Scaling diagnostics in times of COVID-19: Rapid prototyping of 3D-printed water circulators for Loop-mediated Isothermal Amplification (LAMP) and detection of SARS-CoV-2 virus

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Abstract

By the first week of April 2020, more than 1,500,000 positive cases of COVID-19 and more than 50,000 deaths had been officially reported worldwide. While developed countries such as the USA, Italy, England, France, Spain, and Germany struggle to mitigate the propagation of SARS-CoV-2, the COVID-19 pandemic arrived in Latin America, India, and Africa—territories in which the mounted infrastructure for diagnosis is greatly underdeveloped. An actual epidemic emergency does not provide the required timeframe for testing new diagnostic strategies; therefore, the first line of response must be based on commercially and readily available resources. Here, we demonstrate the combined use of a three-dimensional (3D)-printed incubation chamber for commercial Eppendorf PCR tubes, and a colorimetric embodiment of a loop-mediated isothermal amplification (LAMP) reaction scheme for the detection of SARS-CoV-2 nucleic acids. We used this strategy to detect and amplify SARS-CoV-2 DNA sequences using a set of in-house designed initiators that target regions encoding the N protein. We were able to detect and amplify SARS-CoV-2 nucleic acids in the range of ~625 to 2×10^5 DNA copies by this straightforward method. The accuracy and simplicity of this diagnostics strategy may provide a cost-efficient and reliable alternative for use during the COVID-19 pandemics, particularly in underdeveloped regions were the availability of RT-qPCR instruments may be limited. Moreover, the portability, ease of use, and reproducibility of this strategy make it a reliable alternative for deployment of point-of-care SARS-CoV-2 detection efforts during the pandemics.

Key words: LAMP, point-of-care, SARS-CoV-2, COVID-19, diagnostic, portable, isothermal nucleic acid amplification

Introduction

By the end of the first week of April 2020, more than one and a half million positive cases of COVID-19 were officially reported across the globe[1]. Even developed countries, such

as the USA, England, France, and Germany, are struggling to mitigate the propagation of SARS-CoV-2 by implementing social distancing and widespread testing. Less developed regions, such as Latin America, India, and Africa, are now experiencing the arrival of COVID-19, but these—territories are woefully lacking in the finances or the mounted infrastructure for diagnosis of this pandemic infection. Rapid and massive testing of thousands of possibly infected subjects has been an important component of the strategy of the countries that are effectively mitigating the spreading of COVID-19 among their populations (i.e., China[2], South Korea [3], and Singapore [4]). By comparison, developing countries with high demographic densities, such as México [5], India [6], or Brazil [7], may not be able to implement a sufficient number of centralized laboratories for rapid large-scale testing for COVID-19.

Many methodologies have been proposed recently to deliver cost-effective diagnosis (i.e., those based on immunoassays [8–11] or specific gene hybridization assisted by CRISPR-Cas systems [12–14]). While immunoassays are an accurate and efficacious tool for assessing the extent of the infection for epidemiological studies [15], their usefulness is limited to the identification of infected subjects during early phases of infection [11,16], a critical period for infectiveness. For instance, experimental evidence collected from a small number of COVID-19 patients (9 subjects) showed that 100% of them produced specific immunoglobulins G (IgGs) for SARS-CoV-2 within two weeks of infection, but only 50% of them did during the first week post infection [17].

Nucleic acid amplification continues to be the gold standard for the detection of viral diseases in the early stages [18–22], and very small viral loads present in symptomatic or

asymptomatic patients can be reliably detected using amplification based technics, such as PCR[23–25], RPA[26], and LAMP[27–29].

During the last two pandemic events with influenza A/H1N1/2009 and COVID-19, the Centers for Disease Control (CDC) and the World Health Organization (WHO) recommended real-time quantitative PCR (RT-qPCR) methods as the gold standard for official detection of positive cases[16,30]. However, the reliance on RT-qPCR often leads to dependence on centralized laboratory facilities for testing [16,30–33]. To resolve this drawback, isothermal amplification reaction schemes (i.e., loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA)) have been proposed as alternatives to PCR-based methods and devices for point-of-care settings [32,34,35]. The urgency of using reliable molecular-based POC methods for massive diagnostic during epidemiological emergencies has become even more evident during the current COVID-19 pandemics [30,36,37].

In these times of COVID-19 [38], scientists and philanthropists around the globe have worked expeditiously on the development of rapid and portable diagnostics for SARS-CoV-2. Several reports have demonstrated the use of colorimetric LAMP-based methods for diagnosis of pandemic COVID-19 [39–44]. Some of these reports (currently available as preprints) use phenol red, a well-known pH indicator, to assist in the visual discrimination between positive and negative samples [39,40].

In this study, we demonstrate the use of a simple embodiment of a colorimetric Loop-mediated Isothermal amplification (LAMP) protocol for the detection and amplification of synthetic samples of SARS-CoV-2, the causal viral agent of COVID-D. In this LAMP-based strategy, also assisted by the use of phenol red, sample incubation is greatly

facilitated by the use of a three-dimensional (3D)-printed incubator connected to a conventional water circulator, while discrimination between positive and negative samples is achieved by visual inspection. We quantitatively analyze differences in color between positive and negative samples using color decomposition and analysis in the color CIELab space[45]. Moreover, we compare the sensibility of this LAMP colorimetric method versus PCR protocols. This simple strategy is potentially adequate for the fast deployment of diagnostic efforts in the context of COVID-19 pandemics.

Materials and Methods

Equipment specifications: We ran several hundred amplification experiments using a colorimetric LAMP method in a 3D-printed incubation chamber designed in house and connected to a conventional water circulator (Figure 1). The design and all dimensional specifications of this chamber have been made available in Supplementary Information (Figure S1,S2; Supplementary File S1). In the experiments reported here, we used a chamber with dimensions of 20 × 5 × 15 cm^3 and a weight of 0.4 kg (without water). A conventional water circulator (WVR, PA, USA), was used to circulate hot water (set point value at 76 ºC) through the 3D-printed chamber for incubation of the Eppendorf PCR tubes (0.2 mL). In this first chamber prototype, twelve amplification reactions can be run in parallel. This concept design is amenable for fabrication in any STL-3D printing platform and may be scaled up to accommodate a larger number of tubes.

We used a blueGel electrophoresis unit, powered by 120 AC volts, to validate the LAMP amplification using gel electrophoresis. Photo-documentation was done using a smartphone camera. We also used a Synergy HT microplate reader (BioTek Instruments,

VT, USA) to detect the fluorescence induced by an intercalating reagent in positive samples from the PCR reactions.

Validation DNA templates: We used plasmids containing the complete N gene from 2019-nCoV, SARS, and MERS as positive controls, with a concentration of 200,000 copies/µL (Integrated DNA Technologies, IA, USA).

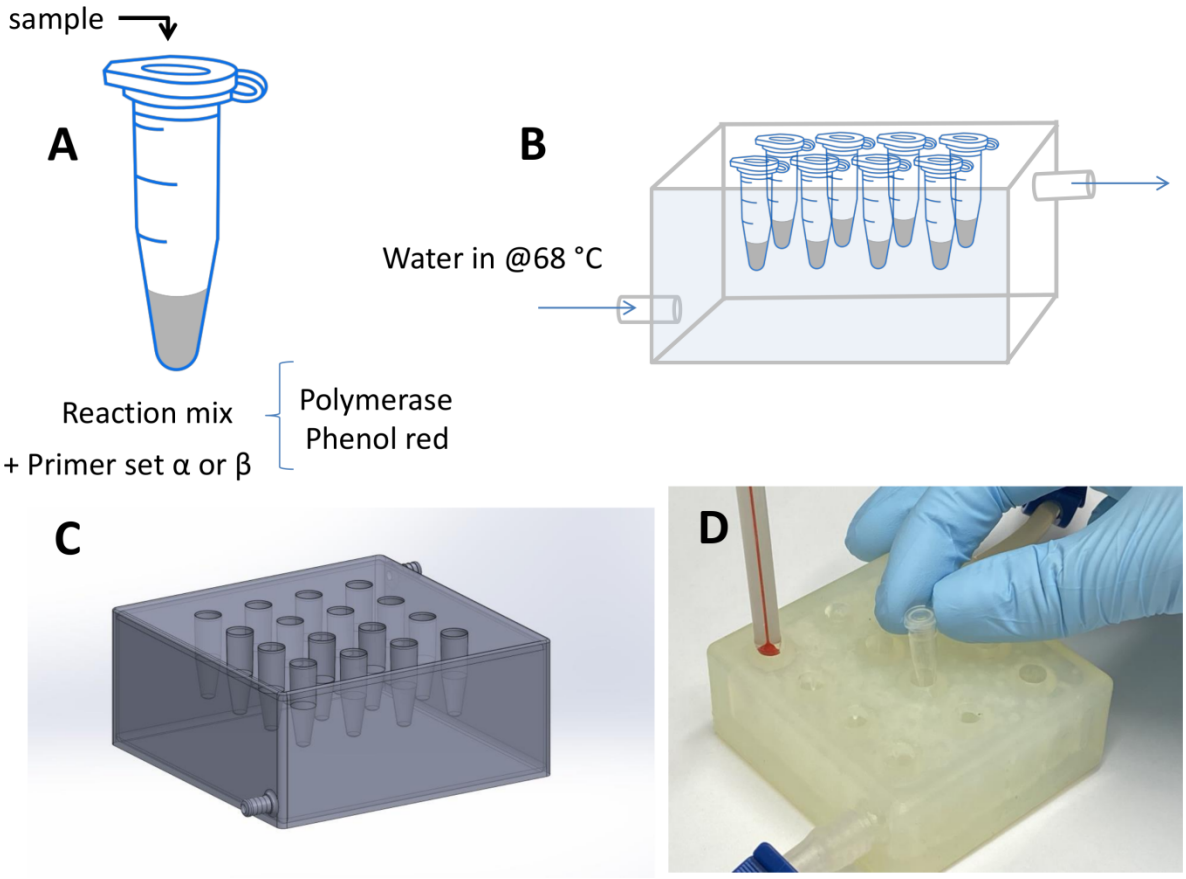


Figure 1. Experimental setup. (A) Commercial 200 microliter Eppendorf PCR tubes, and (B) a 3D-printed incubator were used in amplification experiments of samples containing synthetic SARS-CoV-2 nucleic acid material. (C) 3D CAD model of the LAMP reaction incubator. (D) Actual image of the Eppendorf tube incubator connected to a conventional water circulator.

Samples containing different concentrations of synthetic nucleic acids of SARS-CoV-2 were prepared by successive dilutions from stocks (from 2×10^5 copies to 65 copies). We used a plasmid that contained the gene GP from EBOV as a negative control. The production of this EBOV genetic material has been documented elsewhere by our group [23].

Amplification mix: We used WarmStart® Colorimetric LAMP 2× Master Mix (DNA & RNA) from New England Biolabs (MA, USA), and followed the recommended protocol: 12.5 µL Readymix, 1.6 µM FIP primer, 1.6 µM BIP primer, 0.2 µM F3 primer, 0.2 µM B3 primer, 0.4 µM LF primer, 0.4 µM LB primer, 1µL DNA template (~ 625 to 2×10^5 DNA copies), 1.25 µL EvaGreen® Dye from Biotium (CA, USA), and nuclease-free water to a final volume of reaction 25 µL. This commercial mix contains phenol red as a pH indicator for revealing the shift of pH during LAMP amplification across the threshold of pH=6.8.

Primers used: Two different sets of LAMP primers, referred to here as α and β, were designed in house using the LAMP primer design software Primer Explorer V5 (http://primerexplorer.jp/ lampv5e/index.html). These primers were based on the analysis of alignments of the SARS-Co2 N gene sequences using the software Geneious (New Zealand), downloaded from https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/#nucleotide-sequences.

Each set, containing six LAMP primers, were used to target two different regions of the sequence of the SARS-Co2 N gene. In addition, for comparison purposes, we conducted PCR amplification experiments using the primer sets recommended by the CDC for the standard diagnostics of COVID-19 (i.e., N1, N2, and N3 assays) using RT-qPCR. The

153 sequences of our LAMP primers are presented in Table 1. The sequences of the PCR
154 primers (N1–N3) have been reported elsewhere[24,46].

155

156 *Amplification protocols*: For all LAMP experiments, we performed isothermal heating for
157 30 or 60 min. In our experiments, we tested three different temperatures: 50, 60, and 65 ºC.

158 **Table 1. Primer sequences used in LAMP amplification experiments.** Two different sets of
159 primers were used, directed at the RNA sequence encoding the N sequence of the SARS-CoV-2.

Set	**Description**	**Primers Sequence (5'>3')**
Primer set α	2019-nCoV 1-F3	TGGACCCCAAAATCAGCG
	2019-nCoV 1-B3	GCCTTGTCCTCGAGGGAAT
	2019-nCoV 1-FIP	CCACTGCGTTCTCCATTCTGGTAAATGCACCCCGCATTACG
	2019-nCoV 1-BIP	CGCGATCAAAACAACGTCGGCCCTTGCCATGTTGAGTGAGA
	2019-nCoV 1-LF	TGAATCTGAGGGTCCACCAA
	2019-nCoV 1-LB	TTACCCAATAATACTGCGTCTTGGT
Primer set β	2019-nCoV 2-F3	CCAGAATGGAGAACGCAGTG
	2019-nCoV 2-B3	CCGTCACCACCACGAATT
	2019-nCoV 2-FIP	AGCGGTGAACCAAGACGCAGGGCGCGATCAAAACAACG
	2019-nCoV 2-BIP	AATTCCCTCGAGGACAAGGCGAGCTCTTCGGTAGTAGCCAA
	2019-nCoV 2-LF	TTATTGGGTAAACCTTGGGGC
	2019-nCoV 2-LB	TAACACCAATAGCAGTCCAGATGA

160

161 *Documentation of LAMP products*: We analyzed 10 µL of each LAMP reaction in a
162 blueGel unit, a portable electrophoresis unit sold by MiniPCR from Amplyus (MA, USA).
163 This is a compact electrophoresis unit (23 × 10 × 7 cm) that weighs 350 g. In these
164 experiments, we analyzed 10 µL of the LAMP product using 1.2 % agarose electrophoresis

tris-borate-EDTA buffer (TBE). We used the Quick-Load Purple 2-Log DNA Ladder (NEB, MA, USA) as a molecular weight marker. Gels were dyed with Gel-Green from Biotium (CA, USA) using a 1:10,000 dilution, and a current of 48 V was supplied by the blueGel built-in power supply (AC 100–240V, 50–60Hz).

As an alternative method for detection and reading of the amplification product, we evaluated the amplification products by detecting the fluorescence emitted by a DNA-intercalating agent, the EvaGreen® Dye from Biotium (CA, USA), in a Synergy HT microplate reader (BioTek Instruments, VT, USA). Briefly, 25 µL of the LAMP reaction was placed in separate wells of a 96-well plate following completion of the LAMP incubation. A 125 µL volume of nuclease-free water was added to each well for a final sample volume of 150 µL and the samples were well-mixed by pipetting. These experiments were run in triplicate. The following conditions were used in the microplate reader: excitation of 485/20, emission of 528/20, gain of 75. Fluorescence readings were done from the top at room temperature.

Color determination by image analysis: We also photographically documented and analyzed the progression of color changes in the positive and negative SARS-CoV-2 synthetic samples during the LAMP reaction time (i.e., from 0 to 50 min). For that purpose, Eppendorf PCR tubes containing LAMP samples were photographed using a smartphone (iPhone, from Apple, USA). We used an application for IOS (Color Companion, freely available at Apple store) to determine the components of color of each LAMP sample in the CIELab color space. Color differences between the positive samples and negative controls were calculated as distances in the CIELab coordinate system according to the following formula:

189 $\text{Color Distance}_{\text{sample-negative}} = \text{SQRT}\,[(L_{\text{sample}}-L_{\text{negative}})^2 + (a_{\text{sample}}-a_{\text{negative}})^2 + (b_{\text{sample}}-b_{\text{negative}})^2]$

190

191 Here L, a, and b are the color components of the sample or the negative control in the

192 CIELab color space (Supplementary Figure 4).

193

194 # Results and Discussion

195

196 ## Rationale

197 We have developed a simple diagnostic method for the detection of SARS-CoV-2, the

198 causal agent of COVID-19. The rationale underlying this strategy is centered on achieving

199 the simplest possible integration of easily available reagents, materials, and fabrication

200 techniques to facilitate fast and massive implementation during the current COVID-19

201 pandemics in low- or middle-income regions.

202 This method is based on the amplification of the genetic material of SARS-CoV-2 using a

203 loop-mediated isothermal amplification (LAMP). The amplification is conducted using a

204 commercial reaction mix in commercial and widely available 200 µL Eppendorf PCR

205 tubes. Moreover, we have designed and fabricated a simple 3D-printed chamber (Figure 1)

206 for incubation of the Eppendorf tubes and to enable LAMP at high temperatures (50–65 ºC)

207 and extended times (up to 1 h). We show that this incubation chamber, when connected to a

208 conventional water recirculator, enables the successful amplification of positive samples

209 (i.e., samples containing SARS-CoV-2 nucleic acids).

210 This incubation chamber is one of the key elements that enable rapid and widespread

211 implementation of this diagnostic method at low cost. This 3D-printed incubator can be

212 rapidly printed using standard SLA printers widely available in markets worldwide.

Standard 3D-printing resins can be used. The availability of the original AutoCAD files (included here as supplemental material) enables fast modification/optimization of the design for accommodation of a larger number of samples or larger or smaller tubes, adaptation to any available hoses (tubing), and possible incorporation of an on-line color reading system. Indeed, all this is consistent with the main rationale of our proposed diagnostic strategy for pandemic COVID-19: To enabling a fast and feasible response using widespread, distributed, and scalable diagnostics fabricated with widely available resources.

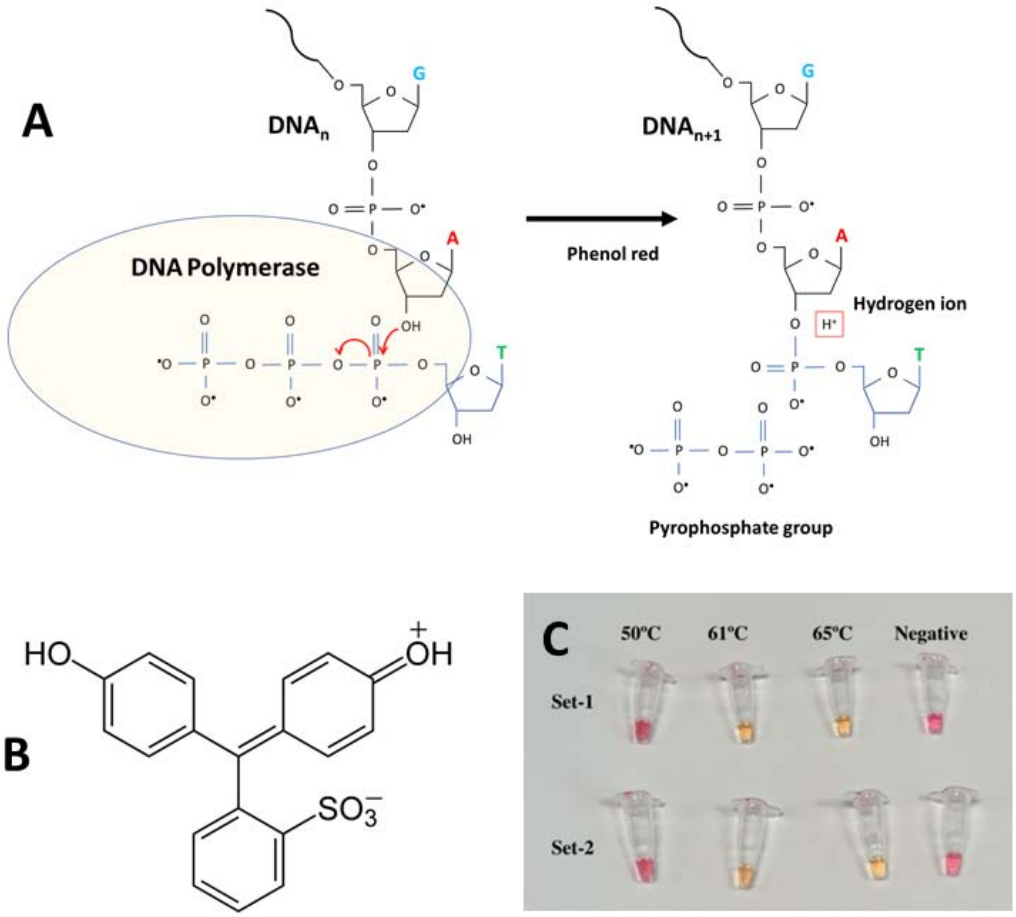
In the following section, we briefly discuss the mechanisms of amplification and visual discrimination between positive and negative samples.

Colorimetric LAMP amplification

The presence of phenol red within the LAMP reaction mix allows for naked-eye discrimination between positive and negative samples. The reaction mix is coupled with the pH color transition of phenol red, a widely used pH indicator, which shifts in color from red to yellow at pH 6.8. During LAMP amplification, the pH of the reaction mix continuously evolves from neutrality to acidic values as protons are produced [27,47]. The mechanism of production of hydrogen ions (H^+) during amplification in weakly buffered solutions has been described [47]. DNA polymerases incorporate a deoxynucleoside triphosphate into the nascent DNA chain. During this chemical event, a pyrophosphate moiety and a hydrogen ion are released as byproducts (Figure 2 A). This release of hydrogen ions is quantitative, according to the reaction scheme illustrated in Figure 2. The caudal of H^+ is high, since it is quantitatively proportional to the number of newly integrated dNTPs. In fact, the

236 quantitative production of H^+ is the basis of previously reported detection methods, such as

237 the semiconductor sequencing technology operating in Ion Torrent sequencers[48].



238

239 **Figure 2**. **Initiators and pH indicator for SARS-Co2 detection using a colorimetric**
240 **LAMP method**. (A) LAMP reaction scheme. (B) Chemical structure of phenol red. (C)
241 Two different sets of LAMP primers were used for successfully targeting a gene sequence
242 encoding the SARS-Co2 N protein. Successful targeting and amplification is clearly
243 evident to the naked eye: positive samples shift from red to yellow.

244

245 In the initially neutral and weakly buffered reaction mixes, the production of H^+ during
246 LAMP amplification progressively and rapidly shifts the pH across the threshold of phenol
247 red (Figure 2B).

Moreover, the pH shift is clearly evident to the naked eye, thereby freeing the user from reliance on spectrophotometric instruments and facilitating simple implementation during emergencies (Figure 2C). Images in Figure 2C show representative colors of the amplification reaction mixes contained in Eppendorf PCR tubes after incubation for 30 min. Three different incubation temperatures were tested (50, 60, and 65 ºC) and two different sets of LAMP-primers (α and β) were used.

Both sets of primers performed equivalently, at least based on visual inspection, in the three temperature conditions tested. Discrimination between positive and negative controls is possible using only the naked eye to discern the reaction products from amplifications conducted at 60 and 65 ºC. No or negligible amplification was evident at 50 ºC or in the control group.

Furthermore, we were able to successfully discriminate between positive and negative samples using LAMP reaction mix already added with primers and kept at room temperature for 48 h or at 4 °C for 72 hours (Figure S3). The stability of the reaction, the isothermal nature of the amplification process, and its independence from specialized equipment greatly simplifies the logistic of implementation of this diagnostic method outside centralized labs.

Analysis of sensitivity

We conducted a series of experiments to assess the sensitivity of the LAMP reactions in the 3D-printed incubation chamber using the two sets of primers (α and β; Table 1). The amplification proceeds with sufficient quality to also allow proper visualization of the amplification products in electrophoresis gels, even at low nucleic acid concentrations. We observed that amplification proceeded successfully in a wide range of viral loads, from 625

to 5×10^5 copies in experiments using synthetic SARS-CoV-2 nucleic acid material (Figure 3A).

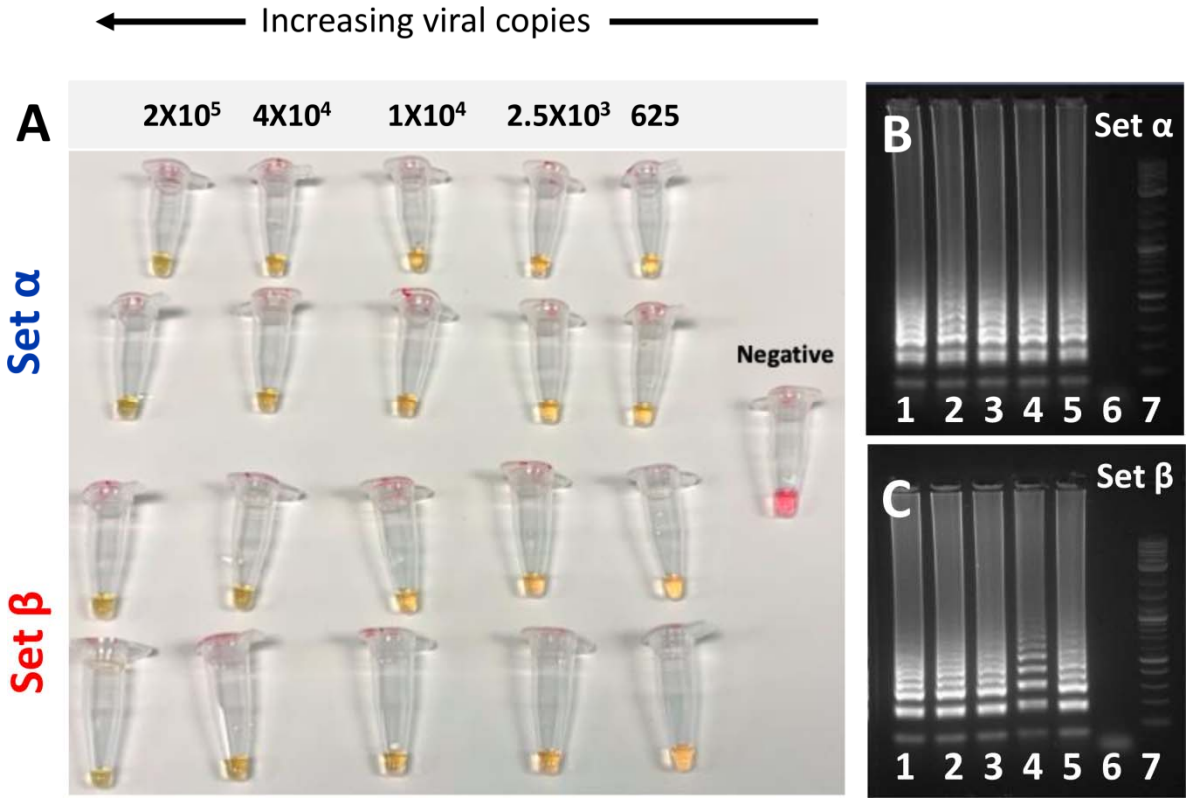


Figure 3. **Two different sets of LAMP-primers were used for successfully targeting of a gene sequence encoding the SARS-Co2 N protein**. (A) LAMP primer sets α and β both enable the amplification of synthetic samples of SARS-CoV-2 nucleic acids in a wide range of template concentrations, from 625 to 2.0×10^5 DNA copies of SARS-CoV-2 when incubated for 50 minutes at a temperature range from 60 to 65 ºC. (B,C) Agarose gel electrophoresis of DNA amplification products generated by targeting two different regions of the sequence coding for SARS-Co2 N protein. Two different primer sets were used: (B) primer set α, and (C) primer set β. The initial template amount was gradually decreased from left to right: 2.0×10^5 DNA copies (lane 1), 4.0×10^4 copies, (lane 2), 1.0×10^4 copies (lane 3), 2.5×10^3 copies (lane 4), 625 copies (lane 5), negative control (lane 6), and molecular weight ladder (lane 7).

We clearly observed amplification in samples containing as few as 625 viral copies after incubation times of 5 min at 65 ºC. If we put this range into a proper clinical context, the actual viral load of COVID-19 in nasal swabs from patients has been estimated to fall within the range of 10^5 to 10^6 viral copies per mL [49]. Discrimination between positive and negative samples (controls) can be clearly established by the naked eye in all reactions incubated for 50 min, regardless of the number of viral copies present. In addition, we did not observe any non-specific amplification in negative samples (i.e., containing synthetic genetic material form EBOV) incubated for 50 min at 65 ºC. Indeed, the identification and amplification of SARS-CoV-2 synthetic material is feasible in samples that contained ~62.5 viral copies using this LAMP strategy (Figure S3) and incubation times of 50–60 min.

We corroborated the amplification by visualizing LAMP products with gel electrophoresis for the different viral loads tested. Figures 3B,C show agarose gels of the amplification products of each one of the LAMP experiments, where two different sets of primers (α and β) were used to amplify the same range of concentrations of template (from 625 to 2×10^5 synthetic viral copies). We were able to generate a visible array of bands of amplification products, a typical signature of LAMP, for both LAMP primer sets and across the whole range of synthetic viral loads. Indeed, both primer sets rendered similar amplification profiles.

In summary, using the primers and methods described here, we were able to consistently detect the presence of SARS-CoV-2 synthetic nucleic acids. We have used a simple 3D-printed incubator, connected to a water circulator, to conduct LAMP. We show that, after only 30 min of incubation, samples containing a viral load in the range of 10^4 to 10^5 copies could be clearly discriminated from negative samples by visual inspection with the naked eye (Figure 2C). Samples with a lower viral load were clearly discriminated when the

LAMP reaction was incubated for 50 min. Incubation periods of up to 1 h at 68 ºC did not induced false positives and were able to amplify as few as ~62 copies of SARS-CoV-2 synthetic genetic material. These results are consistent with those of other reports in which colorimetric LAMP, assisted by phenol red, has been used to amplify SARS-COV-2 genetic material [39,40].

We observe 0 false positive cases in experiments where synthetic samples containing EBOV genetic material were incubated at 65 ºC for 1 h.

In the current context of the COVID-19 pandemics, the importance of communicating this result does not reside in its novelty but in its practicality. Some cost considerations follow. While the market value of a traditional RT-qPCR apparatus (the current gold standard for COVID-19 diagnostics) is in the range of 10,000 to 40,000 USD, a 3D-printed incubator, such as the one described here (Figure S1,S2; Supplementary file S1), could be fabricated for under 200 USD at any 3D printer shop. This difference is significant, especially during an epidemic or pandemic crisis when rational investment of resources is critical. While the quantitative capabilities of testing using an RT-qPCR platform are undisputable, the capacity of many countries to rapidly, effectively, and massively establish diagnostic centers based on RT-qPCR is questionable. The current pandemic scenarios experienced in the USA, Italy, France, and Spain, among others, have crudely demonstrated that centralized labs are not an ideal solution during emergencies. Portable diagnostic systems may provide a vital flexibility and speed of response that RT-qPCR platforms cannot deliver.

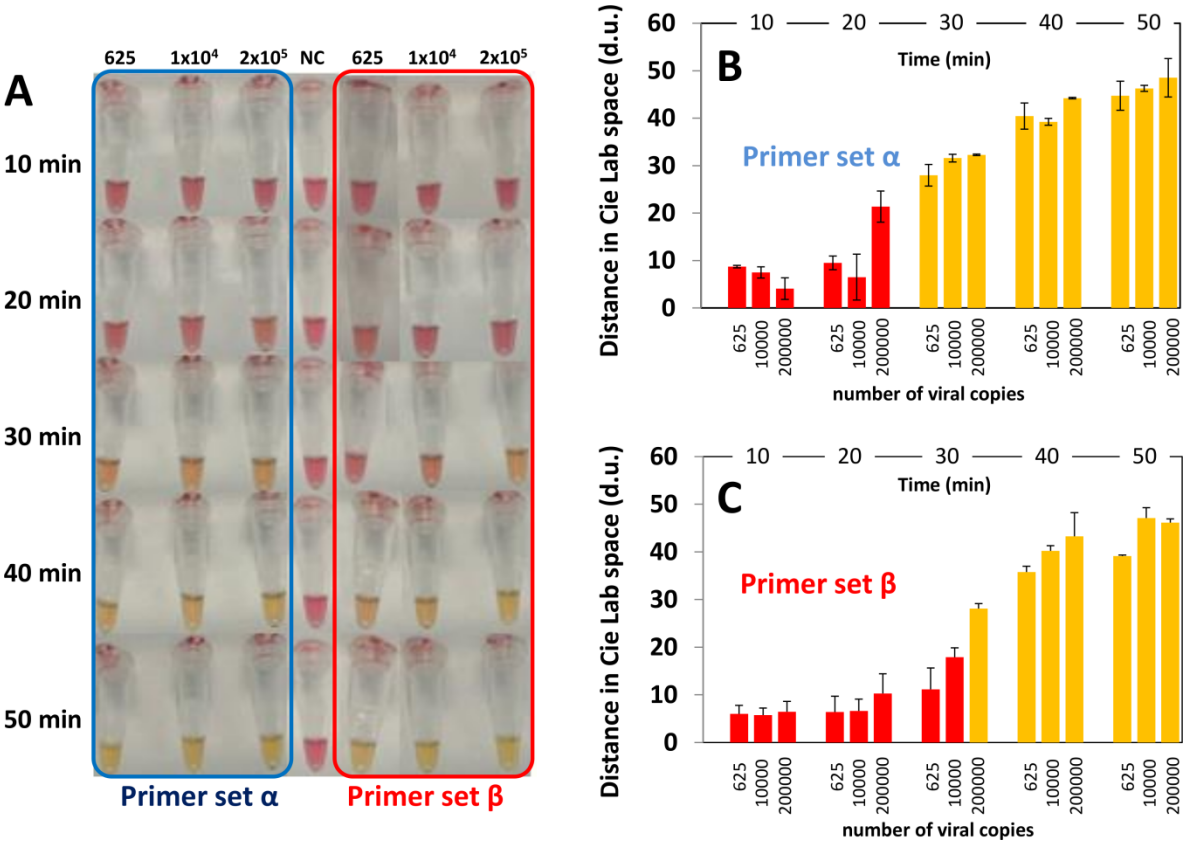
Feasibility of real-time quantification

Here, we further illustrate the deterministic and quantitative dependence between the concentration of the amplification product and the color signal produced during this colorimetric LAMP reaction. For this purpose, we simulated real-time amplification experiments by conducting a series of amplification reactions using initial amounts of 625, 1×10^4, and 2×10^5 copies of synthetic SARS-CoV-2 genetic material in our 3D-printed incubator.

We extracted samples from the incubator after 0, 10, 20, 30, 40, and 50 min of incubation at 65 ºC. The color of these samples was documented as images captured using a smart phone (iPhone 7) against a white background (Figure 4A). The images were analyzed using the free access application Color Companion® for the iPhone or iPad. Briefly, color images were decomposed into their CIELab space components. In the CIELab color space, each color can be represented as a point in a 3D-space, defined by the values **L**, **a**, and **b** [45]. In this coordinate system, **L** is the luminosity (which ranges from 0 to +100), **a** is the blue-yellow axis (which ranges from -50 to 50), and **b** is the green-red axis (which ranges from -50 to 50) (Figure S4).

The difference between two colors can be quantitatively represented as the distance between the two points that those colors represent in the CIELab coordinate system. For the colorimetric LAMP reaction mixture used in our experiments, the spectrum of possible colors evolves from red (for negative controls and negative samples) to yellow (for positive samples). Conveniently, the full range of colors for samples and controls can be represented in the red and yellow quadrant defined by L [0,50], a [0,50], and b [0,50]. For instance, the difference between the color of a sample (at any time of the reaction) and the color of the

359 negative control (red; L=53.72 ± 0.581, a=38.86 ± 2.916, and b=11.86 ± 0.961) can be
360 calculated in the CIELab space.



361

362 **Figure 4.** Evaluation of the sensitivity of the combined use of a colorimetric LAMP method
363 assisted by the use of phenol red. (A) Sensitivity trials using different concentrations of the template
364 (positive control) and two different primers sets: α (indicated in blue) and β (indicated in red).
365 Photographs of the Eppendorf PCR tubes containing positive samples and negative controls were
366 acquired using a smartphone. (C,D) Distance in the color CIELab space between negative controls
367 (red) and samples containing different concentrations of SARS-CoV-2 nucleic acid material (i.e.,
368 625, 10000, and 200000 synthetic copies) analyzed after different times of incubation (i.e., 10, 20,
369 30, 40, and 50 minutes) at 65 °C. The analysis of color distances is presented for amplifications
370 conducted using primer set (B) α and (C) β.

371

372 We determined the distance in the CIELab space between the color of samples taken at
373 different incubation times that contained SARS-CoV-2 genetic material and negative

controls (Figure 4B and C). We repeated this calculation for each of the LAMP primer sets that we used, namely primer set α (Figure 4B) and β (Figure 4C).

These results suggest that the color difference between the samples and negative controls is quantifiable. Therefore, color analysis may be implemented to assist the discrimination between positives and negatives. Furthermore, imaging and color analysis techniques may be implemented in this simple colorimetric LAMP diagnostic strategy to render a real-time quantitative Lamp (RT-qLAMP). Alternatively, the progression of the amplification at different times can be monitored by adding an intercalating DNA agent, (i.e., EvaGreen Dye), and measuring fluorescence on time (Figure S5).

Note that the variance coefficients for the control are 1.08, 7.50, and 8.10% for L, a, and b, respectively. These small values suggest robustness and reproducibility in the location of the coordinates of the control point (reference point). Similarly, the variation in color between negative controls and positive samples incubated for 50 min was reproducible and robust (average of 46.60 +/- 4.02 d.u.; variance coefficient of 8.62%).

Finally, we observed differences in the performance of the two LAMP primer sets used in the experiments reported here (Figure 4B and 5).

Our results suggest that primer set α enabled faster amplification in samples with fewer viral copies. Consistently, this primer set yielded positive discrimination in samples with 625 viral copies in 30 min (Figure 4B). The use of primer set β enabled similar differences in color, measured as distances in the CIELAB 3D-space, in 40 min (Figure 4C). These findings suggest that primer set α should be preferred for final-point implementations of this colorimetric LAMP method. Interestingly, primer set β may better serve the purpose of a real-time implementation. While primer set α produced similar trajectories of evolution of color in samples that contained 1.0×10^4 and 2.0×10^5 viral copies (Figure 5A), primer set

398 β was better at discriminating between amplifications produced from different initial viral
399 loads (Figure 5B).

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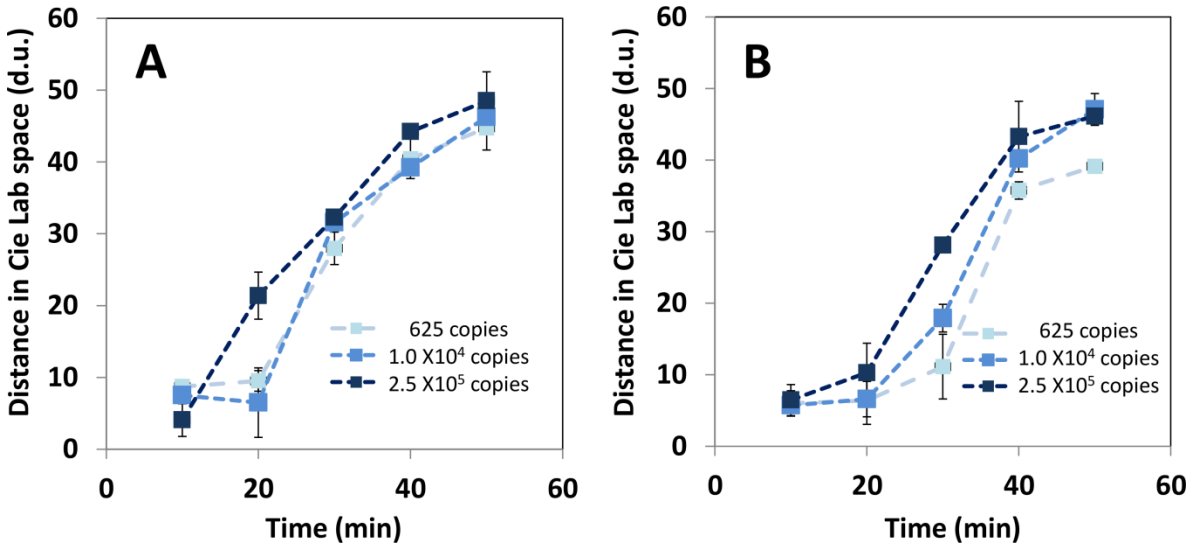

401

402 **Figure 5.** Time progression of the distance in color with respect to negative controls (red
403 color) in the CIELab space for positive SARS-CoV-2 samples containing 625 (light blue,
404 ■), 1×10^4 (medium blue, ■), and 2.5×10^6 (dark blue, ■) copies of synthetic of SARS-
405 CoV-2 nucleic acids. Results obtained in experiments using (A) primer set α, and (B)
406 primer set β.

407

408 ## Conclusions

409 The challenge of point-of-care detection of viral threats is of paramount importance,
410 particularly in underdeveloped regions and in emergency situations (i.e., epidemic
411 outbreaks). In the context of the current COVID-19 pandemic, the availability of testing
412 infrastructure based on RT-qPCR is recognized as a serious challenge around the world. In
413 developing economies (i.e. Latin America, India, and most African countries), the currently
414 available resources for massive COVID-19 testing by RT-qPCR will clearly be insufficient.

Even in developed countries, the time to get diagnostic RT-qPCR results from a COVID-19 RT-qPCR test currently ranges from 1 to 5 days. Clearly, the available PCR labs are overburdened with samples, have too few personnel to conduct the tests, are struggling with backlogs on the instrumentation, and face complicated logistics to transport delicate and infective samples while preserving the cold chain.

Here, we have demonstrated that a simple embodiment of a LAMP reaction, assisted by the use of phenol red as a pH indicator and the use of a simple 3D-printed chamber connected to a water circulator, can enable the rapid and highly accurate identification of samples that contain artificial SARS-CoV-2 genetic sequences. Amplification is visually evident, without the need for any additional instrumentation, even at low viral copy numbers. In our experiments with synthetic samples, we observed 100 percent accuracy in samples containing as few as 625 copies of SARS-CoV-2 genetic material.

Validation of these results using real human samples from positive and negative COVID-19 subjects is obviously needed to obtain a full assessment of the potential of this strategy as an alternative to RT-qPCR platforms. However, our results with synthetic samples suggest that this simple strategy may greatly enhance the capabilities for COVID-19 testing in situations where RT-qPCR is not feasible or is unavailable.

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Supporting Information

Supporting Information

Figure S1. (A) Actual images, and (B) rendering of the 3D- printed incubation chamber used in the LAMP experiments.

Figure S2. Schematic representation of the chamber (different views) showing its relevant dimensions.

Figure S3. (A) The colorimetric LAMP method described here was able to identify and amplify synthetic SARS-CoV-2 genetic material in samples containing as few as ~62 viral copies. (B) Evaluation of the stability and functionality of the LAMP reaction mix at different storage times and temperatures. The reaction mix, which is formulated with LAMP primers and ready for the addition of nucleic acid extracts, is functional and discriminates between positive and negative samples when stored (i) at room temperature for 48 h or (ii) at 4 °C for 72 h.

Figure S4. (A) Color analysis conducted on positive and negative SARS-CoV-2 samples contained in Eppendorf PCR tubes (yellow inset) using Color Companion, a freely available app from Apple (downloadable at Apple Store, USA). This app identifies the components of color in a specific location of an image (black circle in the yellow inset) in the CIELab, RGB, HSB, or CMYK spaces. The image can be uploaded using e-mail, airdrop, or Whatsapp. (B) Schematic representation of the CIELab space, a color system where any color can be represented in terms of a point and its coordinates in a 3D space, where L is luminosity, a is the axis between green and red, and b is the axis between yellow and red.

Figure S5. (A) The amount of amplification product in LAMP experiments was evaluated by measuring the fluorescence emitted by the amplification product in reactions with an added intercalating agent. Fluorescence readings were conducted in standard 96-well plates and using a conventional plate reader. (A) Fluorescence readings, as measured in a

commercial plate reader, for different dilutions of SARS-CoV-2 synthetic DNA templates. Results using two different LAMP primer sets are shown: set α (indicated in blue), and set β (indicated in red).

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640

641

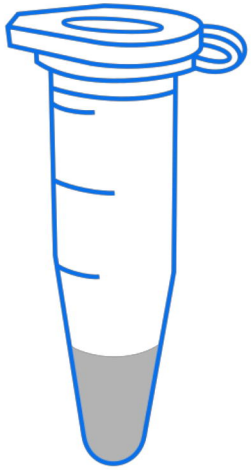
642

643

644

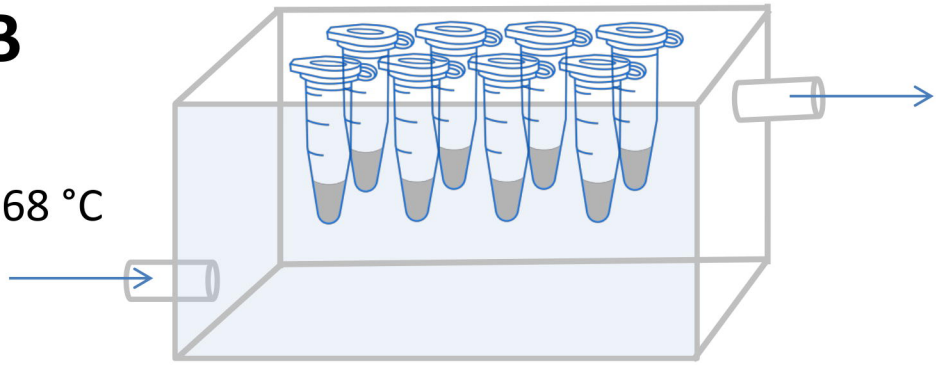
sample

A



B

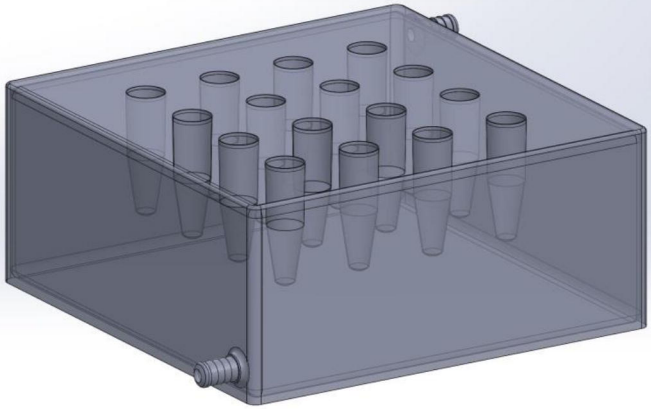
Water in @68 °C



Reaction mix
+ Primer set α or β

Polymerase
Phenol red

C



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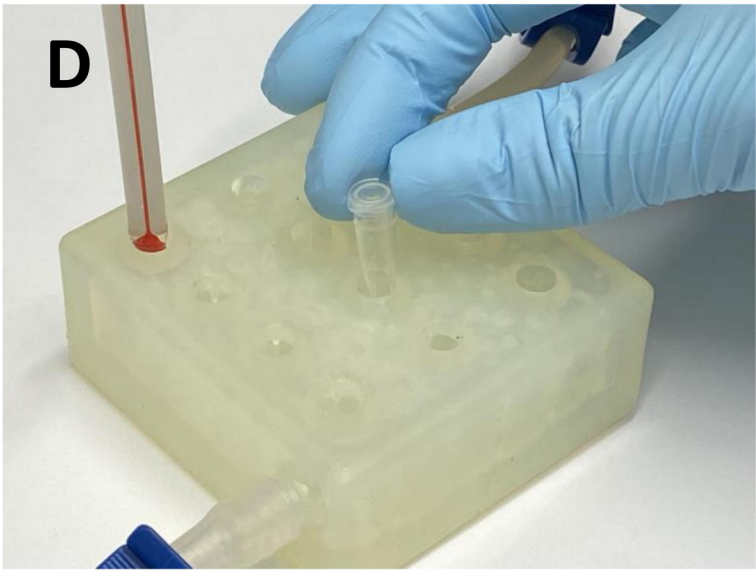
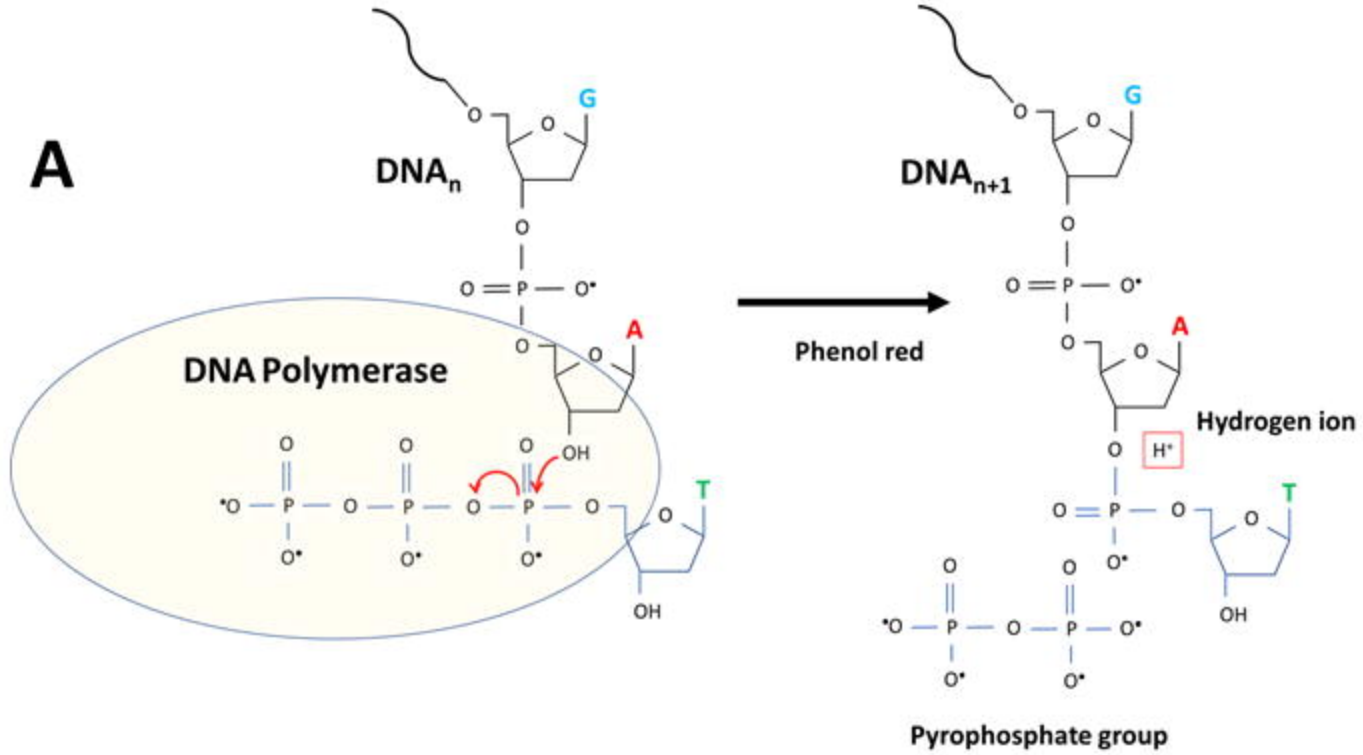
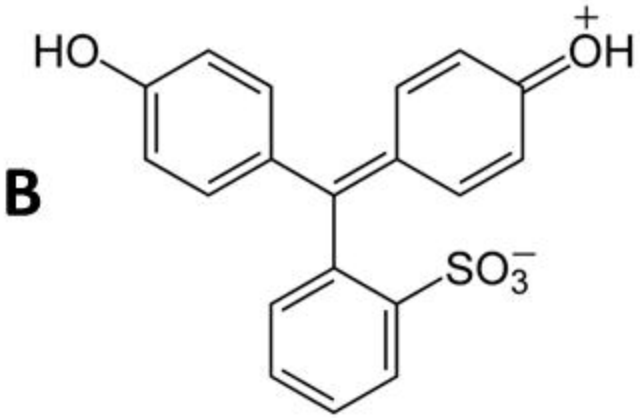
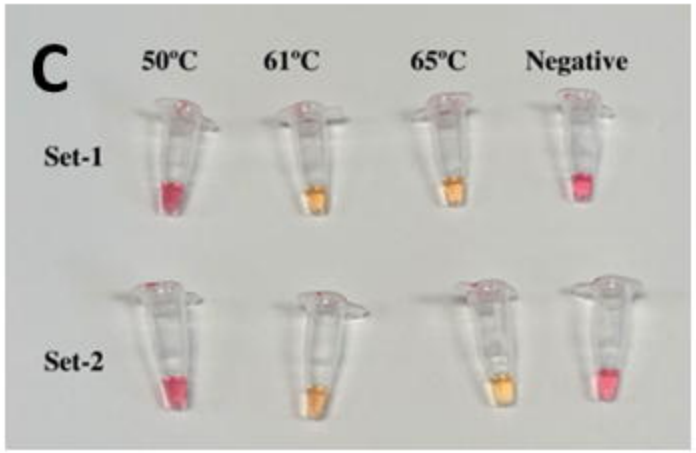
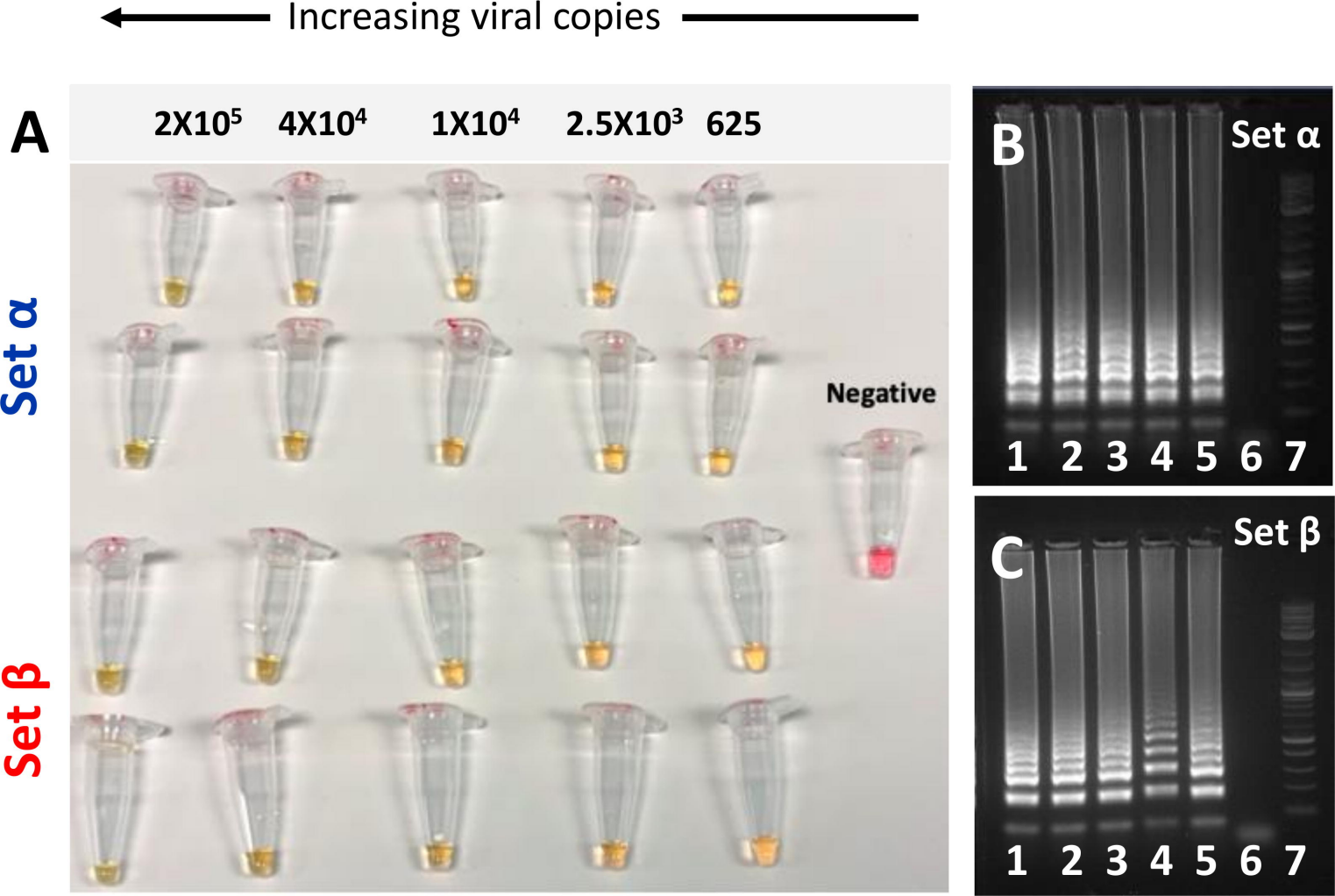
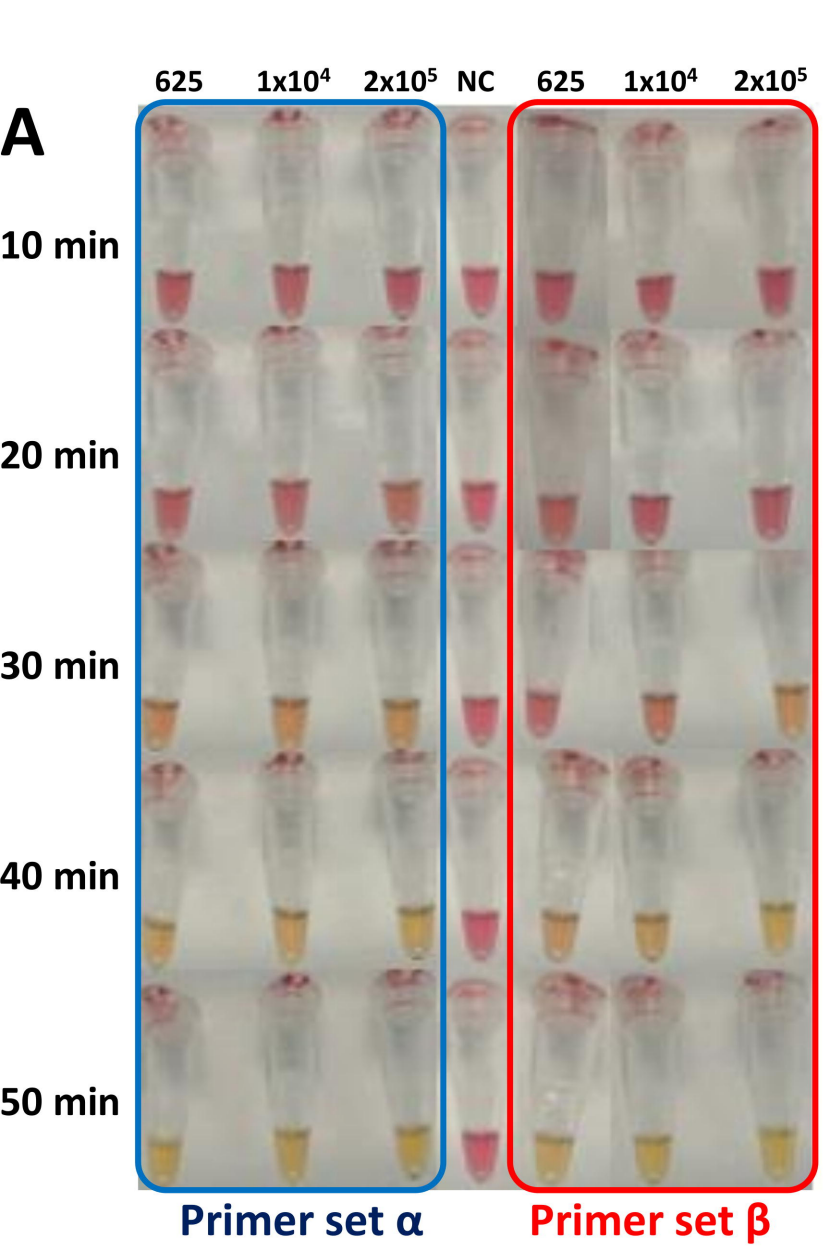


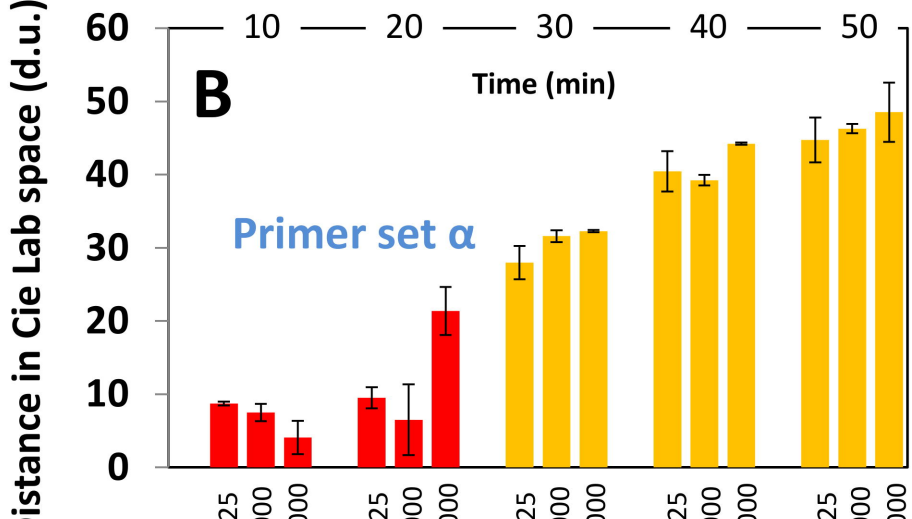

A
DNA$_n$
DNA$_{n+1}$
DNA Polymerase
Phenol red
Hydrogen ion
Pyrophosphate group
G
A
T
OH
H$^+$

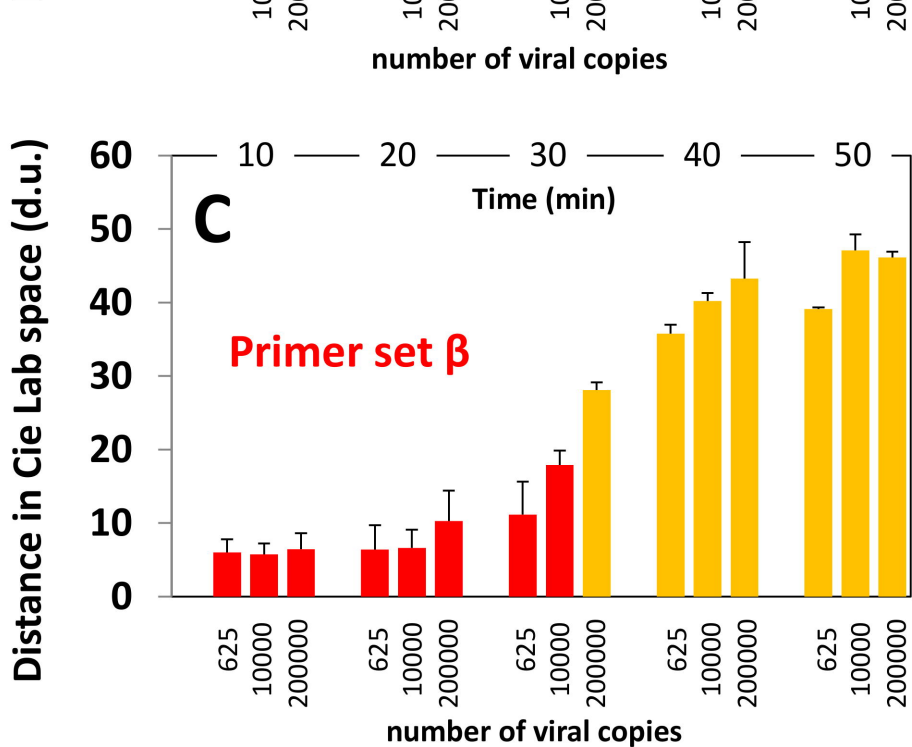

B
HO
OH
SO_3^-


C
50°C
61°C
65°C
Negative
Set-1
Set-2


Increasing viral copies
A
2X10^5
4X10^4
1X10^4
2.5X10^3
625
Negative
Set α
Set β
B
Set α
1 2 3 4 5 6 7
C
Set β
1 2 3 4 5 6 7


A
625
1x10^4
2x10^5
NC
625
1x10^4
2x10^5
10 min
20 min
30 min
40 min
50 min
Primer set α
Primer set β


B
Time (min)
10
20
30
40
50
Primer set α
Distance in Cie Lab space (d.u.)
0
10
20
30
40
50
60
625
10000
200000
number of viral copies


C
Time (min)
10
20
30
40
50
Primer set β
Distance in Cie Lab space (d.u.)
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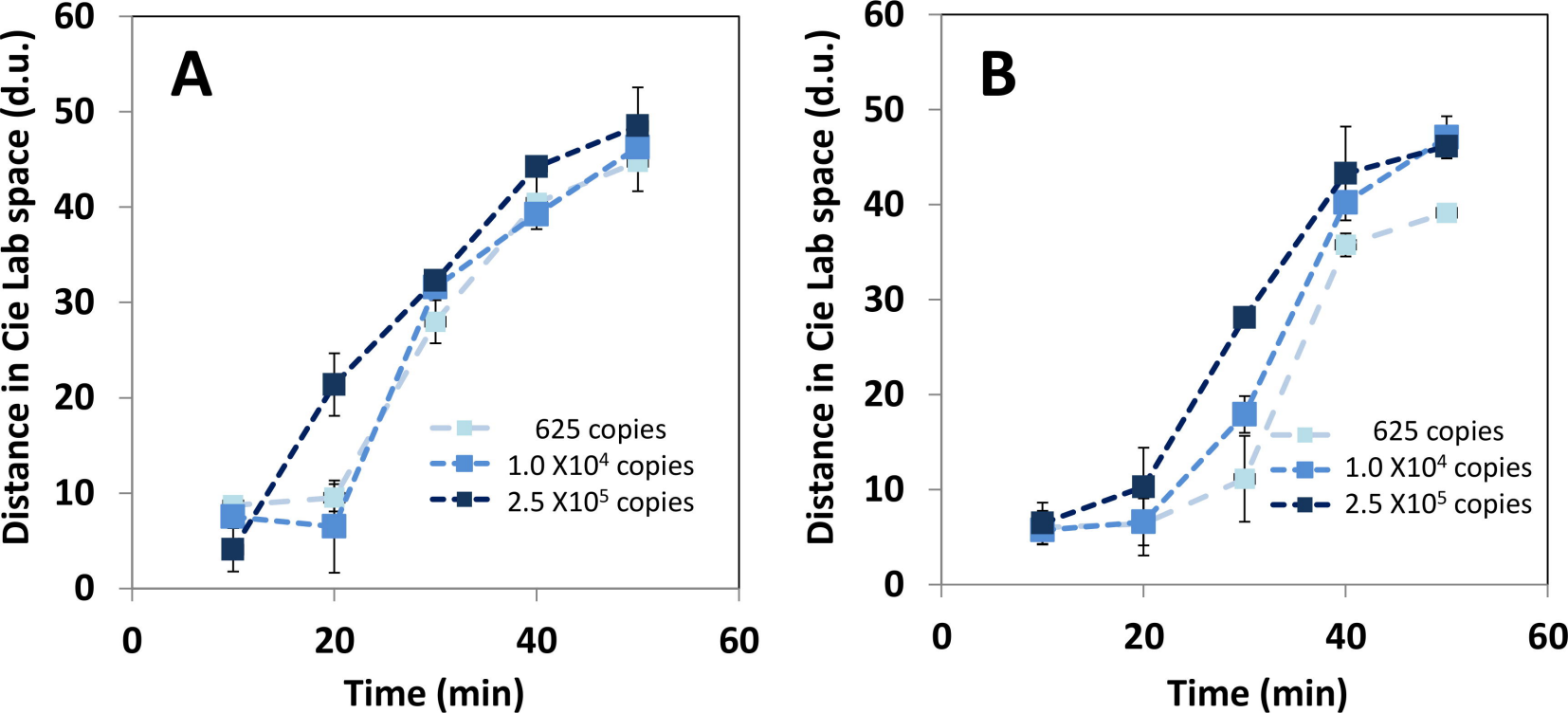
10
20
30
40
50
60
625
10000
200000
number of viral copies


A
B
Distance in Cie Lab space (d.u.)
Time (min)
0
10
20
30
40
50
60
625 copies
1.0 X10^4 copies
2.5 X10^5 copies