WHoTLaMP: A simple, inexpensive, and sensitive molecular test for the detection of SARS-CoV-2 in saliva

David Ng¹, Ana Pinharanda², Merly C. Vogt³, Ashok Litwin-Kumar¹, Kyle Stearns⁴, Urvashi Thopte¹, Enrico Cannavo¹, Armen Enikolopov, Felix Fiederling¹, Stylianos Kosmidis¹, Barbara Noro¹, Ines Rodrigues-Vaz¹, Hani Shayya¹, Peter Andolfatto², Darcy S. Peterka¹, Tanya Tabachnik¹, Jeanine D’Armiento⁶, Monica Goldklang⁴,⁵, Andres Bendesky¹,⁶,*

¹Zuckerman Mind Brain Behavior Institute, Columbia University, New York, NY, 10027, USA
²Department of Biological Sciences, Columbia University, New York, NY, 10027, USA
³Howard Hughes Medical Institute, Columbia University, New York, NY, 10027, USA
⁴Department of Anesthesiology, Columbia University Irving Medical Center, New York, NY, 10032, USA
⁵Center for LAM and Rare Lung Diseases, Columbia University Irving Medical Center, New York, NY, 10032
⁶Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, NY, 10027, USA

† Contributed equally

* Correspondence: a.bendesky@columbia.edu

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
Abstract

Despite the development of effective vaccines against SARS-CoV-2, epidemiological control of the virus is still challenging due to slow vaccine rollouts, incomplete vaccine protection to current and emerging variants, and unwillingness to get vaccinated. Therefore, frequent testing of individuals to identify early SARS-CoV-2 infections, contact-tracing and isolation strategies remain crucial to mitigate viral spread.

Here, we describe WHotLAMP, a rapid molecular test to detect SARS-CoV-2 in saliva. WHotLAMP is simple to use, highly sensitive (3.6 viral RNA copies per microliter of saliva) and specific, as well as inexpensive, making it ideal for frequent screening. Moreover, WHotLAMP does not require toxic chemicals or specialized equipment and thus can be performed in point-of-care settings, and may also be adapted for resource-limited environments or home use. While applied here to SARS-CoV-2, WHotLAMP can be modified to detect other pathogens, making it adaptable for other diagnostic assays, including for use in future outbreaks.
Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus with high transmissibility that causes the Coronavirus Disease of 2019 (COVID-19) (Petersen et al., 2020; C. Wang et al., 2021). Unlike SARS-CoV-1, where infectiousness is mostly restricted to the symptomatic phase (Cheng et al., 2004), ~50% of SARS-CoV-2 transmissions occur 1-2 days before symptom onset or through people who never develop symptoms (Casey et al., 2020; Johansson et al., 2021; Walsh et al., 2020). Thus, screening for symptoms is simply not enough to stop SARS-CoV-2 transmission (Paltiel et al., 2020).

Testing, combined with contact tracing and social isolation, along with physical barriers such as face masks and distancing became staple strategies to reduce community spread (Stuart et al., 2021). However, in many countries, viral spread has been difficult to contain. This is partly due to insufficient testing infrastructure, which leads to long delays in both access to testing and in obtaining test results. This lag greatly reduces the effectiveness of contact-tracing and isolation strategies (Larremore et al., 2020; Mina et al., 2021).

Despite the development of safe and effective vaccines against SARS-CoV-2, the threat of the virus remains high because of the logistical difficulties of global vaccination, limited supply of vaccine doses, and reluctance to get vaccinated (Wouters et al., 2021). Moreover, the emergence of SARS-CoV-2 variants that lower the protection conferred by natural or vaccine-induced immunity, suggests that testing will remain an important tool to reduce viral transmission (Madhi et al., 2021; Muik et al., 2021; Z. Wang et al., 2021). Furthermore, without global vaccination coverage there is potential for future viral outbreaks.

Frequent testing using ‘rapid’ tests has been proposed as an effective strategy to survey the population and identify infectious people (Larremore et al., 2020; Mina et al., 2021), permitting a faster and safer reopening of the economy. A frequent testing strategy is effective if a test is: 1) rapid; 2) inexpensive; 3) simple to use (ideally self-administered for convenience and to minimize health-care resources); 4) sensitive enough to identify most infectious people; and 5) highly specific, so that when prevalence is low, most positives tests are true.

Initial tests to detect SARS-CoV-2 infection used deep nasopharyngeal swabs followed by RT-qPCR and were conducted by specially trained personnel (Pondaven-Letourmy et al., 2020; US Food and Drug Administration, 2020d). To increase testing capacity and reduce time to get a test result, point-of-care (POC) and home-based diagnosis using ‘rapid’ tests were developed to detect viral antigens from shallow nasal swabs (US Food and Drug Administration, 2021). While these tests provide quick results, even the most sensitive of antigen tests can only detect ~20,000 viral RNA copies per microliter (μL) (Corman et al., 2021) and may miss up to 30% of people with the high viral loads associated with infectivity (Baro et al., 2021; Cevik et al., 2021; Corman et al., 2021; Mak et al., 2020; Marc et al., 2021; Marks et al., 2021; Regev-Yochay et al., 2021; Scohy et al., 2020; Singanayagam et al., 2020; Wyllie et al., 2020; Yamayoshi et al., 2020).
Other molecular ‘rapid’ tests detect viral RNA using an isothermal enzymatic reaction to exponentially amplify fragments of the genome (Ben-Assa et al., 2020; Butler et al., 2020; Joung et al., 2020; Rabe & Cepko, 2020; Wei et al., 2021; Zhang et al., 2020), and some have been approved for emergency use for POC and at home (US Food and Drug Administration, 2020a, 2020c). While this type of rapid test is sensitive, they are expensive and/or require specialized equipment (i.e. are not simple to use). Saliva offers several advantages over nasal and nasopharyngeal swabs for the early detection of SARS-CoV-2 infections: it 1) has a higher viral load than swabs early in an infection (Savela et al., 2021; Teo et al., 2021); 2) is easier than swabs to obtain from children, who are often anxious about the swabbing procedure; 3) requires fewer materials to collect, diminishing waste and reliance on resources that can be scarce. Several protocols to detect SARS-CoV-2 from saliva have been described (US Food and Drug Administration, 2020b; Wyllie et al., 2020). However, these approaches require toxic chemicals or specialized equipment (e.g. centrifuges, pipettes, thermocyclers) which make them impractical for POC, home testing, and other resource-limited environments.

Given these considerations, a fast, economical, easy to use test that is both sensitive, specific, and safe, is still required. To this end, we devised WHotLAMP, a rapid molecular test to detect SARS-CoV-2 viral RNA directly from saliva without the need for specialized equipment, with results obtained in 30 minutes. This test extracts RNA from saliva and uses an isothermal enzymatic reaction to amplify and colorimetrically detect SARS-CoV-2 RNA. WHotLAMP is inexpensive, highly sensitive and specific, making it ideal for frequent screening and detection of infectious individuals to limit the spread of SARS-CoV-2.

Results and Discussion

A one-tube saliva test to detect SARS-CoV-2 RNA

To develop a simple procedure to extract SARS-CoV-2 RNA from saliva, we leveraged the nucleic acid binding properties of cellulose paper (e.g. Whatman filter paper (Zou et al., 2017)), and molecular detection of SARS-CoV-2 RNA using Loop-mediated isothermal amplification (LAMP), an enzymatic reaction that exponentially amplifies a target nucleic acid sequence at a constant temperature (Notomi et al., 2000). Inspired by work from Liu et al. (Liu et al., 2011), we sought to develop an inexpensive, sensitive and simplified test that did not require potentially dangerous chemicals, and would be suitable for frequent use at POC and adaptable for home use. We initially tested saliva spiked with naked SARS-CoV-2 control RNA and found that a short exposure of Whatman No. 1 filter paper to saliva, followed by brief washes, could capture sufficient SARS-CoV-2 control RNA to be detected in a LAMP reaction with primers directed against SARS-CoV-2 (Figure 1A). Amplification of the target sequence leads to a drop in pH, which is detected with a pH-sensitive dye as a color change from pink (negative) to yellow (positive) (Zhang et al., 2020). We next tested whether we could capture encapsulated SARS-CoV-2 RNA particles that were spiked into saliva, using Whatman paper and an established lysis buffer (Zou et al., 2017). This procedure can detect as few as ~4 SARS-CoV-2 particles per μL of saliva (Figure 1B). This level of sensitivity is notable, as it has been determined that 90% of COVID-19 patients carry more than 5 copies of SARS-CoV-2 per μL of saliva (Savela et al., 2021; Wyllie et al., 2020). These findings suggest that a strategy using Whatman paper is a viable approach for isolating RNA from SARS-CoV-2 virions in saliva.
Figure 1. Loop-mediated isothermal amplification (LAMP) detection of SARS-CoV-2 RNA captured from saliva using Whatman no. 1 filter. (A) Detection of naked SARS-CoV-2 RNA in saliva. Saliva with spike-in SARS-CoV-2 RNA (tubes 1-3), saliva without RNA spike-in (tubes 4-6), SARS-CoV-2 RNA added directly to LAMP reaction (tube 7), no template control (tube 8). (B) Detection of encapsulated SARS-CoV-2 RNA particles in saliva (tubes 1-4); saliva with spike-in encapsulated RNase P RNA particles (tube 5); saliva with spike-in encapsulated SARS-CoV-2 RNA particles with no extraction treatment (tube 6), saliva alone with no spike-in (tube 7), and no saliva (tube 8). LAMP reactions used N2+E1 primers for detection of SARS-CoV-2 RNA. Concentrations are in copies per microliter of saliva.

Since the original extraction procedure used guanidine hydrochloride (a toxic protein denaturant), we sought an alternative that avoided toxic chemical components. We developed a saliva extraction procedure using only two components, a non-toxic RNA preservative (RNAlater™), and an endopeptidase, Proteinase K. To minimize handling of Whatman paper, we secured a piece of Whatman paper to the bottom of a 1.7 mL centrifuge tube using a small amount of Kwik-Sil™ silicone adhesive. Notably, Kwik-Sil™ did not interfere with the colorimetric pH indicator in the LAMP reaction mixture, whereas other adhesives we examined caused a color change in negative-control reactions without RNA (Table 1).
Table 1: Compatibility of materials with colorimetric LAMP

| Compound                                         | Color change in LAMP (from pink) |
|--------------------------------------------------|----------------------------------|
| Kwik-Sil™ silicone elastomer (World Precision Instruments) | N                               |
| Clear aquarium silicone (Aqueon)                 | Y                                |
| Neutral cure silicone (Dow Corning 737)          | Y                                |
| Ultra-clear polyester plastic sheet (dipstick)   | N                                |
| Glue stick (polyamide, Power Adhesives TEC Bond 7718) | Y                               |
| Glue stick (acrylic, Infinity Bond)              | Y                                |
| Glue stick (high temperature, Allary)            | Y                                |
| Gorilla glue (original, polyurethane)            | Y                                |
| Crazy Glue (Loctite)                             | Y                                |
| Scotch permanent double sided tape               | Y                                |
| Rubber cement (Best-Test paper cement)           | Y                                |

WHotLAMP can be performed entirely in a single 1.7 mL microfuge tube (Figure 2A). In this test, viral RNA is preserved using a non-hazardous RNA stabilizing solution and is extracted by a brief Proteinase K digestion. Heating the sample at 95 °C inactivates both Proteinase K and SARS-CoV-2 virions (Batéjat et al., 2021), thereby increasing the biosafety of the sample. It was critical to include a wash step to remove both RNAlater solution and saliva that were soaked up by the filter paper, as well as particulates bound to the filter paper. We designed LAMP primer sets throughout the SARS-CoV-2 genome (see Methods), and focused on the primer set (ZI-1, targeting ORF 1a) with the lowest predicted propensity for primer-dimer formation. To evaluate the specificity of the ZI-1 primers, we tested a panel of 22 inactivated respiratory pathogens, including SARS-CoV-1, MERS, H1N1 influenza, and common respiratory coronaviruses. We detected SARS-CoV-2 RNA in samples containing encapsulated SARS-CoV-2 RNA particles, but not in samples containing only the other respiratory pathogens (Figure 2B-E), indicating that the primers were specific to SARS-CoV-2.
Figure 2. Specificity of SARS-CoV-2 LAMP primers. (A) Schematic of the WHotLAMP assay. (B) LAMP reactions using ZI-1 LAMP primers with 1x10^5 copies of SARS-CoV-1 DNA (tubes 1-3), MERS DNA (tubes 4-6), SARS-CoV-2 RNA (tube 7), and no template control (tube 8). (C) and (D), same as (B) but with CUFC1 or N2+E1 LAMP primers, respectively. (E) Triplicate LAMP reactions with ZI-1 LAMP primers using WHotLAMP detecting different respiratory pathogens (pools 1-5), no respiratory pathogens (-Ctrl), and with inactivated SARS-CoV-2 virions (+CoV-2 Ctrl).
Assay consistency

To determine the consistency of RNA extraction from saliva using WHotLAMP, we designed intron-spanning LAMP primers to detect human RAB7A mRNA, a transcript expressed at high levels in multiple tissues (Eisenberg & Levanon, 2013). RAB7A LAMP primers led to a yellow color change with saliva from healthy donors, but not when RNase A was added after Proteinase K treatment, indicating the amplification originated from RNA and not genomic DNA (Figure 3A and 3B). In contrast, the reaction control (RPP30) in the CDC-recommended RT-qPCR test panel for SARS-CoV-2 amplifies both genomic DNA as well as cDNA (Dekker et al., 2020). Further testing of additional saliva samples with RAB7A LAMP led to a yellow color change in 20 of 20 samples, indicating that the RAB7A LAMP primers are an appropriate control for benchmarking successful RNA extractions from saliva.

Figure 3. Detection of RAB7A RNA in saliva. (A) LAMP reactions using RAB7A LAMP primers with purified RNA from healthy saliva (tubes 1-3), or purified RNA treated with RNase A (tubes 4-6). (B) LAMP reactions using WHotLAMP detecting RAB7A in saliva (tubes 1-3), or with RNase A treatment (tubes 4-6).
Color variation among healthy donor saliva and automated scoring of assay

To devise a quantitative colorimetric threshold from which to differentiate between positive and negative LAMP results, we photographed LAMP assays under controlled illumination using a custom-made portable photobox (Figure 4A). A potential concern regarding testing saliva using pH-sensitive dyes, rests in how the variability of pH of saliva samples could influence the specificity of this test (Choi et al., 2017). To examine the colorimetric variability of WHotLAMP, we tested saliva samples from 36 healthy volunteers (nasal swab SARS-CoV-2 qPCR negative) (Figure 4B). The range of hues of these healthy salivas did not overlap with the range of SARS-CoV-2 positive samples, indicating unambiguous colorimetric classification of results (Figure 4C-D).

Limit of detection

We next performed a series of dilutions of a SARS-CoV-2 positive saliva sample to estimate the limit of detection (LoD). Using a saliva sample from an individual with a positive nasal swab Ct (threshold cycle) value of 21, we could detect SARS-CoV-2 using WHotLAMP in 20/20 (100%) of saliva samples diluted 1:20,000, and 19/20 (95%) of samples diluted 1:40,000, suggesting a LoD of Ct ~36. To better quantify the LoD of our assay, we purified RNA from this saliva and performed RT-qPCR using CDC 2019 nCoV N1 and N2 PCR primers. Through interpolation to a standard curve ($R^2 = 0.99$) using a dilution series of a standard (IDT N-gene), we determined that the LoD of WHotLAMP with ZI-1 primers corresponds to ~3.6 viral RNA copies/μL saliva. This LoD matches the 4 viral RNA copies/μL of saliva determined using SARS-CoV-2 RNA spiked into saliva (Figure 1B), making it >50x more sensitive than other recent saliva LAMP assays (Yang et al., n.d.).

Specificity and sensitivity

To evaluate the clinical sensitivity of WHotLAMP, we tested saliva from patients who at the same time tested positive for SARS-CoV-2 with a nasal swab qPCR. WHotLAMP with ZI-1 primers detected 36/38 (94.7%) positives with a Ct value up to 34 (Figure 5A). In contrast, CUFC1 primers (Wei et al., 2021) detected only 25/32 (78.1%) positives, amongst the same cohort of SARS-CoV-2 saliva samples (Figure 5B). Previous reports indicate low success in culturing SARS-CoV-2 from patients with a positive nasal swab at a Ct value >34 (Cevik et al., 2021; La Scola et al., 2020), suggesting that the WHotLAMP assay with ZI-1 primers can detect nearly all individuals that carry viral loads considered to be contagious. Furthermore, while the most accurate antigen tests have a false negative rate of ~20% for samples with a Ct <30 (Baro et al., 2021), WHotLAMP with ZI-1 primers detected 21/21 samples that had Ct <31 in nasal swab RT-qPCR.

To evaluate the specificity of WHotLAMP, we tested saliva from asymptomatic individuals who had a negative qPCR result from a nasal swab taken within 24 hours of the saliva collection. Notably, we found no false positives among 40 samples (false-positive rate <1/40; CI=0-0.091) using ZI-1 primers, indicating this primer set offers high specificity (Figure 5C), whereas CUFC1 primers detected 2 false positives out of 37 samples (a subset of the 40 tested with ZI-1; false-positive rate 0.054; CI=0.0097-0.18) (Figure 5D).
Figure 4. Colorimetric quantification of LAMP reactions. (A) Illuminated lightbox with automated image acquisition using Raspberry Pi unit; 1) Raspberry Pi unit; 2) white LED strip; 3) camera unit; 4) test tube rack. (B) LAMP reactions using WHotLAMP with ZI-1 primers on saliva samples from different negative nasal-swab qPCR SARS-CoV-2 individuals (top white box) and SARS-CoV-2 positive (nasal swab) samples (bottom white box). (C) Processed image showing conversion of colorimetric LAMP results to hues. (D) Hue distribution of WHotLAMP saliva results from negative (-) and positive (+) nasal-swab SARS-CoV-2 qPCR donor samples.
Figure 5. Sensitivity and specificity of WHotLAMP. (A and B) Sensitivity and (C and D) specificity of WHotLAMP using (A and C) ZI-1 or (B and D) CUFC1 primers with qPCR SARS-CoV-2 positive saliva. Yellow circles denote positive (+) LAMP reactions and magenta circles denote negative (-) LAMP reactions.

Concluding remarks

We describe WHotLAMP, a simple, inexpensive molecular test (~$3.00 for consumables per reaction at retail prices) that does not require specialized laboratory equipment, to detect SARS-CoV-2 virus in saliva. We show that WHotLAMP can detect low levels of SARS-CoV-2 virus in saliva in 30 minutes. Its low false-positive rate allows for deployment under conditions of low prevalence, where a high test specificity is particularly important to achieve high positive predictive values. The current assay design is already applicable to test at POC settings. Moreover, its single-tube format that requires no centrifugation, is conducive to scaling to 96-well formats, but can also be adapted for home use for frequent self-administered monitoring. While here we focused on a test for SARS-CoV-2, this technology could be used to detect other pathogens that are present in saliva by substituting primers (Bonney et al., 2020), making WHotLAMP a broadly useful diagnostic assay.
Methods

Saliva RNA spike-in assay

For saliva RNA spike-in tests, a 1.7 mL tube with 100 μL of saliva was combined with 100 μL lysis buffer (0.8 M guanidine hydrochloride (G3272, Sigma), 2% Tween-20 (BP337, Fisher Biotech)), mixed, and incubated at room temperature for 5 min. Approximately 1x10^5 copies of SARS-CoV-2 RNA (MT007544.1, TWIST Bioscience) were added to the saliva lysate and mixed. A 2x3 mm piece of Whatman No. 1 filter paper (Cat. 1001-929, GE Healthcare) was added to the lysate and incubated at room temperature for 1 min. The lysate was removed and the filter paper was washed twice. For each wash, 1 mL of wash buffer (1 mM Tris-Cl pH 8.0, 0.1 mM EDTA pH 8.0, 0.1% Tween-20) was added, inverted 20x, incubated for 1 minute at room temperature, and then removed. The filter paper was then transferred to a PCR tube containing 40 μL of 1x LAMP reaction mixture (see below).

For saliva encapsulated SARS-CoV-2 RNA spike-in tests, the extraction was performed as described for the RNA spike-in assay, except that encapsulated RNA controls (either AccuPlex SARS-CoV-2 or human RNaseP, 0505-0168, LGC Sera Care) were added to the saliva lysate mixture instead of naked RNA. For the no extraction control, encapsulated SARS-CoV-2 RNA control was added directly to saliva without treatment with lysis buffer.

WHotLAMP assay

For WHotLAMP assays, a 1.7 mL tube with a piece of Whatman No.1 filter paper (approximately 6 mm^2 surface area) affixed at the bottom using Kwik-Sil™ silicone (see below) was used to carry out the entire assay. For each sample, 100 μL of saliva was loaded into the tube, followed by 50 μL of RNAlater (R0901, Sigma) and 25 μL of Proteinase K (10 mg/mL) (PB0451, BioBasic; or 25530-049, Ambion), and mixed. The tube was incubated at 26 °C for 5 min, then at 95 °C for 5 min, and then returned to room temperature. The saliva mixture was removed and the filter paper was washed twice. For the first wash, 1 mL of wash buffer (1 mM Tris-Cl pH 8.0, 0.1 mM EDTA pH 8.0, 0.1% Tween-20) was added, inverted 20x, incubated for 1 min at room temperature, and then removed. For the second wash, 1 mL of wash buffer was added and the tube was incubated for 1 min at room temperature. The wash buffer was then removed, and 50 μL of 1x LAMP reaction mixture was added (see below).

Assay to test for cross-reactivity of respiratory pathogens

ZI-1, CUFC1 and N2+E1 LAMP primers were used in 25 μL colorimetric RT-LAMP reactions conducted in PCR tubes. 1x10^5 DNA copies of SARS-CoV-1 and MERS, or RNA purified from positive SARS-CoV-2 saliva were added. Reactions were carried out at 65 °C for 45 min. To test whether ZI-1 primers had cross-reactivity with other respiratory pathogens, the WHotLAMP assay was followed, except that the 100 μL of saliva was substituted with 100 μL of respiratory pathogen mixture (20 μL of a respiratory control panel (NATRPP-1, ZeptoMetrix) or SARS-CoV-2 control (NATSARS(COV2)-ERC, ZeptoMetrix) with 80 μL water).
Collection and processing of patient nasal and saliva samples

With informed consent (Columbia University IRB AAAT1974), patients were provided sterile cotton tipped swabs and conical tubes for sample collection. First, patients underwent separate nose and throat swabs for COVID-19 PCR analysis. For nose swabs, patients were instructed to swab 10 circles per nostril at ~1-2 cm from the nasal opening. For throat samples, patients self-swabbed. Swabs were then placed into 500 μL RLT buffer (RNeasy Mini kit, 74106, Qiagen) with 10 μL beta-mercaptoethanol per 1 mL RLT buffer. Participants were instructed, if able, to swish and swallow a small amount of water to clean the mouth. Participants were then asked to produce saliva that naturally pools in their mouth (not expectorated) into a 50 mL Falcon sterile tube. All specimens were assayed within 2 hrs or stored at 4 °C for up to 48 hrs for further analysis.

For RNA isolation and real-time qPCR analysis, RNA was purified using the RNeasy Mini Kit (Qiagen) with minor modifications to the standard protocol: Both the nose and throat lysates were combined on a single column for RNA isolation. In addition, only 1 RPE buffer spin was performed, followed by an 80% ethanol spin. The membrane was dried at full speed centrifugation for 5 min. RNA was eluted with two separate 20 μL RNase-free water 5 min incubations and 1 min full speed spins. RNA was transcribed into cDNA utilizing the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) with the following thermocycler settings: 25 °C for 10 min, 37 °C for 60 min, 85 °C for 5 min, then 4 °C until used.

Real-time qPCR was performed on cDNA according to standard protocols utilizing TaqPath qPCR Master Mix, ThermoScientific Microamp 96-well reaction plates, and the QuantStudio 3 Real Time PCR system. COVID-19 N1 and N2 FAM primers (2019-nCoV_N1-P, FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1; 2019-nCoV_N2-P, FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1) were analyzed out to 40 cycles as compared to 18S rRNA VIC loading control. A 20 μL reaction was performed with 10 μL master mix, 1 μL of COVID-19 N1 or N2 primer sequence, 1 μL of 18s rRNA endogenous control, 4 μL nuclease-free water, and 4 μL RNA were added to each well. All assays were run in duplicate. Each plate was run with a COVID-19 positive control (Integrated DNA Technologies, 2019-nCoV_N_Positive Control, #10006625), and water as a negative control. Samples were deemed negative if by qPCR there was no amplification for N1 or N2. The average Ct of the duplicates was used.

Saliva samples and WHotLAMP assays were handled and processed under BSL-2 containment. To test the sensitivity of WHotLAMP, patient saliva samples and negative control samples were tested under blind conditions. Saliva samples from healthy volunteers used to assess saliva variability were collected without prior food or beverage restrictions. Samples were tested using the WHotLAMP assay with 1.7 mL tubes.

Purification of RNA from saliva for RAB7A LAMP and LoD qPCR

RNA was purified from saliva samples using RNeasy Mini columns (Qiagen). 250 μL of saliva was mixed with 250 μL of RLT buffer and 500 μL of 70% ethanol. 500 μL of the mixture was loaded onto a column and centrifuged at 14,000g for 30 s. A second 500 μL volume was loaded onto the same column and centrifuged. The column was washed with 500 μL of RPE, centrifuged, transferred to a new tube, and
spun to dry. The column was transferred to a fresh tube and eluted in 20 μL of water. Two additional elutions using 20 μL of water were performed, and all eluates were pooled into one tube.

cDNA synthesis for saliva LoD analysis

For cDNA synthesis, total RNA purified from saliva was reverse transcribed using random primers and recombinant M-MuLV reverse transcriptase (E6300S, NEB) according to manufacturer’s instructions. Briefly, 50 ng of total RNA was mixed with random primers and denatured for 5 min at 70 °C, spun briefly, and placed on ice. M-MuLV reaction mix and M-MuLV enzyme were added to the mixture and incubated at 25 °C for 5 min, then incubated at 42 °C for 1 hr and heat inactivated at 80 °C for 5 min. The cDNA was then stored at -20 °C until further use.

qPCR for saliva LoD analysis

cDNA from SARS-CoV-2 positive saliva and positive control SARS-CoV-2 N gene DNA (2019-nCoV N positive control, IDT Cat. 10006625), were diluted in triplicate. qPCR was performed using CDC N1 and N2 gene primers, their respective fluorescent probes (2019-nCoV_N1 Probe: FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1 and 2019-nCoV_N2-Probe: FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1) and Taqman Fast Advanced master mix (ThermoFisher, Cat. 4444551), and a QuantStudio 3 Real-Time PCR system (Applied Biosystems) using recommended CDC 2019 nCoV RT-PCR thermocycling parameters. Ct values falling within the linear amplification range were used to estimate SARS-CoV-2 copy number in the cDNA, and an estimated LoD based on copy number was calculated based on the dilutions.

RT-LAMP Primers and Assay

Primers (desalted, Integrated DNA Technologies and Eurofins Genomics) used for LAMP reactions were prepared in water as 10x stocks (16 μM FIP/BIP, 2 μM F3/B3, 4 μM LF/LB) (see Table 1). ZI LAMP primer sets were identified using the NEB Primer Design Tool (https://lamp.neb.com/#/) and a ~800 bp sliding window across the SARS-CoV-2 genomic sequence (MN908947.3). Primer sets with low primer ΔG values (e.g. <-2.2) were selected for further analysis. One primer set, ZI-1, was selected because it was highly sensitive when tested using positive SARS-CoV-2 saliva. All LAMP reactions consisted of 1x colorimetric RT-LAMP mixture (NEB M1800S), 40 mM guanidine hydrochloride (G3272, Sigma, freshly made) and LAMP primers (1.6 μM FIP/BIP, 0.2 μM F3/B3, 0.4 μM LF/LB). Reactions were carried out at 65°C for 20 minutes (for 1.7 mL tubes) in a heat block or 45 minutes (for PCR tubes) in a thermocycler. Tubes were cooled afterwards to enhance color contrast between positive and negative colorimetric LAMP results.
Table 2: Primers used in study

| Primer         | Sequence                                                                 |
|----------------|--------------------------------------------------------------------------|
| ZI-1-F3        | 5' GGA TAC AAC TAG CTA CAG AGA A 3'                                      |
| ZI-1-B3        | CCA CAA GTT ACT TGT ACC ATA C                                            |
| ZI-1-FIP       | TTG GTA AAG AAC ATC AGA ACC TGA GGC TGC TTG TTG TCA TCT C                |
| ZI-1-BIP       | CCA CCA CAA ACC TCT ATC ACC TAA CCC TCA ACT TTA CCA GAT                 |
| ZI-1-LF        | AAG TCA TTG AGA GCC TTT GC                                              |
| ZI-1-LB        | GTG GTT TTA GAA AAA TGG CAT TCC C                                       |
| CUFC1-F3       | TGG ATA CAA CTA GCT ACA GAG A                                            |
| CUFC1-B3       | AGC CAA AGA CCG TTA AGT GTA                                              |
| CUFC1-FIP      | GTG GTG GTT GTT AAA GAA CAT CAG ACT TGT TGT CAT CTC GCA AAG G            |
| CUFC1-BIP      | CCT CTA CCA CCT CAG CTG TTT TGC TGT ACC ATA CAA CCC TCA ACT T            |
| CUFC1-LF       | ACC TGA GTT ACT GAA GTC ATT GAG A                                       |
| CUFC1-LB       | TGG TTT TAG AAA AAT GGC ATT ACC                                       |
| N2-F3          | ACC AGG AAC TAA TCA GAC AAG                                              |
| N2-B3          | GAC TTG ATC TTT GAA ATT TGG ATCT                                        |
| N2-FIP         | TTC CGA AGA ACG CTG AAG CGG AAC TGA TTA CAA ACA TTG GCC                  |
| N2-BIP         | CGC ATT GGC ATG GAA GTC ACA ATT TGA TGG CAC CTG TGT A                    |
| N2-LF          | GGG GGC AAA TTG TGC AAT TTG                                             |
| N2-LB          | CTT CGG GAA CTT GGT TGA CC                                              |
| E1-F3          | TGA GTA CGA ACT TAT GTA CTC AT                                           |
| E1-B3          | TTC AGA TTT TTA ACA CTA GGA GAG T                                      |
| E1-FIP         | ACC ACG AAA GCA AGA AAA AGA AGT TCG TTT CGG AAG AGA CAG                  |
| E1-BIP         | TTG CTA GTT ACA CTA CCA CCT ATC TTT AGG TTT TAC AAG ACT CAC GT           |
| E1-LB          | GCG CTT CGA TTG TGT GCG T                                               |
| E1-LF          | CGC TAT TAA CTA TTA ACG                                                 |
| RAB7A-F3       | ACA GGC CTG GTG CTA CAG                                                 |
| RAB7A-B3       | CTG CAG CTG TCT GCC GAG                                                 |
| RAB7A-FIP      | CAA TCG TCT GGA ACG CCT GCT CCC TAC TTT GAG ACC AGT GC                  |
| RAB7A-BIP      | AAG CAG GAA ACg GAG GTG GAG GCC CGG TCA TTC TTG TCC                      |
| RAB7A-LF       | ACG TTG ATG GCC TCC TTG                                                 |
| RAB7A-LB       | TGT ACA AGC AAT TTC CTG AAC C                                            |
| 2019-nCoV_N1-F | GAC CCC AAA ATC AGC GAA AT                                               |
| 2019-nCoV_N1-R | TCT GGT TAC TGC CAG TTG AAT CTG                                        |
| 2019-nCoV_N1-P | **FAM-ACC CCG CAT TAC TTG TGG ACC-BHQ1**                                |
| 2019-nCoV_N2-F | TTA CAA ACA TTG GCC GCA AA                                               |
| 2019-nCoV_N2-R | GCG CGA CAT TCC GAA GAA                                                 |
| 2019-nCoV_N2-P | **FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1**                              |
WHotLAMP LoD assay

SARS-CoV-2 positive saliva was initially heat-inactivated at 65 °C for 30 min prior to diluting using negative control saliva. An initial series using 10-fold dilutions was tested using WHotLAMP with ZI-1 primers. A second series using 2-fold dilutions was tested and repeated 20 times to estimate the consistency at the LoD. Saliva from the same SARS-CoV-2 positive sample was used to extract RNA for RT-qPCR analysis.

Adhesive and material handling and testing

Adhesives that could be used to affix Whatman No. 1 filter paper to the bottom of a tube were tested to determine if they were compatible with the LAMP colorimetric assay. A small quantity of liquid adhesives (~2 μL) was spotted at the bottom of a tube and allowed to cure for at least 24 hrs. For solid materials, ~1-2 mm² pieces were used. Tubes containing different adhesives were incubating in 1x LAMP reaction mixture at 65 °C for 20 min (for 1.7 mL tubes) or 45 min (for 0.2 mL PCR tubes). Materials tested were, silicones (Kwik-Sil™ silicone elastomer [World Precision Instruments], aquarium silicone [Aqueon], neutral cure silicone [Dow Corning 737]), liquid glues (Gorilla glue [original, polyurethane], Crazy Glue [Loctite]), glue gun sticks (polyamide [Power Adhesives TEC Bond 7718], acrylic [Infinity Bond], Hot Melt Mini Glue Sticks [Allary]), rubber cement (paper cement [Best-Test]), double-sided tape (Permanent [Scotch, 3M]), plastic strip, (cut from a 175 micron polyester sheet [Grafix Plastics]). To prepare large numbers of 1.7 mL tubes with Whatman No. 1 filter paper glued with Kwik-Sil™, a small aliquot of component A and component B were mixed together and placed on ice to slow the polymerization process. Tear-shaped Whatman filter paper pieces were prepared using a hole-punch. The tapered end of the paper was dipped slightly in Kwik-Sil™ and placed, taper side facing up, at the bottom of the 1.7 mL tube using fine-tipped forceps, and air dried for at least 24 hrs.

Raspberry Pi lightbox

To quantify colorimetric ranges under uniform conditions we embedded an enclosed white box with: 1) a Raspberry Pi 3 (model B+), 2) camera unit (camera v2.1), and 3) white LED lights (DC12V LED strip). The raspistill command line tool was run to capture still images (raspistill --raw -o png). A color chart (Digital Kolor Kard) inside the box was used as reference to calibrate the white balance of images. Images were then used to extract hues to interpret WHotLAMP positive and negative colorimetric results.

Image processing

We developed a proof-of-concept image analysis pipeline that identified sample results. Images of an array of samples were acquired and thresholded based on color saturation to identify regions of interest (ROIs) corresponding to samples. Areas of high or low brightness as well as areas near the image border were excluded from potential ROIs. We found that this method successfully identified correct ROIs and that the average hue within each ROI formed a bimodal distribution that could be used to successfully categorize samples into positives and negatives.
Supplementary information

Supplementary Material 1: Python code for LAMP colorimetric result analysis

Supplementary Material 2: WHotLAMP Protocol

Author contributions

Conceptualization: DN, AB, MV, AP, TT, DP
Methodology: DN, AB, MV, AP, HS, EC, SK, BN, FF
Validation: DN, MV, AP, UT
Formal analysis: DN, AB, MK, ALK
Funding: AB, PA
Investigation: DN, MV, AP, UT, MG, KS, JD’A, HS, EC, SK, IR-V, BN, FF
Resources: MG, KS, JD’A, AE, TT
Software: AB, ALK
Supervision: DN, AB, JD’A
Visualization: DN, AB
Writing – original draft: DN, AB, MG, ALK
Writing – review & editing: DN, AB, MV, AP, MG, JD’A, ALK, PA
Data Curation: AB, MG

Acknowledgements

Natalie Steineman, Marjorie Xie, and Aniruddah Das provided organizational support. The Zuckerman Institute Scientific Platforms provided resources. We thank Rui Costa and the Zuckerman Institute for unwavering support.

Funding

This study was funded by Friends of the Zuckerman Institute and Columbia University and NIH grant GM112758 to PA. AB is a Searle Scholar, Klingenstein-Simons Neuroscience Fellow and a Sloan Neuroscience Fellow.

Conflicts of interest

Patent application 63/088,694 related to this technology was filed by Columbia University, to facilitate that this technology be made widely available. AB became a member of the advisory board of Rapid Diagnostic Systems Limited for which he received options, after the conclusion of experiments and data analyses described here.
443 References

444 Baro, B., Rodo, P., Ouchi, D., Bordoy, A. E., Saya Amaro, E. N., Salsench, S. V., Molinos, S., Alemany, A.,
445 Ubals, M., Corbacho-Monné, M., Millat-Martínez, P., Marks, M., Clotet, B., Prat, N., Estrada, O.,
446 Vilar, M., Ara, J., Vall-Mayans, M., G-Beiras, C., ... Mitjà, O. (2021). Performance characteristics of
447 five antigen-detecting rapid diagnostic test (Ag-RDT) for SARS-CoV-2 asymptomatic infection: a
448 head-to-head benchmark comparison. The Journal of Infection, 82(6), 269–275.

449 Batéjat, C., Grassin, Q., Manuguerra, J.-C., & Leclercq, I. (2021). Heat inactivation of the severe acute
450 respiratory syndrome coronavirus 2. In Journal of Biosafety and Biosecurity (Vol. 3, Issue 1, pp. 1–
451 3). https://doi.org/10.1016/j.jobb.2020.12.001

452 Ben-Assa, N., Naddaf, R., Gefen, T., Capucha, T., Hajjo, H., Mandelbaum, N., Elbaum, L., Rogov, P., King,
453 D. A., Kaplan, S., Rotem, A., Chowers, M., Szwarcwort-Cohen, M., Paul, M., & Geva-Zatorsky, N.
454 (2020). Direct on-the-spot detection of SARS-CoV-2 in patients. Experimental Biology and Medicine,
455 245(14), 1187–1193.

456 Bonney, L. C., Watson, R. J., Slack, G. S., Bosworth, A., Wand, N. I. V., & Hewson, R. (2020). A flexible
457 format LAMP assay for rapid detection of Ebola virus. PLoS Neglected Tropical Diseases, 14(7),
458 e0008496.

459 Butler, D. J., Mozsary, C., Meydan, C., Danko, D., Foox, J., Rosiene, J., Shaiber, A., Afshinneko, E.,
460 MacKay, M., Sedlazeck, F. J., Ivanov, N. A., Sierra, M., Pohle, D., Zietz, M., Gisladottir, U., Ramlall, V.,
461 Westover, C. D., Ryon, K., Young, B., ... Mason, C. E. (2020). Shotgun Transcriptome and Isothermal
462 Profiling of SARS-CoV-2 Infection Reveals Unique Host Responses, Viral Diversification, and Drug
463 Interactions. bioRxiv : The Preprint Server for Biology. https://doi.org/10.1101/2020.04.20.048066

464 Casey, M., Griffin, J., McAloon, C. G., Byrne, A. W., Madden, J. M., Mc Evoy, D., Collins, Á. B., Hunt, K.,
465 Barber, A., Butler, F., Lane, E. A., O’Brien, K., Wall, P., Walsh, K. A., & More, S. J. (2020). Pre-
466 symptomatic transmission of SARS-CoV-2 infection: a secondary analysis using published data. In
Cevik, M., Tate, M., Lloyd, O., Maraolo, A. E., Schafers, J., & Ho, A. (2021). SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *The Lancet. Microbe, 2*(1), e13–e22.

Cheng, P. K. C., Wong, D. A., Tong, L. K. L., Ip, S.-M., Lo, A. C. T., Lau, C.-S., Yeung, E. Y. H., & Lim, W. W. L. (2004). Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. *The Lancet, 363*(9422), 1699–1700.

Choi, J. E., Lyons, K. M., Kieser, J. A., & Waddell, N. J. (2017). Diurnal variation of intraoral pH and temperature. *BJD Open, 3*, 17015.

Corman, V. M., Haage, V. C., Bleicker, T., Schmidt, M. L., Mühlemann, B., Zuchowski, M., Jo, W. K., Tscheak, P., Möncke-Buchner, E., Müller, M. A., Krumbholz, A., Drexler, J. F., & Drosten, C. (2021). Comparison of seven commercial SARS-CoV-2 rapid point-of-care antigen tests: a single-centre laboratory evaluation study. *The Lancet. Microbe*. https://doi.org/10.1016/S2666-5247(21)00056-2

Dekker, R. J., Ensink, W. A., van Leeuwen, S., Rauwerda, H., & Breit, T. M. (2020). Overhauling a faulty control in the CDC-recommended SARS-CoV-2 RT-PCR test. In *bioRxiv* (p. 2020.06.12.147819). https://doi.org/10.1101/2020.06.12.147819

Eisenberg, E., & Levanon, E. Y. (2013). Human housekeeping genes, revisited. *Trends in Genetics: TIG, 29*(10), 569–574.

Johansson, M. A., Quandelacy, T. M., Kada, S., Prasad, P. V., Steele, M., Brooks, J. T., Slayton, R. B., Biggerstaff, M., & Butler, J. C. (2021). SARS-CoV-2 Transmission From People Without COVID-19 Symptoms. *JAMA Network Open, 4*(1), e2035057.

Joung, J., Ladha, A., Saito, M., Kim, N.-G., Woolley, A. E., Segel, M., Barretto, R. P. J., Ranu, A., Macrae, R. K., Faure, G., Ioannidi, E. I., Krajjeski, R. N., Bruneau, R., Huang, M.-L. W., Yu, X. G., Li, J. Z., Walker, B. D., Hung, D. T., Greninger, A. L., ... Zhang, F. (2020). Detection of SARS-CoV-2 with SHERLOCK One-
Larremore, D. B., Wilder, B., Lester, E., Shehata, S., Burke, J. M., Hay, J. A., Milind, T., Mina, M. J., & Parker, R. (2020). Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. medRxiv: The Preprint Server for Health Sciences. https://doi.org/10.1101/2020.06.22.20136309

La Scola, B., Le Bideau, M., Andreani, J., Hoang, V. T., Grimaldier, C., Colson, P., Gautret, P., & Raoult, D. (2020). Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology, 39(6), 1059–1061.

Liu, C., Geva, E., Mauk, M., Qiu, X., Abrams, W. R., Malamud, D., Curtis, K., Owen, S. M., & Bau, H. H. (2011). An isothermal amplification reactor with an integrated isolation membrane for point-of-care detection of infectious diseases. The Analyst, 136(10), 2069–2076.

Madhi, S. A., Baillie, V., Cutland, C. L., Voysey, M., Koen, A. L., Fairlie, L., Padayachee, S. D., Dheda, K., Barnabas, S. L., Bhorat, Q. E., Briner, C., Kwatra, G., Ahmed, K., Aley, P., Bhikha, S., Bhiman, J. N., Bhorat, A. E., du Plessis, J., Esmail, A., … NGS-SA Group and the Wits-VIDA COVID Group. (2021). Efficacy of the ChAdOx1 nCoV-19 Covid-19 Vaccine against the B.1.351 Variant. The New England Journal of Medicine, 384(20), 1885–1898.

Mak, G. C., Cheng, P. K., Lau, S. S., Wong, K. K., Lau, C. S., Lam, E. T., Chan, R. C., & Tsang, D. N. (2020). Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology, 129, 104500.

Marc, A., Kerioui, M., Blanquart, F., Bertrand, J., Mitjà, O., Corbacho-Monné, M., Marks, M., & Guedj, J. (2021). Quantifying the relationship between SARS-CoV-2 viral load and infectiousness. medRxiv. https://doi.org/10.1101/2021.05.07.21256341
Marks, M., Millat-Martinez, P., Ouchi, D., Roberts, C. H., Alemany, A., Corbacho-Monné, M., Ubals, M.,
Tobias, A., Tebé, C., Ballana, E., Bassat, Q., Baro, B., Vall-Mayans, M., G-Beiras, C., Prat, N., Ara, J.,
Clotet, B., & Mitjà, O. (2021). Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort
study. The Lancet Infectious Diseases, 21(5), 629–636.

Mina, M. J., Peto, T. E., García-Fiñana, M., Semple, M. G., & Buchan, I. E. (2021). Clarifying the evidence
on SARS-CoV-2 antigen rapid tests in public health responses to COVID-19. The Lancet, 397(10283),
1425–1427.

Muik, A., Wallisch, A.-K., Sänger, B., Swanson, K. A., Mühl, J., Chen, W., Cai, H., Maurus, D., Sarkar, R.,
Türeci, Ö., Dormitzer, P. R., & Şahin, U. (2021). Neutralization of SARS-CoV-2 lineage B.1.1.7
pseudovirus by BNT162b2 vaccine–elicited human sera. Science, 371(6534), 1152–1153.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000).
Loop-mediated isothermal amplification of DNA. Nucleic Acids Research, 28(12), E63.

Paltiel, A. D., David Paltiel, A., Zheng, A., & Walensky, R. P. (2020). Assessment of SARS-CoV-2 Screening
Strategies to Permit the Safe Reopening of College Campuses in the United States. In JAMA
Network Open (Vol. 3, Issue 7, p. e2016818).

https://doi.org/10.1001/jamanetworkopen.2020.16818

Petersen, E., Koopmans, M., Go, U., Hamer, D. H., Petrosillo, N., Castelli, F., Storgaard, M., Al Khalili, S., &
Simonsen, L. (2020). Comparing SARS-CoV-2 with SARS-CoV and influenza pandemics. The Lancet
Infectious Diseases, 20(9), e238–e244.

Pondaven-Letourmy, S., Alvin, F., Boumghit, Y., & Simon, F. (2020). How to perform a nasopharyngeal
swab in adults and children in the COVID-19 era. European Annals of Otorhinolaryngology, Head
and Neck Diseases, 137(4), 325–327.

Rabe, B. A., & Cepko, C. (2020). SARS-CoV-2 detection using isothermal amplification and a rapid,
inexpensive protocol for sample inactivation and purification. Proceedings of the National Academy
Regev-Yochay, G., Kriger, O., Beni, S., Rubin, C., Mina, M. J., Mechnik, B., Hason, S., Biber, E., Nadaf, B., Kreiss, Y., & Others. (2021). Real World Performance of SARS-CoV-2 Antigen Rapid Diagnostic Tests in Various Clinical Settings. *medRxiv*. https://www.medrxiv.org/content/10.1101/2021.03.02.21252400v1.abstract

Savela, E. S., Winnett, A., Romano, A. E., Porter, M. K., Shelby, N., Akana, R., Ji, J., Cooper, M. M., Schlenker, N. W., Reyes, J. A., Carter, A. M., Barlow, J. T., Tognazzini, C., Feaster, M., Goh, Y.-Y., & Ismagilov, R. F. (2021). SARS-CoV-2 is detectable using sensitive RNA saliva testing days before viral load reaches detection range of low-sensitivity nasal swab tests. *medRxiv: The Preprint Server for Health Sciences*. https://doi.org/10.1101/2021.04.02.21254771

Scohy, A., Anantharajah, A., Bodéus, M., Kabamba-Mukadi, B., Verroken, A., & Rodriguez-Villalobos, H. (2020). Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, 129, 104455.

Singanayagam, A., Patel, M., Charlett, A., Lopez Bernal, J., Saliba, V., Ellis, J., Ladhani, S., Zambon, M., & Gopal, R. (2020). Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. *Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin*, 25(32). https://doi.org/10.2807/1560-7917.ES.2020.25.32.2001483

Stuart, R. M., Abeysuriya, R. G., Kerr, C. C., Mistry, D., Klein, D. J., Gray, R. T., Hellard, M., & Scott, N. (2021). Role of masks, testing and contact tracing in preventing COVID-19 resurgences: a case study from New South Wales, Australia. *BMJ Open*, 11(4), e045941.

Teo, A. K. J., Choudhury, Y., Tan, I. B., Cher, C. Y., Chew, S. H., Wan, Z. Y., Cheng, L. T. E., Oon, L. L. E., Tan, M. H., Chan, K. S., & Hsu, L. Y. (2021). Saliva is more sensitive than nasopharyngeal or nasal swabs
for diagnosis of asymptomatic and mild COVID-19 infection. Scientific Reports, 11(1), 3134.

US Food and Drug Administration. (2020a, August 11). EUA FDA Letter to LumiraDx UK Ltd – LumiraDx SARS-CoV-2 RNA STAR. https://www.fda.gov/media/141054/download

US Food and Drug Administration. (2020b, November 13). EUA FDA Letter to Infinity Biologix LLC – Infinity Biologix TaqPath SARS-CoV-2 Assay. https://www.fda.gov/media/137773/download

US Food and Drug Administration. (2020c, November 17). EUA FDA Letter to Lucira Health, Inc. – Lucira COVID-19 All-In-One Test Kit. https://www.fda.gov/media/143810/download

US Food and Drug Administration. (2020d, December 1). EUA FDA Letter to Dr. Robert R. Redfield, CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel. https://www.fda.gov/media/134919/download

US Food and Drug Administration. (2021, April 1). EUA FDA Letter to Quidel Corporation – Sofia SARS Antigen FIA. https://www.fda.gov/media/137886/download

Walsh, K. A., Jordan, K., Clyne, B., Rohde, D., Drummond, L., Byrne, P., Ahern, S., Carty, P. G., O’Brien, K. K., O’Murchu, E., O’Neill, M., Smith, S. M., Ryan, M., & Harrington, P. (2020). SARS-CoV-2 detection, viral load and infectivity over the course of an infection. The Journal of Infection, 81(3), 357–371.

Wang, C., Wang, Z., Wang, G., Lau, J. Y.-N., Zhang, K., & Li, W. (2021). COVID-19 in early 2021: current status and looking forward. Signal Transduction and Targeted Therapy, 6(1), 114.

Wang, Z., Schmidt, F., Weisblum, Y., Muecksh, F., Barnes, C. O., Finkin, S., Schaefer-Babajew, D., Cipolla, M., Gaebler, C., Lieberman, J. A., Oliveira, T. Y., Yang, Z., Abernathy, M. E., Huey-Tubman, K. E., Hurley, A., Turroja, M., West, K. A., Gordon, K., Millard, K. G., ... Nussenzweig, M. C. (2021). mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature, 592(7855), 616–622.

Wei, S., Kohl, E., Djandjji, A., Morgan, S., Whittier, S., Mansukhani, M., Hod, E., D’Alton, M., Suh, Y., & Williams, Z. (2021). Direct diagnostic testing of SARS-CoV-2 without the need for prior RNA extraction. Scientific Reports, 11(1), 2402.
Wouters, O. J., Shadlen, K. C., Salcher-Konrad, M., Pollard, A. J., Larson, H. J., Teerawattananon, Y., & Jit, M. (2021). Challenges in ensuring global access to COVID-19 vaccines: production, affordability, allocation, and deployment. *The Lancet, 397*(10278), 1023–1034.

Wyllie, A. L., Fournier, J., Casanovas-Massana, A., Campbell, M., Tokuyama, M., Vijayakumar, P., Warren, J. L., Geng, B., Muenker, M. C., Moore, A. J., Vogels, C. B. F., Petrone, M. E., Ott, I. M., Lu, P., Venkataraman, A., Lu-Culligan, A., Klein, J., Earnest, R., Simonov, M., ... Ko, A. I. (2020). Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. *The New England Journal of Medicine, 383*(13), 1283–1286.

Yamayoshi, S., Sakai-Tagawa, Y., Koga, M., Akasaka, O., Nakachi, I., Koh, H., Maeda, K., Adachi, E., Saito, M., Nagai, H., Ikeuchi, K., Ogura, T., Baba, R., Fujita, K., Fukui, T., Ito, F., Hattori, S.-I., Yamamoto, K., Nakamoto, T., ... Kawaoka, Y. (2020). Comparison of Rapid Antigen Tests for COVID-19. *Viruses, 12*(12). https://doi.org/10.3390/v12121420

Yang, Q., Meyerson, N. R., Clark, S. K., Paige, C. L., Fattor, W. T., Gilchrist, A. R., Barbachano-Guerrero, A., Healy, B. G., Worden-Sapper, E. R., Wu, S. S., Muhlrad, D., Decker, C. J., Saldi, T. K., Lasda, E., Gonzales, P. K., Fink, M. R., Tat, K. L., Hager, C. R., Davis, J. C., ... Sawyer, S. L. (n.d.). *Saliva TwoStep for rapid detection of asymptomatic SARS-CoV-2 carriers.*

https://doi.org/10.1101/2020.07.16.20150250

Zou, Y., Mason, M. G., Wang, Y., Wee, E., Turni, C., Blackall, P. J., Trau, M., & Botella, J. R. (2017). Nucleic acid purification from plants, animals and microbes in under 30 seconds. *PLoS Biology, 15*(11), e2003916.