

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Lee, JYH;Best, N;McAuley, J;Porter, JL;Seemann, T;Schultz, MB;Sait, M;Orlando, N;Mercoulia, K;Ballard, SA;Druce, J;Tran, T;Catton, MG;Pryor, MJ;Cui, HL;Luttick, A;McDonald, S;Greenhalgh, A;Kwong, JC;Sherry, NL;Graham, M;Hoang, T;Herisse, M;Pidot, SJ;Williamson, DA;Howden, BP;Monk, IR;Stinear, TP

Title:

Validation of a single-step, single-tube reverse transcription loop-mediated isothermal amplification assay for rapid detection of SARS-CoV-2 RNA

Date:

2020-01-01

Citation:

Lee, J. Y. H., Best, N., McAuley, J., Porter, J. L., Seemann, T., Schultz, M. B., Sait, M., Orlando, N., Mercoulia, K., Ballard, S. A., Druce, J., Tran, T., Catton, M. G., Pryor, M. J., Cui, H. L., Luttick, A., McDonald, S., Greenhalgh, A., Kwong, J. C., ... Stinear, T. P. (2020). Validation of a single-step, single-tube reverse transcription loop-mediated isothermal amplification assay for rapid detection of SARS-CoV-2 RNA. *Journal of Medical Microbiology*, 69 (9), pp.1169-1178. <https://doi.org/10.1099/jmm.0.001238>.

Persistent Link:

<https://hdl.handle.net/11343/252905>

License:

CC BY

EDITOR'S CHOICE

Validation of a single-step, single-tube reverse transcription loop-mediated isothermal amplification assay for rapid detection of SARS-CoV-2 RNA

Jean Y. H. Lee^{1,2}, Nickala Best³, Julie McAuley¹, Jessica L. Porter¹, Torsten Seemann^{1,4}, Mark B. Schultz⁴, Michelle Sait⁴, Nicole Orlando⁴, Karolina Mercoulia⁴, Susan A. Ballard⁴, Julian Druce⁵, Thomas Tran⁵, Mike G. Catton⁵, Melinda J. Pryor⁶, Huanhuan L. Cui⁶, Angela Luttick⁶, Sean McDonald³, Arran Greenhalgh³, Jason C. Kwong^{1,7}, Norelle L. Sherry^{4,7}, Maryza Graham⁸, Tuyet Hoang^{1,4}, Marion Herisse¹, Sacha J. Pidot¹, Deborah A. Williamson^{1,4,9}, Benjamin P. Howden^{1,4,7}, Ian R. Monk^{1†} and Timothy P. Stinear^{1*,†}

Abstract

Introduction. The SARS-CoV-2 pandemic of 2020 has resulted in unparalleled requirements for RNA extraction kits and enzymes required for virus detection, leading to global shortages. This has necessitated the exploration of alternative diagnostic options to alleviate supply chain issues.

Aim. To establish and validate a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay for the detection of SARS-CoV-2 from nasopharyngeal swabs.

Methodology. We used a commercial RT-LAMP mastermix from OptiGene in combination with a primer set designed to detect the CDC N1 region of the SARS-CoV-2 nucleocapsid (N) gene. A single-tube, single-step fluorescence assay was implemented whereby 1 µl of universal transport medium (UTM) directly from a nasopharyngeal swab could be used as template, bypassing the requirement for RNA purification. Amplification and detection could be conducted in any thermocycler capable of holding 65 °C for 30 min and measure fluorescence in the FAM channel at 1 min intervals.

Results. Assay evaluation by assessment of 157 clinical specimens previously screened by E-gene RT-qPCR revealed assay sensitivity and specificity of 87 and 100%, respectively. Results were fast, with an average time-to-positive (Tp) for 93 clinical samples of 14 min (SD±7 min). Using dilutions of SARS-CoV-2 virus spiked into UTM, we also evaluated assay performance against FDA guidelines for implementation of emergency-use diagnostics and established a limit-of-detection of 54 Tissue Culture Infectious Dose 50 per ml (TCID₅₀ ml⁻¹), with satisfactory assay sensitivity and specificity. A comparison of 20 clinical specimens between four laboratories showed excellent interlaboratory concordance; performing equally well on three different, commonly used thermocyclers, pointing to the robustness of the assay.

Conclusion. With a simplified workflow, The N1 gene Single Tube Optigene LAMP assay (N1-STOP-LAMP) is a powerful, scalable option for specific and rapid detection of SARS-CoV-2 and an additional resource in the diagnostic armamentarium against COVID-19.

INTRODUCTION

The current SARS-CoV-2 pandemic has created an unprecedented global demand for rapid diagnostic testing. Most countries are employing reverse transcriptase quantitative PCR (RT-qPCR) for confirmation of infection [1–7]. Consequently, a global shortage of RNA extraction kits as well as RT-qPCR assay kits and their associated reagents has ensued [8–10]. Therefore, alternative diagnostics not dependent on these commonly used materials are required. First described

20 years ago by Notomi *et al.*, the loop-mediated isothermal amplification (LAMP) assay is robust, rapid and straightforward, yet retains high sensitivity and specificity [11]. These features have seen the LAMP assay and the inclusion of a reverse transcriptase (RT-LAMP) implemented for a broad range of molecular diagnostic applications extending from infectious diseases, including detection of the original SARS-CoV-1 virus [12], bacteria and parasites [13–15] to cancer [16]. The advantages of RT-LAMP include using different reagents than RT-qPCR, the potential for direct processing

of samples without the need for prior RNA extraction and an extremely rapid turn-around time. Several groups have now described different RT-LAMP assays for detection of SARS-CoV-2 RNA [17–24].

In this study, we developed a RT-LAMP assay that targets the CDC N1 region of the SARS-CoV-2 nucleocapsid gene (N-gene) [3] and used a commercial mastermix from OptiGene. This mix contains a proprietary reverse transcriptase for cDNA synthesis and the thermophilic GspSSD strand-displacing polymerase/reverse transcriptase for DNA amplification (www.optigene.co.uk) with a dsDNA intercalating fluorescent dye. Detection was achieved by measuring the increase in fluorescence as amplification products accumulate. The N1 gene Single Tube Optigene LAMP assay (hereafter called N1-STOP-LAMP) was assessed for the direct detection of SARS-CoV-2 RNA, following *FDA Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency* against the four parameters and acceptance criteria summarized in the following reference [5]. Validation samples were human upper respiratory tract specimens, collected using nasopharyngeal flocked swabs stored in universal transport media (UTM).

METHODS

Specimen collection and handling

Nasopharyngeal swabs were collected by qualified healthcare professionals from patients meeting the epidemiological and clinical criteria as specified by the Victorian Department of Health and Human Services at the time of swab collection [1] between the 23 March [8] and 4 April 2020. Copan flocked swabs collected and directly inoculated on site in either 1 ml or 3 ml of UTM (Catalogue N^os., 330C and 350C, respectively) were used. Samples were collected at metropolitan hospitals in Melbourne, Victoria, Australia, and transported to the Doherty Institute Public Health Laboratories for further testing as per World Health Organization recommendations [6]. All swabs were processed in a class II biological safety cabinet.

Cell culture and SARS-CoV-2 RNA extraction

Vero cells (within 30 passages from the original American Type Culture Collection [ATCC] stock) were maintained in

Minimal Essential Media (MEM) supplemented with 10% heat-inactivated foetal bovine serum (FBS), 10 μ M HEPES, 2 mM glutamine and antibiotics. Cell cultures were maintained at 37 °C in a 5% CO₂ incubator. All virus infection cultures were conducted within the High Containment Facilities in a PC3 laboratory at the Doherty Institute. To generate stocks of SARS-CoV-2, confluent Vero cell monolayers were washed once with MEM without FBS (infection media) then infected with a known amount SARS-CoV-2 virus originally isolated from a patient [25]. After 1 h incubation at 37 °C in a 5% CO₂ incubator to enable virus binding, infection media containing 1 mg ml⁻¹ TPCK-trypsin was added, and flasks returned to the incubator. After 3d incubation and microscopic confirmation of widespread cytopathic effect (CPE), the supernatants were harvested, and filter sterilized through a 0.45 μ m syringe filter. To assess infectious SARS-CoV-2 viral titres, both Tissue Culture Infectious Dose 50 (TCID₅₀) and plaque assays were performed. Briefly, serial dilutions of the stock virus were added to washed monolayers of Vero cells. After 1 h incubation to allow virus to adhere, for the TCID₅₀ assay, infection media containing 1 μ g ml⁻¹ TPCK-Trypsin was added, while for the plaque assay the infected cell monolayer was overlaid with Leibovitz-15 (L15) media supplemented with 0.9% agarose (DNA grade, Sigma), antibiotics and 2 μ g ml⁻¹ TPCK Trypsin. After 3d incubation, the dilution of stock required to cause CPE in at least 50% of wells (TCID₅₀) was determined via back calculation of microscopic confirmation of CPE in wells for a given dilution. For quantitation via plaque assay, plaques present in the monolayer were macroscopically visualized, individually counted and back calculated for each given dilution to determine the number of p.f.u. per ml in the original stock. Stocks of SARS-CoV-2 used by this study had a TCID₅₀ of 5.4 \times 10⁵ ml⁻¹ and plaque assay gave 9.78 \times 10⁵ p.f.u. ml⁻¹. To heat inactivate the virus, 200 μ l of neat stock was heated to 60 °C for 30 min, then cooled. Inactivation was confirmed via complete lack of CPE and plaque formation using both TCID₅₀ and plaque assays. To prepare SARS-CoV-2 RNA from stocks, 500 μ l aliquots were thawed and RNA extracted using the RNeasy mini Kit (Qiagen) according to the manufacturer's specifications, with an elution volume of 50 μ l. Based on RNA concentrations, the total virus harvested from Vero cell culture was 3.1 \times 10⁹ copies ml⁻¹, suggesting a high number of non-infectious virus particles in the virus stocks.

Received 30 April 2020; Accepted 10 July 2020; Published 05 August 2020

Author affiliations: ¹Department of Microbiology and Immunology, University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia; ²Department of Infectious Diseases, Monash Health, Clayton, Victoria, Australia; ³GenWorks Pty Ltd, Thebarton, South Australia, Australia; ⁴Microbiological Diagnostic Unit Public Health Laboratory, University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia; ⁵Victorian Infectious Diseases Reference Laboratory, Melbourne Health at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia; ⁶360Biolabs, Melbourne, Victoria, Australia; ⁷Department of Infectious Diseases, Austin Health, Heidelberg, Victoria, Australia; ⁸Department of Microbiology, Monash Health, Clayton, Victoria, Australia; ⁹Melbourne Health, Melbourne, Victoria, Australia.

*Correspondence: Timothy P. Stinear, tstinear@unimelb.edu.au

Keywords: nasopharyngeal swabs; RT-LAMP; SARS-CoV-2; universal transport media.

Abbreviations: CDC, Centers for Disease Control; CPE, cytopathic effect; FDA, US Food and Drug Administration; iMonk, Dr Ian Monk; LAMP, loop-mediated isothermal amplification; RT-qPCR, reverse-transcription quantitative polymerase chain reaction.

†These authors contributed equally to this work

Three supplementary tables are available with the online version of this article.

RNA extraction from UTM for RT-qPCR

A 200 µl aliquot of Copan UTM was processed through the QIA Symphony DSP Virus/Pathogen Mini Kit (Qiagen, Cat No. 937036) following the manufacturer's instructions on the QIA Symphony SP instrument. The RNA was eluted in 60 µl of recommended buffer.

RNA extraction from UTM SPRI beads for N1-STOP-LAMP

Solid-phase reversible immobilisation (SPRI) on carboxylated paramagnetic beads (Sera-Mag Magnetic SpeedBeads, from GE Healthcare) were prepared for RNA binding as described (https://openwetware.org/wiki/SPRI_bead_mix). RNA purification was performed in 96-well plates with initial lysis by the addition of 25 µl of 6GTD lysis buffer (7.08 g 10 ml⁻¹ guanidine thiocyanate [6M], made up to 8.2 ml with water, 1 ml Tris HCL [pH 8.0] and 800 µl of 1M dithiothreitol), mixed by pipetting ten times and incubation at room temperature for 1 min [26]. To this, 75 µl of 100% ethyl alcohol and 20 µl of prepared SPRI beads were added, mixed by pipetting ten times and incubated at room temperature for 5 min. The RNA-bead complex was then immobilized by placing the 96-well plate on a magnetic rack and incubated again at room temperature for 5 min. The supernatant was then discarded, and the beads washed twice in 200 µl of freshly prepared 80% ethyl alcohol (v/v) with 30 s room-temperature incubation between each wash. The beads were air-dried for 2 min at room temperature before RNA was eluted by the addition of 20 µl of nuclease-free water.

E-gene RT-qPCR

A one-step RT-qPCR was conducted with the primers as described by Corman *et al.*, targeting the viral envelope E-gene of SARS-CoV-2 E_Sarbeco_F: 5'-ACAGGTACGTTA ATAGTTAATAGCGT-3', E_Sarbeco_R: ATATTGCA GCAGTACGCACACA-3', E_Sarbeco_P: 5'-FAM-ACAC TAGCCATCCTTACTGCGCTTCG-BHQ1-3') [27]. A 20 µl reaction was assembled consisting of 1 × qScript XLT One-Step RT-qPCR ToughMix Low ROX (2 ×) (QuantaBio), 400 nM E_Sarbeco_F, 400 nM E_Sarbeco_R, 200 nM E_Sarbeco_P (Probe) and 5 µl of purified RNA. The following program was conducted on an ABI 7500 Fast instrument: 55 °C for 10 min for reverse transcription, 1 cycle of 95 °C for 3 min and then 45 cycles of 95 °C for 15 s and 58 °C for 30 s.

N1-STOP-LAMP

A 50-reaction bottle of dried Reverse Transcriptase Isothermal Mastermix (Optigene, ISO-DR004-RT) was rehydrated with 750 µl of resuspension buffer and vortexed gently to mix. The mix contains both a proprietary reverse transcriptase and the GspSSD LF DNA polymerase that has both reverse transcriptase and strand-displacing DNA polymerase activity. The N1-STOP-LAMP assay uses six standard LAMP oligonucleotide primers that target the sequence spanning the CDC N1 region of the SARS-CoV-2 nucleocapsid gene. Sequences of the primers are available upon request (GeneWorks). Each N1-STOP-LAMP reaction contained: 15

µl of mastermix, 5 µl of 5 × primer stock and 4 µl of water. Mastermix was reconstituted in a separate biological safety cabinet to that used for template addition. The source of RNA template for the reaction consisted of either 1 µl of purified SARS-CoV-2 RNA, 1 µl of SARS-CoV-2 purified virus or 1 µl of UTM from a nasopharyngeal swab. A no-template control (1 µl water) was included in all runs. Reactions were assembled in either 8-tube Genie strips (OP-00008, OptiGene) or 96-MicroAmp-Fast-Optical reaction plate (Applied Biosystems). Strip tubes reactions were capped, or for 96-MicroAmp-Fast-Optical reaction plates, sealed with MicroAmp Optical adhesive film (Applied Biosystems) and tapped to remove bubbles. Strip tubes were loaded onto the Genie-II or Genie-III (OptiGene Ltd) and 96-well plates run on a QuantStudio seven thermocycler (ThermoFisher). Reactions were incubated at 65 °C for 30 min with fluorescence acquisition every 30 s (Genie instruments) or 1 min (QuantStudio 7). A positive result was indicated by an increase in fluorescence at an emission wavelength of 540 nm (FAM channel) above a defined threshold, recorded as time-to-positive (Tp) expressed in min:sec.

Dilution series of purified SARS-CoV-2

A virus stock with of 5.4 × 10⁵ TCID₅₀ ml⁻¹ was serially diluted to 10⁻⁶ in a generic UTM (comprising per litre: 8 g NaCl, 0.2 g KCl, 1.15 g Na₂HPO₄, 0.2 g KH₂PO₄, 4 ml 0.5% phenol red 0.5%, 5 g gelatin, 950 ml tissue culture water, 1 ml Fungizone [5 mg ml⁻¹], 20 ml penicillin/streptomycin) in biological triplicates, with the 10⁻¹ through to the 10⁻⁶ dilutions tested by N1-STOP-LAMP (1 µl). RNA was extracted from a 200 µl aliquot of each dilution as described above and 5 µl of the purified RNA used as template in E-gene RT-qPCR or N1-STOP-LAMP assay.

Interlaboratory comparison of N1-STOP-LAMP

A panel of 20 blinded clinical samples (13 positive and 7 negative) with cycle threshold (Ct) values previously established by E-gene RT-qPCR were aliquoted and distributed to three different laboratories for independent testing by N1-STOP-LAMP assay (Table S1, available in the online version of this article).

Biological specificity

To test for cross reactivity of the N1-STOP-LAMP assay, a control panel of respiratory pathogens (NATRPC2-BIO, ZeptoMetrix) was screened (Table S2). A 1 µl aliquot of NATrol RP1 or RP2, or samples spiked with SARS-CoV-2 virus were used as template for N1-STOP-LAMP.

In silico nucleotide sequence comparisons of the N1 region of SARS-CoV-2

To assess the inclusivity and exclusivity of the N1 region targeted by the LAMP assay 2755 publicly available SARS-CoV-2 genomes were downloaded and filtered to remove entries of less than 29 kb using *Seqtk seq* (v1.3-r106). Sequence gaps were replaced with 'N' using *Seqkit* (v0.12.0). After this quality-control step, homology searches were then

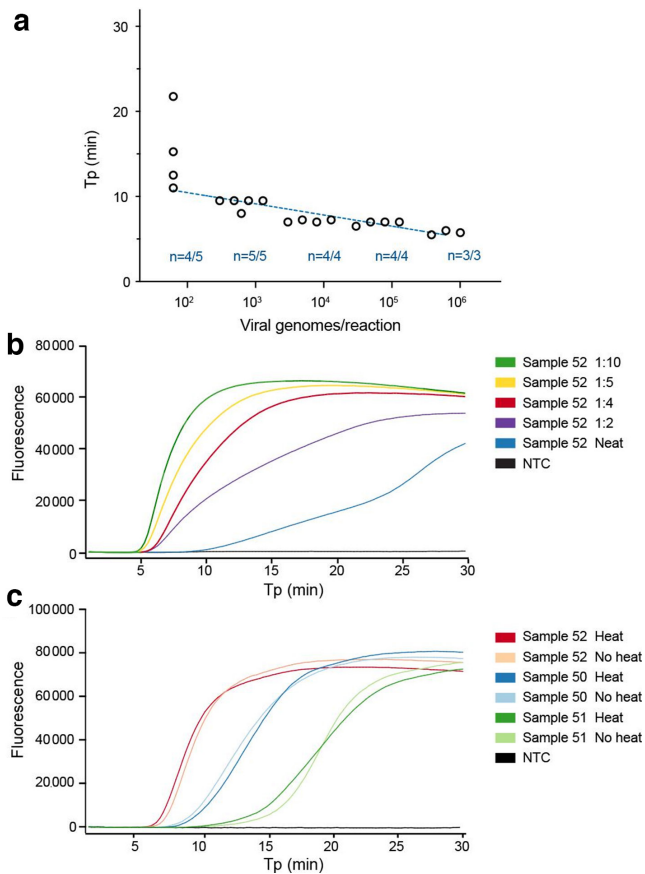


Fig. 1. Limit of detection of N1-STOP-LAMP and establishing optimal sample template volume and inactivation conditions. (a) Plot showing performance of the N1 LAMP assay across 5- \log_{10} dilution of purified viral RNA. Y-axis is time-to-positive (Tp) and the x-axis is an estimate of viral genomes/reaction based on starting RNA concentration. The number of replicates per dilution (n) and number of positive replicates per dilution is indicated. (b) Example N1-STOP-LAMP amplification plots for SARS-CoV-2 positive clinical sample No. 52, showing inhibitory impact of 5 μ l of a neat sample matrix on LAMP and the effect of diluting the UTM in water. (c) N1-STOP-LAMP amplification plots for three SARS-CoV-2 positive clinical samples, showing no loss in detection sensitivity after specimen heat-treatment of 60 °C for 30 min to inactivate virus.

conducted using *NCBI BLAST+BLASTn* using *Genepuller.pl* (<https://github.com/tseemann/bioinfo-scripts/blob/master/bin/gene-puller.pl>) to find the region in each of the remaining 2738 genomes matching the 5' region of the N1 sequence. The resulting sequence coordinates of those hits were then used to extract the 240 bp region from all 2738 genomes using *Genepuller.pl* and aligned with *Clustalo* (v1.2.4). The alignment was visualized with *Mesquite* (v3.61).

Statistical analysis

Data analysis was managed using GraphPad Prism (v8.4.1). Sensitivity and specificity testing were performed using the Wilson–Brown hybrid method as deployed in GraphPad Prism.

RESULTS

Assessing detection sensitivity of N1-STOP-LAMP using purified RNA

We began by assessing the limit of detection (LoD) of N1-STOP-LAMP under optimum conditions using a 10-fold dilution series of purified RNA, prepared from a titred SARS-CoV-2 virus stock. Although LAMP assays are not strictly quantitative, a positive correlation between Tp and RNA concentration was evident. This was indicated by an absolute detection threshold between 50 and 500 viral genome copies per reaction. We achieved a reliable detection of 5/5 replicates at 500 viral genome copies and detection of 4/5 replicates at 50 viral genome copies per reaction (Fig. 1a). Of note, this threshold is likely an underestimate of the true detection limit, as we assumed all RNA yielded from the viral stock generated from the Vero cell supernatant was of viral origin. Using $TCID_{50}$, the absolute LoD of N1-STOP-LAMP was between 0.001 and 0.01 $TCID_{50}$ per reaction (equivalent to 1–10 $TCID_{50}$ ml⁻¹).

Direct detection of SARS-CoV-2 RNA in clinical specimens and specimen inactivation

The nasopharyngeal specimens received by our public health laboratories were collected using Copan flocked swabs in either 1 ml or 3 ml of UTM. To assess the impact of sample matrix on the LAMP assay, we performed pilot experiments with UTM from swabs taken from SARS-CoV-2 negative specimens, adding increasing amounts of UTM to N1-STOP-LAMP. Sterile UTM had no impact on N1-STOP-LAMP (data not shown). However, the nasopharyngeal secretions in patient samples were observed to be inhibitory. Most pronounced when 5 μ l of patient sample was used as the direct RNA template (Fig. 1b), but relieved at a 1/5 dilution of the patient sample, thus 5 μ l of a 1/5 dilution of the patient sample or a 1 μ l of neat patient sample was selected as the optimum template volume for N1-STOP-LAMP (Fig. 2a). Experiments were also conducted using dry swabs eluted in PBS. We tested elution in 0.5, 1.0 and 1.5 ml and found that an elution volume of 1.5 ml of PBS was a good compromise between unnecessary dilution of potential virus in the sample and sufficient dilution to alleviate assay inhibition (data not shown). To reduce the risk associated with handling clinical specimens containing infectious SARS-CoV-2, we also assessed the impact of a heat inactivation step on detection sensitivity. Pre-treatment at 60 °C for 30 min led to $>5\text{-log}_{10}$ inactivation of the virus accessed by p.f.u. and $TCID_{50}$ determination (data not shown). No impact on N1-STOP-LAMP detection sensitivity was observed, with equivalent Tp between each treatment (Table 1, Fig. 1c).

Comparative detection sensitivity of N1-STOP-LAMP versus E-gene RT-qPCR

The N1-STOP-LAMP assay was then directly compared with E-gene RT-qPCR, using a dilution of titred SARS-CoV-2 virus stock in UTM across a 6- \log_{10} dilution series. A RT-qPCR calibration curve was established from triplicate extraction

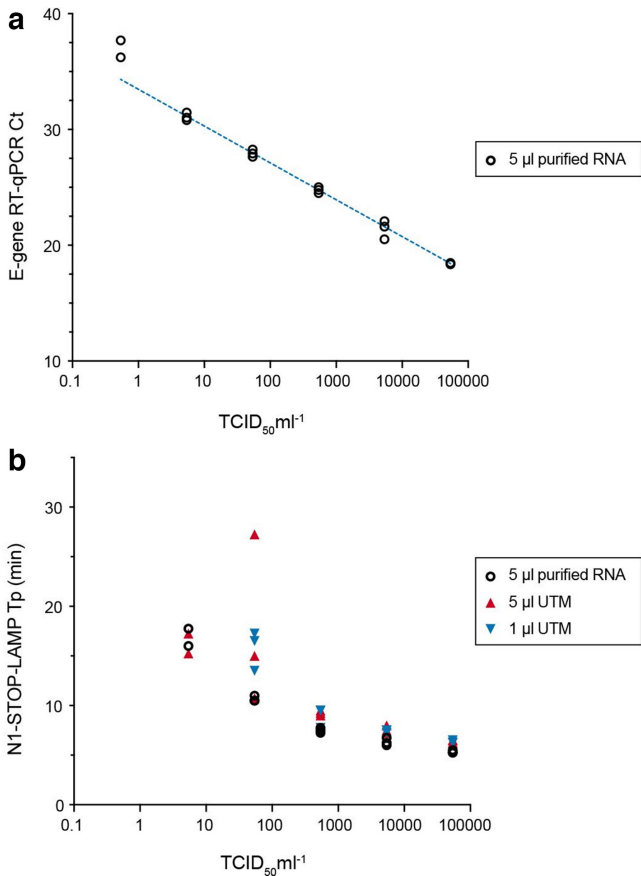


Fig. 2. Comparison of E-gene RT-qPCR with N1-STOP-LAMP and limit of detection. Titrated virus was serially diluted 10-fold in UTM in triplicate and RNA was extracted from each replicate dilution. A 1 µl aliquot of each UTM dilution was also tested directly by N1-STOP-LAMP. (a) Calibration curve for the E-gene RT-qPCR. A curve was interpolated using linear regression. (b) Comparative performance of N1-STOP-LAMP with 5 µl purified RNA versus 1 µl or 5 µl of UTM directly added to the reaction.

experiments for each of the six dilutions (Fig. 2a). The assays were prepared with 5 µl of purified viral RNA as template for both the N1-STOP-LAMP and RT-qPCR assays. A 1 µl and 5 µl aliquot of each virus dilution in UTM was also tested directly in the N1-STOP-LAMP assay (i.e. without RNA

Table 1. Assessment of heat treatment of universal transport medium specimens on detection sensitivity

Sample No.	E-gene RT-qPCR Ct	Treatment	
		No heat Tp (min:s)	Heat Tp (min:s)
50	21.76	10 : 15	10 : 45
51	24.40	17 : 45	15 : 45
52	15.05	07 : 15	07 : 45

Ct, cycle threshold; Tp, time-to-positive

extraction). The side-by-side comparisons showed E-gene RT-qPCR was up to 1-log₁₀ more sensitive than N1-STOP-LAMP, with RT-qPCR detecting 2/3 replicates at the lowest dilution of 0.54 TCID₅₀ ml⁻¹ and N1-STOP-LAMP detecting no viral RNA at this concentration (Table 2, Fig. 2). At 54 TCID₅₀ ml⁻¹, the RT-qPCR and N1-STOP-LAMP detected 3/3 replicates. There was no difference in N1-STOP-LAMP sensitivity using either 5 µl UTM added directly to the test or purified RNA, but the assay lost another 1-log₁₀ sensitivity where 1 µl of neat UTM was added directly to the N1-STOP-LAMP reaction (Fig. 2b).

Establishing N1-STOP-LAMP LoD

The FDA guidelines for implementation of emergency-use diagnostics defines LoD as the lowest concentration at which 19/20 replicates are positive. Informed by the previous experiment (Fig. 2b), we selected a SARS-CoV-2 concentration of 54 TCID₅₀ ml⁻¹ (in UTM) and tested 20×1 µl aliquots by N1-STOP-LAMP. We observed 20/20 positive reactions with an average Tp of 15.8 mins, inter-quartile range 12–19 min and coefficient of variation of 31.18% (Fig. 3a).

Evaluation of N1-STOP-LAMP against FDA criteria

The FDA guidelines for implementation of emergency-use diagnostics also require an assessment of assay performance using at least 30 contrived positive and 30 negative clinical specimens (authentic or contrived), with at least 20 of the positives at a concentration of 1–2 × the LoD. We obtained 30 nasopharyngeal dry swabs from healthy, anonymous volunteers. Swabs were eluted in 1.5 ml of PBS and 1 µl aliquots were tested directly by N1-STOP-LAMP. All eluates were negative. We then spiked the eluates with SARS-CoV-2 virus at 1× and 2× the LoD (approx. 50 and 100 TCID₅₀ ml⁻¹, respectively). Results showed that N1-STOP-LAMP detected 20/20 samples spiked with 1× the assay LoD and 10/10 samples at 2× LoD (Fig. 3b, Table 3). N1-STOP-LAMP thus meets the FDA Clinical Evaluation criteria.

Clinical specimen evaluation of N1-STOP-LAMP against E-gene RT-qPCR gold standard

We then sought to evaluate N1-STOP-LAMP performance against E-gene RT-qPCR, setting the latter assay as the current 'gold standard'. We directly screened 50 negative clinical specimens and 107 positive nasopharyngeal clinical specimens as established by E-gene RT-qPCR (Table S3). Results showed N1-STOP-LAMP assay sensitivity of 87%, specificity of 100%, positive predictive value of 100% and negative predictive value of 78% (Table 4, Fig. 3). As noted during initial sensitivity experiments (Table 2), a positive correlation was observed between N1-STOP-LAMP Tp and E-gene RT-qPCR Ct (Fig. 3c). The average Tp among the 93 N1-LAMP-STOP positive specimens was 14 min (sd±7 min). For the false-negative samples with sufficient clinical material remaining (5 out of 14), RNA extraction using a simple, rapid magnetic bead purification protocol (takes 20 min) was performed, then N1-STOP-LAMP repeated. Four of the five samples returned a positive result with Tp range from 10 :

Table 2. Comparison of N1-STOP-LAMP and RT-qPCR performance using titred virus diluted in universal transport medium

SARS-CoV-2 (TCID ₅₀ ml ⁻¹)	N	N1-LAMP (1 µl direct)		N1-LAMP (5 µl direct)		N1-LAMP (5 µl of 60 µl)		RT-qPCR (5 µl of 60 µl)	
		Tp (Avg)	CV (%)	Tp (Avg)	CV (%)	Tp (Avg)	CV (%)	Ct (Avg)	CV (%)
54000	3	06.33	02.28	06.41	02.25	05.33	02.71	18.41	0.33
5400	3	07.42	01.95	07.58	05.04	06.33	06.03	21.40	3.75
540	3	08.92	11.33	09.25	02.70	07.50	03.33	24.77	1.03
54	3	15.75	12.60	17.67	48.49	10.67	02.71	27.94	1.13
5.4	3	ND	–	16.25	08.70*	16.88	07.33*	31.10	1.17
0.54	3	ND	–	ND	ND	ND	–	36.96	2.79*

*2/3 replicates positive at this dilution.

Tp, time-to-positive; CV, coefficient of variation; ND, not detected.

00 – 25 : 30 min:s (Table S3). Indicating that the reduced sensitivity of N1-STOP-LAMP compared to RT-qPCR can be improved upon by using samples with a higher RNA template concentration and purity with only a small increase in time to results.

Confirmation of N1-STOP-LAMP inclusivity and exclusivity

The FDA guidelines for implementation of emergency-use diagnostics require an assessment of inclusivity and exclusivity of the oligonucleotide primers used in an assay. To assess the former, we performed *in silico* screening of the region spanned by the six primers used for the N1-STOP-LAMP assay against all available SARS-CoV-2 genome sequences (Data File S1). This 240 bp sequence spans the CDC N1 region of the SARS-CoV-2 N-gene [3]. Alignment of this region against 2738 genomes revealed 100% conservation amongst all entries, showing that this is a conserved target sequence and that the primers used will perform equally across all identified lineages of the virus.

To assess the exclusivity criterion, we used a nucleotide BLAST search of the N1 region against the NCBI Genbank *nt* database and observed no non-SARS-CoV-2 sequence matches above 80% nucleotide identity, in-line with FDA cross-reactivity requirement. Further to this analysis, we also performed *in vitro* testing with the NATRPC2-BIO (ZeptoMetrix) specificity panel, a control panel consisting of 22 common respiratory pathogens. None of these pathogens were detected by N1-STOP-LAMP (Table S2). Thus, both *in silico* and *in vitro* testing confirmed the specificity of N1-STOP-LAMP for SARS-CoV-2.

Interlaboratory comparisons

In order to explore the ability of other laboratories to readily deploy N1-STOP-LAMP, we prepared an external-quality-assessment (EQA) panel of 20 clinical specimens (Table S1). These were previous positive and negative clinical samples as assessed by E-gene RT-qPCR that had been heat-inactivated

for safety. These specimens covered a range of virus titres (Ct values 16.3–30.6). The panel was tested simultaneously in four different laboratories (labs A–D). The results showed excellent correspondence between all laboratories for ten positive samples, the majority with E-gene RT-qPCR Ct values <26 (Fig. 3d, Table S1). Above this Ct (i.e. those samples with lower virus titres), the laboratories returned variably positive results (samples 14, 16, 17 and 18), reflecting that these specimens had virus concentrations at or beyond the N1-STOP-LAMP LoD (Table S1). Concordance among the seven negative results was overall very good, however one laboratory (lab C) returned a false-positive result (sample 2), highlighting the issue of contamination with sensitive molecular tests (Table S1). Of note, the laboratories used a range of different instruments for amplification/detection including: OptiGene Genie II and III, BioRad CFX and ThermoFisher Quantstudio 7. This trial suggests that N1-STOP-LAMP is a robust and transferrable assay format.

DISCUSSION

A critical component of an effective COVID-19 pandemic response is the rapid and robust detection of positive clinical samples [28]. This has required massively upscaled SARS-CoV-2 diagnostic capabilities. Particularly, a worldwide focus on developing improved technologies [29]. There have been more than 20 molecular tests recently receiving FDA Emergency Use Authorisations (EUs) [5]. In this current study, we demonstrate the potential of N1-STOP-LAMP. We focused efforts on the LAMP assay because it has been previously employed for the rapid and robust detection of numerous RNA viruses including Zika, Chikungunya, Influenza and SARS-CoV-1 [12, 30–33] and was of great assistance during these outbreaks, particularly in resource constrained settings. Advantages of LAMP testing include rapid turnaround time, ease of implementation, non-standard reagent use and potential utility at point of care [19–21]. To date, there has been limited detail on the performance characteristics of emerging RT-LAMP-based tests in head-to-head comparisons with

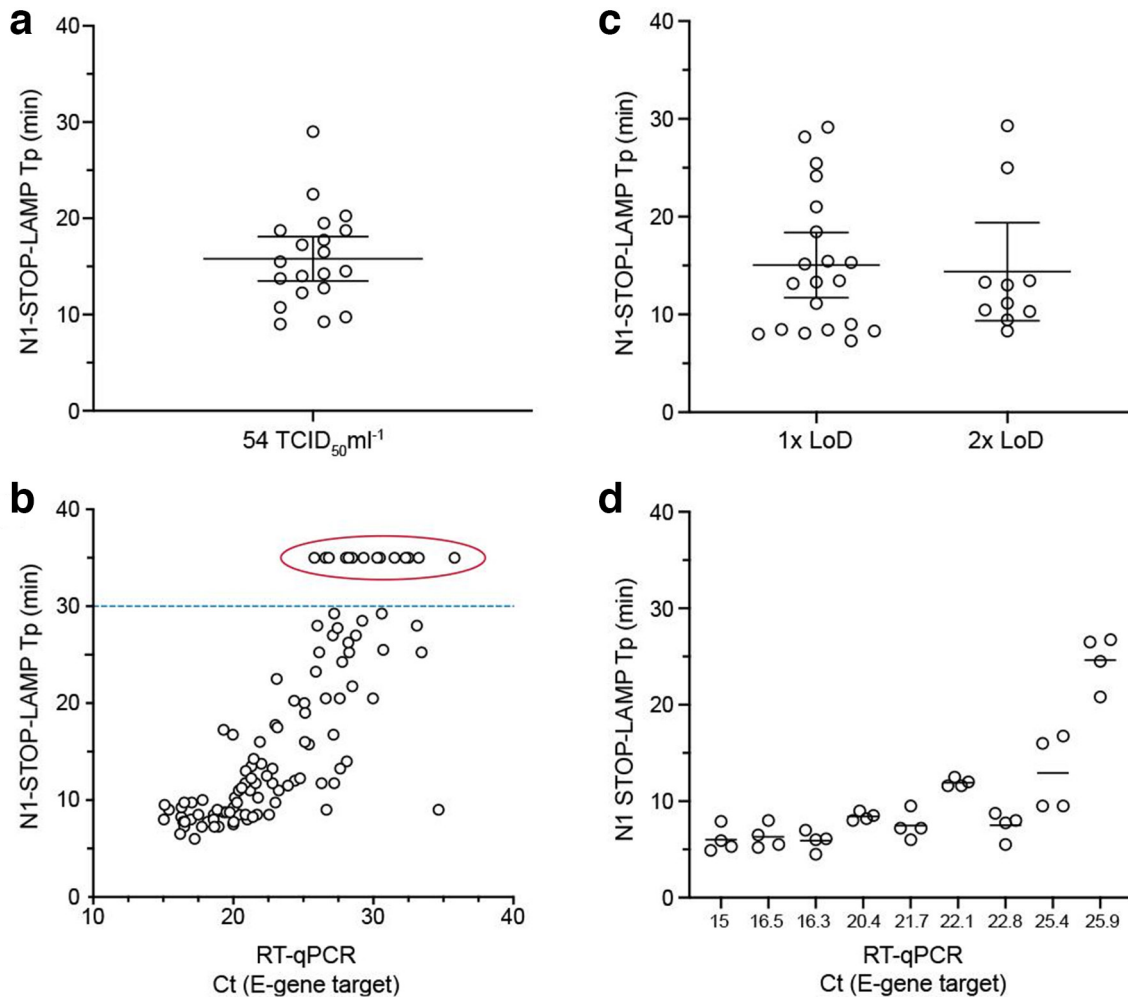


Fig. 3. Clinical evaluation of N1-STOP-LAMP. (a) Establishing limit of detection according to FDA Emergency Use Authorisations (EUA) guidelines. Twenty, 1 µl replicates of SARS-CoV-2 virus in UTM at a concentration of 54 TCID₅₀ ml⁻¹ were tested by direct N1-STOP-LAMP. All data points plotted, with average and 95% CI indicated. (b) Assessment of assay against FDA EUA clinical performance criteria. Thirty healthy volunteer dry nasopharyngeal swabs eluted in 1.5 ml of PBS were screened by N1-STOP-LAMP. Shown are time-to-positives (Tp). Mean and 95% CI are indicated. (c) Plot showing correspondence between 107 E-gene RT-qPCR positive and N1-STOP-LAMP, where 1 µl of clinical specimen in UTM was added directly to the RT-LAMP reaction. The y-axis is LAMP Tp and the x-axis is RT-qPCR cycle threshold (Ct). The dotted line indicates Tp 30 min. Results plotted above this line (encircled) were RT-qPCR positive but N1-STOP-LAMP negative. (d) Results of four-way interlaboratory comparison of N1-STOP-LAMP. Ten clinical samples previously tested positive by E-gene RT-qPCR were used to create a test panel for comparing assay performance between laboratories. The sample with Ct of 15 was the positive control, purified SARS-CoV-2.

Table 3. Summary of clinical evaluation comparisons against FDA criteria

	Negative	1–2x LoD Tp (min:s)	2x LoD Tp (min:s)
N1-STOP-LAMP +	0	20 (07 : 30 – 22 : 00)*	10 (08 : 30 – 21 : 00)*
N1-STOP-LAMP -	30	0	0

*Range indicated within parentheses.
LoD, Limit of detection; Tp, time-to-positive.

Table 4. Comparison of N1-STOP-LAMP versus E-gene RT-qPCR using clinical specimens

	RT-qPCR +	RT-qPCR -	Total
N1-STOP-LAMP +	93	0	93
N1-STOP-LAMP -	14	50	64
Total	107	50	157

gold standard assays. Such information is critical to ensuring the safe deployment of new tests, given the clinical and public health consequences of erroneous test results.

Here we have shown the specific detection of SARS-CoV-2 RNA directly from clinical swabs without the need for RNA purification, using a LAMP assay targeting the N1 region of the SARS-CoV-2 N-gene in conjunction with the reverse transcriptase/GspSSD LF DNA polymerase mastermix from OptiGene. With a detection limit of 54 TCID₅₀ ml⁻¹ the N1-STOP-LAMP assay met each of the four FDA criteria for EUA. Based on reported data, as opposed to head-to-head comparisons, the N1-STOP-LAMP detection limit is higher than several recently reported FDA-EUA COVID-19 tests. Expressed as virus copies per ml the N1-STOP-LAMP LoD is estimated at 54000 virus copies per ml (assumes our TCID₅₀ underestimates virus copy number by 1000-fold, see Methods), compared to Cepheid GeneXpert Xpress 250 virus copies per ml or Abbott ID NOW COVID-19 test 125 virus copies per ml. Other EUA assays reporting LoDs similar to N1-STOP-LAMP include the Luminex ARIES SARS-CoV-2 assay (75000 copies per ml) and the GenMark ePlex SARS-CoV-2 test (100000 copies per ml). Given these performance metrics, and the test's high positive predictive value (100%), we see N1-STOP-LAMP as well suited for widespread screening of large populations when COVID-19 prevalence is low. N1-STOP-LAMP could be used in large-scale, national testing programs to support aggressive COVID-19 contact tracing and disease suppression or elimination activities. The test could also play a role in near-point-of-care settings, such as outbreak investigation in hospitals and nursing homes, providing rapid-turnaround-time of results for health-care workers and highly vulnerable patients. The relatively simple assay format of N1-STOP-LAMP is also suitable for deployment in lower and middle-income countries, where access to sophisticated laboratory infrastructure is limited.

During this evaluation we noted opportunities for improvement of N1-STOP-LAMP. We observed that although 1 µl of UTM was tolerated in the OptiGene RT-LAMP formulation, larger quantities of sample matrix (UTM or PBS containing additional human material from the nasopharyngeal swab) were inhibitory to the RT-LAMP reaction. If more than 1 µl of spiked (purified virus or RNA as template) sample matrix was used in the assay, no amplification was observed (Fig. 1b). However, under ideal conditions (i.e. no sample inhibition from human components), the impact of using an increased template volume was shown, where 5 µl of UTM added directly to the N1-STOP-LAMP reaction was equal to the sensitivity of using purified RNA (Fig. 2b). Furthermore, addition of a simple RNA purification step, that does not require commercial kits, improved the detection sensitivity of N1-STOP-LAMP to rival RT-qPCR. Our follow-up of five false-negative results (Table 4, Fig. 3c), demonstrated the feasibility of including a simple, scalable and rapid magnetic-bead RNA purification step [26], with which we were able to detect SARS-CoV-2 RNA with N1-STOP-LAMP in 4/5 UTM specimens that had

previously tested negative by the assay (Table S3). Other opportunities for improvement include the use of specific fluorescent probes to detect amplification products, thus facilitating multiplex reactions and the inclusion of an internal amplification control within each test [24, 34].

As mentioned, recognized strengths of the LAMP assay include the rapid time to test result, with the majority of the positive clinical samples yielding a result in under 15 min, and the robust test format, testing directly from the swab eluate. Our preferred specimen type for N1-STOP-LAMP is a dry nasopharyngeal swab. Detailed examination of optimum swabs for the detection of influenza and other RNA viruses has shown dry swabs offer superior performance [35]. In the current study we have shown that N1-STOP-LAMP has satisfactory performance using dry swabs eluted in 1.5 ml of PBS (Fig. 3b); a format that potentially doubles the effective concentration of virus in the sample compared to 3 ml of UTM in the Copan format. Another strength of N1-STOP-LAMP is that the assay readily scales from small sample numbers (e.g. 8-well with portable detection unit) to high throughput (run with sample robotics and 96-well thermocyclers in a centralized laboratory).

While establishing the assay, we noted susceptibility to contamination that was easily addressed by the standard precautions used to prevent template carryover for any nucleic acid amplification test, such as physical separation of mastermix preparation from sample inoculation; not opening post-amplification reaction tubes or plates; and inclusion of negative extraction controls. Addition of uracil-N-glycosylase has been reported as a strategy to limit the risk of amplicon contamination, however this is associated with a loss of detection sensitivity [36].

In this report we have shown N1-STOP-LAMP is a robust diagnostic test for the specific and rapid detection of SARS-CoV-2. It is an alternative molecular test for SARS-CoV-2 that can be readily and seamlessly deployed, particularly when access to standard RT-qPCR-based approaches are limited.

Funding information

D.A.W. is supported by an Emerging Leadership Investigator Grant from the National Health and Medical Research Council (NHMRC) of Australia (APP1174555). T.P.S. is supported by NHMRC Research Fellowship (APP1105525). B.P.H. is supported by NHMRC Practitioner Fellowship (APP1105905).

Author contributions

J.L. conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing - original draft writing - review and editing. N.B. conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, visualization, writing - original draft writing - review and editing. J.M.A. conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, writing - review and editing. J.P. data curation, formal analysis, investigation, methodology, writing - review and editing. T.S. conceptualization investigation, methodology, software. M.S. conceptualization, investigation, methodology, software. M.S. formal analysis, investigation, methodology software. N.O. formal analysis, investigation, methodology. K.M. data curation, investigation,

methodology. S.B. conceptualization, data curation, formal analysis, investigation, methodology. J.D. conceptualization, data curation, formal analysis, investigation, methodology. T.T. investigation, methodology. M.C. conceptualization investigation, supervision, writing - review and editing. M.P. conceptualization, investigation, methodology, supervision, writing - review and editing. H.C. investigation, methodology, writing - review and editing. A.L. investigation, methodology, writing - review and editing. S.M. conceptualization, formal analysis, investigation, methodology, writing - review and editing. A.G. conceptualization, formal analysis, funding acquisition investigation, methodology, writing - review and editing. J.K. conceptualization, formal analysis, funding acquisition, methodology, writing - original draft writing - review and editing. N.S. conceptualization, formal analysis investigation, Methodology Validation Writing - original draft Writing - review and editing. M.G., conceptualization, formal analysis investigation, methodology, validation, writing - review and editing. T.H. conceptualization, funding acquisition, investigation, methodology, validation, writing - review and editing. M.H. conceptualization, funding acquisition, investigation, methodology, resources, writing - review and editing. D.W. conceptualization, formal analysis, funding acquisition, investigation, methodology, writing - review and editing. B.H. conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, resources, writing - original draft writing - review and editing. I.M. conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, validation, writing - original draft writing - review and editing. T.S. conceptualization, data curation, formal analysis, funding acquisition investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing - original draft writing - review and editing.

Conflicts of interest

The authors declare the following conflict of interest: Nickala Best, Sean McDonald and Arran Greenhalgh are employees of GeneWorks, a commercial entity that distributes OptiGene reagents in Australia.

Ethical statement

This study was conducted in accordance with the *National Health and Medical Research Council of Australia National Statement for Ethical Conduct in Human Research* 2007 [37]. The study was exempt from requiring specific approvals, as it involved the use of existing collections of data or records that contained non-identifiable data about human beings [37].

References

1. Victorian Department Health and Human Services 2020. Coronavirus disease 2019 (COVID-19) Guidelines for health services and general practitioners - Version 17, 5/04/2020. State Government of Victoria, Australia. <https://www.dhhs.vic.gov.au/health-services-and-general-practitioners-coronavirus-disease-covid-19> [accessed 7 April 2020].
2. UK National Health Service. 2020. Guidance and standard operating procedure: COVID-19 virus testing in NHS laboratories, 16/03/2020. NHS, London. <https://www.england.nhs.uk/coronavirus/publication/guidance-and-standard-operating-procedure-covid-19-virus-testing-in-nhs-laboratories/> [accessed 7 April 2020].
3. Center for Disease Control. 2020. A 2019–Novel Coronavirus (2019-nCoV) real-time RT-PCR panel primers and probes, 24/01/2020. US Department of Health & Human Services, CDC, Atlanta. <https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf> [accessed 7 April 2020].
4. Centre for Disease Control. 2020. Real-time RT-PCR panel for detection 2019–Novel Coronavirus, 24/01/2020. US Department of Health & Human Services, CDC, Atlanta. <https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-for-detection-instructions.pdf> [accessed 7 April 2020].
5. Food and Drug Administration. 2020. Policy for diagnostic tests for Coronavirus disease – 2019 during the public health emergency – Immediately in effect guidance for clinical laboratories, commercial manufacturers, and food and Drug administration staff, 16/03/2020. US Department of Health & Human Services, Food and Drug Administration. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-diagnostic-tests-coronavirus-disease-2019-during-public-health-emergency> [accessed 7 April 2020].
6. World Health Organization. *Laboratory Testing for Coronavirus Disease 2019 (COVID-19) in Suspected Human Cases, 2/03/2020*. Geneva: World Health Organisation; 2020.
7. World Health Organization. *Coronavirus Disease (COVID-19) Technical Guidance: Laboratory Testing for 2019-nCoV in Humans. 3. Molecular Assays to Diagnose COVID-19, 03/2020*. Geneva: World Health Organisation; 2020.
8. Kelly P. Deputy Chief Medical Officer's Press Conference About COVID-19 on 16 March. Australian Government; 2020.
9. Arnold C. COVID-19: biomedical research in a world under social-distancing measures. *Nat Med* 2020.
10. Open for outbreaks. *Nat Biotechnol* 2020;38:377.
11. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K et al. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res* 2000;28:E63
12. Hong TCT, Mai QL, Cuong DV, Parida M, Minekawa H et al. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. *J Clin Microbiol* 2004;42:1956–1961.
13. Iwamoto T, Sonobe T, Hayashi K. Loop-mediated isothermal amplification for direct detection of *Mycobacterium tuberculosis* complex, *M. avium*, and *M. intracellulare* in sputum samples. *J Clin Microbiol* 2003;41:2616–2622.
14. Mori Y, Kanda H, Notomi T. Loop-mediated isothermal amplification (LAMP): recent progress in research and development. *J Infect Chemother* 2013;19:404–411.
15. Poon LLM, Wong BWY, Ma EHT, Chan KH, Chow LMC et al. Sensitive and inexpensive molecular test for falciparum malaria: detecting *Plasmodium falciparum* DNA directly from heat-treated blood by loop-mediated isothermal amplification. *Clin Chem* 2006;52:303–306.
16. Tsujimoto M, Nakabayashi K, Yoshidome K, Kaneko T, Iwase T et al. One-Step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients. *Clin Cancer Res* 2007;13:4807–4816.
17. Zhang Y, Odiwuor N, Xiong J, Sun L, Nyaruaba RO et al. Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric lamp. *medRxiv* 2020.
18. Yu L, Wu S, Hao X, Li X, Liu X et al. Rapid colorimetric detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform: iLACO. *medRxiv* 2020.
19. Yan C, Cui J, Huang L, Du B, Chen L et al. Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. *Clin Microbiol Infect* 2020.
20. Park G-S, Ku K, Baek S-H, Kim S-J, Kim S-I et al. Development of reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays targeting SARS-CoV-2. *J Mol Diagnostics* 2020.
21. Pang J, Wang MX, Ang IYH, Tan SHX, Lewis RF et al. Potential rapid diagnostics, vaccine and therapeutics for 2019 novel coronavirus (2019-nCoV): a systematic review. *J Clin Med* 2020;9:623.
22. Osterdahl M, Lee K, Ni Lochlainn M, Wilson S, Douthwaite S et al. Detecting SARS-CoV-2 at point of care: preliminary data comparing loop-mediated isothermal amplification (LAMP) to PCR. *SSRN Journal* 2020.
23. Lu R, Wu X, Wan Z, Li Y, Zuo L et al. Development of a novel reverse transcription loop-mediated isothermal amplification method for rapid detection of SARS-CoV-2. *Viral Sin* 2020.
24. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V et al. CRISPR-Cas12-based detection of SARS-CoV-2. *Nat Biotechnol* 2020;38:870–874.

25. Caly L, Druce J, Roberts J, Bond K, Tran T *et al.* Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia. *Med J Aust* 2020;212:459–462.
26. He H, Li R, Chen Y, Pan P, Tong W *et al.* Integrated DNA and RNA extraction using magnetic beads from viral pathogens causing acute respiratory infections. *Sci Rep* 2017;7:45199.
27. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 2020;25.
28. Baek YH, Um J, Antigua KJC, Park J-H, Kim Y *et al.* Development of a reverse transcription-loop-mediated isothermal amplification as a rapid early-detection method for novel SARS-CoV-2. *Emerg Microbes Infect* 2020;9:998–1007.
29. Nguyen T, Duong Bang D, Wolff A. 2019 novel coronavirus disease (COVID-19): paving the road for rapid detection and point-of-care diagnostics. *Micromachines* 2020;11:306.
30. FIND. SARS-CoV-2 diagnostic pipeline, 2020 2020.
31. Jayawardena S, Cheung CY, Barr I, Chan KH, Chen H *et al.* Loop-mediated isothermal amplification for influenza A (H5N1) virus. *Emerg Infect Dis* 2007;13:899–901.
32. Lopez-Jimena B, Wehner S, Harold G, Bakheit M, Frischmann S *et al.* Development of a single-tube one-step RT-LAMP assay to detect the Chikungunya virus genome. *PLoS Negl Trop Dis* 2018;12:e0006448.
33. Silva SJRda, Paiva MHS, Guedes DRD, Krokovsky L, Melo FLde *et al.* Development and validation of reverse transcription loop-mediated isothermal amplification (RT-LAMP) for rapid detection of ZIKV in mosquito samples from Brazil. *Sci Rep* 2019;9:4494.
34. Bhadra S, Riedel TE, Lakhota S, Tran ND, Ellington AD. High-surety isothermal amplification and detection of SARS-CoV-2, including with crude enzymes. *BioRxiv* 2020.
35. Moore C, Corden S, Sinha J, Jones R. Dry cotton or flocced respiratory swabs as a simple collection technique for the molecular detection of respiratory viruses using real-time NASBA. *J Virol Meth* 2008;153:84–89.
36. Kim E-M, Jeon H-S, Kim J-J, Shin Y-K, Lee Y-J *et al.* Uracil-DNA glycosylase-treated reverse transcription loop-mediated isothermal amplification for rapid detection of avian influenza virus preventing carry-over contamination. *J Vet Sci* 2016;17:421–425.
37. National Health and Medical Research Council. *National Statement on Ethical Conduct in Human Research 2007 (Updated 2018)*. The National Health and Medical Research Council, the Australian Research Council and Universities Australia. Canberra: Commonwealth of Australia; 2018.

Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as 'excellent' or 'very good'.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.