Saliva-Dry LAMP: A Rapid Near-Patient Detection System for SARS-CoV-2.

Noah Toppings
University of Calgary
Abu Mohon
University of Calgary
Yoonjung Lee
University of Calgary
Hitendra Kumar
University of Calgary
Daniel Lee
University of Calgary
Raitk Kapoor
University of Calgary
Gurmukh Singh
University of Calgary
Lisa Oberding
University of Calgary
Omar Abdullah
University of Calgary
Keekyoung Kim
University of Calgary
Byron Berenger
University of Calgary
Dylan Pillai (drpillai@ucalgary.ca)
University of Calgary

Research Article

Keywords: infectious, SARS-CoV-2, COVID-19

Posted Date: January 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-141322/v1

Abstract

The highly infectious nature of SARS-CoV-2 necessitates the use of widespread testing to control the spread of the virus. Presently, the standard molecular testing method (reverse transcriptase-polymerase chain reaction, RT-PCR) is restricted to the laboratory, time-consuming, and costly. This increases the turnaround time for getting test results. The study sought to develop a rapid, near-patient saliva-based test for COVID-19 with similar accuracy to that of standard RT-PCR tests. A lyophilized dual-target reverse transcription-loop-mediated isothermal amplification (RT-LAMP) test with fluorometric detection by the naked eye. The assay relies on dry reagents that are room temperature stable. A device containing a centrifuge, heat block, and blue LED light system was manufactured to reduce the cost of performing the assay. This test has a limit of detection of 1 copy/µL and achieved positive percent agreement of 100% [95% CI 88.43% to 100.0%] and negative percent agreement of 96.7% [95% CI 82.78% to 99.92%] on saliva. Saliva-Dry LAMP can be completed in 105 minutes. Precision, cross-reactivity, and interfering substances analysis met international regulatory standards. The combination of ease of sample collection, dry reagents, visual detection, low capital equipment cost, and excellent analytical sensitivity make Saliva-Dry LAMP particularly useful for resource-limited settings.

Introduction

Due to the highly infectious nature of SARS-CoV-2 and its ability to be transmitted by asymptomatic individuals[1], widespread testing for COVID-19 is critically important to preventing the spread of the virus[1]. The COVID-19 pandemic has put immense demands on molecular testing infrastructure[2,3]. Presently, the standard method for COVID-19 testing is real-time polymerase chain reaction (RT-PCR)[4–6]. This method cannot be deployed outside of a laboratory. However, for the sake of contact tracing and self-isolation, the utility of a test relates to how quickly one can receive the results of the test after the sample is obtained[7,8]. Tests which require transporting samples to a centralized laboratory increases this time. Not surprisingly, governments have pushed for the immediate development of rapid, near-patient tests for COVID-19[9,10]. Near-patient tests must yield straight-forward results which are easy to interpret. For remote settings, these tests should not be reliant on cold-chains and sophisticated equipment. To meet this immediate need, we developed Saliva-Dry LAMP, a rapid, near-patient saliva test for COVID-19 that uses lyophilized dual-target reverse transcriptase-loop-mediated isothermal amplification (RT-LAMP) with fluorometric detection by the naked-eye[11]. This test can be performed on a portable and low-cost device that we manufactured.

Methods

Patient Samples and Ethics:

Clinical specimens used in this study were anonymized saliva from individuals in Alberta collected between May and September 2020. No clinical information was obtained. Saliva was collected in UTM®-

RT (COPAN Diagnostics Inc., Murrieta, USA) for ease of use[12]. The research involves human participants and was performed in accordance with relevant guidelines/regulations. Informed consent was obtained from all participants and/or their legal guardians, and was approved by Conjoint Health Research Ethics Board (CHREB) at the University of Calgary (REB20-0402/0444).

RNA Extraction:

Saliva diluted in universal transport media (140 µL total) was mixed with 560 µL of a concentrated preparation of lysis buffer and spiked with 2 µL of 50,000 pfu/µL MS2 bacteriophage (Zeptometrix, Buffalo, NY). Buffers used are described previously by Zainabadi *et al.*[13]. This lysate was hand shaken, then incubated at 61 ºC for 5 minutes. The lysate was applied to a spin column (Omega Bio-Tek Inc., Norcross, USA) and spun in a mySPIN™ 12 (Thermo Fisher Scientific Inc., Waltham, USA) for 110 seconds at a peak speed of 11,300 RPM. The flow-through was discarded and 500 µL of wash 1 was applied to the column. The column was centrifuged again (110 seconds, 11,300 RPM) and the flow-through discarded. Next, 500 µL of wash 2 from was applied to the column before centrifugation for 170 seconds at a peak speed of 11,300 RPM. Columns were then transferred to new collection tubes and 50 µL of elution buffer was added. RNA was eluted with a final spin (110 seconds, 11,300 RPM).

Lyophilized RT-LAMP Reactions(“Dry LAMP”):

Lyophilized RT-LAMP reactions for the detection of SARS-CoV-2 were prepared by Pro-Lab Diagnostics Inc. (Richmond Hill, Canada) using patented dual-target primers[14] and the GspSSD2.0 Isothermal Mastermix (ISO-004) (OptiGene Ltd., Horsham, UK). These pellets were dissolved in 10 µL resuspension buffer R1 (Pro-Lab Diagnostics Inc.) and 0.5 µL of dye mix (5.95 mM hydroxynapthanol blue trisodium salt, 69.5 X GelGreen®) (Biotium, Fremont, USA). Next, 14.5 µL of extracted RNA was added to each dissolved pellet. Reactions were mixed and then 30 µL of mineral oil was added on top. Reactions were incubated for 45 minutes at 61 ºC in an IncuBlock™ Mini Dry Bath (Thomas Scientific, Swedesboro, USA) then visualized under an LED transilluminator (MaestroGen Inc., Hsinchu City, ROC). Positive reactions appear bright green while negative reactions appear orange (Fig. 2).

MS2 external amplification controls were ran in parallel in separate lyophilized RT-LAMP reactions prepared by Pro-Lab Diagnostics Inc. using the primers from Benzine *et al.*[15]. Lyophilized reactions were dissolved with 15 µL of resuspension buffer R1, 0.5 µL dye mix and 4.5 µL elution buffer. Reactions contained 5 µL of extracted RNA. Reactions were run simultaneously with SARS-CoV-2 reactions at 61 ºC for 45 min and visualized as described above. These lyophilized MS2 reactions were used with both the Biobox and commercially-available instruments.

Reference RT-PCR:

The US Centres for Disease Control and Prevention N1/N2-gene RT-PCR was performed according to CDC-006-00019, Revision: 01[16] on the corresponding nasopharyngeal swab collected concomitantly with the saliva sample on which LAMP was performed.

Droplet Digital-PCR:

A high titre positive sample was quantified using a Bio-Rad QX200™ Droplet Digital™ (dd) PCR system relying on the Bio-Rad SARS-CoV-2 ddPCR Kit (Bio-Rad Laboratories, Hercules, CA)[17]. The ddPCR master mix consisted of (per sample) 2.5 µL One-Step RT–ddPCR reverse transcriptase, 6.25 µL One-Step RT–ddPCR Supermix, 1 µL 300 mmol/L dithiothreitol, 1 µL of each forward and reverse primers, 0.5 µL probe (20 µM primers and 10 µM probe), 7.5 µL RNase-free water, and 5 µL of extracted RNA. A 20 µL aliquot of each template mastermix was added to the sample well of the droplet generation cartridge, with 70 µL of droplet generation oil for probes. Thermocycling was done with the Bio-Rad C1000 Touch™ Thermal Cycler before measurement with the QX200™. Cycling conditions were 50 °C for 1 hour, 95 °C for 10 min, 40 cycles of 95 °C for 30 sec and 60 °C for 60 sec, then 98 °C for 10 min. Ramp rates were 2 °C/sec.

Limit of Detection Studies:

The limit of detection using commercially-available instruments and the Biobox was determined using a patient sample (nasopharygeal swab diluted in 25% saliva, 75% UTM[18]) which was quantified by the Bio-Rad SARS-CoV-2 ddPCR Kit (Bio-Rad Laboratories, Hercules, CA). This sample was serially diluted to achieve a range from 1 to 0.25 copies/µL.

Cross-reactivity and interfering substance studies:

Potentially cross-reactive respiratory pathogens were tested with Saliva-Dry LAMP using inactivated stocks from Zeptometrix (Buffalo, USA) (Table S2). For interference testing (Table S3), negative samples and samples contrived to 9X LOD were spiked at the indicated concentrations with substances expected to be commonly found in saliva.

Clinical Validation:

Saliva is not collected routinely for COVID-19 diagnosis in Alberta. Given the low prevalence of COVID-19 in Alberta during this study, saliva and corresponding NP swab samples had to be collected from individuals who previously tested positive by RT-PCR. Clinical saliva samples were selected to reflect the natural distribution of viral loads in the population during early infection (See Figure S1). Plots, and 95% confidence intervals (Clopper-Pearson) were performed using MATLAB R2020b (The Mathworks Inc., Natick, USA).

Biobox fabrication:

A custom-made device, termed “Biobox” (Fig. 3), was developed to execute the sequence of steps for Saliva-Dry LAMP – centrifugation, isothermal incubation and naked-eye fluorescent detection respectively. The Biobox comprised of three components – centrifuge, heating block and transilluminator

(470 nm light emitting diode, LED, arrays). The design was prepared using Solidworks™ 2020 (Dassault Systems, Waltham, USA). All housing parts/fixtures were fabricated using a fused deposition modeling (FDM) 3D printer (Anycubic C, Commerce, USA) with poly-lactic acid (PLA) filament unless specified. The centrifuge rotor was fabricated using polycarbonate filament. The transilluminator consists of two LED arrays – a 6 x 8 LED array mounted inside the Biobox and a pair of 2 x 8 LED arrays mounted on the sides of the cap to provide illumination from the sides. A second cap was placed on the transilluminator with acrylic sheet window to block the wavelengths emitted by the LED's but not the intercalating dye. The aluminum heating block was machined to house both 2 mL and 1.5 mL microcentrifuge tubes. The temperature of the heating block was maintained at 61 ^{o}C using three heating elements and three thermocouple sensors. The centrifuge was made with a direct current (DC) powered brushless motor (T-motor F40 Pro3 2600Kv, Nanchang, P.R.C.) mounted on an aluminum bracket. The centrifuge rotor was mounted on the brushless motor and achieved 8000 RCF. All components were controlled by an ESP32 microprocessor. The device is operated through the user interface using an LCD display and pushbuttons. A DC power supply of 21–23 V was used to power the device.

Saliva-dry LAMP performed on the Biobox:

The lyophilized RT-LAMP reagents for the amplification of SARS-CoV-2 on the Biobox were obtained from Illucidx Inc. (Calgary, Canada). Lyophilized reactions consisted of the master mix described previously[14] but employed the dye mix described above. A proprietary excipient mix was also added. For extractions on the Biobox, conditions were identical as those on the commercially-available instruments with the exception that centrifugation times were 50 seconds shorter (due to faster ramping rates). Lyophilized reactions were resuspended with 25 µL of extracted RNA, mixed, then 30 µL of mineral oil was added on top. LAMP was run for 45 minutes at 61 ^{0}C and visualized with the Biobox LED transilluminator.

Results

Analytical study of Saliva-Dry LAMP:

The saliva dry-LAMP kit's workflow, on the Biobox, is depicted in Fig. 1. The limit of detection was determined with commercially-available instruments and the Biobox using a dilution series of a quantified contrived sample which spanned 1-0.25 copies/µL (Table 1). All replicates tested positive at 0.5 copies/µL when using commercially available centrifuge and dry bath, whereas 4/5 were positive on the Biobox. A limit of detection confirmation was conducted using 20 replicates at 0.5 copies/µL prepared in the same way as described previously. Limit of detection confirmation was then retried successfully at 1 copy/µL (19/20 positive) (Table S1).

Table 1

Limit of detection determined by dilution series for Saliva-Dry LAMP using commercially-available instruments and the Biobox manufactured in this study. A single experiment performed in quadruplicate or quintuplicate is shown at each concentration using a contrived saliva sample containing SARS-CoV-2.

Sample Concentration	Positive Reactions	
	Commercially-available Instruments	Biobox
1 copies/µL	4/4	4/5
0.5 copies/µL	4/4	3/5
0.25 copies/µL	1/4	0/5

Clinical Validation:

Clinical validation was conducted on 60 unique clinical saliva samples (~ 25% saliva, ~ 75% UTM). Considering that no gold standard method exists for saliva yet, positive percent agreement (PPA) and negative percent agreement (NPA) was calculated (Table 2). The CDC reference RT-PCR were run on the corresponding NP swab as reference methods (Figure S1). Saliva-Dry LAMP achieved a PPA of 100% [95% CI 88.43–100.0%] and an NPA of 96.7% [95% CI 82.78–99.92%] (Table).

Table 2

Saliva Dry-LAMP clinical validation using paired saliva and NP swabs obtained compared to the reference standard CDC RT-PCR method. PPA: positive percent agreement, NPA: negative percent agreement, S: spike, RdRP: RNA-dependent RNA polymerase.

Saliva-Dry LAMP (S + RdRP)	CDC RT-PCR on paired NP swab		Total
	Positive	Negative	
Positive	30	1	31
Negative	0	29	29
Total	30	30	60
PPA	100% [95% CI 88.43–100.0%]		
NPA	96.7% [95% CI 82.78–99.92%]		

Assay Precision, Cross-reactivity, and Interference:

None of the 11 potentially cross-reactive respiratory pathogens tested showed any cross reactivity with Saliva-Dry LAMP *in vitro* (Table S1). None of the 18 potentially interfering medicines/substances tested showed any interference with Saliva-Dry LAMP *in vitro* (Table S2). The assay precision was confirmed with two samples twice a day for 20 days (Table S3). Variation arising from equipment was determined adequate with five samples per day on three different sets of instruments for five days (Table S4).

Performance of Saliva-Dry LAMP on the Biobox:

In order to demonstrate functionality of the instrument designed and manufactured by our group (Biobox, Fig. 3), a limit of detection study was performed with Saliva-Dry LAMP. The limit of detection was determined using a dilution series of a quantified contrived sample which spanned 1-0.25 copies/µL. Four out of five replicates tested positive at 1.0 copies/µL.

Discussion

In this study, we have developed a rapid, near-patient, saliva test for COVID-19 using lyophilized LAMP reagents with fluorometric detection by the naked-eye. Experiments were designed to satisfy regulatory standards. Saliva-Dry LAMP showed no cross-reactivity or interference from any tested respiratory pathogens or medicines, respectively (Table S1, S2). In silico analysis by Mohon et al. did not identify any primer cross-reactivity in 13 relevant respiratory pathogens either[14]. As an RT-LAMP test, this method uses different reagents than RT-PCR, thus averting some supply chain bottlenecks and export restrictions[2,3,19]. Saliva-Dry LAMP detects SARS-CoV-2 from saliva, instead of the specimens from the standard nasopharyngeal swab, as there is a higher likelihood of detecting virus in saliva than detecting virus in nasopharyngeal swab specimens during the early phase of infection when diagnostic testing is most useful[20]. Saliva can be collected without a healthcare worker[20,21] and self-collection does not induce coughing, sneezing or bleeding[21,22]. Therefore, saliva collection avoids depleting critical supplies of PPE and swabs while reducing healthcare worker demand and exposure[20,21]. Saliva is also favourable for testing children as NP swabs are invasive[21].

An important area of ongoing development for point-of-care nucleic acid tests is rapid RNA extraction. Standard laboratory RNA extractions are very time-consuming; however, replacing an RNA purification step with a simple inactivation step can compromise assay sensitivity [14,23,24] . RNA purifications result in the concentration of viral RNA and the removal of amplification inhibitors, both of which increase sensitivity. Some rapid RNA extraction methods exist, but many of them require a cold-chain [25–28] . The streamlined, column-based RNA extraction developed for Saliva-Dry LAMP purifies RNA in under 30 minutes while concentrating RNA 2.8-fold and costing only $3.15 (CAD) per preparation. Using commercially-available equipment, this test has a throughput of 10 samples per batch. Manufacture of the Biobox instrument reduced the capital equipment cost 5-fold from US $1977.44 to US $386.72 (Table S5 and S6), while still achieving an excellent limit of detection. Saliva-Dry LAMP has a capital equipment cost an order of magnitude less than RT-PCR when using commercially-available instruments, and a capital equipment cost nearly two orders of magnitude less than RT-PCR when using the Biobox (S. Rudgar, personal communication). Either of these options enables the deployment of Saliva-Dry LAMP in resource-limited settings.

The performance characteristics, limit of detection, and ease-of-use of Saliva-Dry LAMP is comparable to other commercial near-patient nucleic acid COVID-19 tests. STOPCovid is a rapid diagnostic test that

employs LAMP and CRISPR technology but relies on nasopharyngeal swabs[25]. Saliva-Dry LAMP achieved similar sensitivity as STOPCovid (93.1% sensitivity, 188/202) and specificity (98.5% specificity, 197/200)[25]. Lalli *et al.* developed an extraction-free RT-LAMP test for COVID-19 using saliva with colorimetric detection[28]. Lalli *et al.* achieved a slightly higher limit of detection than Saliva-Dry LAMP (59 copies/reaction or 21.6 copies/µL of saliva) but the clinical validation achieved a sensitivity of 85% (17/20) and a specificity of 90% (9/10) The test performed well compared to an internationally recognized reference method. The one false positive was likely a sampling error for the NP swab as the concomitant saliva sample was positive (CDC N2 RT-PCR positive, Ct 25.97). However, the clinical validation set did not contain more samples with very late Ct values in the 35–40 range which in our experience fail to amplify by LAMP. Due to the paucity of *in vivo* studies, the clinical and epidemiological significance of high Ct value (> 35), low viral copy, individuals remains unclear[29,30]. *In vitro* studies suggest individuals with low viral loads are rarely infectious or not infectious[29,30]. The analytical sensitivity of a diagnostic test is not the only measure by which it should be judged[8]. In terms of limiting the transmission of SARS-CoV-2, the advantages of Saliva-Dry LAMP are its superior sample-to-result time (~ 105 minutes) compared to RT-PCR, near-patient deployment, ease of application, and cost[8].

This method has its limitations. Firstly, the positive controls used in this study are not room-temperature stable reagents and require cold chain. Second, while the sample-to-result time near the patient is useful, the time required to perform the test is in approximately 105 minutes with a minimal throughput of four samples per run. Finally, the equipment developed still requires electricity and further refinements to increase the portability. A second prototype of the Biobox relying on a lithium-ion battery is feasible and is currently being evaluated. Future studies will aim to port Saliva-Dry LAMP onto a microfluidic cartridge, improving speed, and point-of-care feasibility.

Declarations

Competing Interests:

DRP is scientific advisor to Illucidx Inc., a University of Calgary start-up company supported by Innovate Calgary, which holds patents related to LAMP technology. All other authors declare no conflicts of interest.

Funding:

Funds were provided through the Canadian Institutes for Health Research (NFRFR-2019-00015, DRP) and Fast Grants (Thistledown Foundation, 10028555).

Acknowledgements:

We thank Cody Doolan and Claire Kamaliddin for research technical support. We also thank Kanti Pabbaraju and Linda Ward for assistance with specimen collection. We thank Doru Gorzo and Elite Machining Ltd. For providing aluminum.

Author Contributions:

The first author named is the lead author. Conceptualization: DRP, KK; Methodology: NBT, AMN, BB, YL, HK, DL, RK, GS, LO, DRP, KK; Software: DL, RK, GS, NBT; Formal Analysis: DL, RK, GS, HK, YL, NBT, LO; Investigation: NBT, AMN, DL, RK, BB, GS, HK, YL, LO, OA; Data Curation: NBT; Writing – Original Draft: NBT, DRP; Writing – Reviewing & Editing: All authors; Visualization: YL, HK, OA, NBT; Supervision: DRP, BB, KK; Funding Acquisition: DRP.

Data Availability:

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

References

1. Hu, Z. *et al.* Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. *Sci. China Life Sci.* **63**, 706–711 (2020).
2. Ivanov, D. Predicting the impacts of epidemic outbreaks on global supply chains: A simulation-based analysis on the coronavirus outbreak (COVID-19/SARS-CoV-2) case. *Transp. Res. Part E Logist. Transp. Rev.* **136**, 101922 (2020).
3. Sarata, A. K. COVID-19 Testing: Key Issues.*Congr. Res. Serv.*1–3(2020).
4. Centres for Disease Control and Prevention. Real-time RT-PCR Primers and Probes for COVID-19 | CDC. *cdc.gov* (2020). Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html. (Accessed: 3rd November 2020)
5. Corman, V. M. *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance.* **25**, 2000045 (2020).
6. Freire-Paspuel, B. *et al.* Evaluation of nCoV-QS (MiCo BioMed) for RT-qPCR detection of SARS-CoV-2 from nasopharyngeal samples using CDC FDA EUA qPCR kit as a gold standard: An example of the need of validation studies. *J. Clin. Virol.* **128**, 104454 (2020).
7. Smith, D. S., Richey, E. A. & Brunetto, W. L. A Symptom-Based Rule for Diagnosis of COVID-19. *SN Compr. Clin. Med.* 1–8 https://doi.org/10.1007/s42399-020-00603-7 (2020).
8. Larremore, D. B. *et al.* Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. *Sci. Adv.* https://doi.org/10.1126/sciadv.abd5393 (2020).
9. Public Health Agency of Canada. Government of Canada's research response to COVID-19 - Canada.ca. (2020). Available at: https://www.canada.ca/en/public-health/services/publications/diseases-conditions/covid-19-government-canada-research-response.html. (Accessed: 3rd June 2020)
10. National Institutes of Health. Rapid Acceleration of Diagnostics (RADx). *nih.gov* (2020). Available at: https://www.nih.gov/research-training/medical-research-initiatives/radx/radx-programs. (Accessed: 5th November 2020)

11. Notomi, T. *et al.* Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **28**, e63 (2000).
12. Berenger, B. M. *et al.* Saliva collected in universal transport media is an effective, simple and high-volume amenable method to detect SARS-CoV-2. *Clin. Microbiol. Infect.* https://doi.org/10.1016/j.cmi.2020.10.035 (2020).
13. Zainabadi, K. *et al.* A novel method for extracting nucleic acids from dried blood spots for ultrasensitive detection of low-density Plasmodium falciparum and Plasmodium vivax infections. *Malar. J.* **16**, 377 (2017).
14. Mohon, A. N. *et al.* Optimization and clinical validation of dual-target RT-LAMP for SARS-CoV-2. *J. Virol. Methods.* **286**, 113972 (2020).
15. Benzine, J. W. *et al.* Molecular diagnostic field test for point-of-care detection of ebola virus directly from blood. *J. Infect. Dis.* **214**, S234–S242 (2016).
16. Centers for Disease Control and Prevention. *CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel For Emergency Use Only Instructions for Use*. (2020).
17. Taylor, S. C., Laperriere, G. & Germain, H. Droplet digital PCR versus qPCR for gene expression analysis with low abundant targets: from variable nonsense to publication quality data. *Sci. Rep.* **7**, 1–8 (2017).
18. Lagerlöf, F. & Dawes, C. The volume of saliva in the mouth before and after swallowing. *J. Dent. Res.* **63**, 618–621 (1984).
19. Nkengasong, J. Let Africa into the market for COVID-19 diagnostics. *Nature.* **580**, 565–565 (2020).
20. Wyllie, A. L. *et al.* Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N. Engl. J. Med.* **383**, 1283–1286 (2020).
21. López-Martínez, B. *et al.* Saliva as a promising biofluid for SARS-CoV-2 detection during the early stages of infection. *Bol. Med. Hosp. Infant. Mex.* **77**, 228–233 (2020).
22. To, K. K. W. *et al.* Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect. Dis.* **20**, 565–574 (2020).
23. Ben-Assa, N. *et al.* Direct on-the-spot detection of SARS-CoV-2 in patients. *Exp. Biol. Med.* **245**, 1187–1193 (2020).
24. Yoshikawa, R. *et al.* Development and evaluation of a rapid and simple diagnostic assay for COVID-19 based on loop-mediated isothermal amplification. *PLoS Negl. Trop. Dis.* **14**, e0008855 (2020).
25. Joung, J. *et al.* Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing. *N. Engl. J. Med.* **383**, 1492–1494 (2020).
26. Vogels, C. B. *et al.* SalivaDirect: A simplified and flexible platform to enhance SARS-CoV-2 testing capacity. *medRxiv* 2020.08.03.20167791 (2020). doi:10.1101/2020.08.03.20167791
27. Ladha, A. *et al.* A 5-min RNA preparation method for COVID-19 detection with RT-qPCR. medRxiv 1–3(2020). doi:10.1101/2020.05.07.20055947

28. Lalli, M. A. *et al.* Rapid and extraction-free detection of SARS-CoV-2 from saliva by colorimetric reverse-transcription loop-mediated isothermal amplification. Clin. Chem. hvaa267(2020).
29. Singanayagam, A. *et al.* Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. *Eurosurveillance.* **25**, 1–5 (2020).
30. La Scola, B. *et al.* 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.* **39**, 1059–1061 (2020).

Figures

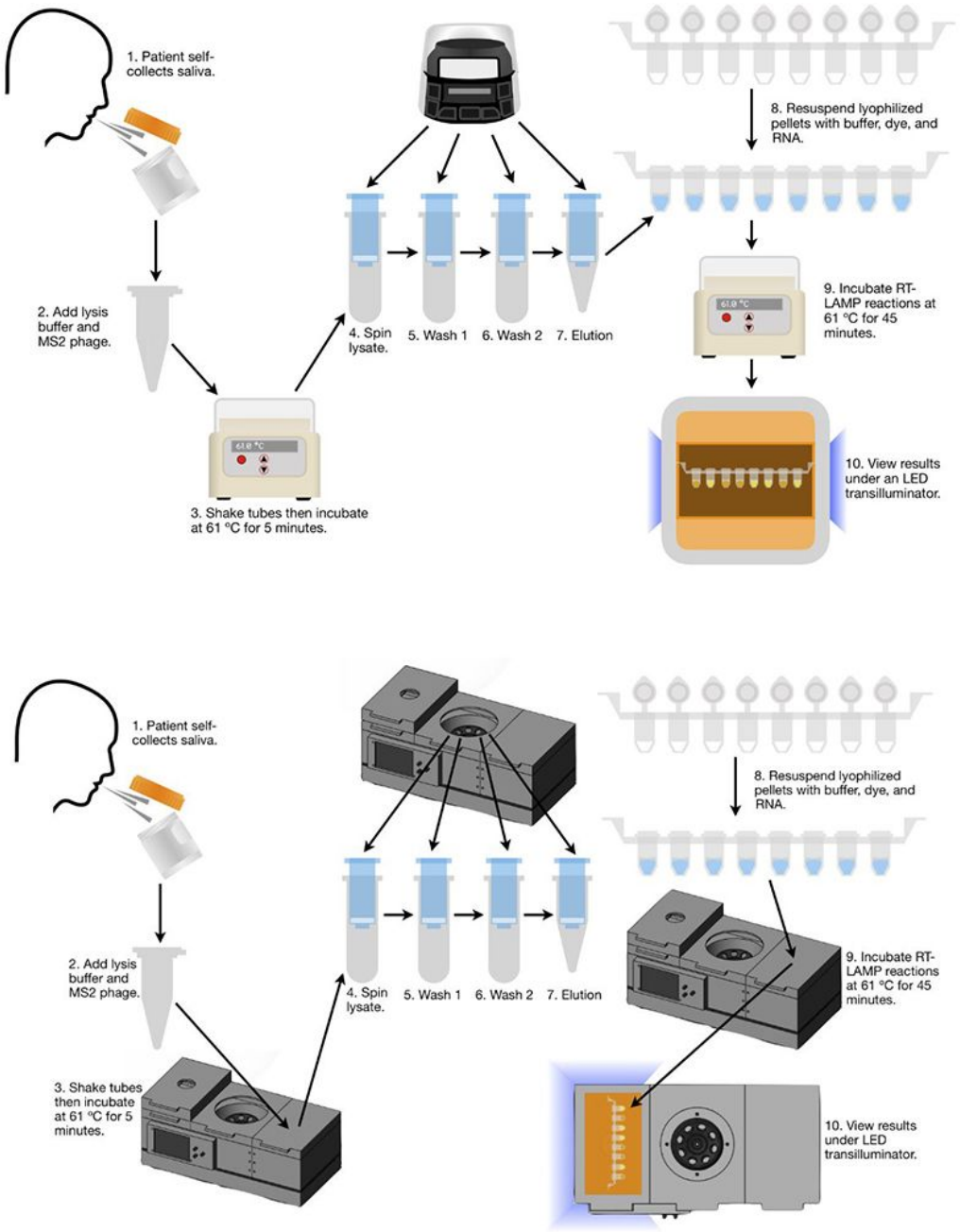

Figure 1

Saliva-Dry LAMP workflow diagram and equipment requirements. The sample processing workflow for conducting Saliva-Dry LAMP on (A) commercially-available instruments and (B) the Biobox.

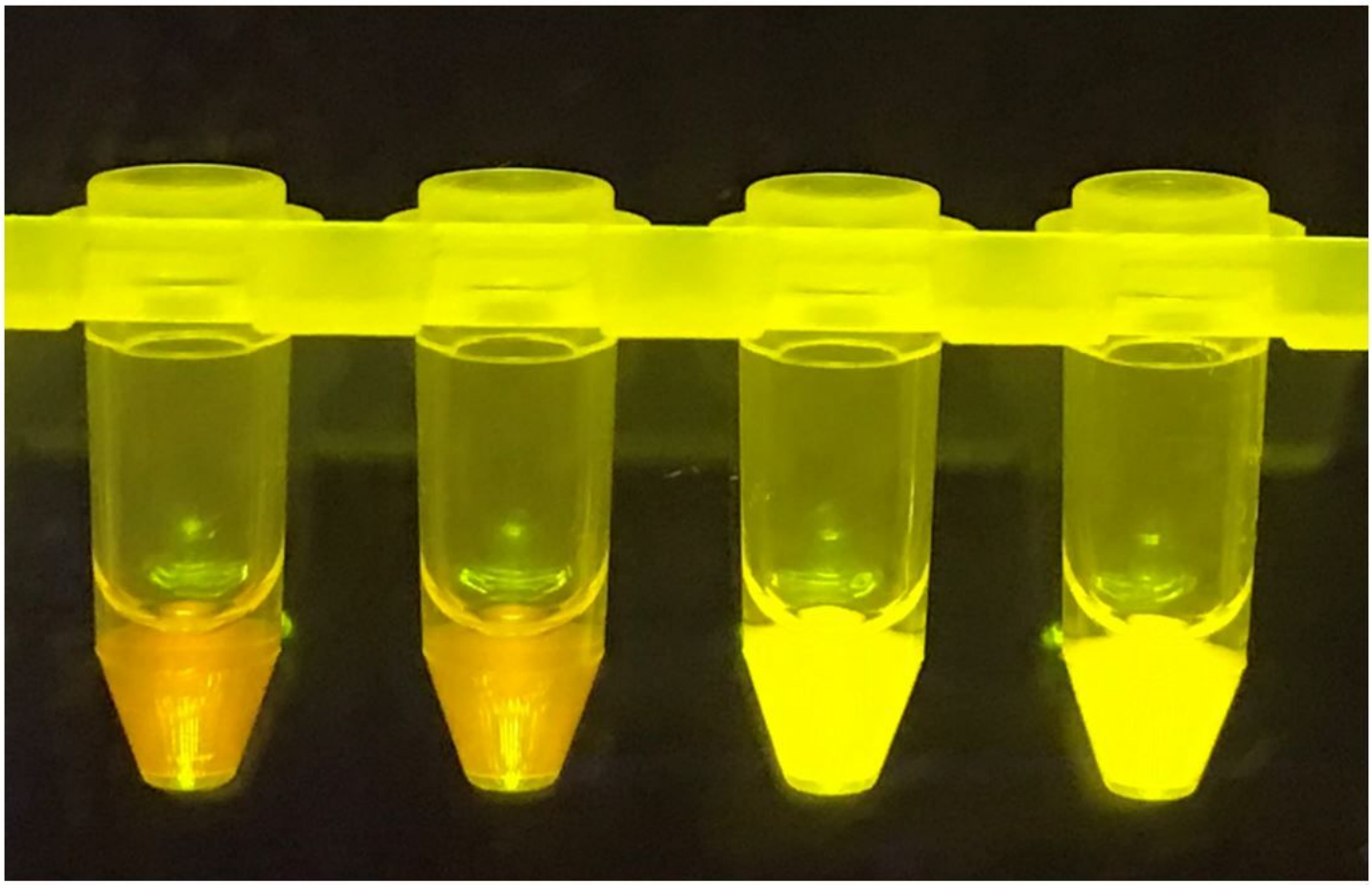

Figure 2

Visual results of Saliva-Dry LAMP reactions as viewed under a blue light transilluminator. The two reactions on left are negative (orange) and the two reactions on the right (green) are positive read outs.

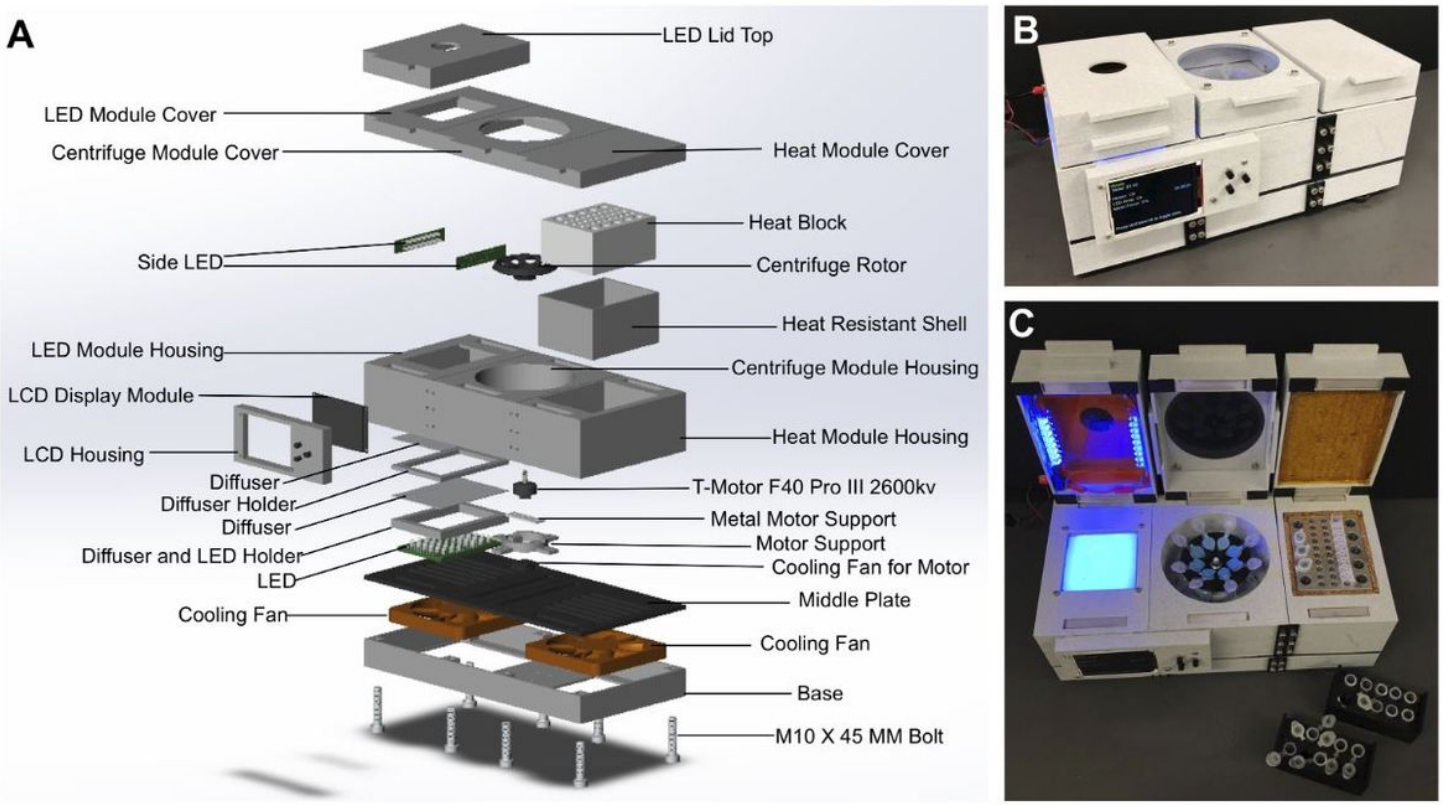

Figure 3

“Biobox” manufactured to perform the Saliva-Dry LAMP experiments. A) Computer-aided design drawing with exploded view of the “Biobox”. The device is comprised of a heat block, centrifuge, and blue LED transilluminator which meet specifications to perform the Saliva-Dry LAMP reaction. Photographs of the side (B) and top (C) view of the Biobox are shown for reference.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SciRepSupplementalMaterialJan132021.pdf