Article

Fast detection of SARS-CoV-2 RNA directly from respiratory samples using a loop-mediated isothermal amplification (LAMP) test

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Abstract: The availability of simple SARS-CoV-2 detection methods is crucial to contain the COVID-19 pandemic. This study examined whether a commercial LAMP assay can reliably detect SARS-CoV-2 genomes directly in respiratory samples without having to extract nucleic acids (NA) beforehand. Nasopharyngeal swabs (NPS, n = 220) were tested by real-time reverse transcription (RT)-PCR and with the LAMP assay. For RT-PCR, NA were investigated. For LAMP, NA from 26 NPS in viral transport medium (VTM) were tested. The other 194 NPS were analyzed directly without prior NA extraction [140 samples in VTM; 54 dry swab samples stirred in phosphate buffered saline]. Ten NPS were tested directly by LAMP using a sous-vide cooking unit. The isothermal assay demonstrated excellent specificity (100%) but moderate sensitivity (68.8%), with a positive predictive value of 1 and a negative predictive value of 0.65 for direct testing of NPS in VTM. The use of dry swabs, even without NA extraction, improved the analytical sensitivity; up to 6% of samples showed signs of inhibition. The LAMP could be performed successfully with a sous-vide cooking unit. This technique is very fast, requires little laboratory resources and can replace rapid antigen tests or verify reactive rapid tests on site.

Keywords: SARS-CoV-2; COVID-19; RT-PCR; nucleic acids; direct testing; loop-mediated isothermal amplification; LAMP

1. Introduction

The ongoing pandemic caused by the *severe acute respiratory syndrome coronavirus* 2 (SARS-CoV-2) is a major challenge for humankind. The virus has infected more than 120 million people worldwide with a death toll of more than two million (1). The impact of SARS-CoV-2 on health care systems and economy is huge. From the beginning of the pandemic, the demand for rapid, reliable, cheap and easy-to-use diagnostics has been great and, even taking into account the progress made, there is still a big gap between supply and demand. This is especially true in low-resource settings, where specialized equipment and personnel is often lacking (2).

Real-time reverse transcription polymerase chain reaction (RT-PCR) has been considered the gold standard for the detection of SARS-CoV-2 due to its high specificity and sensitivity (3). It is, however, not without drawbacks, as it requires cost-intensive instruments (specialized equipment) and laboratory skills. Additionally, it is time-consuming

and the consumables are expensive, making it an unattractive option for low-resource settings. Furthermore, the reagents and materials required for RT-PCR are often scarce or unavailable, so that the development and evaluation of alternative methods is urgently required (4). In addition to the now widespread rapid antigen tests, loop-mediated isothermal amplification (LAMP) could be a useful alternative. This method can be performed without the use of thermocyclers and tends to be faster than RT-PCR. Several LAMP assays for SARS-CoV-2 detection have been developed (4), a limited number of them have been evaluated using clinical samples with promising initial results (5-10).

Here we evaluate the performance of a commercially available LAMP assay for the detection of SARS-CoV-2 in respiratory tract samples compared to RT-PCR. Among other things, it is investigated to what extent the LAMP method is suitable for the direct detection of SARS-CoV-2-RNA without prior extraction of the nucleic acids (NA). Furthermore, the test results are compared depending on the sampling system used.

2. Materials and Methods

Loop-mediated isothermal amplification for SARS-CoV-2 detection

The performance of a research use only LAMP assay (SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit, New England Biolabs, Ipswich, USA) was tested under different conditions as indicated below. The LAMP reaction was conducted as recommended by the manufacturer. In brief, 2 µl of NA or 2 µl of the original respiratory material (swab in viral transport medium, VTM or dry swab stirred in 500 µl phosphate-buffered saline, PBS; 20 µl pre-incubated for 10 min at 99°C) were pipetted together with 23 µl LAMP-Reaction-Mix consisting of 12.5 µl WarmStart Colorimetric LAMP 2X Master Mix (containing Uracil-DNA Glycosylase), 2.5 µl SARS-CoV-2 LAMP Primer Mix (targeting parts of SARS-CoV-2 E and N-genes), 5.5 µl nuclease-free water and 2.5 µl guanidine hydrochloride in a 100 µl PCR reaction vessel. For each sample, a second separate vessel was prepared in order to demonstrate the presence of human NA and absence of inhibition of reaction. For this purpose, 2.5 µl of the Internal Control LAMP Primer mix (rActin) were used instead of the SARS-CoV-2 LAMP Primer Mix. Furthermore, a separate positive control (2 µl SARS-CoV-2 Positive Control) and a non-template control (2 µl Nuclease-free Water) were included in each experimental setting. Thereafter, all vessels were incubated for 30 minutes at 65°C in a thermocycler (unless otherwise stated) and immediately placed at room temperature for 5 min followed by recording of the color of the reaction mixture. Yellow color was valued as positive while pink was valued as negative.

Deep nasopharyngeal swab samples (NPS) stirred in PBS

We tested 54 dry NPS for the detection of SARS-CoV-2 in the Labor Krause MVZ GmbH, Kiel, Germany. The swabs were stirred in 500 µL sterile PBS without Calcium and Magnesium (Lonza, Basel, Switzerland) and tested for SARS-CoV-2 RNA using the LAMP assay and a triplex in-house RT-PCR designed for the detection of SARS-CoV-2 N-gene RNA as described before (11). This RT-PCR uses primers and probes for the simultaneous detection of SARS-CoV-2 N-gene, Pseudomonas phi6 phage, and human glyceraldehyde-3-phosphate dehydrogenase. In the case of the RT-PCR, NA extraction was preferentially performed with the KingFisher Flex system and with the ABI7500 real time thermocycler (both Thermo Fisher Scientific, Waltham, MA USA) as described previously (11).

Deep NPS in VTM with and without NA extraction for LAMP

We included 140 NPS for the detection of SARS-CoV-2 tested in the Institute for Virology of University Hospital Essen. The swab collection kits contained VTM (Yocon virus sampling kit, Yocon Biology, Beijing, China), which was

used for both the PCR and the LAMP as described above. Detection of the SARS-CoV-2 genomes via RT-PCR was performed using the RealStar® SARS-CoV-2 RT-PCR kit 1.0 (Altona Diagnostics, Hamburg, Germany) on CFX Connect Real-Time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA), and the NA extraction was performed using the MagNA Pure 96 System (Roche, Mannheim, Germany) according to the manufacturer´s instructions. In the case of 26 additional samples prior to performing the LAMP, instead of heat inactivation, we applied the same NA extraction protocol as for the RT-PCR: 18 NPS were tested at the Insitute of Virology, Essen using the methods described above, while 8 NPS were tested at the Institute of Virology, University Hospital Bonn, after NA extraction (AltoStar AM16, Altona Diagnostics) with both LAMP and RT-PCR (Altona Diagnostics) on CFX Connect Real-Time PCR detection system.

Viral load in different RT-PCRs used by the laboratories

The number of RT-PCR cycles required for the fluorescent signal to exceed the background level (threshold) was recorded as threshold cycle (Ct). In order to achieve comparability between the results produced by the three laboratories, we tested two SARS-CoV-2 standard samples previously provided by INSTAND e.V. (Düsseldorf, Germany) as well as dilutions of them. The 10^4, 10^5, 10^6 and 10^7 SARS-CoV-2 copies/ml corresponded to Ct values of 31.4, 26.3, 23.1 and 20.2 (E-gene, Altona, Essen), 36.4, 32.8, 29.6 and 26.6 (N-gene, in-house triplex RT-PCR, Kiel) and 29.5, 26.1, 22.9, and 19.3 (E-gene, Altona, Bonn) respectively. Linear regression was performed for both series of values and the viral load in copies/ml was calculated based on equation deriving from it.

To evaluate crossreactivity, samples that tested positive by RT-PCR for endemic coronaviruses (HCoV NL63, HCoV OC43, HCoV 229E, HCoV HKU1), influenza A virus, respiratory syncytial virus, human metapneumovirus, human rhinovirus, or human bocavirus 1, or several bacterial species (*Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Haemophilus influenzae*, *Bordetella pertussis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes, Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*), were tested using the LAMP assay. Analyses for the above mentioned viruses were performed by using the FTD Respiratory pathogen 21 assay (Fast Track Diagnostics, Mikrogen, Munich, Germany). Most samples containing viral pathogens were collected before the emergence of SARS-CoV-2. In case of sampling from 2020 onwards, SARS-CoV-2 RT-PCR was negative. The viral samples were present in VTM or in some cases only as NA. For the bacteria, 100 µl of a SARS-CoV-2 free NPS stirred in 500 µl PBS were spiked each with a fresh colony of an ATCC or clinical strain of *Haemophilus influenzae, Bordetella pertussis, Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*, respectively. For *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, 10 µl of inactivated and resolved external quality assessment samples were spiked into 90 µl of the NPS. All samples were mixed, heat-inactivated for 10 min at 99°C (except for those only available as NA) and tested as described by LAMP. Amplification of rActin was noted as absence of inhibition.

Assessment of test samples for the improvement of diagnostic procedures has been approved by the ethics committees of the medical faculties of the University of Duisburg-Essen (20-9512-BO) and Kiel University (D467/20). Statistical analysis was performed using SPSS software (v23, SPSS Inc., Chicago, IL, USA), GraphPad Prism 6.0 (GraphPad, CA, USA) and the platform VassarStats (http://vassarstats.net).

3. Results

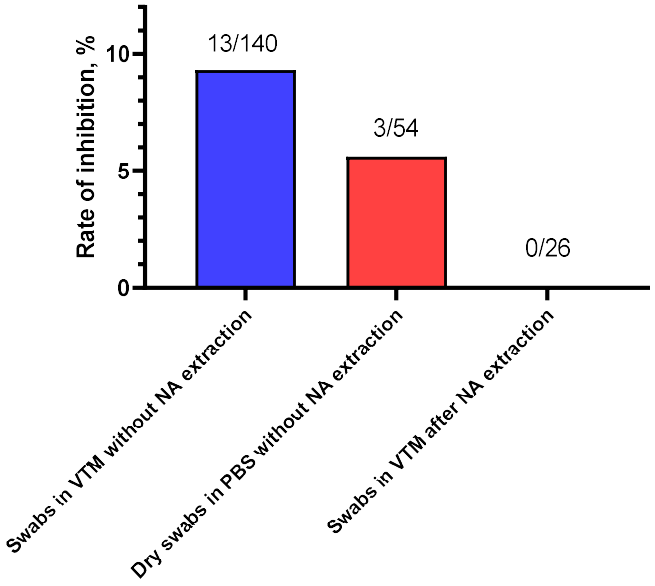
Swabs in VTM: moderate sensitivity, good specificity, frequent inhibition

We tested 140 consecutive NPS for the presence of SARS-CoV-2 RNA. The samples were acquired from the community through the public health department and included patients with possible/suspected SARS-CoV-2 infection based on clinical and/or epidemiological reasons. The samples were tested with RT-PCR after NA extraction (reference method) and LAMP after incubation at 99°C for 10 min but without chemical extraction. Inhibition in LAMP was

frequent (13 samples with no evidence of rActin signal, 9.3%) (Figure 1A). We observed no false positive results, but had 25 (19.7%) false negative samples. True positive results could be observed in 55 (43.3%) samples and true negatives in 47 (37.0%), amounting to a sensitivity of 68.8 % (CI 95% 57.3-78.4), a specificity of 100% (CI 95% 90.6-100), a positive predictive value of 1 (CI 95% 0.92-1), and a negative predictive value of 0.65 (CI 95% 0.53-0.76). A total of 64.7% (n = 55/85) of the samples were correctly identified as positive, while 5.9% (n = 5/85) of the positive samples showed signs of LAMP inhibition. The sensitivity of the method improved, when taking into account samples with increasingly higher viral load (Figure 1B).

All samples (n=33) with a viral load over 10^7 copies/ml, 83.3% (n=15/18) of the samples with a viral load between 10^6 and 10^7 copies/ml, 50.0% (n=5/10) of the samples with a viral load between 10^5 and 10^6 copies/ml and 20.0% (n=2/10) of the samples with a viral load between 10^4 and 10^5 copies/ml were detectable with LAMP. None of the nine samples with viral load less than 10^4 copies/ml could be detected with LAMP (Figure 1C).

A



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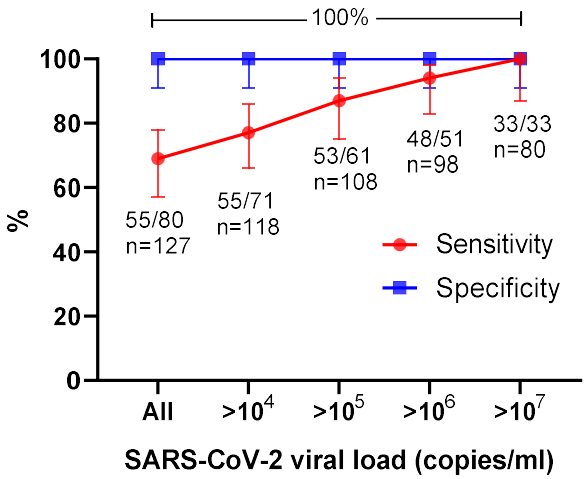

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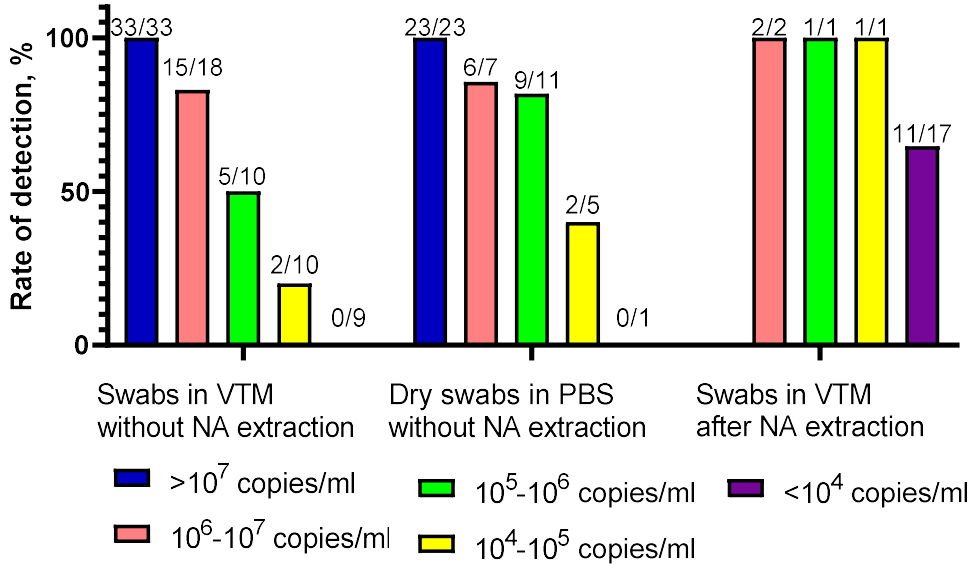

Figure 1: **Inhibition rate and diagnostic performance of the loop-mediated isothermal amplification reaction (LAMP) assay.** A) The inhibition rate of the LAMP assay (i.e. failure in detection of rActin) was relatively high in nasopharyngeal swab samples tested without nucleic acid (NA) extraction. The numbers above the columns indicate the number of inhibited samples / number of samples tested using the LAMP assay. B) The sensitivity and specificity of the LAMP assay without prior NA extraction was compared to RT-PCR after NA extraction (n=140). Stratification was performed with the inclusion of all or a fraction of the samples according to their viral load in copies/ml. Inhibited samples were excluded. The sensitivity of the LAMP assay increased with increasing viral load. The specificity amounted to 100% irrespective of stratification. The error bars show the 95% confidence intervals. The numbers under the lines indicate the number of samples tested positive using the LAMP assay / number of samples tested positive using RT-PCR, n= number of negative and positive samples above the below mentioned viral load threshold per RT-PCR. C) The rate of detection differed amongst the three groups of samples. Dry swabs without NA extraction outperformed swabs in viral transport medium (VTM) without NA extraction. Performing NA extraction prior to testing increased the detection rate for samples with low viral loads considerably. The numbers above the columns indicate the number of samples tested positive using the LAMP assay / number of samples tested positive using RT-PCR.

Dry swabs in PBS: higher detection rate in samples with low viral load compared to swabs in VTM

We tested 54 consecutive NPS for the presence of SARS-CoV-2 RNA. NPS were collected as dry swab and stirred in PBS as described above. The samples were tested with both RT-PCR after NA extraction, serving as the reference method, and LAMP after viral inactivation but without extraction. Three samples were inhibited (5.6%) (Figure 1A). All four SARS-CoV-2 free samples were correctly identified, while seven (14.9%, n=7/47) of the positive samples were misidentified as false negative. Samples with low viral load in the RT-PCR were more often undetectable with LAMP than samples with high viral load. All samples (n=23) with a viral load exceeding 10^7 copies/ml were detectable and nearly all samples (85.7%, n=6/7 and 81.8%, n=9/11) with a viral load between 10^6 and 10^7 and 10^5 and 10^6 copies/ml, respectively. The detection rate for samples with a viral load between 10^4 and 10^5 copies/ml fell to 40.0% (n=2/5), while one sample with less than 10^4 copies/ml was undetectable with LAMP (Figure 1C).

Additionally, we tested 18 samples (eight with a SARS-CoV-2 load of over 10^7 copies/ml and ten negative for SARS-CoV-2) in parallel with a rapid lateral flow antigen test (NADAL® COVID-19 Ag Test, Nal von Minden, Moers, Germany), with RT-PCR serving as reference method. Both methods could correctly identify all samples as positive or negative. Furthermore, two false-positive samples by another lateral flow antigen test (SARS-CoV-2 Rapid Antigen Test, Roche) were tested negative by LAMP following the 99°C inactivation protocol.

Swabs in VTM after NA extraction: enhanced detection rate for samples with a low viral load, no inhibition

We tested 26 NPS in VTM after NA extraction with both LAMP and RT-PCR. Fifteen (57.7%) samples tested positive in both methods and four (15.4%) samples tested negative in both methods. One sample (3.8%) tested positive in LAMP but not in the RT-PCR, while six (23.1%) tested positive in the PCR but not the LAMP. There was no evidence of LAMP inhibition for these samples (Figure 1A). All samples with a viral load higher than 10^4 copies/ml and 64.7% (n=11) of samples with a viral load less than 10^4 copies/ml were detectable with LAMP (Figure 1C).

Sous-vide and LAMP: a thermocycler is not required

We tested the performance of the LAMP assay using a commercially available sous-vide cooking unit for households (Russell Hobbs 25630-56 Sous-Vide, Russel Hobbs, Failsworth, UK). We used heat-inactivated samples (without NA extraction), including a total of three negative samples and seven SARS-CoV-2 positive samples. The samples were also tested using RT-PCR (RealStar® SARS-CoV-2 RT-PCR kit 1.0) after NA extraction (MagNA Pure, Roche). The SARS-CoV-2 viral load of the positive samples ranged from 10^5 to 10^7 copies/ml. The samples were immersed in the unit for 30 minutes, which was set at 65°C. During the 30 minutes period the sous-vide cooking unit managed to retain this temperature plus/minus 1°C. Reaction vessels were placed into a plastic bag during amplification. All samples were correctly identified as positive or negative with the LAMP assay (Figure 2).

A	SARS-CoV-2 copies/ml (RT-PCR)	LAMP
1	$2.3*10^7$	positive
2	$4.2*10^6$	positive
3	$3.1*10^6$	positive
4	$3.9*10^5$	positive
5	$2.4*10^5$	positive
6	$1.2*10^5$	positive
7	$1.1*10^5$	positive
8	negative	negative
9	negative	negative
10	negative	negative

RT-PCR= Real-time reverse transcription polymerase chain reaction.

Figure 2: **Loop-mediated isothermal amplification (LAMP) assay without a thermocycler.** A) Comparison of RT-PCR *vs* the LAMP assay performed in a household sous-vide cooking unit. B) Picture of the sous-vide cooking unit during testing, and (C) of the samples after completion of the assay.

No crossreactivity with frequent respiratory viruses and bacteria

Samples positive for endemic coronaviruses (HCoV NL63, HCoV OC43, HCoV 229E, HCoV HKU1), different influenza A virus types, respiratory syncytial virus, human rhinovirus, human metapneumovirus, human bocavirus-1 and nine bacterial species were tested with LAMP. None of these pathogens was detected by the LAMP assay. The results of the crossreactivity evaluation for the viral pathogens are shown in Table 1 in more detail.

Table 1: Results of the Loop-mediated isothermal amplification (LAMP) assay performed on respiratory samples positive for common pathogens.

Pathogen	Ct of the RT-PCR (if available)	Number of samples tested directly	Number of samples tested after preparation of NA	Result of the LAMP for SARS-CoV-2
HCoV HKU1	18.6-28.5	4	-	negative
HCoV NL63	18.3-28	4	10	negative
HCoV 229E	17.1-27.8	3	-	negative
HCoV OC43	18.6-24.9	2	1	negative
IAV H1N1	18.8-24.3	3	-	negative
IAV H3N2	23.2	1	-	negative
IAV non H1N1, non H3N2	19.5-24.7	5	-	negative
RSV	18-22.3	12	-	negative
RSV + HRV	20.7-23 + 30.1-35	3	-	negative
RSV + HBoV1	18.8-20.6 + 35-35	2	-	negative
HMPV	15.3-23.6	3	3	negative
HMPV + HRV	24.5 + 20.2	1	-	negative
HBoV1	13.1-14.6	2	-	negative
Haemophilus influenzae	n.a.	1	-	negative
Bordetella pertussis	n.a.	1	-	negative
Streptococcus pneumoniae	n.a.	1	-	negative
Streptococcus pyogenes	n.a.	1	-	negative
Staphylococcus aureus	n.a.	1	-	negative
Escherichia coli	n.a.	1	-	negative
Klebsiella pneumoniae	n.a.	1	-	negative
Mycoplasma pneumoniae	n.a.	1	-	negative
Chlamydia pneumoniae	n.a.	1	-	negative

Ct: Cycle threshold; NA: nucleic acids; RT-PCR: reverse transcription real-time PCR; n.a., not available; HCoV: human coronavirus; RSV: respiratory syncytial virus; HBoV1: human bocavirus; HMPV: human metapneumovirus; HRV: human rhinovirus; IAV: influenza A virus

4. Discussion

Improving and expanding our diagnostic capacity in light of the COVID-19 pandemic is crucial. Several LAMP assays for SARS-CoV-2 detection have been developed (4), a limited number of them have been evaluated using clinical samples with promising initial results (5-10). A previous study presenting the performance of a LAMP assay indicated excellent specificity (99.5%) for NPS samples but moderate sensitivity (86%) when foregoing NA extraction (5). Another study evaluating 106 leftover NPS showed that 63% could be correctly identified as SARS-CoV-2 positive by a LAMP assay without NA extraction, the percentage went up to 83% when including samples with a viral load greater than 10^6 copies/ml (12). Performing LAMP using QuickExtract® lysates directly prepared from crude patient respiratory tract

swab samples led to the correct identification as positive of 50 to 64% of the samples, depending on the swab volume (8).

We tested 140 NPS samples in VTM, taken in a community setting through the public health department from individuals with possible/suspected SARS-CoV-2 infection due to clinical and/or epidemiological reasons. The LAMP assay carried out from VTM without NA preparation demonstrated excellent specificity (100%), but only moderate sensitivity (69%). Only 64% of the samples were correctly identified as positive, while 6% of the positive samples were inhibited. The detection rate of the method increased considerably for samples with high(er) viral load. These results indicate that without NA extraction the LAMP assay cannot compete with the combination of RT-PCR and NA extraction in terms of analytical sensitivity. However, it can reliably identify individuals with high viral load, which is relevant, when aiming to reduce transmission in a community setting.

We evaluated different test strategies in terms of both usability and diagnostic performance. Focusing on a NA extraction-free approach, we demonstrated that using dry swabs suspended in PBS led to a better detection rate for samples with lower viral loads compared to swabs in VTM. Compared to the PBS (without magnesium and calcium), VTM contains a number of additives. We suspect that these could interfere with direct testing of low viral loads. Previous studies have shown that dry swabs are appropriate for PCR detection of respiratory viruses, including SARS-CoV-2 (13, 14). This is relevant, since using dry swabs could lower the cost of the sampling kit and provide an alternative in a setting, where procuring VTM is difficult. Furthermore, viral isolation in cell-culture is also successful from dry swabs stirred in PBS (11). Unsurprisingly, implementing NA extraction prior to the performance of LAMP increased the analytical sensitivity of the method considerably compared to the extraction free protocols.

The greatest advantage of LAMP compared to RT-PCR is that it can be carried out quickly. Direct testing of NPS takes around 45 minutes per sample. Furthermore, isothermal amplification can be readily implemented without use of specialized laboratory equipment as demonstrated in our study, making it an attractive option in low-resource settings and in the case of RT-PCR reagents not being available. The LAMP assay is also scalable, allowing for the testing of a small number of samples to high throughput using 96-well thermoblocks.

On-site testing with rapid SARS-CoV-2 antigen tests is assigned an important role in combating the COVID-19 pandemics. The greatest advantage of this kind of point-of-care (POCT) tests is their ease of use and the extremely short time span of 15 to 20 minutes until the test result is available. However, their performance varies markedly (9, 15, 16) and test sensitivities depend on the viral load, the time-point of sampling and the presence of symptoms (15, 17, 18). Importantly, although many POCTs demonstrate high specificity, false positive results have been reported (15, 17, 19). Thus, positive POCT results should be confirmed by RT-PCT (20). The analytical sensitivity and specificity of the LAMP assay is likely to be superior, when compared with different POCTs (11, 17, 19) and largely meets requirements of the World Health Organization (21), especially when using dry swabs. Because of these advantages, the LAMP could replace rapid tests. However, if fast testing is in the foreground, it would alternatively be conceivable to use the LAMP for on-site confirmation of reactive rapid tests. To do this, however, another NPS would have to be taken.

A strength of our study is the comprehensive evaluation of a LAMP assay for the detection of SARS-CoV-2 RNA under different conditions. Yet, our study has also limitations. Sampling was performed through different healthcare professionals, so that a standardization beyond following the instructions given by the health authorities was not possible. Furthermore, the samples were tested in three laboratories, using different RT-PCR assays. A comparison of the results with one another was made possible by using nationally available standard samples. Nevertheless, we believe that our results are relevant as they reflect the everyday testing routine and can be of use to the scientific community. The demand for SARS-CoV-2 diagnostics remains unabated and bottlenecks in resources are frequent.

Expanding the diagnostic capacity and diversifying the diagnostic methods to fit different circumstances, for example low-resource settings, seems prudent.

In conclusion, the LAMP assay demonstrated excellent specificity, but moderate sensitivity compared to RT-PCR. The isothermal amplification method can be implemented without the use of specialized laboratory equipment and may be useful for replacement of POCTs or the immediate confirmation of reactive antigen tests.

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Institutional Review Board Statement: Assessment of test samples for the improvement of diagnostic procedures has been approved by the ethics committees of the medical faculties of the University of Duisburg-Essen (20-9512-BO) and Kiel University (D467/20).

Data Availability Statement: The data presented in this study can become in part available on request from the corresponding author. The data are not publicly available due to data protection issues.

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