Reverse transcription loop-mediated isothermal amplification has a high performance in the detection of SARS-CoV-2 in saliva samples and nasal swabs from asymptomatic and symptomatic individuals

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Abstract. The detection of coronavirus disease 2019 cases represents a significant challenge at the epidemiological level. Limitations exist in effectively detecting asymptomatic cases, achieving good follow-up in hospitals without the infrastructure for reverse transcription-quantitative PCR (RT-qPCR) or in difficult-to-access areas and developing methods with the need for less invasive sampling procedures. Therefore, the present study evaluated the performance of the direct reverse transcription loop-mediated isothermal amplification (RT-LAMP) test for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the saliva and nasal samples of asymptomatic individuals belonging to the university population. In addition, this test was also assessed for effectiveness in symptomatic individuals referred from a hospital with poor infrastructure in molecular biology and located outside the urban area. The RT-LAMP assay was compared with the results obtained from the RT-qPCR nasopharyngeal swab test, where the diagnosis was confirmed by lateral flow immunoassay test for rapid antigen detection. A total of 128 samples were analyzed, of which 43% were symptomatic positive individuals, 25% were asymptomatic positive individuals and 32% were SARS-CoV2-negative control individuals. Among positive individuals, no differences were found between the Cq values determined by RT-qPCR. A sensitivity of 96.5% and a specificity of 97.6% was reported for the detection of SARS-CoV-2 in symptomatic individuals by salivary and nasal RT-LAMP, as well as a sensitivity of 100% and a specificity of 97.6% for the detection of SARS-CoV-2 in asymptomatic individuals. These findings indicated that performance of the direct RT-LAMP test using saliva and nasal samples has high sensitivity and specificity, which in turn suggest that it is a viable and reliable alternative for use in epidemiological monitoring.

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), first emerged in Wuhan, China in December 2019 (1). According to the World Health Organization (WHO), the COVID-19 pandemic is the result of the rapid international spread of SARS-CoV-2. From the start of the pandemic until September 2022, ~601 million people have been infected and 6 million deaths have been recorded worldwide (database accessed on September 13, 2022; covid19.who.int/) (2). Notably, infection with SARS-CoV-2 can either remain asymptomatic or can result in severe conditions requiring hospital medical care (3,4). The transmission route of SARS-CoV-2 is typically by contact, by inhalation of respiratory droplets or by aerosols emitted by an infected individual (5). Although the number of severe cases has decreased due to vaccination, the number of cases continues to increase, although the rate of increase of cases is decreasing (6,7). Asymptomatic and symptomatic patients have similar viral loads, this is reported by Miguel *et al* (2022), who performed a RT-qPCR study and observed that the Ct's showed marginal differences (8). They are an important source of transmission, rendering their identification and isolation essential for containing the spread of the virus (9).

The gold standard for detecting SARS-CoV-2 is reverse transcription-quantitative PCR (RT-qPCR), a test with high sensitivity and specificity (10). However, the global increase in the number of cases revealed that, in low- and middle-income countries, there was limited accessibility to RT-PCR due to problems with sample collection, operating procedures and a lack of reagents for viral RNA extraction. The cost of the equipment, the need for trained personnel and the installation in difficult-to-access areas were some of the variables that slowed down timely detection, which consequently increased the spread of cases (11,12). As a result, other diagnostic methods have emerged, including the reverse transcription loop-mediated isothermal amplification (RT-LAMP) method. Since 2000, the advantages of RT-LAMP as a diagnostic tool in infectious diseases, such as Zika virus, dengue and malaria, have been described (13-15).

The RT-LAMP reaction is typically performed in a single step at an isothermal temperature due to the presence of the enzyme *Bst* or *Bsm* in the reaction that allows for the amplification of products together with the template RNA, reverse transcriptase and primers, without the need for specialized equipment (16). This technique uses four to six primers that bind to six regions of the specific DNA fragment, making the technique specific and sensitive. Amplified end products can then be visualized by turbidity, fluorescence, luminescence or metal-sensitive colorimetry; thus, RT-LAMP is a rapid detection method, Amaral *et al* (17) report that time elapsing from sample collection to delivery of results is ~30 min, depending on whether it is an RT-LAMP without RNA extraction or with RNA extraction (the latter plus the time it takes to perform RNA extraction) (17). These are more evident especially in places where the necessary equipment to perform molecular tests such as TR-qPCR is not available. Currently to perform a RT-qPCR in these places requires that the sample is taken and sent to a central laboratory for processing so it can take 2 to 5 days for diagnosis (18). RT-LAMP is considered an excellent option for rapid and continuous epidemiological follow-up in the community, in reference or specialty hospitals, in outpatients or in those living in difficult-to-access areas.

For the diagnosis of SARS-CoV-2 by RT-LAMP, a sensitivity of >80% has been reported based on saliva, nasal or nasopharyngeal samples with or without the previous step of viral RNA extraction (19,20), which holds an advantage over other tests. In Mexico, there are few diagnostic studies regarding the detection of SARS-CoV-2 with RT-LAMP. In this regard, Cisneros-Villanueva *et al* (21) previously reported a sensitivity and specificity of >90% from nasopharyngeal swab samples with a previous RNA extraction step. Therefore, due to the advantages offered by the RT-LAMP technique, the objective of the present study was to determine the sensitivity and specificity of the RT-LAMP test for the detection of SARS-CoV-2 in the saliva and nasal samples from symptomatic individuals derived from a hospital located far from the urban area and from asymptomatic individuals belonging to the university population. The RT-LAMP assay was compared with the results obtained by RT-qPCR of nasopharyngeal swab tests and the diagnosis was confirmed by lateral flow immunoassay (LFIA) for the rapid detection of antigens.

Materials and methods

Study design and population. In this observational and cross-sectional multi-center study, adults aged 18-70 years (mean 29.3±12.4 age) with a confirmatory diagnosis of SARS-CoV-2 determined by RT-qPCR were invited to participate, the samples were collected from January to May 2022 and samples were eliminated if there was insufficient material or if the RT-qPCR test was inconclusive. The present study had three study groups: Symptomatic patients 21 men and 35 women (mean 38.4±13.7 age), asymptomatic patients 15 men and 17 women (mean 22±5.7 age) and healthy individuals patients 19 men and 22 women (41 individuals, mean 29.7±12.4 years). Symptomatic patients were diagnosed according to the operational definition for COVID-19 issued by the Ministry of Health (México) and the WHO (22,23), which establishes that they are patients who present at least one of the following symptoms: Fever, cough or headache. In addition, they should be accompanied by at least two of the following symptoms: Dyspnea, arthralgia, myalgia, odynophagia, rhinorrhea and conjunctivitis (22). They would also have to be confirmed positive for SARS-CoV-2 by commercial RT-qPCR and antigen test (*LFIA*, with this test the SARS-CoV-2 antigens are recognized for antibody anti-SARS-CoV-2.),. The asymptomatic group is comprised of individuals who did not present any symptoms of COVID-19 but were tested positive for SARS-CoV-2, as determined by commercial RT-qPCR and antigen test (LFIA). The control group without COVID-19 were individuals without symptoms and who were tested negative for SARS-CoV-2, as determined by RT-qPCR and antigen test (LFIA).

Symptomatic patients were recruited from the Institute of Social Security and Services for State Workers (ISSSTE) Hospital (Huauchinango, Mexico). The asymptomatic individuals were recruited from random screening carried out in the community of the Puebla Popular University of the State (UPAEP; Puebla, Mexico). For the samples of healthy individuals were also randomly collected at UPAEP.

Sample collection and transportation. All samples including the controls were collected between January 12 and May 1, 2022. A total of five samples were taken from each participant: Two nasopharyngeal swab samples for RT-qPCR and antigen testing (LFIA), two nasal samples for RT-LAMP and antigen testing, and one saliva sample for RT-LAMP, The samples were collected when the patients presented less than 9 days of symptoms. The nasopharyngeal swab samples for RT-qPCR was placed in 15-ml tubes with 3 ml viral transport medium, whereas nasal and saliva samples were placed in 15-m tubes without any buffer. All samples were stored at a temperature of 7˚C for further processing. The samples were placed in a viral transport medium (sterile phosphate-buffered saline PBS Cat. No. 70011044 Gibco) and transported with triple packaging as established in the 'Operative Guide for the Clinical Management of Severe Acute Respiratory Infection by COVID-19' of the Mexican government in 2020 (23).

RNA extraction for RT-qPCR. RNA was extracted from the nasopharyngeal swabs using the commercial RNeasy kit (cat. no. 74104; Qiagen GmbH) according to the manufacturer's protocol. Briefly, lysis buffer (Buffer RLT) and 10 μl proteinase K were added and sample was incubated at 55˚C for 30 min, after which 70% ethanol was added in a 1:1 volume. The sample was then transferred to the RNeasy Mini spin column, centrifuged for 15 sec at 4,000 x g at 4˚C, Subsequently 700 μl RW1 buffer (Qiagen) was added to the RNeasy column and centrifuged for 15 sec at 4,000 x g at 4˚C, For washing, the column was transferred to a new tube and 500 μl of RPE buffer (Qiagen) was added, after which it was centrifuged at 4,000 x g for 15 sec at a temperature of 4˚C , this wash was performed twice, but in the second wash it was centrifuged for 2 min. Finally the column was transferred to a 1.5 ml collection tube, 50 μl of water were added, incubated for 1 min at room temperature and finally centrifuged at 4,000 x g for 1 min. RNA quantification was performed by spectrophotometry using the Nanodrop 2000 (Nanodrop; Thermo Fisher Scientific, Inc.), to verify the integrity, an electrophoresis was performed in gel of agarose at 1.5% and SYBR® Green was used for visualization (SYBR® Green I nucleic acid gel stain; cat no. 163795-75-3 Sigma-Aldrich; Merck KGaA).

RT-qPCR for SARS-CoV-2. Samples from the ISSSTE Hospital were processed at the state laboratory of public health of Puebla, Mexico. For the detection of SARS-CoV-2, VIASURE SARS-CoV-2 Real Time PCR Detection Kit (cat. no. VS-NCO213H; Certest Biotec) was used according to the manufacturer's manual. 15 μl rehydration buffer solution was added to the PCR plates (cat. no. VS-NCO213H, the tubes contained a mixture of enzymes, primers-probes, buffer and dNTPs), 5 μl each sample, the positive control and negative control were added and centrifuged briefly for 10 sec. PCR tubes were placed in the thermal cycler and the conditions for RT-qPCR were as follows: One cycle of 15 min at 4˚C, 1 cycle of 2 min at 95˚C, 45 cycles of 10 sec at 95˚C and 50 sec at 60˚C. before the results were uploaded onto the SISVER database (sisver.sinave.gob.mx/influenza/search_patients.php). Samples from the UPAEP were processed at the UPAEP Molecular Diagnostic Laboratory, samples were processed using a commercial kit (Logix Smart™ 2019-nCoV Kit cat. no. COVID-K-001-250-I; Co-Diagnostics), To perform RT-qPCR, 5 microliters master mix was added to the PCR tubes, 5 μl of the sample was added and centrifuged for 10 sec. The tubes were then placed in the thermal cycler (Applied Biosystems™ 7500 Fast Dx Real-Time PCR; Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following conditions, 1 cycle at 45˚C for 15 min, 1 cycle at 95˚C for 2 min, and 45 cycles at 95˚C for 3 sec and 55˚C for 32 sec. In all cases, those results with a Cq value <35 were considered positive. This kit is Food and Drug Administration (US FDA) approved for the diagnosis of SARS-CoV-2 infections in the clinic, which is a RT-qPCR technique using patented CoPrimers™ technology (24).

RT-LAMP assay. For the colorimetric RT-LAMP test for detecting SARS-CoV-2, a commercial test was used, RT-LAMP SARS-CoV-2 (cat. no. BMLAMP01; Amunet) that can detect the Nucleocapsid (N) gene and open reading frames 1a (ORF1a) gene of SARS-CoV-2. A set of six primers were used for each gene tested.

For the N gene, the following primers were used: Forward outer primer F3, 5'-AGATCACATTGGCACCCG-3' and reverse outer primer B3, 5'-CCATTGCCAGCCATTCTAGC-3'; loop forward primer, 5'-GCAATGTTGTTCCTTGAGGAAGTT-3' and loop backward primer, 5'-TCGTTCCTCATCACGTAG TCGC-3' and forward inner primer, 5'-TGCTCCCTTCTGCGT AGAAGCCAATGCTGCAATCGTGCTAC-3' and backward inner primer, 5'-GGCGGCAGTCAAGCCTCTTCCCTACTG CTGCCTGGAGTT-3'.

For the ORF1a gene, the following primers were used: Forward outer primer F3, 5'-AACATGGAGGAGGTGTTG C-3' and backward outer primer B3, 5'-CAAGTAGAACTT CGTGCTG-3'; loop forward primer, 5'-GTAGCTATGTAA TCATCAGA-3' and loop backward primer, 5'-TTGTCGGCC CAAATGTTAAC-3' and forward inner primer, 5'-ACTACC ACCCACTTTAAGTGTAACAATGCCATGCAAGTTG-3' and backward inner primer, 5'-ATCTTGCTAAACACTGTC TTCAGAAGTTGAATGTCTTCACC-3'.

Briefly, 400 μl isotonic saline solution (0.9% PISA CS Solution) was added to the nasal sample. Subsequently, 400 μl LAMP buffer was added to the saliva and nasal swab samples and mixed by vortexing for 10 sec. A microtube was prepared, which contained 5 μl 5X Master Mix LAMP and 2.5 μl primers (primer mix for gene 1 ORF1a), 2.5 μl primers (primer mix for 1 N gene), to which 5 μl sample was added and incubated for 30 min at 65˚C in a thermoblock. Subsequently, the microtube was placed in an ice bath for 30 sec. This is a colorimetric test, so it is performed by obtaining a color change (yellow is positive) and no change is negative (pink). All trials were double-blind.

LFIA test. The LFIA assay for detecting SARS-CoV-2 was performed using the SARS CoV-2 Antigen in Saliva Rapid Test (cat. no. SKU:89355; Amunet) according to the manufacturer's instructions. Nasopharyngeal swab samples were resuspended in 10 drops of the provided running solution. Subsequently, three drops of the preparation were incorporated into the sample area of the test cartridge, before the results were interpreted after 15 min. The appearance of two lines (control and test) was considered a positive result, whereas the appearance of only the control line was a negative result.

Detection limit assessment. To determine the detection limit of the RT-LAMP assay, the NATtrol™ SARS-CoV-2 [catalog number NATSARS(COV2)-ST), pGEM-ORF1a gene (cat. no. 0810624CFHI, ZeptoMetrix LLC, ORF1a gene, USA/PHC658/2021 lineage) and pGEM-N gene (cat. no. 0810624CFHI, ZeptoMetrix LLC, N gene, USA/PHC658/2021 lineage)] was used. RT-qPCR assay was performed using the Logix Smart™ 2019-nCoV commerce kit (cat. no. COVID-K-001-250-I; Co-Diagnostics), with the conditions previously described, as a control for this test, however we start from known concentrations emitted by the supplier. The minimum detection concentration was determined by using different dilutions (10^8, 10^6, 10^5, 10^4, 10^3, 10^2, 50 and 25 copy number), with 20 replicates performed for each serial dilution. RT-LAMP kit SARS-CoV-2 (cat. no. BMLAMP01) was used to perform the RT-LAMP test. The master mix was prepared with the corresponding primers as previously described in the RT-LAMP methodology and 5 microliters of sample were added with the aforementioned concentrations.

Statistical analysis. Counts and percentages were calculated for the categorical variables. The comparison of the Cq values among the groups was carried out using an individual value plot, We perform the unpaired t-student test. The efficiency of RT-LAMP as a diagnostic test was determined by calculating its sensitivity=True Positive/(True Positive + False Negative) and specificity=True Negative/(True Negative + False Positive), considering the RT-qPCR test as the gold standard. χ^2 test was used to compare the asymptomatic and symptomatic patients, and Fisher's test to compare the symptoms in female and male patients. Statistical analyses were performed using SPSS v.28 (IBM Corp.) and GraphPad Prism v. 8.0.0 for Windows (Dotmatics).

Results

Characteristics of the study population. A total of 128 samples, distributed as follows, were analyzed in the present study: 55 samples from symptomatic patients with COVID-19, 32 samples from asymptomatic patients and 41 control samples from disease-free individuals. A total of 74 (57.8%) of the participants were women. Classified by age, the majority of the participants 86 (67.1%), were aged between 18 and 39 years (Table I), 75% were between 18 and 39 years old (Table I; data not shown). The asymptomatic group did not present any symptoms or signs of the disease, whereas the three most frequent symptoms were cough, headache and odynophagia in the symptomatic group (Table I).

Cq values of symptomatic and asymptomatic individuals. A total of 87 samples were analyzed and confirmed to be positive for SARS-CoV-2 by RT-qPCR. Analyzing the Cq values, it was revealed that all positive symptomatic patients had Cq values <35, where 9.09% of the samples had Cq values between 31 and 35, 63.63% of the samples had Cq values between 21 and 30 and 27.27% of the samples had Cq values <20. Notably, 90.09% of the samples presented with Cq values <30 (Table II).

In the case of asymptomatic patients, the majority of the RT-qPCR Cq values were <30. Specifically, 3.1% of the samples had Cq values >30, 84.3% of the samples had Cq values between 31 and 30 and 12.5% of the samples had Cq values <20 (Table II). When comparing the distribution of the Cq values between the symptomatic and asymptomatic patients, reduced dispersion of data was observed in the asymptomatic patients, with the majority being concentrated around a Cq value of 25 (Fig. 1). No significant difference could be found between the Cq values of the symptomatic group and the asymptomatic group.

When analyzing all of the positive samples as a whole, it was revealed that 6.9% of the samples had Cq values >30, 71.3% had Cq values between 21 and 30 and 21.8% had Cq values <20 (Table II).

Results by type of test. A total of 128 samples were analyzed by RT-qPCR, of which 68% were positive for SARS-CoV-2 and 32% were negative. For the salivary and nasal RT-LAMP assays, 67.2% of samples were positive and 32.8% were negative, but one false positive and two false negatives were found. Regarding the LFIA test of saliva samples, 60.2% of the samples were positive and 39.8% were negative, where 10 false negatives were found (Table III).

Sensitivity and specificity of salivary and nasal RT-LAMP. The sensitivity and specificity of the salivary and nasal RT-LAMP test was determined using the following formula: Sensitivity=TP/(TP + FN); Specificity=TN/(TN + FP), TP are the true positive samples (positive samples by RT-qPCR), FN are the false negatives (Positive samples RT-qPCR but were negative RT-LAMP) and FP are false positives (Negative samples by RT-qPCR but were RT-LAMP positive). According to these data, for all samples (symptomatic and asymptomatic) a sensitivity of 97.7% and a specificity of 97.60% was obtained for salivary and nasal RT-LAMP (Table IV). When the analysis was specifically performed on the symptomatic or asymptomatic group, a sensitivity of 96.5% and a specificity of 97.6% for the detection of symptomatic individuals was found for salivary and nasal RT-LAMP, whereas a sensitivity of 100% and a specificity of 97.6% was obtained for the detection of asymptomatic individuals (Table IV).

Detection capacity assessment. To determine the detection capacity of SARS-CoV-2 by RT-LAMP assay, different concentrations of viral particles and NATtrol™ SARS-CoV-2 was used. It was observed that, when viral particles at concentrations of 10^8, 10^6, 10^5, 10^4, 10^3 and 10^2 copy number were used, the detection of NATtrol™ SARS-CoV-2, pGEM-ORF1a gene and pGEM-N gene was 100% by RT-LAMP. When viral particles at a concentration of 50 copy number were used, detection was 95% for the pGEM-ORF1a gene, 90% for the pGEM-N gene and 95% for NATtrol. Finally, when viral particles at a concentration of 25 copy number were used, detection was 75% for the pGEM-ORF1a gene, 70% for the pGEM-N gene and 85% for NATtrol (Table V).

Discussion

The present study analyzed the performance of the commercial RT-LAMP test in detecting SARS-CoV-2

Table I. Distribution of patients.

Characteristic	Total	Female	Male	P-value[a]
Group	n (%)	n (%)	n (%)	0.3236
Positive with symptoms	55 (43.0%)	35 (47.2%)	20 (37.0%)	
Positive without symptoms	32 (25.0%)	17 (22.9%)	15 (27.7%)	
Negative control	41(32.0%)	22 (29.7%)	19 (35.8%)	
Age				
18-39 years	86 (67.1%)	45 (60.8%)	41 (75.9%)	
40-59 years	36 (28.1%)	24 (32.4%)	12 (22.3%)	
≥60 years	6 (4.6%)	5 (6.7%)	1 (1.8%)	
Symptoms				P-value[b]
Cough	51 (92.7%)	33 (97.0%)	18 (85.7%)	0.6162
Headache	40 (72.7%)	26 (76.4%)	14 (66.6%)	0.7608
Myalgia	42 (76.3%)	27 (79.4)%	15 (71.4%)	0.7659
Odynophagia	43 (78.1%)	28 (82.3%)	15 (71.4%)	0.7401
Runny nose	33 (60.0)%	23 (67.6%)	10 (47.6%)	0.2702
Fever	17 (30.9%)	10 (29.4%)	7 (33.3%)	0.7630
Anosmia	6 (10.9%)	5 (14.7%)	1 (4.7%)	0.3995
Diarrhea	7 (12.7%)	4 (11.7%)	3 (14.2%)	0.6960
Dyspnea	1 (1.8%)	1 (2.9%)	0 (0.0%)	1.0000

A total of 128 patients (74 female and 54 men) were recruited to the present study, of which 55 patients had symptoms (34 women and 21 men). [a]χ^2 test, [b]Fisher's test.

Table II. Cq values of patients, as determined by reverse transcription-quantitative PCR.

Cq value range	All samples (n=87)	Symptomatic (n=55)	Asymptomatic (n=32)	P-value[a]
11-20.9	19 (21.8%)	15 (27.2%)	4 (12.5%)	0.1424
21-30.9	62 (71.2%)	35 (63.6%)	27 (84.3%)	
31-35	6 (6.8%)	5 (9.0%)	1 (3.1%)	

[a]Fisher's test.

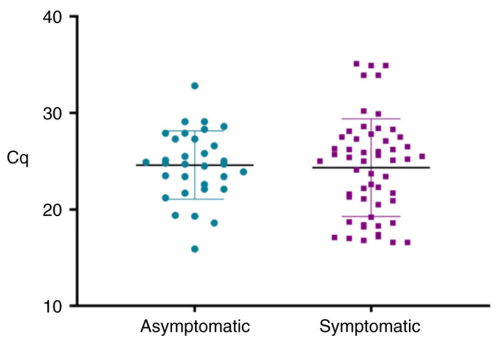

Figure 1. Dot plot of the distribution of Cq values obtained by reverse transcription-quantitative PCR. Unpaired Student's t-test was used to analyze data.

in symptomatic and asymptomatic patients from nasal and saliva samples without viral RNA extraction (direct RT-LAMP). RT-LAMP was shown to have high specificity and sensitivity (>96%) in detecting SARS-CoV-2 in both symptomatic and asymptomatic patients, suggesting it to be a robust and effective test for epidemiological monitoring, detecting cases and for preventing outbreaks. The present results are in agreement with those from other studies. González-González *et al* (25) previously reported a sensitivity of 92.8% and a specificity of 81% for RT-LAMP in nasopharyngeal samples, whereas Schneider *et al* (19) reported a sensitivity of 85.9% and a specificity of 99.5% in 443 saliva samples.

Notably, in the present study, the sensitivity and specificity values were similar for both salivary and nasal RT-LAMP tests, symptomatic DSe=96.5 asymptomatic DSe=100 and DSp=97.6. However, for the nasal sample a saline solution is also added. Therefore, these findings may be attributed to the fact that the RT-LAMP tests used in the present study consisted of a set of six primers that detect two genes, N and ORF1a, of SARS-CoV-2. This was verified by Rödel *et al* (26), who analyzed several commercial

Table III. Distribution of asymptomatic and symptomatic positive patients by type of test.

Test	Asymptomatic patients (n=32)	Symptomatic patients (n=55)	Total (n=87)
Reverse transcription-quantitative PCR	32 (100.0%)	55 (100.0%)	87 (100.0%)
Salivary RT-LAMP	32 (100.0%)	53 (96.4%)	85 (67.2%)
Nasal RT-LAMP	32 (100.0%)	53 (96.4%)	85 (67.2%)
Salivary lateral flow immunoassay	24 (75.0%)	53 (96.4%)	77 (88.5%)

Data are presented as n (%). RT-LAMP, reverse transcription loop-mediated isothermal amplification.

Table IV. Sensitivity and specificity of nasal RT-LAMP test for the detection of severe acute respiratory syndrome coronavirus 2 compared with RT-quantitative PCR.

Category	All samples (overall result)	Symptomatic patients	Asymptomatic patients	Negative controls
A, Salivary RT-LAMP				
Positive	85 (97.7%)	53 (96.4%)	32 (100.0%)	1 (2.4%)
Negative	2 (2.2%)	2 (3.6%)	0 (0.0%)	40 (97.6%)
Total	87 (100.0%)	55 (100.0%)	32 (100.0%)	41 (100.0%)
DSe/DSp	DSe=97.7	DSe=96.5	DSe=100	DSp=97.6
B, Nasal RT-LAMP				
Positive	85 (97.7%)	53 (96.4%)	32 (100%)	1(2.4%)
Negative	2 (2.2%)	2 (3.6%)	0 (0.0%)	40 (97.6%)
Total	87 (100.0)	55 (100.0%)	32 (100.0%)	41 (100.0%)
DSe/DSp	DSe=97.7	DSe=96.5	DSe=100.0	DSp=97.6
C, Lateral flow immunoassay (nasal)				
Positive	75 (86.2%)	51 (92.7%)	24 (75%)	1 (2.4)
Negative	12 (13.7%)	4 (7.2%)	8 (25%)	40 (97.6%)
Total	87 (100.0%)	55 (100.0%)	32 (100.0)	41 (100.0)
DSe/DSp	DSe=87.8	Dse=93.2	Dse=80.0	Dsp=97.6

Data are presented as the n (%). Dse, diagnostic sensitivity; DSp, diagnostic specificity; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RT, reverse transcription.

Table V. Reverse transcription loop-mediated isothermal amplification sensitivity for the detection of each control plasmid corresponding to its respective gene.

Gene (copy number)	10^8	10^6	10^5	10^4	10^3	10^2	50	25
ORF1a gene	100%	100%	100%	100%	100%	100%	95%	75%
N gene	100%	100%	100%	100%	100%	100%	90%	70%
NATtrol™ SARS-CoV-2	100%	100%	100%	100%	100%	100%	95%	85%

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; open reading frames a1 gene (ORF1a), Nucleocapsid (N).

RT-LAMP tests for detecting SARS-CoV-2, who concluded that the sensitivity and specificity tended to improve when the N and ORF1ab genes are analyzed in combination. On the other hand, Artik in 2021, also found high sensitivity and specificity of the RT-LAMP test, using the SARS-CoV-2 ORF 8 and N genes, without the need for a previous RNA extraction step (27). For this reason we decided to use an RT-LAMP test kit that analyses two genes.

RT-LAMP test in saliva and nasal swabs showed a high sensitivity (97.7%) and specificity (97.6%) compared with the RT-qPCR test in the present study. Saliva sampling presents a minimally invasive test that avoids uncomfortable

nasopharyngeal sampling. Furthermore, saliva has been shown to present viral loads similar to those found in nasopharyngeal samples (28-30). Cook *et al* (31) recommended the salivary RT-LAMP test to monitor COVID-19 in the general population outside of health care facilities, who also revealed that the detection capacity of SARS-CoV-2 in saliva by RT-LAMP does not differ significantly from the detection in nasopharyngeal samples, even in asymptomatic patients. In addition, it has been recommended that the RT-LAMP test be performed using saliva samples within the first 9 days of disease onset (32). In the present study, all tests of symptomatic patients were performed during this interval, this being an important variable that may be associated with the high specificity and sensitivity found in the present study.

In the present study, the commercial test did not require a viral RNA extraction step, which may facilitate the logistics in the processing of samples in places with poor access to public health services and infrastructure. The present study performed screening in asymptomatic outpatients from a university and individuals from a hospital far from the urban area with little infrastructure for the detection of SARS-CoV-2. This implemented methodology, without purification of genetic material, has also been reported for RT-LAMP in other previous studies. Kidd *et al* (33) reported minimal differences in the sensitivity and specificity of RT-LAMP for saliva samples, compared with those from nasal and oropharyngeal swabs. Furthermore, this previous study reported that the sensitivity and specificity values of salivary RT-LAMP were 84.62 and 100, respectively, for Cq <45, compared to sensitivity of 99.1 and specificity of 100 for Cq values <25 detected by RT-qPCR of ORF1ab (33).

Fowler *et al* (34) performed RT-LAMP for SARS-CoV-2 with nasopharyngeal samples, reporting a sensitivity of 97% and a specificity of 99% in samples with RNA extraction, and a sensitivity of 75% and a specificity of 97% in samples without RNA extraction. This research group associates these results with the performance of using Cq values, since they reported 100% specificity and 97% sensitivity in Cq values <25 in samples without RNA extraction, likewise they report that in samples with extraction of RNA a specificity of 100% and a sensitivity of 99%.

Furthermore, Baba *et al* (35) reported 98% sensitivity with Cq values <30 and demonstrated that the sensitivity decreases as Cq values increase. However, this change was not drastic, since in this previous study, a sensitivity of 97% was identified at Cq <35 (35). In the present study, the analyzed samples presented Cq values between 11 and 35, and we found high efficiency of the RT-LAMP test to detect patients positive for SARS-CoV-2 . This is likely because the commercial kit in the present study detects two genes instead of one, unlike the study by Fowler *et al* which only detects the *ORF1ab* gene (34).

Notably, the present study detected 100% sensitivity for salivary and nasal RT-LAMP in the detection of asymptomatic cases, which is essential to prevent the spread of the virus, especially in those with high viral loads. These results support the findings of other groups, such as Brown *et al* (36), who also reported 100% sensitivity using direct RT-LAMP for the detection of SARS-CoV-2 in saliva samples, confirming that it is a viable alternative technique to RT-qPCR for detection of new cases.

Previous studies have shown the efficacy of RT-LAMP concerning the gold standard RT-qPCR technique, it is also an economical technique and fast that do not require specialized personnel (16,25,27). The advantage of not requiring prior RNA extraction is that it allows minimal handling and does not require specialized personnel. Therefore, RT-LAMP could be self-applied at home, applied in the community or in hospitals lacking in facilities for detecting SARS-CoV-2. The present study screened outpatients from the academic community and patients from a hospital located in a rural area which does not have specialized equipment to perform RT-qPCR. Additionally, the present study revealed that salivary RT-LAMP has high sensitivity and specificity. The use of this type of sample represents an advantage by reducing invasiveness (32,33). In addition, pilot studies could be implemented in difficult-to-access geographical areas for the identification of vulnerable groups (20).

In conclusion, the present study revealed that RT-LAMP of SARS-CoV-2 may be considered a promising, low-cost tool that can be applied in regions that do not have specialized equipment. In addition, RT-LAMP had high sensitivity and specificity in both symptomatic and asymptomatic patients. Finally, no difference was detected between nasopharyngeal samples and saliva samples, so it is more comfortable for patients to take a saliva sample for an RT-LAMP test to detect SARS-CoV-2 virus infection in comparison to taking a nasopharyngeal swab for an RT-qPCR test.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RRM and PSI performed the experiments, analyzed and interpreted data, and wrote the manuscript, MNQ and EBR confirm the authenticity of all the raw data. DVR, DIG, CFA, GSC, AOS, NPS, VRR, EEK, JES, LCM, KLM and CAM conducted nasal, saliva and pharyngeal sample collection and performed the routine LFIA. MRD, EBR and MNQ acquired, analyzed and interpretated the data. MRD made substantial contributions to the conception of the study. MRD, EBR and NQM designed the experiments and wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This protocol was approved by the Research Ethics Committee of the Dean of Health Sciences of the Popular Autonomous University of the State of Puebla (approval no. CONBIOETICA21CEI00620131021) and the Institute of Security and Social Services for Service Workers (approval no. 09-CEI-019-01607729). Patients provided written informed consent for participation. All procedures in studies involving human participants were performed in accordance with the ethical standards of the institutional and national research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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