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SARS-CoV-2 viral RNA detection using the novel CoVradar device associated with the CoVreader smartphone app

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ABSTRACT

The COVID-19 pandemic has highlighted the need for innovative approaches to its diagnosis. Here we present CoVradar, a novel and simple colorimetric method that combines nucleic acid analysis with dynamic chemical labeling (DCL) technology and the Spin-Tube device to detect SARS-CoV-2 RNA in saliva samples. The assay includes a fragmentation step to increase the number of RNA templates for analysis, using abasic peptide nucleic acid probes (DGL probes) immobilized to nylon membranes in a specific dot pattern to capture RNA fragments. Duplexes are formed by labeling complementary RNA fragments with biotinylated SMART bases, which act as templates for DCL. Signals are generated by recognizing biotin with streptavidin alkaline phosphatase and incubating with a chromogenic substrate to produce a blue precipitate. CoVradar results are analysed by CoVreader, a smartphone-based image processing system that can display and interpret the blotch pattern. CoVradar and CoVreader provide a unique molecular assay capable of detecting SARS-CoV-2 viral RNA without the need for extraction, preamplification, or pre-labeling steps, offering advantages in terms of time (~3 h/test), cost (~€1/test manufacturing cost) and simplicity (does not require large equipment). This solution is also promising for developing assays for other infectious diseases.

1. Introduction

Over the course of the COVID-19 crisis, the importance of reliable, accessible testing to screen for the disease has become increasingly apparent. Depending on the target detected, there are three main types of tests relevant for COVID-19 detection: a) nucleic acid tests, based on reverse transcription polymerase chain reaction (RT-qPCR), allowing to detect and analyze of the viral genome of SARS-CoV-2 (Jiang et al., 2021; Tombuloglu et al., 2022); b) lateral flow antigen tests detect the presence of a viral antigen (surface proteins) on SARS-CoV-2 (Aguilar-Shea et al., 2021; Pekosz et al., 2021); c) antibody tests by serological methods that detect antibodies generated against SARS-CoV-2 (James

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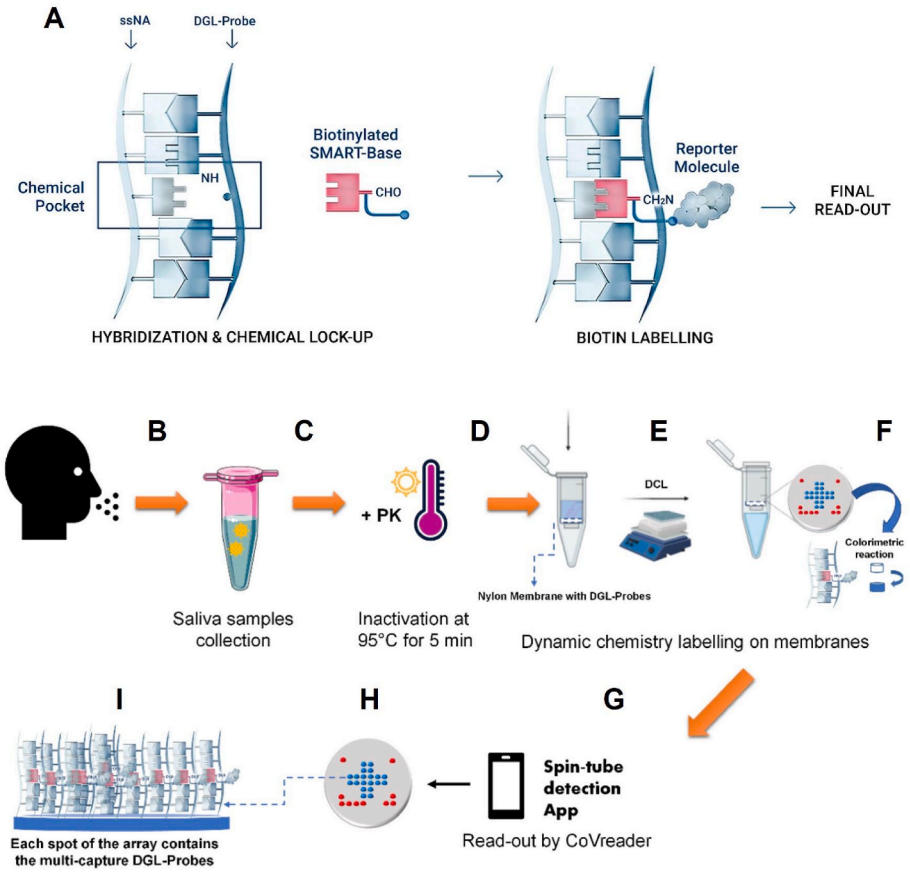

Fig. 1. (A) Dynamic Chemical Labelling workflow: DGL-Probe captures the complementary single-strand nucleic acid (ssNA) forming the Chemical Pocket. When the target ssNA hybridisation is complete, the biotinylated SMART-Base is covalently attached to the backbone of the DGL-Probe (Chemical Lock-up). The duplex is read using a Reporter Molecule that recognizes the biotin tag. (B–I) ***Schematic diagram of CoVradar assay***. *(B) Saliva samples collection in plastic tubes. (C) Addition of proteinase K, inactivation of Proteinase K at 95°C for* 5 min *(fragmentation). (D) Addition to CoVradar detection system. (E) Nucleic acid analysis by DCL technology. (F) Detection by a colourimetric reaction (blue precipitate) in spots. (G) Read-out by CoVreader. (H) Defined array layout. (I) schematic representation of the multi-capture DGL-probes spots included in the nylon membranes of the CoVradar detection system. DCL: Dynamic Chemistry Labelling; PK: Proteinase K.*

et al., 2021). Antibodies can persist in the blood for many months, but there is insufficient evidence to know how long they last. Therefore, antibody testing is not recommended to assess immunity to SARS-CoV-2 after COVID-19 vaccination or to assess the need for immunisation in an unvaccinated person.

RT-qPCR is considered the gold standard for COVID-19 testing as it provides a more definitive answer as to whether an individual has the virus in their body, and genomic sequencing has become vital for tracking variants (Budd et al., 2023). However, although RT-qPCR is a precious test, its demanding high complexity requires skilled operators working in properly equipped laboratories with significant lag times to provide a result (>24 h), and therefore, it is not suitable for its deployment and use in many primary care centres (Yüce et al., 2021). RT-qPCR faces a range of significant limitations, including: i) RNA extraction variability, with loss of target RNA; ii) RNA instability with variable degradation of viral RNA, resulting in false negatives; iii) Longish time to obtain results (Parupudi et al., 2021) and (iv) contamination due to high level of amplicons found within labs established to run RT-qPCR assays in large numbers per day.

Among the molecular technologies commonly used, RNA analysis by Dynamic Chemistry Labelling (DCL) is one of the most promising and game-changing for nucleic acid testing. DCL technology is based on combining biotinylated aldehyde-modified nucleobases (SMART-Bases) with modified PNA capture probes synthesised with an abasic position (DGL-Probes) to interrogate single-strand nucleic acid (ssNA). As shown in Fig. 1A, DCL uses a single biotinylated SMART-Base to label the duplex formation with the ssNA sequence. The DGL-Probe captures the complementary ssNA sequence, forming the Chemical Pocket (Fig. 1A). When the target ssNA hybridization is complete, the biotinylated SMART-Base is covalently attached to the backbone of the DGL-Probe Chemical Lock-up (Fig. 1A). The duplex is read using a Reporter Molecule that recognizes the biotin tag. DCL technology has been successfully demonstrated in various studies (Bowler et al., 2010; Pernagallo et al., 2012; Venkateswaran et al., 2016; Luque-Gonzalez et al., 2018; Delgado-Gonzalez et al., 2019; Robles-Remacho et al., 2023) (Marin-Romero et al., 2018; Detassis et al., 2019).

In this work, our group has tackled the need for novel nucleic acid testing solutions to interrogate SARS-CoV-2 viral RNA by developing the **"CoVradar"** assay (Fig. 1B–I). The methodology behind CoVradar lies in implementing DCL technology with the Spin-Tube device, a simple micro-tube with a nylon membrane, where the principle of reverse-dot blot hybridization can be performed upon DCL reaction to give rise to a colourimetric end-point assay for nucleic acid testing. Our group first designed, constructed, and used the Spin-Tube device to detect and identify trypanosomatid parasites (Tabraue-Chávez et al., 2019). More recently, the Spin-Tube was also the platform chosen by the multi-million-euro EU-funded ARREST-TB project to deliver accurate, low-cost, portable and easy-to-use tests for the detection of tuberculosis (Grant agreement ID: 825,931) (Smirnova et al., 2020).

Within the framework of this work, we have also developed "CoVreader", a dedicated portable Image Processing System (IPS) for interpreting CoVradar's results (Fig. 1G). CoVreader is an implementation of our previous smartphone-based system for Spin-Tube devices (Escobedo et al., 2019). It is the combination of hardware and software elements which enables a digital image processing of CoVradar results by a mobile phone. CoVreader allows the acquisition, process and transmission of the images coming from the CoVradar to reference clinicians in remote locations for advice and treatment decisions with the vision of removing the need for centralised facilities.

2. Material and methods

2.1. Materials and reagents

All chemicals were obtained from Sigma Aldrich and used as received. SCD buffer was prepared from 2x saline sodium citrate (SSC) and 0.1% sodium dodecyl sulphate (SDS) with the pH adjusted to 6.0 using HCl. All synthetic DNA oligomers (desalted) were purchased from Integrated DNA Technologies, BV (Leuven, Belgium). Abasic PNA probes (DGL-probes) were provided by DESTINA Genomica SL (Granada, Spain), HPLC purified. The concentration of DGL-probes was determined using the FLUOstar Omega multidetector microplate reader from BMG LABTECH (Offenburg, Germany) using extinction coefficient values (Ɛ260) either 6.6, 8.8, 13.7 and 11.7 (mM−1 cm−1) for C, T, A, G, respectively. As reported elsewhere, SMART-C-Biotin was prepared by DESTINA Genomica SL (Granada, Spain) (Bowler et al., 2010). Buffers and dilution reagents I to III were provided by DESTINA Genomica SL (Granada, Spain) and buffers IV to VI were purchased from Vitro SA. (Granada, Spain). Biodyne C Membranes were purchased from Pall Corporation (US). Membranes were preactivated by DESTINA Genomica SL (Granada, Spain) using proprietary chemistry. Spin-Tube plastic elements were purchased from Ciro Manufacturing Corporation (FL, US). Spin-Tube devices were manually assembled by DESTINA Genomica SL (Granada, Spain).

2.2. DGL-probes to target SARS-CoV-2 genomic RNA

Fifteen DGL-probes were synthesised with amino-PEGylated groups at their N-terminal end using Fmoc/Bhoc solid phase chemistry and purified by reverse HPLC. DGL-probes presented in certain positions units containing propanoic acid residues at their gamma positions (Venkateswaran et al., 2016; Rissin et al., 2017). DGL-probes' design was made to target different regions of SARS-CoV-2 genomic RNA sequence published by the WHO and available at GenBank: MN908947. Targeted regions were determined considering optimal hybridization matching stability of DGL-Probe versus complementary sequences (see Table S1 in supplementary data).

N-amino-pegylated DGL-Probes were covalently immobilized onto the preactivated Biodyne C nylon membranes as previously described by Tabraue-Chávez et al. (2019). DGL-Probes were solubilised in $NaHCO_3$ buffer (pH 8.3, 0.125M) with amaranth dye (0.2025 mg/mL) and DMSO (30%) as additives and dispensed by solution into the preactivated nylon membranes either by (a) manual spotting with a P2 Gilson Pipette or (b) an automatic immobilisation with a piezoelectric dispenser (sciFlexarrayer) by SCIENION AG (Berlin, Germany).

2.3. In vitro generation of ssRNA templates for the assay development

A library of five single-stranded RNA (or ssRNA) fragments of different sizes (0.9–2.2 Kb) containing the complementary regions of the ten selected DGL-probes was generated *in vitro*. The library was created from viral genomic RNA extracted from a COVID-19 positive patient saliva sample. Twelve saliva samples from healthy adult subjects were used as controls for spiking-in experiments. Saliva samples were provided by the Biobank of the Public Health System of Andalusia (reference S2000262). Informed consent was given by all the individuals enrolled in this study. The library generation is described in supplementary data "C. A library of five single-stranded RNA".

2.4. Assay validation

2.4.1. Dynamic chemistry labelling (DCL) with SMART-C-biotin

1. The reaction mixture (final volume of 200 μL) was prepared by combining: i) 20 μL of template in water or saliva (either ssDNA or ssRNA) - only water or saliva with water for negative controls; ii) 5 μL of SMART-C-Biotin (200 μM); iii) 10 μL of sodium cyanoborohydride ($NaBH_3CN$) - 20 mM in water; and (iv) 165 μL of SCD buffer.

2. The protocol carried out consisted of a) Blocking of nylon membranes containing DGL-Probes with 400 μL of reagent I; b) Washing of membranes with 600 μL of reagent II; c) Incubation of the membranes for 2 min at 45 °C with 200 μL of SCD buffer; d) Incubation of the reaction mixture (from point 1) with the membranes (or CoVradar) at 45 °C for 60 min to perform the dynamic chemistry labelling (DCL); e) Washing of the membranes using 600 μL of reagent II (pre-heated at 45 °C); f) A blocking step in which membranes were incubated with 200 μL of reagent III for 5 min at room temperature (RT); g) Incubation of the membranes with 200 μL of reagent IV at RT for 5 min; h) One post-enzymatic reaction washing step with 600 μL of reagent V; i) Incubation with 200 μL of the chromogenic substrate (reagent VI) at 45 °C for 10 min; j) Finally, one post-chromogen washing step with 200 μL of reagent II. In all steps, the Spin-Tubes were centrifuged at 6000 rpm for 15 s to discard the solutions between each step.

2.4.2. Spike-in studies

Saliva samples from healthy adult subjects were treated with Proteinase K (Sigma Aldrich) to a final concentration of 0.5 mg/mL, 1 mg/mL and 2 mg/mL, vortex for 1 min at 3000–5000 RPM and heated at 95 °C for 5 min to inactivate the proteinase K (untreated saliva sample was also included). Samples were spiked with the complementary DNA oligomer (ssDNA) mix at a ratio of 1:10 in saliva and were kept on ice until the moment of adding them to the reaction to a final concentration of 100pM. In addition, different concentrations of spike-in ssDNA were studied (1 nM, 100pM, 50pM, 25pM, 10pM and 1pM) using the optimized Proteinase K concentration of 1 mg/mL at the same experimental conditions. The same process was carried out for spiking the ssRNA mix produced by *in vitro* transcription but with a fragmentation step of ssRNA spiked samples ahead of the incubation. For the fragmentation process, the ssRNA mix was incubated at 95 °C for either 5 min, 15 min or 30 min and analysed by capillary electrophoresis using the Agilent 2100 Bioanalyzer and the Agilent RNA 6000 Nano Kit (Agilent Technologies). Moreover, the samples spiked with the ssRNA mix were also incubated at 95 °C for either 5 min, 15 min or 30 min and analysed by DCL on membranes with colourimetric readout. The DCL was carried out using saliva samples spiked with the ssRNA fragments of the produced library mixed at a final concentration of 400 pM.

2.5. Design of CoVreader's hardware and software

Our group fabricated a custom-designed accessory to accommodate the CoVradar device by combining a commercial camera lens clip-on plus a 3D-printed holder manufactured by our group. The lens coupled to the clip-on accessory is a macro lens of 12x with a wide lens of 0.67x. The 3D holder was designed using the open-source software Wings 3D v2.2.9 and fabricated with a 3D printer Creality3D model CR-X using black PLA filament. The 3D holder is integrated with a small Printed Circuit Board (PCB) with four white LEDs (NSDL570GS-K1, Nichia Corporation, Tokushima, Japan). Android Studio Artic Fox 2020.3.1 Patch 3 was the Integrated Development Environment (IDE) used to program the mobile phone application. Three different smartphones were used to test the developed application so that it was verified running on devices with different Android versions and distinct camera specifications: a Samsung Galaxy S7, a Xiaomi Mi6, and a OnePlus 8 mobile phone. More information can be found in the supplementary data.

3. Results

3.1. Design and synthesis of DGL-probes

In an antiparallel fashion, we designed and synthesised fifteen DGL-Probes with sequences complementary to SARS-CoV-2 RNA viral genome (N-terminal end of the DGL-Probes face 3′-end of the target RNA

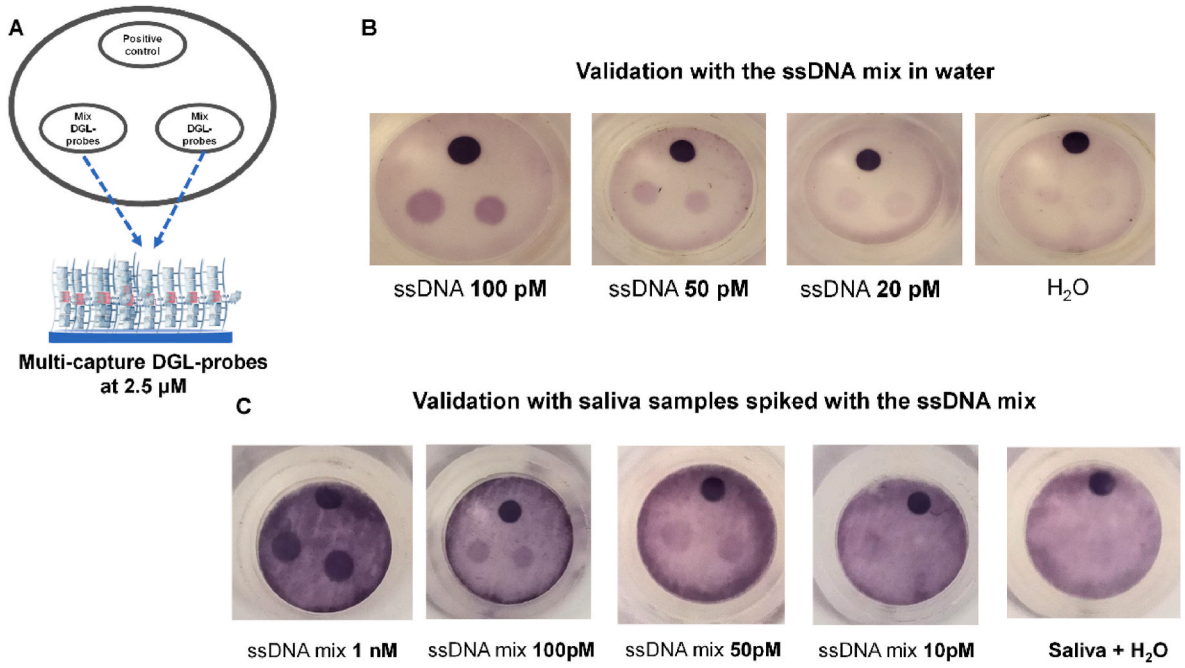

Fig. 2. Multi-capture DGL-probes study. (A) Spotting layout: arrays with two side spots with the ten multi-capture DGL-Probes spotted at 2.5 µM concentration and a middle spot with the solution of biotin-labelled DNA oligomers to control the colourimetric reaction. (B) Sensitivity in water: three decreasing concentrations of ssDNA in water, respectively 100 pM, 50 pM and 20 pM. Only H_2O as negative control. (C) Sensitivity in saliva: four decreasing concentrations of ssDNA in saliva, respectively 1 nM, 100 pM, 50 pM and 10 pM. Only saliva with H_2O as negative control.

and the C-terminal the 5'end). The fifteen DGL-Probes targeted SARS-CoV-2 regions are listed in the supplementary data (Table S1). The performance of the produced fifteen DGL-probes was initially checked on nylon membranes by dynamic chemistry labelling (DCL) with the cytosine SMART-Base (SMART-C-Biotin) incorporation followed by a colourimetric read-out (data not shown). The ten best-performant DGL-probes (indicated with a YES in Table S1 and Fig. S1) were selected for the subsequent experimental validation (section 3.2).

To create CoVradar, DGL-Probes, were covalently immobilized onto nylon membranes of the Spin-Tube device following specific spot patterns. For this implementation, several DGL-Probes were designed to hybridize multi regions of SARS-CoV-2 viral genomic RNA. The assay uses a saliva specimen's pre-treatment to isolate and fragment the viral RNA (Fig. 1B–C). The solution with RNA fragments is added into the Spin-Tube device and interrogated with DCL reaction (Fig. 1D–E). DGL-Probes hybridize with complementary RNA fragments, the duplexes are formed and DCL reaction takes place between the secondary amine of the DGL-probe and the aldehyde group of SMART-Base which is covalently linked to the DGL-Probe (Marin-Romero et al., 2020; Marín-Romero et al., 2021: Marín-Romero et al., 2022). Remarkably, CoVradar demonstrates high specificity. The DCL technology requires two specific molecular events for a signal generation: (i) perfect hybridisation between RNA fragment and DGL-Probe, and (ii) specific molecular recognition through Watson–Crick base-pairing rules between the cytosine SMART Nucleobase and the complementary guanine on the RNA fragment template. Therefore, in the absence of the targeted RNA fragment or if the RNA fragment lacks guanine on the Chemical Pocket, the DCL reaction does not occur (López-Longarela et al., 2020; Garcia-Fernandez et al., 2019; Rissin et al., 2017).

3.2. Multi-capture DGL-Probes to boost the colourimetric signal

The ten selected DGL-Probes were mixed into a single solution and spotted together (multi-capture DGL-probes spot) manually, as indicated in section 2.2. As shown in Fig. S2, arrays containing spots with individual DGL-Probes and multi-capture DGL-Probes at 2.5 µM and 10 µM were prepared. The study was carried out following the protocol described in section 2.4.1. The performances of multi-capture DGL-probes spots were compared with individual DGL-Probe spots within the same concentrations (see Fig. S2). It was observed in all membranes tested that the signal obtained with the multi-capture DGL-probes spots showed a greater intensity than spots with individual DGL-Probes. A concentration of 2.5 µM multi-capture DGL-Probes spots was selected based on its best signal-to-background ratio.

3.3. Signal intensity study of multi-capture DGL-Probes

The array layout for studying the assay's sensitivity is shown in Fig. 2A. As shown in Fig. 2B, within the two multi-capture DGL-Probes spots, signal intensities lowered while the ssDNA mix concentration decreased. A substantial difference was observed for multi-capture DGL-probes spots in which 100 pM and 50 pM of ssDNA mixtures were used as templates, whereas the signal for 20 pM was pretty similar to the background (signal with only water). Signal intensity was also challenged by spiking complementary ssDNAs in saliva samples. Saliva samples from healthy individuals were treated with Proteinase K (Fig. S3) and spiked with four decreasing concentrations of ssDNA oligomer mixes, respectively 1 nM, 100 pM, 50 pM and 10 pM. As shown in Fig. 2C, a progressive reduction of the colourimetric signals was observed as a coherent decrease in target ssDNA concentrations. This indicates that the complex matrix (saliva) is not impacting the DCL assay or the platform's sensitivity but improving it since the background signals (only saliva) are almost absent.

3.4. CoVradar device fabrication

The novel CoVradar tool was created by implementing the Spin-Tube device previously developed by our group (Tabraue-Chávez et al., 2019) with a new set of reagents specific for interrogating the SARS-CoV-2 viral genome. As shown in Fig. 3A, it consists of: i) a centrifuge collection tube; ii) an internal column for the assay; iii) a frit; and iv) a nylon membrane, pre-spotted with DGL-Probes, onto the bottom of the column

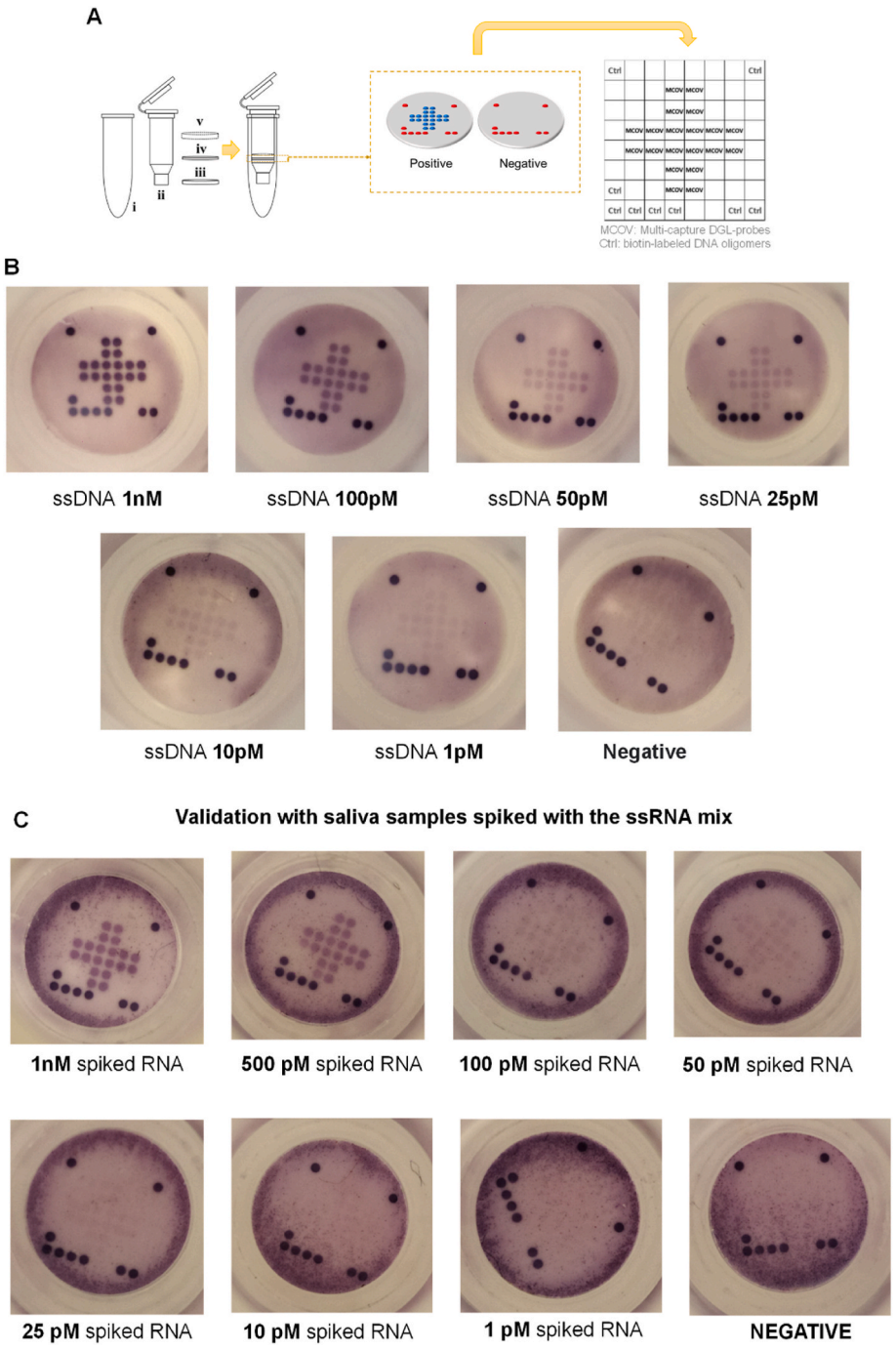

Fig. 3. Implementation and validation of CoVradar assay. (A) The Spin-Tube device structure: i) a centrifuge collection tube; ii) an internal column for the assay; iii) a frit; iv) a nylon membrane (pre-spotted with abasic PNA probes) immobilized onto the bottom of the column via a plastic pressure ring (v). (B) Validation of the assay with saliva samples spiked with a mix of ssDNA templates. (C) Validation of the assay with saliva samples spiked with a mix of ssRNA templates.

via a plastic pressure ring (v). Multi-capture DGL-Probes were printed with an automatic dispenser on the nylon membrane to create an array (Fig. 3A). The array consists of two perpendicular rows of twelve dots each (containing the multi-capture DGL-Probes). In addition, biotin-labelled DNA oligomers were printed at the four corners of the array (nine dots) to control the array's orientation and the colourimetric reaction.

3.5. CoVradar assay validation

The validation step with the ssDNA mix spiked in saliva samples provided similar results compared to the results obtained with the manual spotting of the multi-capture DGL-probes solution (section 3.3). We also observed a slight signal lowering as the ssDNA mix amounts decreased (Fig. 3B). In addition, CoVradar was validated using ssRNA templates generated in-house from RNA extracted from COVID-19 positive patients (see supplementary data). CoVradar performance for ssRNA detection was validated with and without a fragmentation step. Results in Fig. S5 indicated that the fragmentation of the ssRNA mix samples ahead of the incubation in CoVradar provided the highest intensity signal after the DCL and colourimetric readout (Fig. S5). Furthermore, different concentrations of the ssRNA fragments mix ranging from 1 nM to 1 pM were spiked into saliva samples to check the sensitivity of CoVradar. The effect observed was similar to the

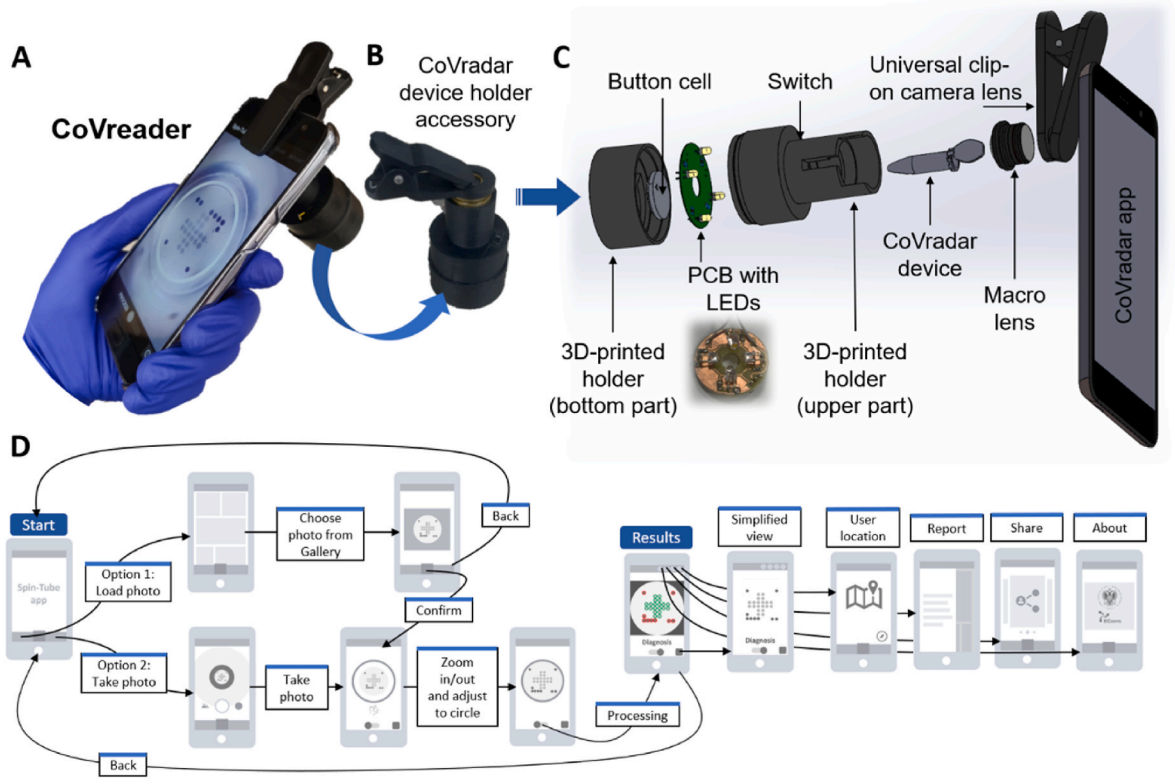

Fig. 4. CoVreader for the reading of CoVradar. (A) Smartphone with a clip-on accessory. (B) Photograph of the complete CoVradar holder accessory. (C) Deployed view of the hardware platform with all components. (D) Software with application user flow showing the user's path through the smartphone app interface for the acquisition and processing of CoVradar.

experimental validation with the saliva samples spiked with the ssDNA mix (Fig. 3C). As the ssRNA mix amounts decreased, we observed a lowering of the signal provided after the colourimetric readout. However, signal intensities were slightly lower in the ssRNA mix spiked samples than in ssDNA mix spiked samples.

3.6. The CoVradar data processing system

For accurate data collection and analysis of CoVradar results, we have developed a dedicated system of image capture and processing called CoVreader. CoVreader is made of hardware and software parts. The hardware part consists of two components: *i)* the mobile phone for acquiring the image using the rear phone camera (Fig. 4A), and *ii)* a custom-designed accessory to accommodate the CoVradar device aligned with the mobile phone camera (Fig. 4B). This accessory consists of a camera lens clip-on plus a 3D-printed holder where the CoVradar is inserted and integrated with the clip-on (Fig. 4C). Combined with the lens clip-on, the 3D-printed holder ensures correct alignment and positioning of the mobile phone with the CoVradar. The 3D-printed holder contains four white LEDs to illuminate the CoVradar (Fig. 4C). While the opaque plastic accessory is useful to block ambient light, the LEDs are used to provide constant illumination so that the platform can be used regardless of the external lighting conditions. The software consists of a 'user-friendly' mobile phone app. It has several screens that guide the user through the steps required to acquire and process a test result image. The whole process is depicted in the application user flow of Fig. 4D. In the first place; there is a welcome screen with a menu with two options: *a)* Option 1: Load a picture from the gallery; *b)* Option 2: Take a picture. If the latter option is chosen, the application starts the device's camera so that the user can photograph the CoVradar device. To obtain correct processing, the user needs to position the CoVradar array properly, with the reference points aligned vertically/horizontally, in either of the four possible positions that meet this requirement. Image processing has been implemented and optimized based on multiple CoVradar and samples simulating different conditions regarding signal intensity, positioning, and background noise.

4. Discussion

Our research group has developed CoVradar, a diagnostic test for SARS-CoV-2 viral RNA detection. The critical drivers to create CoVradar have been the need for test speed, ease of use, robustness, accuracy of the results, and affordability to address WHO criteria and expectations for the ideal diagnostic test.

CoVradar uses a set of novel DGL-probes to interrogate different regions of the SARS-CoV-2 viral genome, improving the platform's sensitivity. We studied using a mixture of the ten best DGL-probes immobilized onto the same spot (multi-capture DGL-Probes spot) to simultaneously target multiple regions of the viral RNA. We have coined it "inverse amplification", in which the signal amplification is not given by enzymatic amplification but by using multiple RNA regions within the same RNA molecule targeted after a fragmentation step. Using multi-capture DGL-probes immobilized within the same spot leads to an inverse amplification by a factor of 10 (an order of magnitude). Multi-capture DGL-Probes spots hybridizes to ten complementary regions of targeted RNA, thus enabling ten other template molecules from a viral RNA copy to incorporate SMART-C-biotin tags via DCL rather than one per viral RNA copy.

We have selected saliva as the sample of choice for CoVradar. It offers the advantage of being easy, non-invasive, more acceptable for repeated testing, and can be performed by non-healthcare professionals or individuals who are properly instructed. Several studies have shown that SARS-CoV-2 can be detected in the saliva of asymptomatic individuals and outpatients (Kojima et al., 2021; Pasomsub et al., 2021; Williams et al., 2020). Clinical studies are warranted on the sensitivity of saliva as sample material for testing symptomatic and asymptomatic

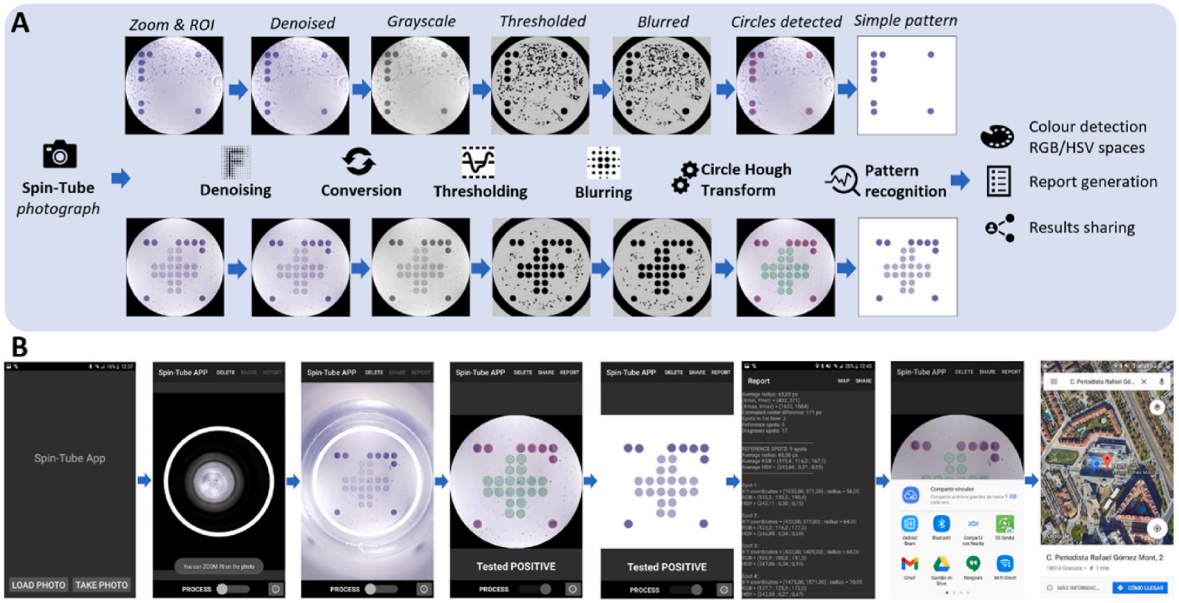

Fig. 5. CoVreader for reading the CoVradar device. (A) Flow diagram describing the image processing sequence conducted by the smartphone application. (B) Screen captures of the smartphone application corresponding to different steps of the related user flow.

individuals and to standardize the sampling collection methods. We studied the behaviour of the saliva matrix in our CoVradar assay. When saliva is enrolled for our spike-in studies, a reduction of background signals in negative controls is observed compared to using water as a matrix for the study (Figs. 2C and 3B). This gave us the confidence to continue developing CoVradar by using saliva as a diagnostic specimen.

CoVradar was optimized and validated using long ssRNA to mimic viral genomic RNA. To have sufficient fragments of ssRNA for the development of CoVradar, we generated a library of *in vitro* transcribed ssRNA molecules containing sequences complementary to our multi-capture DGL-probes (Fig. S1). We designed and set up the conditions for medium-scale production of five specific ssRNA molecules. Afterwards, gradient concentrations of ssRNA molecules were spiked in saliva samples and used to validate the CoVradar assay (Fig. 3C).

We also designed the scheme of an array layout to create a cross-shaped array considering the membrane diameter of 8 mm, indicating the positive symbol for SARS-CoV-2 infected patients (Fig. 3A). Moreover, reference spots at the four corners of the array with biotin-labelled DNA oligomers were printed to control both the orientation of the array and the colorimetric protocol, acting as a positive control for the colorimetric reaction as well as reference spots for the orientation of the array for a correct readout.

To analyze the results of CoVradar, we developed CoVreader, a dedicated IPS for the analysis of CoVradar. CoVreader consists of hardware and software parts. The hardware part includes a mobile phone for acquiring the image and an accessory to accommodate the CoVradar device aligned with the mobile phone camera (Fig. 4A–C). While the software consists of a user-friendly mobile phone app with simple imagery instructions to facilitate its use given language diversity across countries (Fig. 4D). The app allows the user to pinch-to-zoom in and out to align/fit the template grid (white circle) with the CoVradar array. To obtain correct processing, the user needs to position the CoVradar array properly, hence why CoVradar was constructed with biotin-labelled DNA oligomer spots at the four corners of the array (nine spots) to allow the orientation and good alignment for CoVreader (Fig. 5A. CoVreader processes the area of interest, discarding the rest of the image to improve the reliability of the processing. Image processing has been implemented and optimized based on multiple CoVradar testing with different signal intensity, positioning, and background noise characteristics. CoVreader detects, stores in the report, and displays the user geolocation. The results can be shared through social networks, email, messaging, and/or cloud services.

5. Conclusions

In this proof of concept, we have developed CoVradar, a colourimetric molecular assay for testing SARS-CoV-2 RNA in saliva specimens. The limit of detection was the lowest concentration of RNA that could be observed from colourimetric images with reasonable confidence. CoVradar is the first step COVID-19 detection method based on the detection of viral RNA without the need for prior RNA extraction or the use of polymerases to pre-amplify. CoVradar can be adapted to detect new variants of SARS-CoV-2 by re-designing and changing the sequences of DGL-Probes. CoVradar result analysis is straightforward and unambiguous thanks to its dedicated CoVreader, a portable smartphone-based IPS to acquire and process results from CoVradar. CoVreader offers remote primary care diagnosis and telemetry for "cloud-based" notification to ensure the public health and surveillance interventions. We believe that the combination of CoVradar and CoVreader represents a unique solution for testing SARS-CoV-2 viral RNA that offers benefits in terms of time-to-results (~3 h/test), cost (~€1/test manufacturing cost) and simplicity (no large equipment required). This new solution opens the possibility of a repertoire of assays for other infectious diseases, such as malaria and tuberculosis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: JJDM is a founder, shareholder and Director of DESTINA Genomics Ltd. SP is a shareholder of DESTINA Genomics Ltd. DESTINA Genomica SL is a wholly owned subsidiary of DESTINA Genomics Ltd. DESTINA is interested in the exploitation of the technology developed here.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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