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Article

Low-Cost, Open-Source Device for High-Performance Fluorescence Detection of Isothermal Nucleic Acid Amplification Reactions

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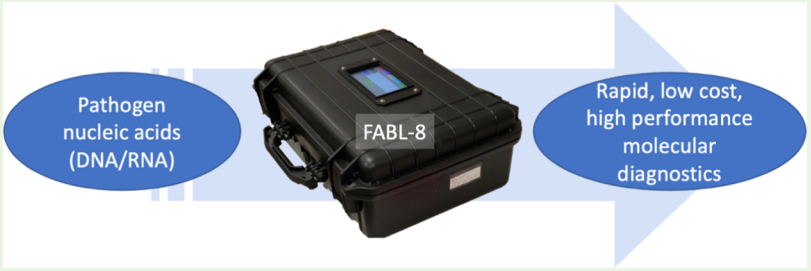

ABSTRACT: The ability to detect SARS-CoV-2 is critical to implementing evidence-based strategies to address the COVID-19 global pandemic. Expanding SARS-CoV-2 diagnostic ability beyond well-equipped laboratories widens the opportunity for surveillance and control efforts. However, such advances are predicated on the availability of rapid, scalable, accessible, yet high-performance diagnostic platforms. Methods to detect viral RNA using reverse transcription loop-mediated isothermal amplification (RT-LAMP) show promise as rapid and field-deployable tests; however, the per-unit costs of the required diagnostic hardware can be a barrier for scaled deployment. Here, we describe a diagnostic hardware configuration for LAMP technology, named the *FABL-8*, that can be built for approximately US$380 per machine and provide results in under 30 min. Benchmarking showed that *FABL-8* has a similar performance to a high-end commercial instrument for detecting fluorescence-based LAMP reactions. Performance testing of the instrument with RNA extracted from a SARS-CoV-2 virus dilution series revealed an analytical detection sensitivity of 50 virus copies per microliter—a detection threshold suitable to detect patient viral load in the first few days following symptom onset. In addition to the detection of SARS-CoV-2, we show that the system can be used to detect the presence of two bacterial pathogens, demonstrating the versatility of the platform for the detection of other pathogens. This cost-effective and scalable hardware alternative allows democratization of the instrumentation required for high-performance molecular diagnostics, such that it could be available to laboratories anywhere—supporting infectious diseases surveillance and research activities in resource-limited settings.

KEYWORDS: *loop-mediated isothermal amplification, rapid diagnostic test, point of care diagnostics, SARS-CoV-2 detection, decentralized diagnostics, low-cost molecular diagnostics*

INTRODUCTION

Prompt detection and physical isolation of infected individuals is a crucial intervention to break SARS-CoV-2 transmission chains, actions which are heavily predicated upon the availability of rapid and high-performance diagnostics.[1−4] Quantitative polymerase chain reaction (qPCR) has become the gold standard methodology for diagnosing infectious diseases, as this platform offers exceptional sensitivity and specificity. Despite its proven diagnostic utility, qPCR remains mostly restricted to centralized and well-resourced laboratories and diagnostic facilities, owing to the high cost per unit and necessity for trained operators. Another major disadvantage of the centralized diagnostic model is lengthy turnaround times of 24−48 h.[2] Longer SARS-CoV-2 test turnaround times equate to a longer period in which infected individuals circulate in the community and unknowingly infect others. Hence, the argument that test turnaround time and frequency of testing appear to be more important than high analytical sensitivity in the population control of SARS-CoV-2.[2]

To overcome the issues linked to diagnostic delays, there has been growing research interest focused on the development of rapid and accessible SARS-CoV-2 diagnostic tests for screening regimes, such as those targeting viral antigen,[5,6] specific

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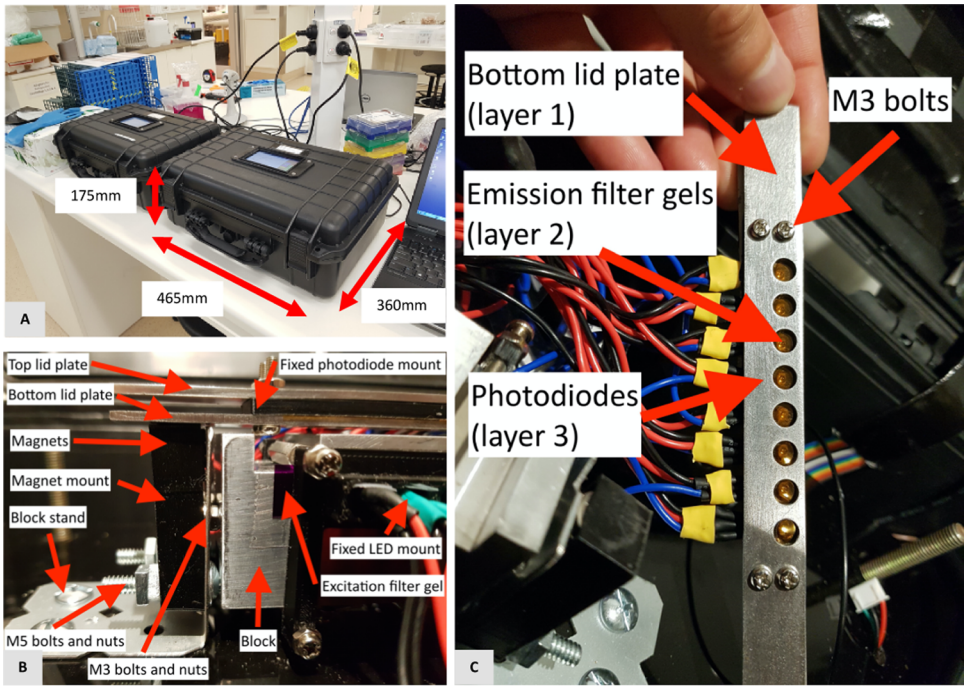

Figure 1. Overview of the FABL-8. (A) Dimensions of the FABL-8 when housed in a tough case. (B) Side aspect of the heating block depicting the array of excitation LEDs, excitation filter gel, and magnetically secured lid that contains an array of photodiodes. (C) Block lid containing eight fixed photodiodes with an overlaid emission filter gel.

antiviral antibodies,[7,8] and genetic material of SARS-CoV-2 with loop-mediated isothermal amplification (LAMP).[9,10] LAMP technology is a genetic amplification approach that does not require sophisticated thermocycling hardware.[11,12] LAMP methods are comparably faster than qPCR, providing increased capacity for rapid point-of-care diagnostics in decentralized clinical situations[10,13] and coupling of LAMP with reverse transcription offers applicability for this technology to detect RNA targets.[12,14−16] Despite the reduction in hardware complexity, commercial LAMP instrumentation capable of running multiple samples, with network connectivity and measuring fluorescence signals are priced upwards of US$5000 and available from relatively few manufacturers, meaning—supply is unassured in times of global demand for molecular diagnostics.

Here, we describe the development of an open-source diagnostic hardware alternative, which we named the *FABL-8*, that is simple to manufacture, is built from readily available parts and is near equivalent in performance to a leading commercial isothermal amplification and fluorescence detection instrument. Using a recently developed RT-LAMP assay known as N1-STOP-LAMP targeting the N-gene of SARS-CoV-2[10] and using a DNA intercalating fluorescent dye to report on the accumulation of LAMP reaction products, we benchmarked performance against a high-end commercial unit and assessed the detection sensitivity and specificity of the *FABL-8*. This development effort was in response to the COVID-19 global pandemic to allow for a reliable, rapid, and scalable point-of-care diagnostic platform of actionable public health utility. Building upon the work of previous innovative, open-source diagnostic efforts,[10,17,18] our system provides a robust and cloud-connected LAMP diagnostic platform that can be built for approximately US$380 per machine.

The code used to control the system, circuit diagrams, and designs of three-dimensional (3D)-printed parts have been deposited in the public domain. It is hoped that the dissemination of this work will allow for the mass deployment of such systems to provide enhanced diagnostic capacity in the fight against COVID-19. In addition to the detection of SARS-CoV-2, the system can be configured to detect virtually any genetic target and therefore has diagnostic utility beyond this pandemic.

METHODS

General System Description. Overall, the system is a robust and portable, briefcase-sized instrument that requires access to mains power and has a run duration of 25 min (Figure 1A). At the heart of the device is a computer numerical control (CNC), milled aluminum block (prepared by Embrey Attachments: https://www.embreyattachments.com.au/) with eight reaction wells that can be heated up to a stable incubation temperature of 65 °C while allowing passage of excitation and emission light through extrusions in the block (Figure 1B). The block was designed to accept an eight-well OptiGene LAMP reaction strip (OptiGene Cat No: OP-0008-50, www.optigene.co.uk). The block is heated by two, 10 Ω 10 W ceramic resistors that are proportional, integral, and derivative (PID) controlled (see the Control section below) through feedback, read by a digital temperature sensor using the I2C communication protocol (Figure 2). The block temperature sensor was calibrated using a digital thermometer with a K-Type thermocouple (Jaycar: QM1602), modified to sit within a 25 μL volume of glycerol in a single OptiGene LAMP reaction tube. The PID control was optimized to rapidly stabilize at 65 °C following possible temperature disruption from the opening and closing of the instrument case for the initiation of a run. The block has a detached lid housing the emission reading system that is held in place by neodymium magnets (Figure 1B). Fluorescence measurements are taken at user-defined regular intervals during the reaction by exciting at 470 nm and measuring emission at 530 nm. The excitation source is a 470 nm 5 mm clear light-emitting diode (LED) with a theatrical lighting gel excitation filter (LEE Filters Cat No: 126-Mauve) (Figure 1B). The emission is read by a light-to-frequency converter photodiode that is covered with a theatrical lighting gel emission filter (LEE Filters Cat No: 015—

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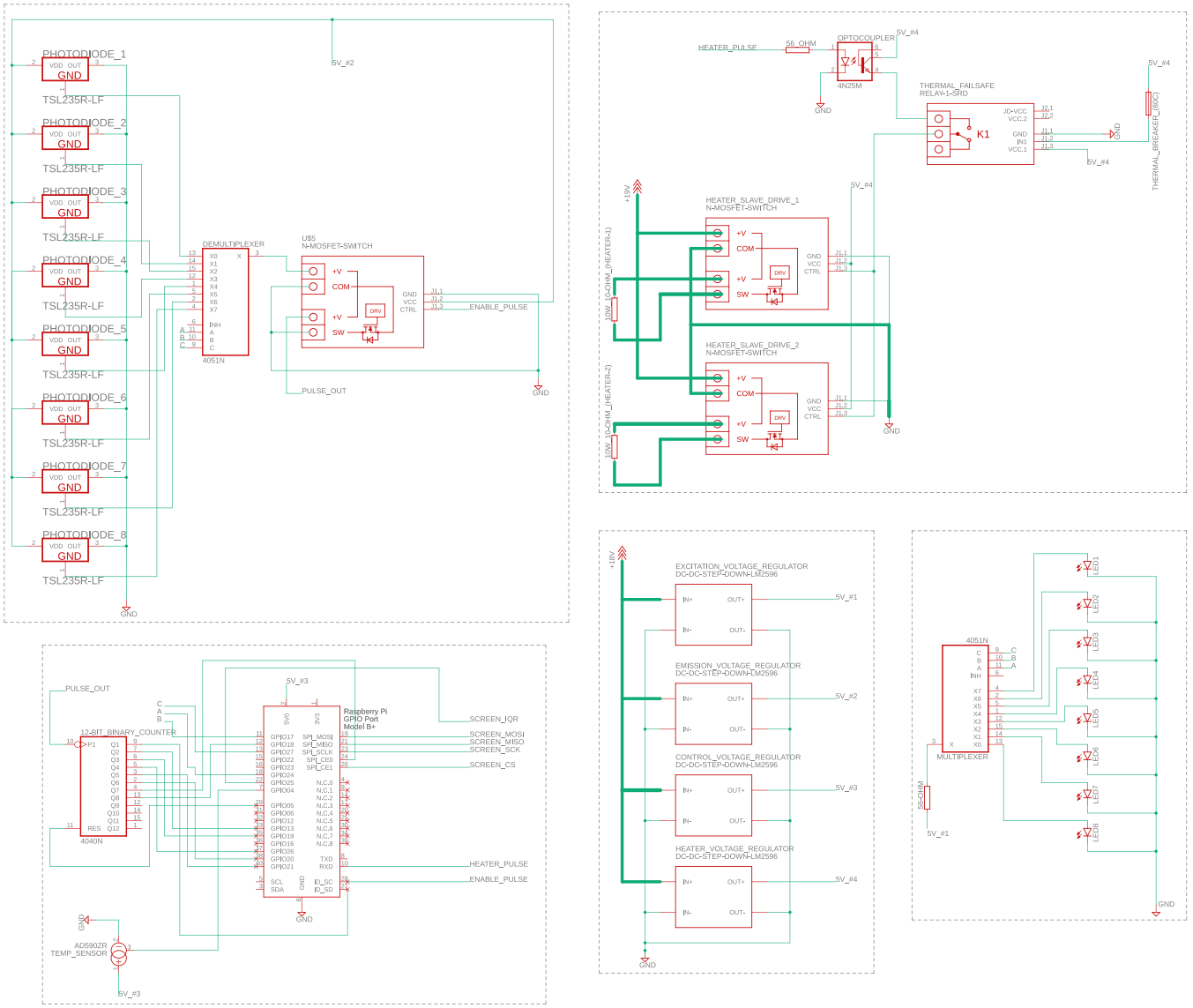

Figure 2. FABL-8 circuit schematic. The circuit consists of five main systems: Voltage regulation—provides individual step down 5 V DC power supplies from the input 19 V DC for the control, heater, excitation, and emission optical systems. Control—Raspberry pi that orchestrates the heater, excitation, and emission optical systems. The control system uses a 12-stage binary counter to accurately quantify clock pulses from the emission system. Heater—provides high-current on/off switching of two, 10 W wire wound resistors that form the heating element. The heating system utilizes a thermal breaker that acts as a failsafe to shutoff the heating element in the event of a control malfunction. Excitation—sequentially illuminates LEDs in an eight LED array using an eight-channel analogue multiplexer. Emission—sequentially reads clock pulses from light-to-frequency photodiodes in an 8x photodiode array using an eight-channel analogue demultiplexer.

Deep Straw and 767—Oklahoma Yellow) (Figure 1C). The optimal combination of gel filters was determined by comparing the optical absorption spectrum information provided by the manufacturer as well as experimentation with an LED and photodiode to maximize the signal-to-noise ratio. The entire system is housed in a plastic tough case with a touchscreen user interface and powered with a 120 W 19 V DC power supply (Figure 1A).

***FABL-8* Instrument Control.** A Raspberry Pi 3 Model B+ was used to control the system with code written in both *Python* and *BASH* programming languages. The main user interface consists of a 5 in. 800 × 480 pixel resolution screen with a resistive touch interface (Figure 3C). The temperature of the block was precisely controlled with PID using temperature readings from a digital sensor and actuated via pulse width modulation (PWM) to energize ceramic resistors mounted inside the block (Figure 2). The heater loads were slave driven by two 5A metal−oxide−semiconductor field-effect transistors using PWM control signals. A peripheral barcode scanner was used to assign sample labels to wells via a Universal Serial Bus port (Figure 3A). A LAMP reaction strip is inserted into the block and held in place by the photodiode-containing lid that is secured by a magnetic mount (Figure 3B). During a reaction, the second derivative of the florescence signal across time was used to determine the inflection point of the curve and was reported as the time-to-positive (TTP) (Figure 3D). Specific build details of the *FABL-8* are included in a dedicated GitHub repository (https://github.com/abuultjens/FABL-8-Open-Isothermal-Platform).

COVID-19 Specimen Collection and Handling. Bilateral deep nasal oropharyngeal swabs were collected by qualified healthcare professionals from patients meeting the COVID-19 epidemiological and clinical criteria as specified by the Victorian Department of Health and Human Services at the time of swab collection between July 10 and Aug 18, 2020. A range of commercial respiratory swabs and transport media were used by the collecting centers during this time (Copan 321C, Copan 346C, Copan 155C, Kang Jian KJ502-19D). Samples were collected in Melbourne, Victoria, Australia, and transported to the Microbiological Diagnostic Unit Public Health

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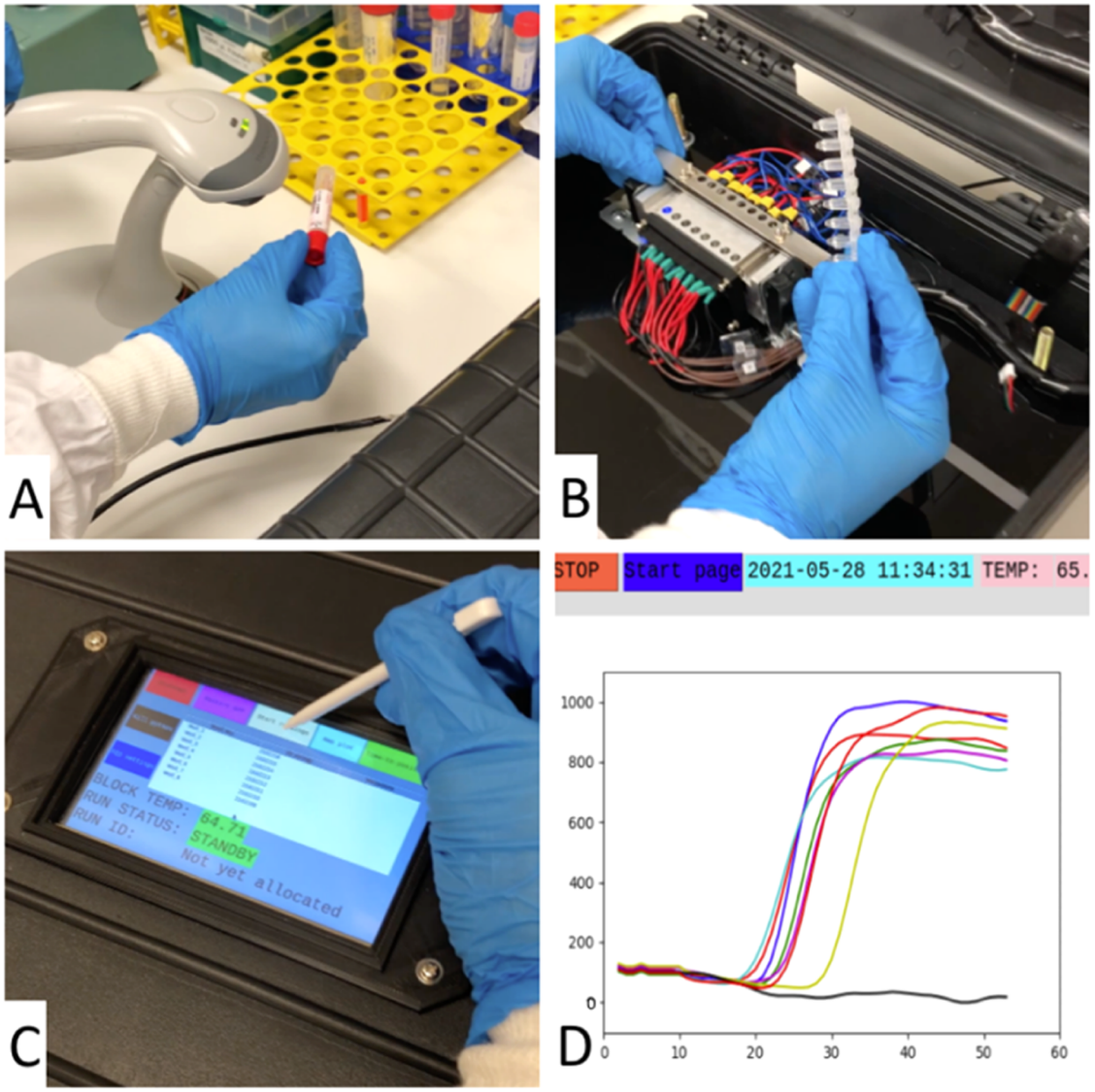

Figure 3. Four key steps in running the *FABL-8* instrument. (A) Unique specimen details are recorded via a barcode scanner and are assigned to specific wells in the reaction strip. (B) LAMP reaction strip (held in the right hand) is inserted into the heating block and the block lid (held in the left hand) is replaced and the instrument case is closed. (C) Run is initiated in the python application via the user interface touchscreen display. (D) Fluorescence is monitored throughout the run via the live plotting window of the application.

Table 1. Oligonucleotide Primers for Bacterial LAMP Assays

target	primer	sequence 5′−3′
M. ulcerans (**IS*2404* gene**)[20]	F3	CATCTCGTGTCGGTGTTC
	B3	TTGGCTTGGTTGGACTTG
	FIP	GGCACGTACGCAGGGAATGATTGGTGCTCGGTCAAC
	BIP	TGGTCACTGTGGATGCGATGCATCAGGTAGTGCGACTTC
	LoopF	CATTGCTTTTCTCGGCGAC
	LoopB	TCACCGCGAAGTTGATCTG
Legionella (**16S rRNA gene**)[19]	LPF3	CGTTACCCACAGAAGAAGC
	LPB3	ACCCTCTCCCATACTCGA
	LPFIP	AGTAATTCCGATTAACGCTCGCAACCGGCTAACTCCGTGC
	LPBIP	GGCGTAAAGGGTGCGTAGGTGACCAGTATTATCTGACCGTCC

Laboratory, Doherty Institute for further testing as per World Health Organization recommendations.[10] All swabs were processed in a class II biological safety cabinet according to standard diagnostic and microbiological practices. RNA was extracted from a total of 85 specimens using the Chemagic Viral DNA/RNA kit on a Perkin Elmer (PE) Chemagic 360 instrument according to the manufacturer's instructions, using 200 μL of a primary sample, eluting in 60 μL of the final volume and then tested for SARS-CoV-2 E-gene by RT-qPCR as described[10] and by N1-STOP-LAMP as described below (Table S1). Titrated stocks of heat-inactivated SARS-CoV-2 virus used as positive control material were prepared as described previously.[10]

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Loop-Mediated Isothermal Amplification (LAMP) Assays. Performance testing of the FABL-8 included using a previously validated and published LAMP assay for SARS-CoV-2 called N1-STOP-LAMP.[10] This assay uses six oligonucleotide primers to detect a 200 bp region of the SARS-CoV-2 nucleocapsid gene (primer sequences available upon request: geneworks.com.au) and a commercial mastermix (Reverse Transcriptase Isothermal Mastermix, ISO-DR004-RT, www.optigene.co.uk).[10] The mix contains a proprietary reverse transcriptase for cDNA synthesis and the thermophilic GspSSD strand-displacing polymerase/reverse transcriptase for DNA amplification with a dsDNA intercalating fluorescent dye. Detection was achieved by measuring the increase in fluorescence as amplification products accumulate. NI-STOP-LAMP reactions were assembled in eight-tube Genie strips (OP-00008, OptiGene). Each reaction contained 15 μL of mastermix, 5 μL of 5 × primer stock and 5 μL of template RNA, following the manufacturer's instructions. A no-template control (5 μL water) was included in all runs. LAMP reactions were run on the FABL-8 and the *Genie II* (www.optigene.co.uk). Reactions were incubated at 65 °C for 30 min with fluorescence acquisition every 40 s (*FABL-8*) or 30 s (*Genie II* instrument). A positive result was indicated by an increase in fluorescence at an emission wavelength of 530 nm (FAM channel) above a defined threshold, recorded as time-to-positive (TTP) expressed in min:sec.

We also used two additional LAMP assays targeting the 16S rRNA gene of the human pathogenic bacterium *Legionella pneumophila*[19] and the IS2404 insertion sequence of the human pathogenic bacterium *Mycobacterium ulcerans*.[20] The oligonucleotide primer sequences for these assays are shown in Table 1. These assays used the same Reverse Transcriptase Isothermal Mastermix (ISO-DR004-RT) as for N1-STOP-LAMP, but genomic DNA instead of RNA was used as template. Each reaction contained 15 μL of mastermix, 5 μL of 5 × primer stock, and 5 μL of template DNA, following the manufacturer's instructions.

XPRIZE Proficiency Panel. Performance testing of the FABL-8 included using the N1-STOP-LAMP assay with a proficiency panel provided as part of the XPRIZE rapid covid testing competition (https://www.xprize.org/prizes/covidtesting). For limit-of-detection testing, the panel consisted of a dilution series of inactivated SARS-CoV-2 particles (ZeptoMetrix—NATSARS(COV2)-ERC) spiked in different sample types, including synthetic nasal and saliva matrices and phosphate-buffered saline (PBS). For specificity testing, the panel included 30 nontarget RNA samples from different human viruses (Twist Biosciences, Table S2). RNA was extracted with the Perkin Elmer Chemagic Viral DNA/RNA kit on a Chemagic 360 instrument (Chemagen, Baesweiler, Germany) (200 μL of extraction volume and 60 μL of elute) and 5 μL of template RNA was added to the N1-STOP-LAMP reactions.

Ethics. 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 (Updated 2018). The study was exempt from requiring specific approvals, as it involved the use of existing collections of data or records that contained nonidentifiable data.[21]

Statistical Analysis. Data analysis was managed using GraphPad Prism (v8.4.1).

RESULTS

Initial Performance Assessments. Maintaining 65 °C is a key requirement for the LAMP assay. We first checked that the PID-controlled aluminum block of the FABL-8 stably held 65 °C for the required time. Assessment of outputs from the on-board digital temperature sensor (Figure 2) showed very stable temperature profiles after a 10 min warm-up period across three runs. Temperature fluctuations were <0.05 °C once 65 °C had been reached (Figure S1).

When a DNA intercalating dye is incorporated into an assay, LAMP reactions can be monitored in real time,[22] with the rate of fluorescence signal growth used as a semiquantitative indication of initial template concentration. To confirm that the FABL-8 optics were performing as expected, we prepared eight identical N1-STOP-LAMP assays that were then run in triplicate with a heat-inactivated stock of SARS-CoV-2 virus at a concentration of 0.05 TCID50/mL. The time-to-positive (TTP) values between each of the eight wells after 25 min at 65 °C were then compared. Concordant TTP values were observed across the eight wells with an overall mean TTP of 10:38 min:s and a standard deviation of 60 s (Figure S2). Satisfied that the FABL-8 was operating as expected, we next compared FABL-8 performance against a commercial isothermal amplification instrument.

Benchmarking the FABL-8 against a High-End Commercial Instrument. To benchmark the *FABL-8* against a high-end, commercial isothermal amplification device, the OptiGene *Genie II*, we compared the time-to-positive (TTP) values for a panel of 61 previously confirmed, COVID-19 positive clinical specimens and 24 confirmed COVID-19 negative clinical specimens that were run on both instruments (Figure 4, Table S1). There were 48 of the 61 positive specimens that returned a positive result with the *FABL-8* compared to 47 of 61 positive specimens with the Genie II. This result is expected, with the N1-STOP-LAMP having a positive predictive value of 79% when used in the *FABL-8* and 77% when used in the *Genie II*. These performance values reflect previously reported reduced detection sensitivity of this LAMP assay compared to RT-qPCR.[10] While there was almost complete correspondence in positive results between the FABL-8 and Genie II, the FABL-8 had a longer average TTP value than that of the *Genie II* (14.38 min versus 9.14 min, respectively, Figure 4). However, there was no difference in overall clinical detection sensitivity between the instruments (Figure 4).

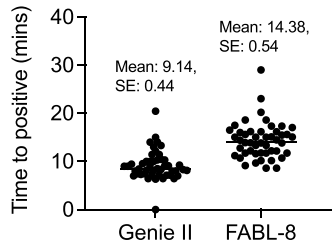

Figure 4. Benchmarking of TTP measurements of the *FABL-8* against a high-end commercial LAMP diagnostic platform (OptiGene *Genie II*). The instruments were run with a paired panel of 48 COVID-19 positive clinical samples using the N1-STOP-LAMP molecular detection assay. Comparison of TTP measurement depicts concordance between instruments with the *FABL-8* having longer TTP values than that of the *Genie II* (14.38 vs 9.14 min, respectively). Means were significantly different ($p < 0.0001$, Wilcoxon matched-pairs signed-rank test).

Assessment of FABL-8 Analytical Performance with N1-STOP-LAMP. To determine the absolute sensitivity and LoD of the *FABL-8* when paired with the N1-STOP-LAMP assay, testing was performed on a twofold dilution series of a defined concentration of viral RNA (Figure 5). A paired set of reactions was also run on the Genie instrument for comparison. RNA for the N1-STOP-LAMP assay was prepared using the Chemagic RNA extraction platform. The head-to-head comparison of the performance of the instruments across the viral RNA target concentration range indicated that reliable

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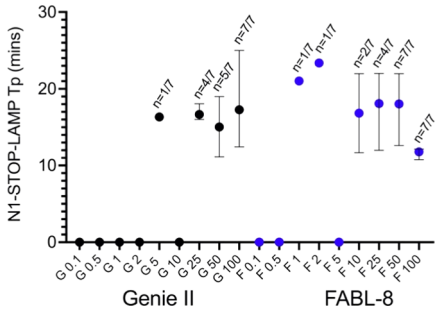

Figure 5. *FABL-8* and *OptiGene Genie II* analytical performance using N1-STOP-LAMP. RNA was extracted into 200 μL and eluted in 60 μL with 5 μL of template added to each RT-LAMP reaction. The *X*-axis depicts the number of target viral genome copies per μL. The TTP measurements were conducted in replicates of seven. Solid circles depict the mean and error bars report the minimum and maximum values for each dilution. Reliable detection (7/7 replicates detected) was achieved at 50 and 100 copies per microliter for the *FABL-8* and the *OptiGene Genie II*, respectively.

detection was achieved when there were 50 and 100 virus copies or more per microliter, for the *FABL-8* and the *Genie II*, respectively (Figure 5). In comparison, the LoD for RT-qPCR was 1−2 virus copies per microliter (Table S3).

Negative Predictive Performance of FABL-8 with N1-STOP-LAMP. Crucial to satisfactory diagnostic test performance is the ability to correctly call negative samples, that is, to reject samples that do not contain the target. To assess *FABL-8* negative predictive performance, we screened RNA from 24 RT-qPCR-confirmed COVID-19 negative specimens using the N1-STOP-LAMP assay. All 24 specimens were N1-STOP-LAMP negative on both the FABL-8 and the Genie II (Table S1), indicating 100% negative predictive performance. We further evaluated the FABL-8 against a panel of RNA samples extracted from humans, influenza H3N2 virus, measles virus, MERS-coronavirus, and mumps virus samples as part of the XPRIZE proficiency panel (Table S2). The *FABL-8* returned negative results for all of the nontarget RNA panels, again indicating excellent negative predictive performance.

Versatility of the FABL-8 for Use with Other LAMP Assays. To demonstrate the versatility of the *FABL-8* to detect different genetic targets, we used it to run LAMP molecular detection assays targeting both *M. ulcerans*[20] and *L. pneumophila*.[19] Here, using the same LAMP reaction mastermix as N1-STOP-LAMP, the platform was able to correctly detect dilutions of genomic DNA for each pathogen, while the no-template controls were negative for both LAMP assays (Figure 6). The same testing panel was also run with the *Genie II* instrument. There was no difference in detection sensitivity between the two instruments, although, as observed for the N1-STOP-LAMP assay, the *FABL-8* displayed a longer TTP across all template dilutions, between 1 and 2 min (Figure 6).

DISCUSSION

The COVID-19 pandemic has resulted in mass disruption driving innovative responses, opening an opportunity for the development of cost-effective, rapid, and scalable diagnostic solutions. The high cost of most commercial molecular diagnostic instrumentation and global supply chain disruption can limit diagnostic capacity, particularly in lower- and middle-income countries (LMIC). Specifically, commercial LAMP instrumentation capable of running multiple samples, with network connectivity and measuring fluorescence signals, is priced upwards of US$5000 and available from relatively few manufacturers, meaning—supply is unassured in these times of global demand for molecular diagnostics. In response to these challenges, we have developed and validated a low-cost, simple-to-fabricate and scalable molecular diagnostic hardware device with a total per unit build cost of $380 USD that we named the *FABL-8*.

Benchmarking of the *FABL-8* against a high-end isothermal fluorescence detection instrument revealed that the *FABL-8* had near-equivalent performance, with the commercial instrument having slightly faster times to a positive result. This discrepancy might be due to the superior optical sensitivity of the interference filters that are used in commercial units. While interference filters offer high performance, they are relatively expensive components that dramatically inflate the per unit cost. Our use of lower performance theatrical gel combinations for both excitation and emission filters offers a cost-effective solution, with a per unit total filter cost of less than $1 USD. The slightly longer TTP of the *FABL-8* compared to the *Genie II* does not represent any practical disadvantage and does not impact the instrument's limit of detection.

Our analytical sensitivity testing revealed that the *FABL-8* in conjunction with the N1-STOP-LAMP assay could achieve

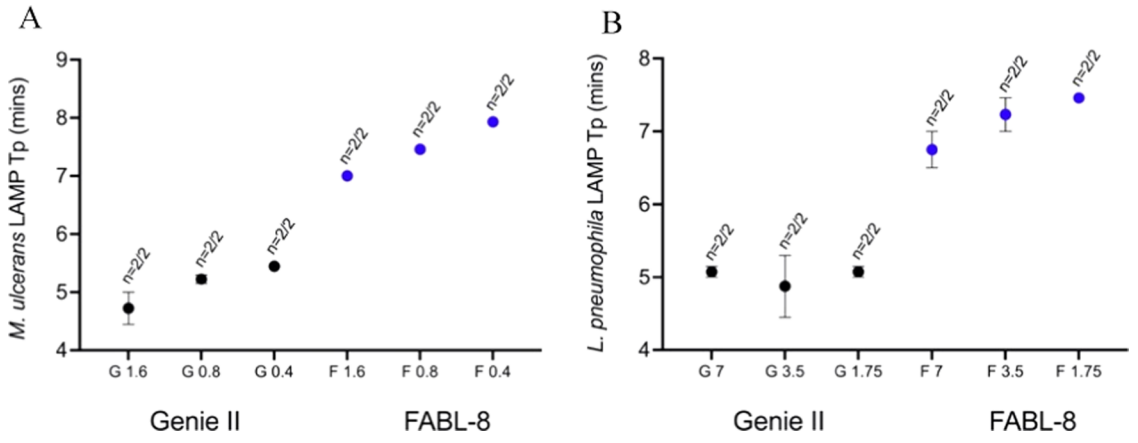

Figure 6. Comparison of *FABL-8* and *OptiGene Genie II* TTP diagnostic performance with LAMP molecular detection assay targeting (A) *M. ulcerans* and (B) *L. pneumophila*. The *X*-axes depict the number of nanograms of each target copy per microliter. The TTP measurements were conducted in duplicate. Solid circles depict the mean and bars report the minimum and maximum values for each dilution.

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reliable detection of 50 SARS-CoV-2 virus copies per microliter, while the *Genie II* with the same LAMP assay obtained reliable detection at 100 viral genome copies per microliter. This difference in performance between instruments at the 50 copies per microliter dilution is likely explained by the assay operating close to its limit of detection, noting too that LAMP assays are not strictly quantitative.

Although the LAMP assay has a higher LoD than the gold standard RT-qPCR assay, rapid assays such as RT-LAMP can still be deployed for the reliable diagnosis of SARS-CoV-2 infections. The main clinical utility of LAMP is for rapid and frequent testing during the early phases of infection (days 3−7 post symptom onset) when viral loads are highest and transmission is most likely to occur.[23−25] Timely identification of such infectious individuals would minimize the likelihood of further community transmission. Furthermore, while RT-qPCR is a more sensitive method of detection, it may be argued that the additional patients captured by this method and missed by RT-LAMP are more likely to be those at a later stage of illness, whom although shedding detectable RNA may no longer be infectious.[23]

It should also be noted that the *FABL-8* analytical sensitivity is largely governed by the kinetics of the particular LAMP assay employed and is therefore not a reflection of the instrument performance itself. In this way, there is a possibility for the *FABL-8* to be paired with a potentially more sensitive test to achieve a lower LoD, such as the opvCRISPR LAMP assay.[26]

Considering the generic applicability of LAMP technology to virtually any genetic target, there is great scope for a system like the *FABL-8* to afford enhanced diagnostic capacity in LMICs during and beyond the COVID-19 pandemic. We showed that the *FABL-8* can be paired with LAMP molecular detection assays that target diagnostic sequences of both *M. ulcerans* and *L. pneumophila*. The *FABL-8* could therefore have utility to detect any pathogen of pressing public health importance as well as genetic markers of significance in sectors beyond medical microbiology including agriculture, conservation, and antibioterrorism.[27−30]

Our use of a cloud-based repository permits for results to be instantly deposited in a centralized database. Despite the decentralizing of the point-of-care diagnostic testing, cloud integration ensures that results are deidentified and redundantly stored in secure and encrypted centralized databases, with real-time data available to inform epidemiological insights.

Owing to the simple design of the *FABL-8*, the units are straightforward to troubleshoot and repair. Often, commercial diagnostic equipment is accompanied by expensive servicing contracts leading to abandonment of instrumentation in low-resourced settings due to prohibitive cost. The ability for a diagnostic platform to be repaired by persons adept in basic electronics enables the ongoing and long-term use of diagnostic systems in LMICs.

Notwithstanding our best efforts to develop and freely disseminate the information necessary to build *FABL-8* units, we recognize that such activities may be limited to groups adept at basic electrical engineering and general hardware manufacture. Despite this hurdle, in the last decade, there has been a global emergence of community makerspaces that engage participants in hands-on electrical hardware development, often involving Raspberry pi systems, that also possess the necessary equipment for *FABL-8* fabrication. Makerspaces, along with university electrical engineering departments, may provide adequate local manufacture capacity to deploy *FABL-8* units at scale. However, for any diagnostic platform used in human medicine, there will be additional costs associated with quality control and quality assurance requirements that will need to be deployed if using the FABL-8. These requirements will vary by country and jurisdiction and users will need to comply with their relevant accreditation bodies and processes.

While the availability of a LAMP diagnostic platform, such as described here, provides an economically viable option for running reactions, there also exists a need for the hardware required to extract and purify RNA from clinical samples. Not dissimilar to the innovative efforts to develop rapid LAMP platforms, there have been numerous recent approaches to decentralize and eliminate the cost barriers for rapid RNA extraction.[31,32] A concurrent project in our research group has been the repurposing of 3D printers to function as high-performance and low-cost RNA extraction robotic systems.[33]

CONCLUSIONS

We have demonstrated that the *FABL-8* platform can detect SARS-CoV-2 RNA with equivalent performance to a commercial instrument. Combined with affordability at approximately US$380 per machine and readily available parts, the *FABL-8* provides a viable option for accessible diagnostics. Such an alternative is particularly valuable during the COVID-19 pandemic as it enables for both scaled and decentralized testing efforts, particularly for LMICs. In addition to lowering analytical costs, the *FABL-8* is a diagnostic platform that can be produced locally, which is important as the supply of diagnostic instrumentation from international vendors is not always assured. As the *FABL-8* is agnostic to the LAMP molecular detection assay employed, it can also be used to detect virtually any genetic target when paired with the appropriate detection assay.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsbiomaterials.1c01105.

Figure S1: FABL-8 block temperature profiles; Figure S2: FABL-8 intra- and inter-run reproducibility; Table S1: Clinical analytical performance comparison of FABL-8 versus Genie II: Table S2: FABL-8 specificity; and Table S3: XPRIZE COVID-19 testing proficiency sample results (PDF)

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Author Contributions

A.H.B. and T.P.S. conceived the study. A.H.B., K.V., L.K.S., J.Y.H.L., I.R.M., and T.P.S. conducted experiments. A.H.B., B.P.H., and T.P.S. wrote the manuscript. All authors read and approved the final draft of the manuscript.

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Notes

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■ ABBREVIATIONS

COVID-19	coronavirus disease 2019
E-gene	viral envelope protein gene
CNC	computer numerical control
LAMP	loop-mediated isothermal amplification
LMIC	lower- and middle-income countries
LoD	limit-of-detection
NTC	no-template control
PBS	phosphate-buffered saline
PID	proportional, integral and derivative
PWM	pulse width modulation
qPCR	quantitative polymerase chain reaction
RT-LAMP	reverse transcription loop-mediated isothermal amplification
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TTP	time-to-positive

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