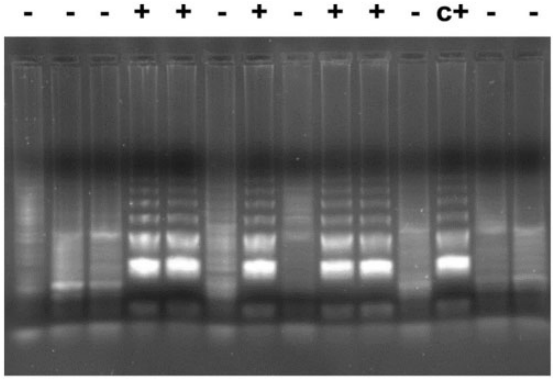

Figure 4. RT-LAMP reaction product was analyzed on a 2% agarose gel. The typical band pattern was observed in samples with a successful RT-LAMP reaction. These samples turned yellow after 40 min of incubation at 65°C. + symbol indicates samples with RT-LAMP positive reaction, – symbol indicates samples with RT-LAMP negative reaction.

step to ensure high specificity and sensitivity of this method. In this study, we address the potential of the RT-LAMP assay for viral detection in fresh RNA samples of patients suspected to have COVID-19. Our experiments were performed using RNA subjected to freeze/thaw cycles following extraction (group 0) and fresh RNA extracts (group 1). We validated our RT-LAMP results using the standard RT-qPCR method.

Once we established the optimal conditions for the RT-LAMP technique, the results showed a high sensitivity to detect synthetic SARS-CoV-2 until Ct∼35. When the test was transferred to RNA from real cases, this sensitivity was reduced to Ct ∼25 in non-fresh samples (group 0) and Ct ∼35 in fresh RNA samples (group 1). As the Ct value increased, the detection capacity for true-positive samples by the RT-LAMP technique decreased. Sensitivity of the RT-LAMP technique decreased by approximately 10 Ct when RNA samples from group 0 (frozen samples) were used. Therefore, the use of fresh RNA samples enables the detection of infections in patients with a lower viral load.

It is important to isolate the RNA from specific NP swabs because not all samples are compatible with RT-LAMP techniques. Some NP sample types may contain inhibitors in the transport media that make the RT-LAMP test less effective. Moreover, the RT-LAMP approach was only able to detect SARS-CoV-2 in samples with high levels of virus and Ct values less than 24 when NP swabs were tested directly without an RNA isolation step.[5,13] Furthermore, Bruce *et al.* recently published that RT-qPCR directly from NP swabs results in a 4-Ct drop in sensitivity.[13]

There have been some attempts to perform RT-LAMP assays directly in clinical samples other than NP swabs. Some studies reported that the RT-LAMP protocol can detect SARS-CoV-2 directly from saliva, but only from patients with severe to very severe disease[14] or when the saliva sample is collected in the early phase of symptom onset, when the viral load of SARS-CoV-2 is high.[15] Consequently, there are studies that demonstrate increased sensitivity of the RT-LAMP technique in combination with RNA extraction from any clinical sample.[5,16]

Understanding the duration of infectivity can help us to control the pandemic. The duration of infectivity and its correlation with viral load remain poorly understood. Recently, Scola *et al.* found that positive culture growth decreased progressively accordingly to viral load. They observed a strong correlation between Ct value and infectivity in a cell culture model. Patients with Ct values equal to or above 34 did not excrete infectious viral particles and were therefore not infectious.[17] Thus, it is important to be able to rapidly detect positive samples with a Ct below 35 with the RT-LAMP protocol. We also demonstrated the importance of using fresh RNA samples to detect all patients with the ability to infect others.

We conclude that the colorimetric RT-LAMP technique can be useful in identifying COVID-19 patients using fresh RNA extracts. Moreover, these results suggest that the RT-LAMP test is less sensitive with RNA samples that have undergone some cycles of freezing/thawing than with fresh RNA extracts. Although this protocol requires a prior RNA isolation step, the results obtained from fresh RNA extracts have a sensitivity of 94.14% and can detect patients with a lower viral load.

In the current work, we optimized the RT-LAMP approach using fresh RNA samples to visually detect SARS-CoV-2 and proposed a schematic protocol for COVID-19 testing (Supplemental Figure 1). Importantly, this technique could be extended to rapidly and efficiently detect other emerging pathogens as well.

AUTHORS' CONTRIBUTIONS

MP, MC, CF, RB participated in conceptualization. MP, MC, AP designed and analyzed all experiments, MPA, FP, LL, NN, MC, MP participated in methodology (primer design, RT-LAMP experiments, RT-qPCR experiments, and agarose gel electrophoresis). MB performed statistical analyses, FXQ provided patient samples, MP wrote the paper with the help and approval of RB.

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DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

1. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y, Ng DYM, Wan CKC, Yang P, Wang Q, Peiris M, Poon LLM. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. *Clin Chem* 2020;**66**:549–55
2. Zhang Y, Ren G, Buss J, Barry AJ, Patton GC, Tanner NA. Enhancing colorimetric loop-mediated isothermal amplification speed and sensitivity with guanidine chloride. *Biotechniques* 2020;**69**:178–85
3. Dao Thi VL, Herbst K, Boerner K, Meurer M, Kremer LP, Kirrmaier D, Freistaedter A, Papagiannidis D, Galmozzi C, Stanifer ML, Boulant S, Klein S, Chlanda P, Khalid D, Barreto Miranda I, Schnitzler P, Krausslich HG, Knop M, Anders S. A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. *Sci Transl Med* **12**: eabc7075
4. Ben-Assa N, Naddaf R, Gefen T, Capucha T, Hajjo H, Mandelbaum N, Elbaum L, Rogov P, King DA, Kaplan S, Rotem A, Chowers M, Szwarcwort-Cohen M, Paul M, Geva-Zatorsky N. Direct on-the-spot detection of SARS-CoV-2 in patients. *Exp Biol Med (Maywood)* 2020;**245**:1187–93
5. Lamb LE, Bartolone SN, Ward E, Chancellor MB. Rapid detection of novel coronavirus/severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by reverse transcription-loop-mediated isothermal amplification. *PLoS One* 2020;**15**:e0234682
6. Tanner NA, Zhang Y, Evans TC. Jr., Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes. *Biotechniques* 2015;**58**:59–68
7. Notomi T, Mori Y, Tomita N, Kanda H. Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. *J Microbiol* 2015;**53**:1–5
8. Carter C, Akrami K, Hall D, Smith D, Aronoff-Spencer E. Lyophilized visually readable loop-mediated isothermal reverse transcriptase nucleic acid amplification test for detection ebola Zaire RNA. *J Virol Methods* 2017;**244**:32–8
9. Wan J, Guo J, Lu Z, Bie X, Lv F, Zhao H. Development of a test kit for visual loop-mediated isothermal amplification of Salmonella in spiked ready-to-eat fruits and vegetables. *J Microbiol Methods* 2020;**169**:105830
10. Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, Warren JL, Geng B, Muenker MC, Moore AJ, Vogels CBF, Petrone ME, Ott IM, Lu P, Venkataraman A, Lu-Culligan A, Klein J, Earnest R, Simonov M, Datta R, Handoko R, Naushad N, Sewanan LR, Valdez J, White EB, Lapidus S, Kalinich CC, Jiang X, Kim DJ, Kudo E, Linehan M, Mao T, Moriyama M, Oh JE, Park A, Silva J, Song E, Takahashi T, Taura M, Weizman OE, Wong P, Yang Y, Bermejo S, Odio CO, Omer SB, Dela Cruz CS, Farhadian S, Martinello RA, Iwasaki A, Grubaugh ND, Ko AI. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N Engl J Med* 2020;**383**:1283–6
11. Buck MD, Poirier EZ, Cardoso A, Frederico B, Canton J, Barrell S, Beale R, Byrne R, Caidan S, Crawford M, Cubitt L, Gamblin S, Gandhi S, Goldstone R, Grant PR, Gulati K, Hindmarsh S, Howell M, Hubank M, Instrell R, Jiang M, Kassiotis G, Lu WT, MacRae JI, Martini I, Miller D, Moore D, Nastouli E, Nicod J, Nightingale L, Olsen J, Oomatia A, O'Reilly N, Rideg A, Song OR, Strange A, Swanton C, Turajilic S, Wu M, E Sousa CR, Crick COVID-19 Consortium. SARS-CoV-2 detection by a clinical diagnostic RT-LAMP assay. *Wellcome Open Res* 2021:**6**:9
12. Kellner MJ, Ross JJ, Schnabl J, Dekens MPS, Heinen R, Grishkovskaya I, Bauer B, Stadlmann J, Menéndez-Arias L, Fritsche-Polanz R, Traugott M, Seitz T, Zoufaly A, Födinger M, Wenisch C, Zuber J, Pauli A, Brennecke J, Vienna Covid-19 Diagnostics Initiative (VCDI). A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing. *bioRxiv* 2020; 2020.06.23.166397.
13. Bruce EA, Huang ML, Perchetti GA, Tighe S, Laaguiby P, Hoffman JJ, Gerrard DL, Nalla AK, Wei Y, Greninger AL, Diehl SA, Shirley DJ, Leonard DGB, Huston CD, Kirkpatrick BD, Dragon JA, Crothers JW, Jerome KR, Botten JW. Direct RT-qPCR detection of SARS-CoV-2 RNA from patient nasopharyngeal swabs without an RNA extraction step. *PLoS Biol* 2020;**18**:e3000896
14. Azzi L, Carcano G, Gianfagna F, Grossi P, Gasperina DD, Genoni A, Fasano M, Sessa F, Tettamanti L, Carinci F, Maurino V, Rossi A, Tagliabue A, Baj A. Saliva is a reliable tool to detect SARS-CoV-2. *J Infect* 2020;**81**:e45–e50
15. Nagura-Ikeda M, Imai K, Tabata S, Miyoshi K, Murahara N, Mizuno T, Horiuchi M, Kato K, Imoto Y, Iwata M, Mimura S, Ito T, Tamura K, Kato Y. Clinical evaluation of self-collected saliva by quantitative reverse transcription-PCR (RT-qPCR), direct RT-qPCR, reverse transcription-loop-mediated isothermal amplification, and a rapid antigen test to diagnose COVID-19. *J Clin Microbiol* 2020;**58**:e01438–20
16. Klein S, Muller TG, Khalid D, Sonntag-Buck V, Heuser AM, Glass B, Meurer M, Morales I, Schillak A, Freistaedter A, Ambiel I, Winter SL, Zimmermann L, Naumoska T, Bubeck F, Kirrmaier D, Ullrich S, Barreto Miranda I, Anders S, et al. SARS-CoV-2 RNA extraction using magnetic beads for rapid Large-Scale testing by RT-qPCR and RT-LAMP. *Viruses* 2020;**12**:863
17. Scola B, La Bideau M, Le Andreani J, Hoang VT, Grimaldier C, Colson P, Gautret P, Raoult D. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *Eur J Clin Microbiol Infect Dis* 2020;**39**:1059

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