A lab-on-a-chip platform for integrated extraction and detection of SARS-CoV-2 RNA in resource-limited settings

Pablo Rodriguez-Mateos,[a] Bongkot Ngamsom,[a] Cheryl Walter,[b] Charlotte E. Dyer,[b] Jesse Gitaka,[c] Alexander Iles,[a] Nicole Pamme[*a]

[a]*Department of Chemistry and Biochemistry, University of Hull, UK*
[b]*Department of Biomedical Sciences, University of Hull, UK*
[c]*Mount Kenya University, Thika, KENYA*

*Corresponding author: **Nicole Pamme**

Department of Chemistry, Faculty of Science and Engineering, University of Hull,

Cottingham Road, Hull, East Riding of Yorkshire, HU6 7RX, UK.

E-mail: n.pamme@hull.ac.uk

Highlights:

- A lab-on-a-chip device integrating RNA extraction and isothermal amplification based on IFAST RT-LAMP.
- Specific and rapid detection of 470 copies mL^{-1} genomic SARS-CoV-2 RNA in 1 h by the naked eye.
- Potential use for point-of-care testing in resource-limited settings.

Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused the unprecedented global pandemic of coronavirus disease-2019 (COVID-19). Efforts are needed to develop rapid and accurate diagnostic tools for extensive testing allowing for effective containment of the infection, via timely identification and isolation of SARS-CoV-2 carriers. Current gold standard nucleic acid tests require many separate steps that need trained personnel to operate specialist instrumentation in laboratory environments, hampering turnaround time and test accessibility, especially in low-resource settings. We devised an integrated on-chip platform coupling RNA extraction based on immiscible filtration assisted by surface tension (IFAST), with RNA amplification and detection via colorimetric reverse-transcription loop mediated isothermal amplification (RT-LAMP), using two sets of primers targeting open reading frame1a (ORF1a) and nucleoprotein (N) genes of SARS-CoV-2. Results were identified visually, with a colour change from pink to yellow indicating positive amplification,

and further confirmed by DNA gel electrophoresis. The specificity of the assay was tested against HCoV-OC43 and H1N1 RNAs. The assay based on use of gene N primers was 100% specific to SARS-CoV-2 with no cross-reactivity to HCoV-OC43 nor H1N1. Proof-of-concept studies on water and artificial sputum containing genomic SARS-CoV-2 RNA showed our IFAST RT-LAMP device to be capable of extracting and detecting 470 SARS-CoV-2 copies mL^{-1} within 1 h (from sample-in to answer-out). IFAST RT-LAMP is a simple-to-use, integrated, rapid and accurate COVID-19 diagnostic platform, which could provide an attractive means for extensive screening of SARS-CoV-2 infections at point-of-care, especially in resource-constrained settings.

1. Introduction

An outbreak of Coronavirus Disease 2019 (COVID-19) caused by a novel human betacoronavirus SARS-CoV-2 was reported in Wuhan, Hubei province of China, in late December 2019 [1, 2]. Due to the rate of transmission and possible fatal progression, the World Health Organisation (WHO) declared a global health emergency on 31 January 2020 and a pandemic situation on 11 March 2020 [3]. The clinical spectrum of COVID-19 ranges from asymptomatic infection to acute respiratory distress syndrome with multisystem failures [4, 5]. The epidemiological update as of 4 February 2021 reported over 100 million cumulative cases and 2.2 million deaths globally since the start of the pandemic [6]. The increasing gravity of the situation has been attributed to the highly contagious nature of the disease from both asymptomatic and pre-symptomatic cases [7, 8], in combination with the lack of effective point-of-care testing for rapid and accurate identification of SARS-CoV-2 carriers [9].

Among rapid diagnostic tests developed for point-of-care and community purposes are the antigen lateral flow tests targeting the nucleocapsid protein of SARS-CoV-2. Despite showing great promise for ease-of-use and ≤ 30 min turnaround time, the low sensitivity (10^6 copies mL^{-1}) is a major disadvantage of this approach [10]. Current gold standard COVID-19 tests still rely on nucleic acid amplification tests measuring viral nucleic acids based on quantitative reverse transcription polymerase chain reaction (RT-qPCR). Samples from the upper respiratory tract (nasopharyngeal swab, nasal aspirate, or pharyngeal swab) or lower respiratory tract (sputum, tracheal aspirate) are taken from suspected cases for RNA extraction followed by reverse transcription and cDNA amplification of a genomic specific region [4, 5]. The

analytical limits of detection of RT-qPCR are usually around 10^3 viral RNA copies mL^{-1} with sample-to-result times of 24-48 h [11]. Several SARS-CoV-2 RT-PCR detection kits have been developed by different companies and institutions [9]. However, such assays are mostly limited to highly specialized laboratories with trained personnel [4, 12]. As opposed to conventional PCR assays, isothermal nucleic acid amplification tests utilise a single temperature and involve no expensive instrumentation, requirement for calibrated internal controls nor trained personnel for operation and result interpretation. Specifically, loop-mediated isothermal amplification (LAMP) relies on auto-cycling strand displacement DNA synthesis using four to six primers, resulting in exponential amplification [13]. Colorimetric RT-LAMP based on pH change can offer rapid and sensitive detection of viral RNAs and requires only a heat source [14, 15]. RT-LAMP has been applied for detection of many viral infectious diseases [15]. Several groups have explored RT-LAMP for SARS-CoV-2 RNA detection, showing comparable results to the gold standard RT-PCR with lowest sensitivity being ca. 20 – 200 RNA copies per reaction, while achieving faster turnaround times (30 – 40 min) [16-22]. Despite the successful detection of SARS-CoV-2 RNA from crude cell lysates reported by Zhang *et al*. [16], the majority of the reported RT-LAMP assays required RNA extraction and purification steps to be performed on clinical samples prior to amplification using laboratory based techniques and procedures. A lower sensitivity of the assays was also reported when clinical samples were directly subjected to RT-LAMP without an RNA isolation step [23]. In addition, investigations on direct RT-LAMP of respiratory samples without RNA extraction using the Variplex™ system [24] demonstrated high false negative rates as well as failure to reliably detect SARS-CoV-2 [25].

A lab-on-a-chip process for a combined workflow of nucleic acid extraction and purification, namely 'IFAST', utilises 'pinned' aqueous/organic liquid interfaces in microchannels to streamline the extraction mechanism, replacing all washing steps with a single traverse of an immiscible fluid barrier [26]. By attaching magnetically-responsive particles to a target cell/ molecule via immunocapture, the target bound magnetic particles can be selectively transported across the immiscible barrier and into a separate solution. Contaminants are prevented from inadvertently crossing the immiscible phase by the high interfacial energy associated with the immiscible phase/aqueous phase boundaries [27]. IFAST has been successfully employed for preconcentration and purification of DNAs [26, 28, 29] and RNAs [29, 30] from various samples. The platform is simple and requires no additional laboratory infrastructure, and yet maintains comparable or better purity, yield and scalability to existing methods [26]. Previously, our group combined the versatility of the IFAST device

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with sensitive molecular detection via adenosine triphosphate (ATP) bioluminescence assay, and devised on-chip IFAST/ATP platforms for rapid detections of *Escherichia coli* O157:H7 from wastewater samples [31], and Group B *Streptococcus* from urine samples [32] for point-of-need testing in resource-limited settings. Very recently, Wimbles *et al*. reported the use of IFAST RT-LAMP platform for on-site extraction of DNA from animal dung, enabling identification of *Ceratotherium simum*, a near threatened species [33].

In this communication, we present for the first time, a lab-on-a-chip device based on IFAST RT-LAMP for rapid detection of genomic SARS-CoV-2 RNAs, integrating consecutive steps of (I) RNA extraction, (II) purification and (III) amplification (RT-LAMP) with colorimetric readout for qualitative result interpretation (Fig. 1). The platform permits simple identification of SARS-CoV-2 infection via the naked eye, fast turnaround from sample-in to answer-out (within 1 h) and requires only chip loading and manipulation of magnetic particles that can be performed by minimally trained staff, enabling widespread screening of the infection for optimised prevention and treatment at the point of care in low-resource settings.

2. Materials and methods

2.1 Reagents

Genomic SARS-CoV-2 RNA (2019-nCoV/USA-WA1/2020, ATCC VR-1986D), HCoV-OC43 (ATCC VR-1558D) and H1N1 (ATCC VR-1736D) were supplied by LGC standards, UK. WarmStart® DNA and RNA polymerase (MS1800S) was purchased from New England Biolabs. Primers were purchased from Integrated DNA technologies (IDT). Oligo (dT)-coated magnetic beads, SYBR Safe and nuclease-free water were supplied by Thermofisher Scientific, UK. Mineral oil and guanidine hydrochloride (GuHCl) were purchased from Sigma-Aldrich.

2.2 Chip design, fabrication and preparation.

This work utilised polymethyl methacrylate (PMMA) chips fabricated via CNC machine milling (Datron M7, Milton Keynes, UK), rather than the polydimethylsiloxane (PDMS) chips employed previously for IFAST/ATP platforms [31, 32]. The chip features a large sample chamber 1 (26 mm wide, 26 mm long); wash chambers 2, 4, 6 (3 mm wide × 3 mm long); wash chamber 3 (3 mm wide× 14 mm long); wash chambers 5, 7, 8 (3 mm wide × 8.5 mm long); and a detection chamber 9 (3 mm wide × 3 mm long). All chambers used had a depth of 3.8 mm, and were interconnected via gates (3 mm to 0.5 mm wide, 3 mm long, 0.2 mm deep) as shown in Fig. 2. To reduce carry-over and provide more effective washing, the previous chip designs were modified to include more wash chambers (Section B,

Supplementary Data). To prevent contamination with RNases, devices were sprayed with RNase decontamination solution (ThermoFisher Scientific), followed by rinsing with nuclease-free water (ThermoFisher Scientific) and were left to dry prior to use. The bottom of the chip was sealed with PCR adhesive film (ThermoFisher Scientific).

2.3 RT-LAMP

Tube-based RT-LAMP was firstly performed targeting ORF1a and N genes using two sets of primers as described by Zhang *et al.*, (Table S1, Supplementary Data [16]). Ten-fold serial dilutions of genomic SARs-CoV-2 RNA (4.7×10^3 copies μL^{-1}) were performed in nuclease-free water. The tube-based RT-LAMP reaction was conducted with the following composition: 16 µM forward inner primer (FIP) and backward inner primer (BIP), 2 µM forward outer primer (F3) and backward outer primer (B3), 4 µM forward loop primer (FLP) and backward loop primer (BLP), 1X LAMP reaction mix, 1-3 µL of the target RNA, and nuclease-free water to make up to a final volume of 20 µL. RT-LAMP products were electrophoresed in 1% w/v agarose gels stained with SYBR Safe at 80 V for 45 min. Gels were imaged in a molecular imager (Chemidoc XRS+, BioRAD). The specificity of the assay was tested by replacing the genomic SARS-CoV-2 RNA with equal volumes of genomic HCoV-OC43 or H1N1 RNAs. For comparison, RT-LAMP of HCoV-OC43 and H1N1 RNAs with their respective primers (Tables S2 and S3, Supplementary Data) were employed for positive amplifications. Colour was visible directly on removal from incubation temperature. However, the reaction was cooled to room temperature to allow colour intensification prior to photographing. Tubes or IFAST devices were placed on an A4 white printing paper to provide a clear background. Images were captured using a mobile phone camera (SAMSUNG Galaxy A3) taken from above the tubes/IFAST devices, under normal laboratory lighting. For comparison, images of tube/IFAST device with negative control were taken in the same frame as the investigated samples.

For on-chip RT-LAMP, the PMMA chambers were alternately filled with aqueous solutions (chambers 1, 3, 5, 7) and mineral oil (chambers 2, 4, 6, 8). RT-LAMP reaction mix was prepared in a PCR tube (20 µL; 8 µL H_2O, 2 µL of primer mix and 10 µL of RT-LAMP MasterMix) and added to detection chamber 9 overlaying with 10 µL mineral oil to prevent evaporation during LAMP. The device was placed on a pre-warmed block heater (SBH200D, Stuart) set at 65 °C for 30-45 min. Visualisation of colour change of the solution in chamber 9 was employed for result verification prior to gel electrophoresis of amplicons for confirmation.

2.4 On-chip RNA extraction

For RNA extraction in the IFAST devices, 1 µL of RNA at the corresponding dilution was diluted with 999 µL nuclease-free water containing Tween 20 (final concentration = 0.005% w/v, to prevent oligo (dT)-coated magnetic beads (MB) from sticking to the adhesive tape used to seal the bottom of the device) in the sample chamber. Subsequently, 20 µL of MB were added and the device was either manually agitated or placed on a rotator (SB3, Stuart) at 40 rpm for 10 min. Afterwards, the downstream chambers 2, 4 and 6 were filled with mineral oil, and chambers 3, 5 and 7 with 0.005% Tween 20. Collection of RNA-bound MB from the sample chamber was achieved by placing a neodymium iron boron (NdFeB) magnet assembly at the bottom of the chip [31]. The assembly featured a 4 mm diameter × 2 mm height disc magnet and a 20 mm × 10 mm × 5 mm bar magnet (Magnet Sales, UK), providing a magnetic strength of 0.4 Tesla. Washing of MB was then carried out by dragging the beads through the aqueous/oil barriers and briefly storing in chamber 7. The magnetically isolated RNA in 0.005% Tween 20 was pipetted from chamber 7 into a PCR tube where RT-LAMP was to be performed to verify successful magnetic isolation of RNA from the on-chip extraction. After removing the supernatant, the beads with captured RNA were resuspended in 20 µL of LAMP reaction mix and incubated in a block heater set at 65 °C for 30-40 min.

2.5 Integrated on-chip RNA extraction and on-chip RT-LAMP

On-chip RNA extraction was similarly performed as described above, followed by the addition of LAMP mix (20 µL) in chamber 9 into which the RNA-captured beads were mixed and the chamber overlaid with 10 µL mineral oil. The chip was placed on a block heater for RT-LAMP at 65 °C for 40 min. The complete workflow is demonstrated in Section E, Supplementary Data. A similar process was carried out with artificial sputum samples spiked with genomic RNAs, except that the 0.005% w/v Tween 20 was replaced with sputum samples formulated according to Kaur *et al*. [34]. To prepare a negative control of RNA-spiked samples, artificial sputum (250 µL) was spiked with HCoV-OC43 (1 µL, 5 pg µL^{-1}) and H1N1 (1 µL, 0.029 ng µL^{-1}) genomic RNAs and the mix was directly added to the sample chamber of the device. The positive sample was prepared similarly to the negative control, except that the sample was also spiked with 1 µL of 0.095 ng µL^{-1} SARS-CoV-2 RNA to afford a final concentration of 470 SARS-CoV-2 copies mL^{-1}. RNA-spiked artificial sputum was diluted with 748 µL of 5 M GuHCl (final concentration = 3.7 M) and 2 µL of 2.5% w/v Tween 20 (final concentration = 0.005% w/v) inside the sample chamber. On-chip processes were subsequently performed as described above for the SARS-CoV-2 spiked water samples. The full workflows were independently tested by two operators.

3. Results and discussion

3.1 On-chip RT-LAMP for genomic SARS-CoV-2 RNA detection

Utilising the commercially available colorimetric LAMP kit and two primer sets targeting ORF1a and N genes [16], the effectiveness of tube-based RT-LAMP for SARS-CoV-2 RNA detection was firstly assessed on a series of ten-fold dilutions performed on the initial genomic RNA (4.7×10^3 copies μL^{-1}). The assay was capable of amplifying ≥ 470 copies of genomic RNA after 30 min (Fig. 3), a reduced sensitivity compared with 120 copies being reported for RNA fragments using the same primer sets reported by Zhang e*t al.* [16]. Being amongst the largest viral genomes, with 30 kb size [35], a longer time is expected for primers and enzymes to find the target sequences within the genome compared with much shorter RNA fragments.

In order to check the feasibility of performing on-chip RT-LAMP as a consecutive step after RNA extraction, it is vital to verify that (i) on-chip amplification occurs similarly to the tube-based assay, and (ii) the magnetic beads utilised for RNA extraction do not interfere with amplification. Consequently, for on-chip RT-LAMP, oligo (dT)-functionalised magnetic beads were added to the sample chamber and directed through the immiscible phases to combine with the RT-LAMP reaction mix in the last chamber prior to heating. Successful on-chip amplification was achieved with no interference from the magnetic beads.

3.2 Specificity of RT-LAMP assays for SARS-CoV-2 detection

The specificity of the RT-LAMP primers for SARS-CoV-2 RNA detection was tested against Betacoronavirus HCoV-OC43, a ubiquitous human coronavirus in the environment responsible for up to one third of common colds [36, 37], and influenza A virus H1N1, which shares substantial similarities in viral shedding, transmission dynamics and clinical features of viral respiratory illnesses [38]. RT-LAMP assays conducted on genomic HCoV-OC43 and H1N1 using their respective primers showed positive amplifications (Fig. 4a).

Although demonstrating a slightly faster amplification than Gene N primers, ORF1a primers showed cross-reactivity with both HCoV-OC43 and H1N1 RNAs (Fig. 4b), and were excluded from further investigations. In contrast, only samples with SARS-CoV-2 RNA resulted in positive amplifications exploiting Gene N primers, while HCoV-OC43 and H1N1 remained negative (Fig. 4b, c), demonstrating specific pairing of Gene N primers to SARS-CoV-2 RNA, but not to HCoV-OC43 or H1N1 RNAs. This indicates the possibility of simultaneously diagnosing infection(s) of COVID-19 (SARS-CoV-2), a common cold virus (HCoV-OC43), and influenza A virus (H1N1) by paralleling on-chip RT-LAMP at a single amplification temperature and time, using primer sets specific to target viral genomes.

3.3 On-chip SARS-CoV-2 RNA extraction via IFAST

The microscale IFAST was next explored as a platform for one-step isolation and purification of SARS-CoV-2 RNA. Typically, multi-step solid phase extraction (SPE) processes for RNA extraction (e.g., Qiagen TurboCapture, Invitrogen FastTrack MAG 96) are labour-intensive and require expensive automated systems to facilitate the extensive washing that must be performed on individual samples [26]. The IFAST approach simplifies and expedites the cumbersome RNA extraction process, and enables direct interfacing with the amplification process, reducing overall labour and time-consuming pre-amplification steps. Oligo (dT)-functionalised magnetic beads were employed for selective isolation of polyadenylated RNA species. This specific capture discriminates ribosomal RNA, DNA, proteins and small RNA molecules. Although RNA fragmentation may occur during extraction, primers targeting the N gene region near to the 3' poly-A tail were used to ensure that the captured zone could be amplified and detected. One further advantage of using oligo (dT) magnetic beads is that this approach can also provide an opportunity to include a positive swab control, such as detection of RNAse P mRNA [39]. This abundant mRNA is an excellent sample control that is currently a typical internal standard for RT-PCR diagnostics, but is not incorporated in point-of-care COVID-19 lateral flow testing devices [40, 41].

The successful use of IFAST was demonstrated for extraction and purification of genomic SARS-CoV-2 RNA from aqueous samples containing 470 copies mL^{-1} within 10 min, validated by positive amplification of bead-bound isolated RNA via off-chip RT-LAMP assays (Fig. 5). In these experiments, the magnetically isolated RNAs would be between 470 and 47 copies, as suggested by Fig. 3 (the same reaction time amplified 470 copies, Gene N primers). The level of detected genomic RNA isolated on-chip was significantly lower than the reported median viral loads of 7.99×10^4 copies mL^{-1} and 7.52×10^5 copies mL^{-1} in throat swab and sputum samples, respectively [42]. This on-chip IFAST purification process uses only minute quantities of mineral oil that can effectively filter contaminants from clinical samples in a single step, thereby eliminating multiple washing or centrifugation normally needed for RNA purification. The positive amplifications of the magnetically-isolated RNA by off-chip RT-LAMP confirmed successful purification with no adverse effect on RNA integrity. The current protocol was performed manually, demonstrating its simplicity with no requirement for additional laboratory infrastructure. However, improved capacity can be achieved by automation [43]. This on-chip RNA extraction platform is not limited by the use of oligo (dT) magnetic beads, it can also be applied with other suitable surface chemistries for magnetic

isolation, e.g., silica paramagnetic particles [30, 33, 44], and can be further explored for RNA extraction from clinical samples.

3.4 On-chip integration of RNA extraction and detection via IFAST RT-LAMP for detection of SARS-CoV-2 RNA

Having shown the two on-chip processes separately, i.e. RNA extraction via IFAST and RT-LAMP, the combined workflow for on-chip extraction and on-chip RT-LAMP was next investigated with water samples containing genomic RNA (Fig. 6a). The platform was capable of detecting 470 RNA copies from 1 mL sample in 40 min. The entire process took less than 1 h to complete (2 min sample loading, 10 min RNA extraction and 40 min amplification), with the negative/positive results being clearly distinguishable by the naked eye.

The performance of the platform was further tested with artificial sputum spiked with genomic RNAs due to clinical sample inaccessibility during the investigation. The device is ultimately aimed for point-of-care testing, with swab samples being directly loaded into the sample chamber to mix with lysis reagents, followed by RNA extraction, amplification and visual detection for negative/positive results. With this in mind, the RNA-spiked artificial sputum samples were diluted with GuHCl, a chaeotropic reagent commonly used for isolation of intact mRNA from cells [45], as lysis buffer containing strong surfactant can destroy the immiscible interfaces of the IFAST device. In addition, GuHCl can act as Ribonuclease (RNase) inhibitor which helps to maintain RNA integrity, a common challenge in analysis of clinical samples. By using GuHCl, mRNAs could be isolated from potential viral capsids without any additional steps, extracted with oligo (dT) magnetic beads and amplified via RT-LAMP. The compatibility of the GuHCl with RNA extraction by oligo (dT) magnetic beads, as well as RT-LAMP, was shown by successful specific detection of SARS-CoV-2 from samples containing SARS-CoV-2, H1N1 and HCoV-OC43 RNAs (Fig. 6b). This demonstrates the feasibility of implementing the platform with patient samples; clinical validation will be conducted in Kenya when COVID-19 travel restrictions are lifted.

We delivered a proof-of-concept IFAST RT-LAMP platform for detection of genomic SARS-CoV-2 from water and artificial sputum samples, integrating RNA extraction and colorimetric RT-LAMP in one device. This lab-on-a-chip platform offers a ≤1 h turnaround time exploiting a single device that includes all essential steps required for rapid, sensitive and specific detection of SARS-CoV-2 RNA. This qualitative technology with distinct colour change from pink (negative) to yellow (positive) can be easily visualised under normal ambient light conditions. Although not pursued here, quantification of colour and hence RNA copy

number could be achieved by image analysis of parameters, e.g., hue, saturation or brightness. This would be beneficial for visually colour-compromised operators. At the current proof-of-concept stage, all samples and reagents are manually pipetted into the device. Nevertheless, we anticipate pre-storage of immiscible liquids in wash chambers as well as lyophilised RT-LAMP reagents for the ready-to-use device. This would reduce the number of required pipetting steps where end users would only introduce the sample and magnetic beads, making the platform more deployable for point-of-care testing.

The present setup successfully isolated and detected 470 copies of genomic SARS-CoV-2 from 1 mL sample in 60 min; a superior sensitivity to the 4×10^3 copies mL^{-1} reported from the iAMP® COVID-19 Detection Kit [9]. Other small footprint rapid test equipment being developed for the point-of-care diagnosis of COVID-19 include the ID NOW^{TM} COVID-19 Test Kit (Food and Drug Administration under Emergency Use Authorizations, Abbott), and microchip RT-PCR COVID-19 detection kit (Luminex). Despite achieving the excellent ≤13 min turnaround time, with minimal reagent consumption, diminished contamination and occasional human errors, special instrumentation is still required for result interpretation. Additionally, these platforms have low sensitivities (2×10^4 and 9×10^3 copies mL^{-1}, for Abbott and Luminex systems, respectively) compared to conventional RT-qPCR [46]. The herein proposed RNA-based platform is also much more sensitive than the rapid antigen-based lateral flow assays aiming for community and point-of-care testing, whose positive results normally require confirmation from nucleic acid amplification test(s) [9, 47].

The cost of our device is currently ca. $10 (small scale device fabrication = $1.8, reagents = $8.3; the FDA EUA approved Abbott BinaxNOW™ COVID-19 Ag Card rapid test costs $5). This estimation excluded the cost of a block heater and NdFeB magnet assembly as they can be reused. This figure is anticipated to be substantially reduced by mass production, i.e., using injection moulding process to replace the CNC-machined fabrication.

4. Conclusion

We have devised a low complexity, high sensitivity and specificity lab-on-a-chip platform based on IFAST RT-LAMP for SARS-CoV-2 RNA detection, integrating two consecutive steps of RNA extraction and amplification into a single device. The current setup allowed detection of as little as 470 copies of genomic RNA within 1 h. This platform has the potential to increase COVID-19 screening speed and expand testing capacity for disease surveillance as well as point-of-care testing in resource-limited settings, enabling timely isolation prior to unwitting viral transmission.

CRediT authorship contribution statement

Pablo Rodriguez-Mateos & Bongkot Ngamsom: Conceptualisation, Validation, Investigation, Formal Analysis, Visualisation, Writing – Original Draft – Review & Editing. **Cheryl Walter:** Conceptualisation, Supervision, Writing – Review & Editing. **Charlotte E. Dyer:** Conceptualisation, Funding acquisition, Supervision, Writing – Review & Editing. **Jesse Gitaka:** Conceptualisation, Funding acquisition, Writing – Review & Editing. **Alexander Iles:** Conceptualisation, Funding acquisition, Supervision, Writing – Review & Editing. **Nicole Pamme:** Conceptualisation, Funding acquisition, Supervision, Writing – Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Appendix A. Supplementary Data

Details for RT-LAMP primers for SARS-CoV-2, HCoV-OC43 and H1N1 RNAs. Washing efficiency of the microfluidic devices. On-chip IFAST RT-LAMP using PDMS and PMMA devices. Immiscible interfaces inside PMMA devices during amplification. Video demonstration of IFAST RT-LAMP workflow.

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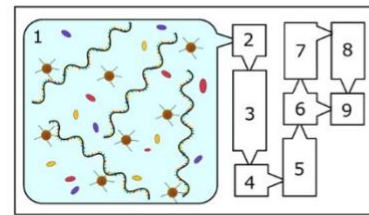
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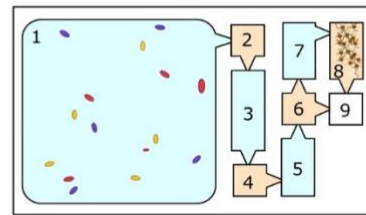[47]   CDC Coronavirus Antigen Test Guidelines, 2020.

Figure legend

Fig. 1 Conceptual scheme for the microfluidic IFAST RT-LAMP device for SARS-CoV-2 RNA detection comprising three consecutive steps: (I) RNA extraction via oligo (dT)-functionalised magnetic beads; (II) separation and purification of magnetic bead-captured RNA through a series of immiscible liquids; and (III) colorimetric RT-LAMP for detection of extracted RNA. Visible change of colour from pink to yellow indicates SARS-CoV-2 positive.

Fig. 2 (a) Design of the IFAST RT-LAMP device featuring a sample chamber (1) interconnected to wash chambers (2 – 8) and detection chamber (9) via gates. (b) Photograph of an IFAST RT-LAMP microfluidic device for SARS-CoV-2 RNA detection.

Fig. 3 Tube-based and on-chip RT-LAMP assays for detection of genomic SARS-CoV-2 RNA using primers targeting ORF1a and N genes: (-) = no template control; (1), (2) and (3) are 47, 470 and 4.7×10^3 RNA copies, respectively. Reactions performed at 65 °C and photographs taken at 30 min for ORF1a primers and 40 min for Gene N primers.

Fig. 4 Specificity investigations: (a) Tube-based RT-LAMP assays of HCoV-OC43 and H1N1 RNAs with their respective primers. (b) RT-LAMP assays of SARS-CoV-2, HCoV-OC43 and H1N1 RNAs using ORF1a and Gene N primers. (c) RT-LAMP assays using Gene N primers: 1 = no template control, 2 = HCoV-OC43 + H1N1 RNAs, and 3 = SARS-CoV2 + HCoV-OC43 + H1N1 RNAs. All assays performed at 65 °C for 30 min; HCoV-OC43 = 0.005 ng, H1N1 = 0.0289 ng, and SARS-CoV-2 = 0.095 ng (n=3).

Fig. 5 On-chip extraction of SARS-CoV-2 RNA via IFAST, followed by tube-based RT-LAMP. The process involved mixing (by gentle agitation for 10 min) of oligo (dT)-coated magnetic beads with RNA sample (470 RNA copies mL^{-1}) in the sample chamber of the device and separating the extracted RNA through wash chambers (n=2). Amplification of the bead-bound isolated RNA was conducted via tube-based RT-LAMP with Gene N primers at 65 °C for 40, and compared with control RT-LAMP where RNA was directly added into the reaction mix. Each RNA extraction was compared with a no template control (NTC) sample where functionalised magnetic beads were incubated with aqueous solution containing no RNA. (-) = NTC, (+) = magnetic bead-isolated RNA from on-chip RNA extraction experiments.

Fig. 6 Proof-of-concept investigations of on-chip integrated steps of RNA extraction via IFAST and RT-LAMP for detection of SARS-CoV-2 RNA. (a) Two independent experiments of integrated on-chip steps for extraction and detection of genomic SARS-CoV-2 from spiked water; (-) = no template control, (+) = 470 RNA copies mL^{-1} (10 min extraction, 65 °C RT-LAMP with Gene N primers and 40 min amplification). (b) The IFAST RT-LAMP platform implemented for detection of SARS-CoV-2 from artificial sputum spiked with genomic RNAs diluted in 5 M GuHCl (aq); (-) = sample spiked with HCoV-OC43 and H1N1 RNAs, (+) = sample spiked with SARS-CoV-2, HCoV-OC43 and H1N1 RNAs (10 min extraction, 65 °C RT-LAMP with Gene N primers and 40 min amplification).
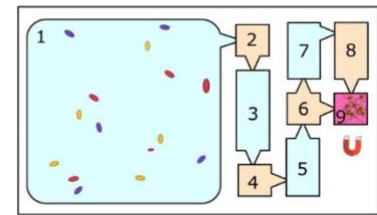
Fig. 1

a)

2 7 8

1

3 6 9

gate

4 5

3 mm

b)

3 mm

Fig. 2

Fig. 3

Fig. 4

I. RNA capture

II. RNA separation

III. Off-chip detection (RT-LAMP)

LAMP mix
65 °C

On-chip extracted RNA

Control

Control

Replicate 1

Replicate 2

On-chip extracted RNA

Replicate 1

Replicate 2

L   (−)   (+)

500 bp

Fig. 5

Fig. 6

I. RNA capture

II. RNA separation

III. Detection via RT-LAMP

LAMP mix
65 °C

- Oligo (dT) magnetic bead
- SARS-CoV-2 RNA
- Contaminants

- Aqueous phase
- Oil phase
- Magnet

- ☐ → ☐ SARS-CoV-2 negative
- ☐ → ☐ SARS-CoV-2 positive

10 min   1 min   30-40 min

Graphical Abstract

Supplementary Data

A lab-on-a-chip platform for integrated extraction and detection of SARS-CoV-2 RNA in resource-limited settings

Pablo Rodriguez-Mateos,[a] Bongkot Ngamsom,[a] Cheryl Walter,[b] Charlotte E. Dyer,[b] Jesse Gitaka,[c] Alexander Iles,[a] Nicole Pamme[*a]

[a]Department of Chemistry and Biochemistry, University of Hull, UK

[b]Department of Biomedical Sciences, University of Hull, UK

[c]Mount Kenya University, Thika, KENYA

*Corresponding author: **Nicole Pamme**
Department of Chemistry, Faculty of Science and Engineering, University of Hull,
Cottingham Road, Hull, East Riding of Yorkshire, HU6 7RX, UK.
E-mail: n.pamme@hull.ac.uk

Section A: RT-LAMP primers

Table S1. LAMP primers for SARS-CoV-2 detection [1].

Name	Sequence (5' to 3')
ORF1a-C-B3	GATCAGTGCCAAGCTCGTC
ORF1a-C-F3	CTGCACCTCATGGTCATGTT
ORF1a-C-FIP	GAGGGACAAGGACACCAAGTGTGGTAGCAGAACTCGAAG GC
ORF1a-C-BIP	CCAGTGGCTTACCGCAAGGTTTTAGATCGGCGCCGTAAC
ORF1a-C-LF	ACCACTACGACCGTACTGAAT
ORF1a-C-LB	GCTCCTTTATTACCGTTCTTACGAA
GeneN-A-F3	TGGCTACTACCGAAGAGCT
GeneN-A-B3	TGCAGCATTGTTAGCAGGAT
GeneN-A-FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG
GeneN-A-BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT
GeneN-A-LF	GGACTGAGATCTTTCATTTTACCGT
GeneN-A-LB	TGTATTCAAGGCTCCCTCAGT

Table S2. LAMP primers for HCoV-OC43 detection.[2]

Name	Sequence (5' to 3')
ORF-1b Rep-F3	AATATGTTTATCACCCGCG
ORF-1b Rep-B3	CTCTGGTGAATTCTGTGTT
ORF-1b Rep-Loop F	CAAAGCCAATCCACGCA
ORF-1b Rep-Loop B	CCAGCTAGGATTTTCTACAGG
ORF-1b Rep-F1 c	GTTGCATGACAGCCCTCTAC
ORF-1b Rep-B1 c	GCTGTGGGTACTAACCTACCT

Table S3. LAMP primers for H1N1 detection.[3]

Name	Sequence (5' to 3')
H1-F3	AGCAAGAAGTTCAAGCCG
H1-B3	CGTGAACTGGTGTATCTGAA
H1-FIP	GGCCTACTAGTGTCCAGTAATAGTAAATAGCAATAAGACCCAAAGTG
H1-BIP	ATAACATTCGAAGCAACTGGAAATCTGATAATACCAGATCCAGCATT
H1-LF	TCTCCCTTCTTGATCCC
H1-LB	TAGTGGTACCGAGATATGCA

Section B: Washing efficiency 9-chamber design vs 5-chamber design

The new IFAST chip (9-chamber design) was designed to have extra wash chambers (Figure S1a). This design was compared its washing performance with the previously reported 5-chamber design used in the IFAST/ATP assay for detection of Group B *streptococcus* (GBS) from urine samples [4]. Here, GBS (10^4 CFU mL^{-1}) was extracted using anti-GBS magnetic beads and purified through wash chambers of the two chip designs. The supernatant of the last chamber were plated after removing the GBS-captured magnetic beads. Much fewer number of colonies was observed from supernatant from the new IFAST chips, suggesting that the more efficient washing was achieved in the new 9-chamber design (Figure S1).
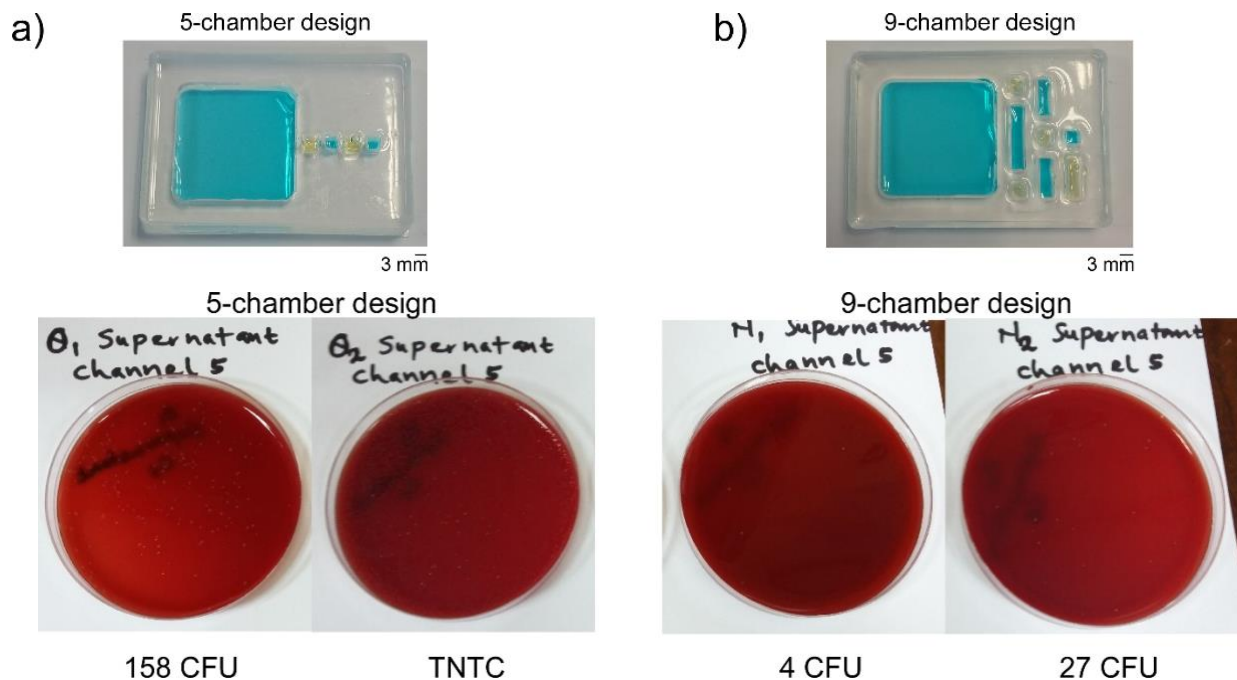

Figure S1. Group B *streptococcus* colonies on blood agar (×2) plated from supernatant from the last chambers of (a) 5-chamber design and (b) 9-chamber design. TNTC = Too numerous to count.

Section C: PDMS and PMMA devices for on-chip IFAST RT-LAMP

The microfluidic devices for on-chip IFAST and RT-LAMP were firstly fabricated in Polydimethoxysilane (PDMS). However, the RT-LAMP reaction usually completed over a period of 60 min to observe change of colour from pink to yellow in positive samples. The next generation IFAST RT-LAMP chips were therefore fabricated from polymethyl methacrylate (PMMA), which not only demonstrate faster amplifications (Figure S2), but are also compatible with mass manufacturing process of injection moulding.
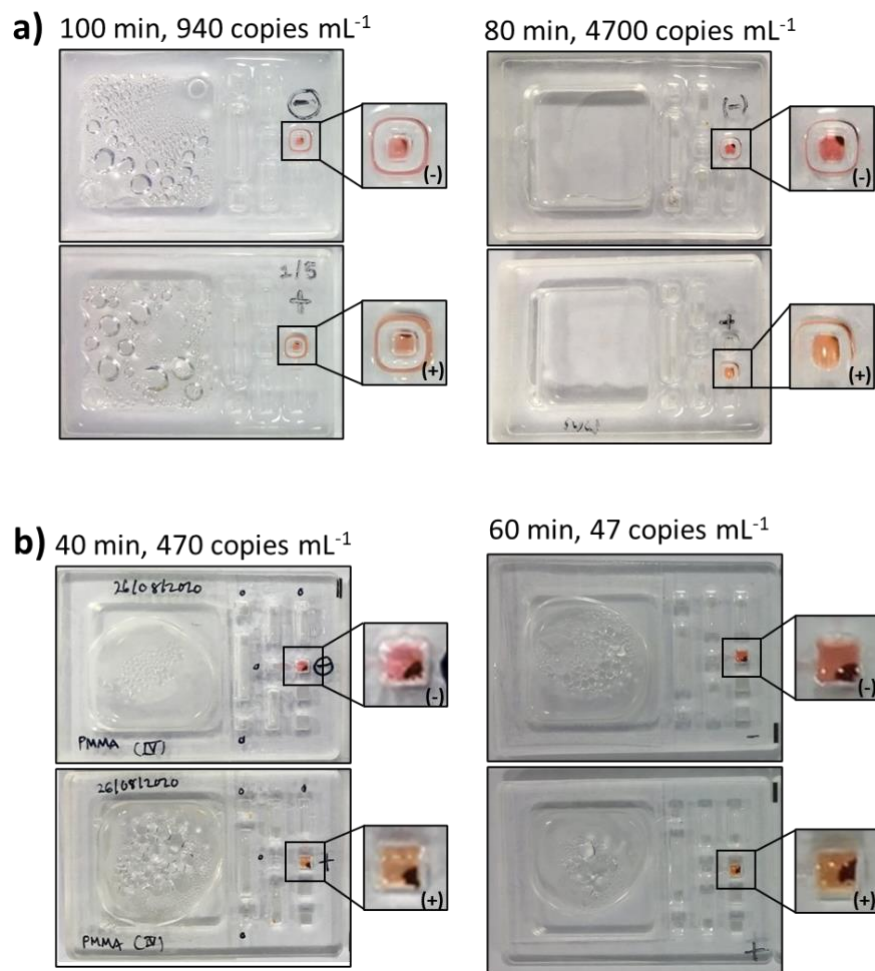

Figure S2. On-chip IFAST RT-LAMP performed using (a) PDMS and (b) PMMA devices. (-) = Negative control, (+) = samples containing genomic SARS-CoV-2 RNA; reactions performed using Gene N primers.

Section D: Immiscible interfaces inside PMMA devices during amplification

Immiscible interfaces between mineral oil and RT-LAMP substrate in the PMMA device subjected to heating at at 65 °C were assessed under a microscope over a period of 50 min (Figure S3).
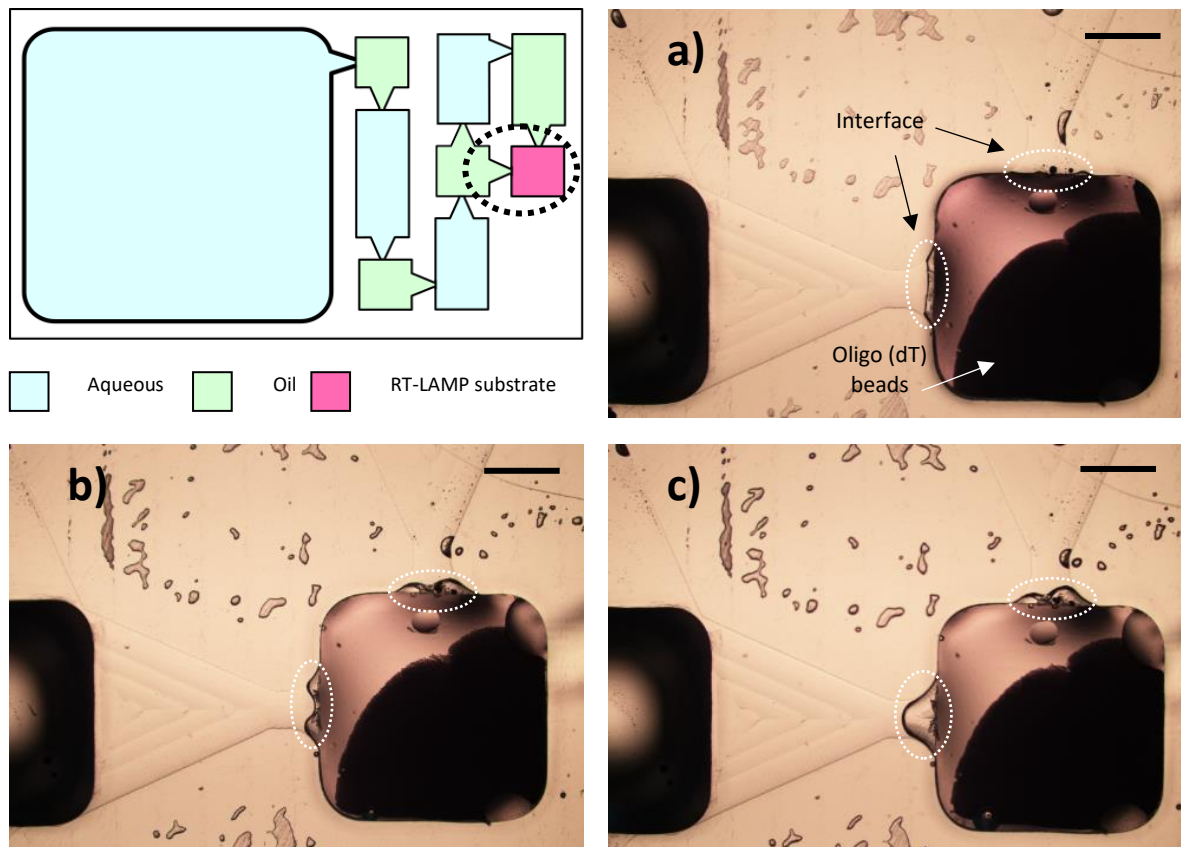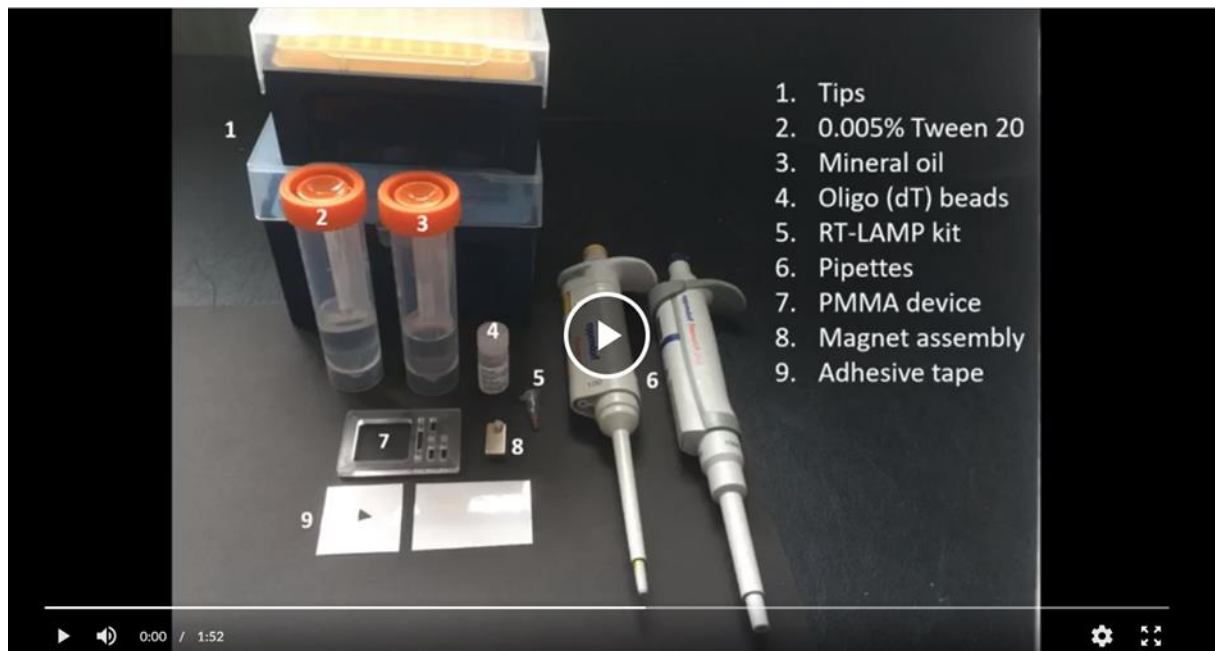

Figure S3. Microscopic images taken from the last chamber and the adjacent gates of the PMMA device: (a) 0 min, (b) 20 min and (c) 50 min after subjected to heating at 65 °C. Scale bar = 1 mm.

Section E: Video demonstration of IFAST RT-LAMP workflow

1. Tips
2. 0.005% Tween 20
3. Mineral oil
4. Oligo (dT) beads
5. RT-LAMP kit
6. Pipettes
7. PMMA device
8. Magnet assembly
9. Adhesive tape

Video demonstration of IFAST RT-LAMP workflow: 2× real speed.