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Research Article

Amplification-Free COVID-19 Detection by Digital Droplet REVEALR

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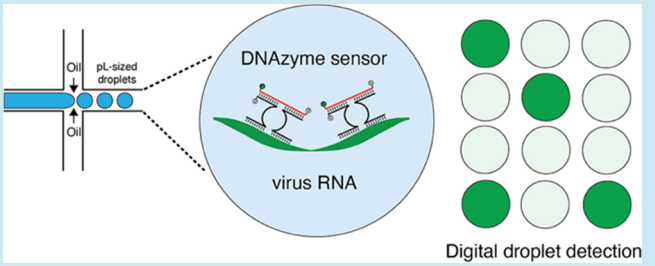
ABSTRACT: The COVID-19 pandemic, caused by the SARS-CoV-2 virus, exposed a pressing need for new public health tools for pathogen detection, disease diagnosis, and viral genotyping. REVEALR (RNA-encoded viral nucleic acid analyte reporter) is an isothermal DNAzyme-based point-of-care diagnostic that functions with a detection limit of ~10 copies/μL when coupled with a preamplification step and can be utilized for viral genotyping of SARS-CoV-2 variants of concern through base pair mismatch recognition in a competitive binding format. Here, we describe an advanced REVEALR platform, termed digital droplet REVEALR (ddREVEALR), that can achieve direct viral detection and absolute sample quantitation utilizing a signal amplification strategy that relies on chemical modifications, DNAzyme multiplexing, and volume compression. Using an AI-assisted image-based readout, ddREVEALR was found to achieve 95% positive predictive agreement from a set of 20 nasal pharyngeal swabs collected at UCI Medical Center in Orange, California. We propose that the combination of amplification-free and protein-free analysis makes ddREVEALR a promising application for direct viral RNA detection of clinical samples.



KEYWORDS: *RNA diagnostics, droplet microfluidics, SARS-CoV-2, REVEALR, multicomponent DNAzyme*

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic caused by the spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) fostered unprecedented growth in the development of novel methods for nucleic acid detection.[1] As of November 2022, 275 diagnostic tests have received emergency use authorization by the U.S. Food and Drug Administration (FDA), the vast majority of which rely on the technique of reverse transcription polymerase chain reaction (RT-PCR) for analyte detection. A much smaller subset (~10%) utilize isothermal amplification strategies, such as loop-mediated isothermal amplification (LAMP)[2] and recombinase polymerase amplification (RPA),[3] to increase sample copy number by avoiding the thermocycling requirements of RT-PCR. However, despite their widespread use in FDA-approved diagnostics, amplification-based approaches developed for routine healthcare monitoring suffer from problems associated with nonspecific DNA amplification, elevated costs due to the need for specialized enzymes, and bottlenecks in product development caused by poor supply chain resilience.[4] These difficulties raise an important challenge in the field of nucleic acid biochemistry of how to develop a nucleic acid detection assay that can accurately quantify pathogen levels in patient samples without the need for sample amplification.

Recent advances in nucleic acid technologies have led to the development of a broad range of amplification-free approaches for pathogen detection.[5] Several of these methods have been shown to function with analytical limits of detection (LoD) in the low attomolar range (10^{-18} M), corresponding to ~1000 copies of a viral genome/mL.[6] However, many of these techniques require user expertise and expensive analytical instrumentation that is highly customized for a particular assay, and as such may not be readily available for broad deployment as a public health screening tool.[6] One trend to overcome these limitations is CRISPR-based approaches, which have attracted considerable attention due to their vast signal amplification based on strong sequence-specific recognition that allows for precise detection of pathogen-specific nucleic acid signatures in a way that is more adaptable to standard readout modalities (electrical, electrochemical, coulometric, and fluorescent readout) than other competing methods.[6,7] Such examples include a graphene field-effect transistor for targeted DNA detection[8] and a multiplex strategy that is compatible with mobile phone microscopy.[9] Given the success of CRISPR-based systems toward the problem of amplification-free detection, we wondered if this paradigm could be made even simpler by establishing a protein-free approach for direct pathogen detection that relies on the inherent catalytic activity of DNA enzymes (DNAzymes).

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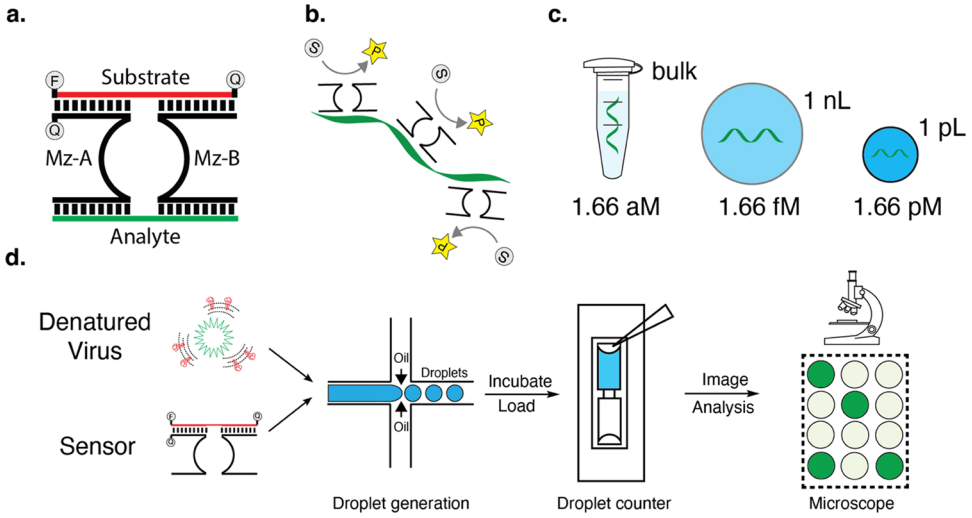

Figure 1. Amplification-free COVID-19 detection by digital droplet REVEALR. (a) Cartoon representation of a multicomponent DNAzyme sensor developed for SARS-CoV-2 detection. The catalytic core self-assembles in the presence of a viral RNA analyte (green) to produce a functionally active enzyme that is capable of generating an optical signal by cleaving a quenched fluorescent RNA reporter (red). (b, c) Signal amplification. The optical signal produced by the DNAzyme can be increased by multiplexing (b) and volume compression (c). Multiplexing involves targeting the viral analyte with sets of DNAzymes that recognize different regions of the viral genome. Volume compression increases analyte concentration through microfluidic encapsulation in uniform water-in-oil droplets of defined size. (d) Workflow. Reaction mixture containing denatured virus and multiple DNAzymes is encapsulated in microdroplets, incubated, transferred to a droplet counter, and imaged. The droplet population is then digitally assessed and fitted to a calibration curve. Abbreviations: S, substrate; P, product; Mz, multicomponent enzyme; F, fluorophore; Q, quencher.

REVEALR (RNA-encoded viral nucleic acid analyte reporter) is a rapid and highly sensitive DNAzyme-based point-of-care diagnostic developed for SARS-CoV-2 detection.[10] The technology is based on a multicomponent nucleic acid enzyme (Mz) that self-assembles in the presence of a target analyte to produce a functional DNAzyme capable of generating an output signal by cleaving a quenched fluorescent oligonucleotide reporter.[11] REVEALR maintains high sequence specificity through complementary Watson−Crick base pairing between the target binding arms of the multicomponent enzyme and viral RNA sequence (Figure 1), allowing for continuous signal amplification while the DNAzyme is bound to the viral RNA analyte. When coupled to an RPA-preamplification step, REVEALR can achieve an analytical LoD (≤20 aM, ∼10 copies/μL), which is comparable to the SHERLOCK and DETECTR systems based on CRISPR-Cas enzymology.[12,13] In a competitive binding format, REVEALR was shown to successfully genotype sequence-verified clinical samples spanning the complete set of SARS-CoV-2 variants of concern observed in the U.S. in 2021, matching local and national surveillance efforts.[14]

Here, we report the conversion of our standard REVEALR assay into a digital droplet format, termed digital droplet REVEALR (ddREVEALR), that can achieve absolute RNA quantitation through direct single-molecule viral RNA detection in uniform water-in-oil (w/o) droplets generated by a standard microfluidic device. Through a combination of chemical optimization, volume compression, and reagent multiplexing, ddREVEALR was found to achieve 95% positive predictive agreement from a set of 20 PCR-verified patient-derived nasal pharyngeal swabs collected at UCI Medical Center in Orange, California. We suggest that the simplicity of the design, coupled with its use of low-cost renewable DNA reagents, makes ddREVEALR a promising approach for personalized diagnostics.

RESULTS

Assay Design. We envisioned the development of an amplification-free detection assay for COVID-19 that would enable absolute quantitation of the viral genome in a digital droplet format that is sensitive, rapid, and affordable. As outlined in Figure 1, a small aliquot of the patient-derived sample, reaction buffer, and DNAzyme sensors targeting different regions of the viral genome are encapsulated in uniform w/o microcompartments. Since the average viral load of patients infected with SARS-CoV-2 ranges from 80−752 copies per μL,[15] a Poisson distribution dictates that a large droplet population will consist of mostly unoccupied droplets, while a small fraction of occupied droplets would contain only 1 copy of the viral genome. However, it should be noted that it is statistically possible for patients infected with a higher viral load to have a small number of droplets containing two copies of the RNA genome, meaning that analyte detection in a microcompartment would require single-molecule or near-single-molecule sensitivity. Following incubation to facilitate target recognition and catalysis, the droplet population is transferred to a droplet counter and imaged by confocal microscopy. AI-assisted image analysis produces a droplet segmentation pattern that is used to determine positive and negative results based on the fluorescence signal of each droplet. Similar to digital droplet PCR, absolute quantitation is achieved by converting the percentage of positive droplets (PPD) into a precise analyte concentration based on Poisson statistics (Figure S1).[16]

Critical to the success of this workflow was the need to increase the LoD of our REVEALR assay. In bulk solution, direct detection of the SARS-CoV-2 genome requires an analytical LoD of 1.6 aM, which is 10^8-fold lower than the analytical LoD previously measured for a REVEALR assay performed using a modified DNAzyme sensor without sample preamplification.[10] Assay miniaturization can be used to reduce

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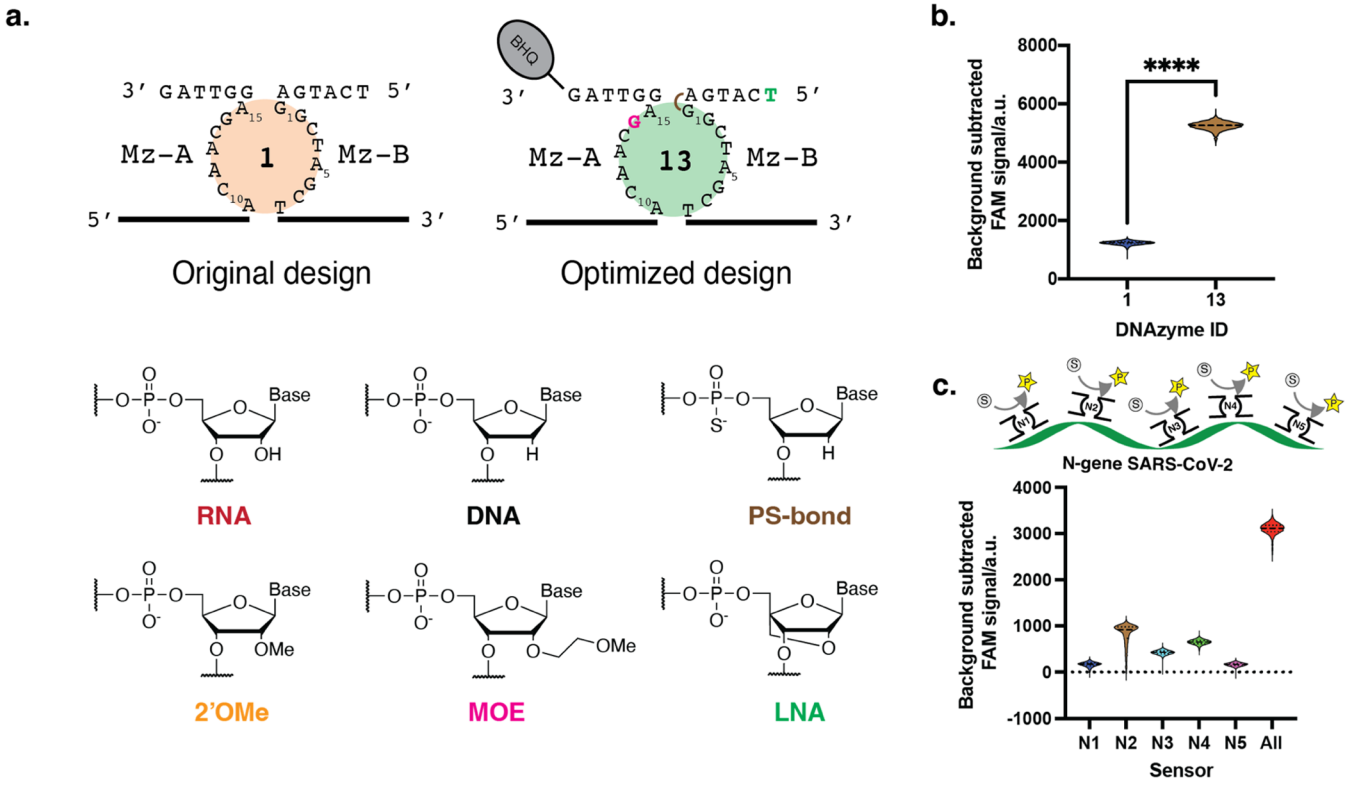

Figure 2. Sensor optimization. (a) Comparison of original DNAzyme 1 and chemically optimized DNAzyme 13. Chemical modifications are indicated by color, and residue is shown at the bottom. (b) Analyte detection. Quantitative analysis comparing the original all-DNA design (Mz-1) to the most active design (Mz-13) (c) SARS-CoV-2 detection using DNAzyme sensors individually and collectively in a multiplex format. Assays were performed in Bio-Rad droplets generated from buffer containing 200 mM $MgCl_2$, 50 mM Tris (pH 8.5), 150 mM NaCl, 500 nM FAM-labeled RNA substrate and 500 nM DNAzyme (for the multiplexed reaction, each DNAzyme is present at 100 nM concentration), 100 pM of in vitro transcribed RNA analyte, and 1× Bio-Rad ddPCR Supermix. Droplet populations were evaluated after a 1 h incubation using a Bio-Rad ddPCR droplet reader. Abbreviations: 2′-*O*-methylribonucleic acid (2′-OMe), 2′-*O*-methoxyethylribonucleic acid (MOE), locked nucleic acid (LNA), phosphonothioate (PS), and black hole quencher (BHQ).

the magnitude of this problem, as analyte concentration increases through the effects of volume compression when analytical samples are compartmentalized in microfluidic droplets, with smaller-size droplets poising the RNA analyte at higher concentrations. As an example, droplets produced with an average diameter of 12 μm increase the viral RNA concentration by 10^6-fold relative to bulk solution (Figure 1). In compartments of this size, the analytical LoD required to achieve single viral RNA genome detection is 1.6 pM, which is only ∼150-fold lower than the LoD observed for the REVEALR assay described above.[10] Based on previous experience,[10,14] we reasoned that it may be possible to increase the sensitivity of our assay through optimization of the DNAzyme sensor and reagent multiplexing. However, it was unclear if such steps would be sufficient to enable direct viral RNA detection of patient samples in a digital droplet format, as single-molecule nucleic acid detection by a DNA enzyme has not been achieved previously.

Assay Miniaturization and Optimization. To explore the benefit of performing REVEALR-based nucleic acid detection assays in a digital droplet format, we compared the analytical LoD of an all-DNA version of a DNAzyme sensor targeting in vitro transcribed RNA encoding a region of the S-gene from the SARS-CoV-2 genome in a bulk aqueous solution to reagents encapsulated in uniform microdroplets. For simplicity, this assay was performed using a commercial Bio-Rad digital droplet generator and digital droplet reader designed to produce and analyze medium size droplets (1 nL volume, 124 μm diameter) that are stable and easy to generate from commercial reagents. After 1 h of incubation at 25 °C, the original all-DNA design (Mz-1, multicomponent enzyme 1) was observed to function with an analytical LoD of 5 nM in bulk solution as previously reported,[10] which improved to 50 pM when the assay was performed in a digital droplet format (Figure S2). Although this result demonstrated the ability for volume compression to increase the sensitivity of REVEALR-based RNA detection, it also revealed the challenges of achieving direct viral RNA detection as the analytical LoD of our assay was still above the limit required to permit direct pathogen detection.

Substrate binding is widely viewed as the rate-limiting step of DNAzyme-mediated RNA strand cleavage.[17] Based on our prior experience with chemically modified DNAzymes,[18] we postulated that the sensitivity of our assay could be increased by enhancing the catalytic turnover of the enzyme using chemical modifications introduced at key structural positions along the backbone architecture of the DNAzyme scaffold. Following a systematic analysis of diverse chemotypes, we discovered several DNAzyme sensors with improved activity (Figures 2a and S3) under optimized incubation temperature (Figure S4), buffer conditions (Figure S5), and substrate design (Figure S6). The most active sensor, Mz-13, contains a black hole quencher (BHQ) at the 3′ terminus of Mz-A, a locked nucleic acid (LNA) residue at the 5′ terminus of Mz-B,

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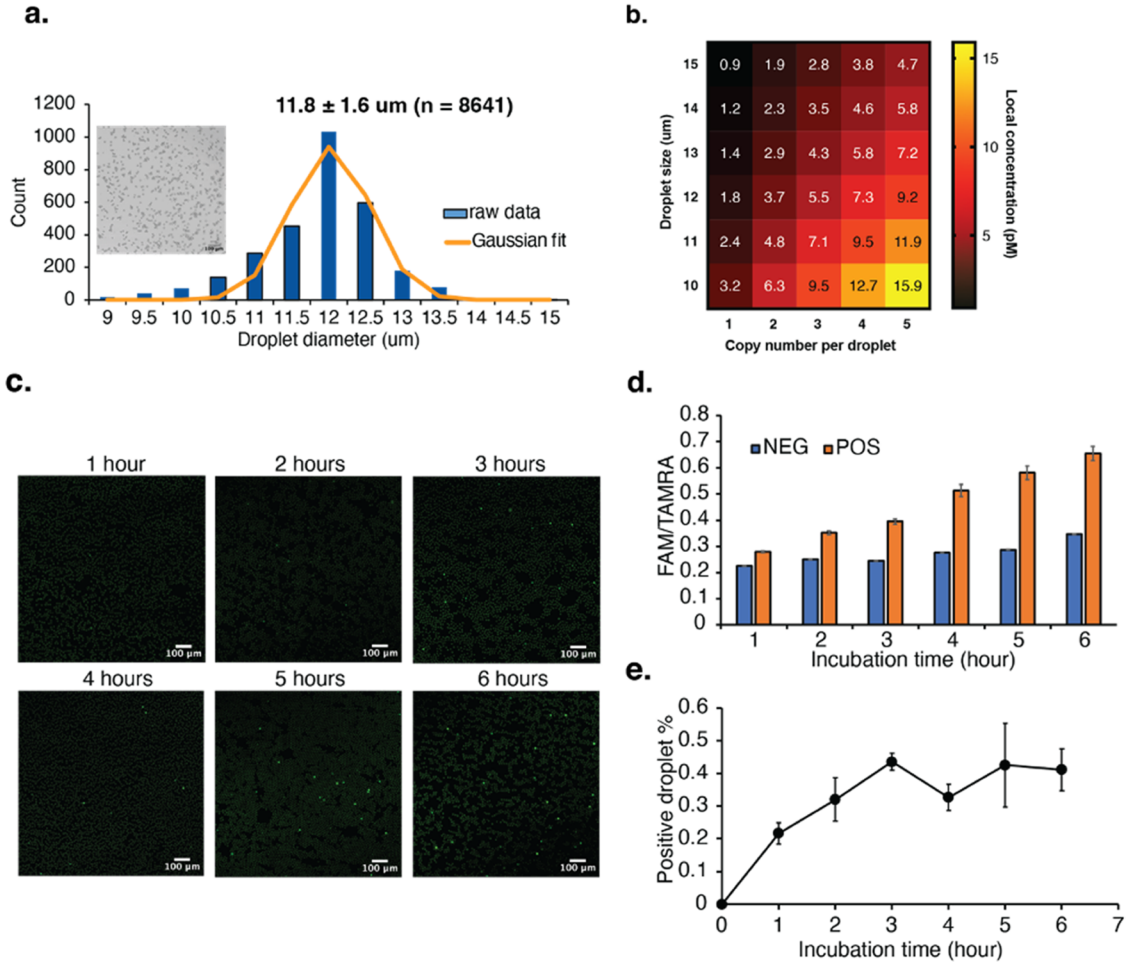

Figure 3. Digital droplet REVEALR in custom microfluidic droplets. (a) Droplet size distribution for custom in-house microfluidic droplet production. (b) Simulation of analyte concentration across a range of droplet sizes. (c) Digital time course analysis of SARS-CoV-2 signal generation produced from 10 fM in vitro transcribed RNA analyte. (d) Average positive and negative droplet signal observed after 1−6 h of incubation at 34 °C. (e) Positive drop percentage observed as a function of time. Error bars denote ± standard error of the mean for 3 independent replicates. Reactions were performed in droplets generated from buffer containing 200 mM $MgCl_2$, 50 mM Tris (pH 9.0), 150 mM NaCl, 1 uM FAM-labeled RNA substrate, 500 nM TAMRA labeled reference oligo, 20 nM of each multicomponent DNAzyme, 10 fM in vitro transcribed RNA analyte, and 1× Bio-Rad ddPCR Supermix. Droplet populations were imaged by confocal microscopy, with data analysis performed using Biodock.

a phosphorothioate linkage between residues A_0 and G_1 of the catalytic loop, and a 2′-*O*-methoxyethylribonucleic acid (MOE) substitution for the standard 2′-deoxyguanosine residue at position 14 of the catalytic loop. The BHQ and LNA modifications enable improved contact quenching, which minimizes the background FAM signal of the uncleaved substrate (Figure S7) and increases substrate hybridization, respectively, while modifications made to the catalytic loop are designed to increase the catalytic activity of the multicomponent enzyme. Compared to the all-DNA version of the sensor, the functionally enhanced sensor established for Mz-13 exhibits an increase (>4-fold) in catalytic activity (Figure 2b and Table S3), indicating that alternative chemotypes can significantly improve the performance of multicomponent DNA enzymes.

Next, we attempted to increase the sensitivity of our assay using a multiplexing strategy that involved the application of multiple DNAzyme sensors targeting different regions of the viral genome (Figure 1). Five DNAzyme sensors (N1−N5) were designed to recognize different regions of the N-gene, which is a highly conserved region of the SARS-CoV-2 genome.[19] To improve the overall activity of the assay, each DNAzyme was prepared using the optimal chemistry developed for Mz-13 (Figure 2), and reactions were performed and analyzed in 1 nL Bio-Rad droplets, as described above. After 3 h of incubation at 34 °C, the multiplex assay afforded an ∼5-fold higher signal than the average signal obtained using each DNAzyme sensor individually with the same total concentration of DNAzyme sensors and substrate (Figure 2c). However, despite improvements imbued through the multipronged approach of chemical optimization, multiplexing, and volume compression, the analytical LoD of 250 fM (Figure S8) remained above the 1.6 fM level required for single RNA detection in a 1 nL droplet. This observation suggested that an effective single-molecule DNAzyme strategy would require smaller volume compartments.

Digital Droplet REVEALR. In an effort to achieve direct viral RNA detection, we turned to microdroplets produced in-house using a custom fluorocarbon-coated poly(dimethylsiloxane) (PDMS) microfluidic device previously developed for enzyme engineering applications.[20] By adjusting the flow rate of the aqueous component, we discovered that it was possible to generate populations of $\sim 10^7$ droplets in 6 min. The resulting confocal microscopy image indicates that these

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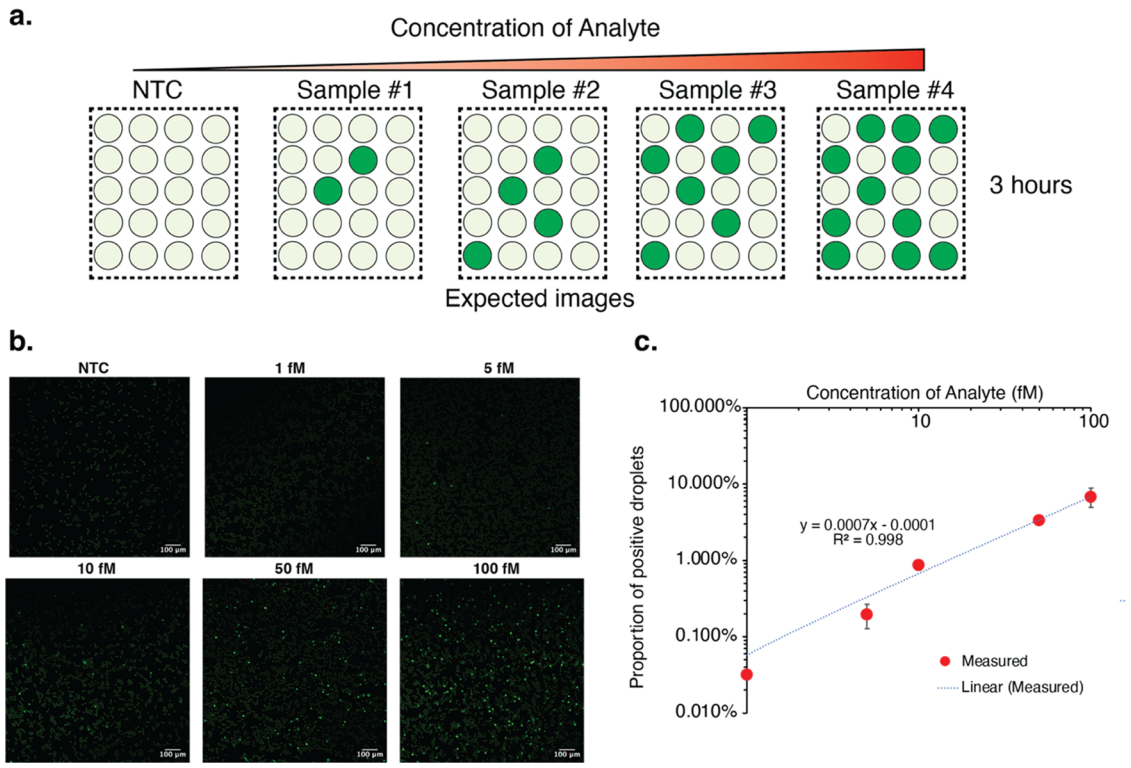

Figure 4. Sensitivity of digital droplet REVEALR. (a) Illustration of droplet signal across a gradient of analyte concentrations. (b) Images of digital droplets collected after 3 h of incubation. (c) Calibration curve defining analyte concentration as a function of positive droplets with 100, 50, 10, 5, and 1 fM in vitro transcribed RNA analyte or a water-only no template control (NTC). Red dots indicate positive percent values. The dashed line is a linear fit of experimental data. Error bars denote ± standard deviation of the mean for two independent replicates. Reactions were performed in droplets generated from buffer containing 200 mM $MgCl_2$, 50 mM Tris (pH 9.0), 150 mM NaCl, 1 uM FAM-labeled RNA substrate, 500 nM TAMRA labeled reference oligo, 20 nM of each DNAzyme, in vitro transcribed RNA analyte, and 1× Bio-Rad ddPCR Supermix. Droplet populations were evaluated after 3 h of incubation at 34 °C by confocal microscopy, with data analysis performed using Biodock.

conditions allowed the PDMS device to produce uniform w/o droplets that were much smaller in size (average diameter: 12 μm, average volume: 1 pL) than the droplets produced using a commercial Bio-Rad instrument (Figure 3a). Simulation of analyte concentrations across a range of droplet sizes (Figure 3b) indicates that droplets containing a single copy of the viral genome would have a concentration of 1.8 pM, which is ~10^3-fold higher than the concentration predicted for 1 nL size Bio-Rad droplets.

To explore the potential for smaller-size droplets to achieve direct viral RNA detection, we performed a time course analysis with in vitro transcribed RNA encoding a region of the SARS-CoV-2 genome. Reactions were performed at a fixed analyte concentration of 10 fM over a time period of 6 h at 34 °C. This analyte concentration is the range expected for a patient-derived clinical sample.[15] At this analytic concentration, we would expect (Figure S9) most of the generated droplets to be empty (~99.5%). However, a small fraction of droplets (~0.5%) would contain a single copy of the viral genome, and an even smaller fraction (~0.001%) would contain two copies of the viral genome (Figure S9). The resulting images obtained by confocal microscopy reveal that the fluorescent signal plateaus after 3 h (Figure 3c–e), indicating that 3 h is sufficient to generate a fluorescence signal from a single-molecule DNAzyme-mediated detection assay. Analysis of the images by AI-assisted software reveals close concordance between the experimental and predicted values (~0.4% vs ~0.5%, respectively) for the fluorescent droplets (Table S2). This result implies that 12 μm compartments should be sufficient in size to enable direct viral RNA detection of SARS-CoV-2 from patient-derived samples.

Sensitivity Test. We evaluated the sensitivity of ddREVEALR by measuring the fluorescence signal of individual droplets produced across a range of analyte concentrations. As illustrated in Figure 4a, in vitro transcribed RNA spanning a concentration gradient of 1−100 fM, or no template control (NTC) were individually encapsulated in 12 μm microdroplets. After 3 h of incubation at 34 °C, analyte concentration was measured by digital detection (Figure 4b), and the resulting values were compared to a linear regression plot of Ct values obtained by qRT-PCR. Comparison of the ddREVEALR data to qRT-PCR results provides a close correlation between the positive droplet percentage and authentic values (Figure 4c), indicating that droplet population analysis of DNAzyme-mediated fluorescence offers a viable path for quantifying biological samples. In this case, we evaluated three randomly selected fields of droplets, which allowed us to evaluate ~10,000 droplets per sample or ~0.05% of the droplet population. Analysis of the no template control revealed a false positive rate of ≤0.01% (Figure S10), which we attribute to either degradation of the RNA fluorophore or imperfect oligonucleotide synthesis. Using this approach, the current limit of detection is ~1 fM (Figure 4c), which corresponds to ~5 positive droplets observed within a 10,000-droplet population by simulation (Table S2) (Figure 5).

Clinical Validation. To establish ddREVEALR as an amplification-free diagnostic for COVID-19 detection, we evaluated 20 patient-derived nasal pharyngeal samples

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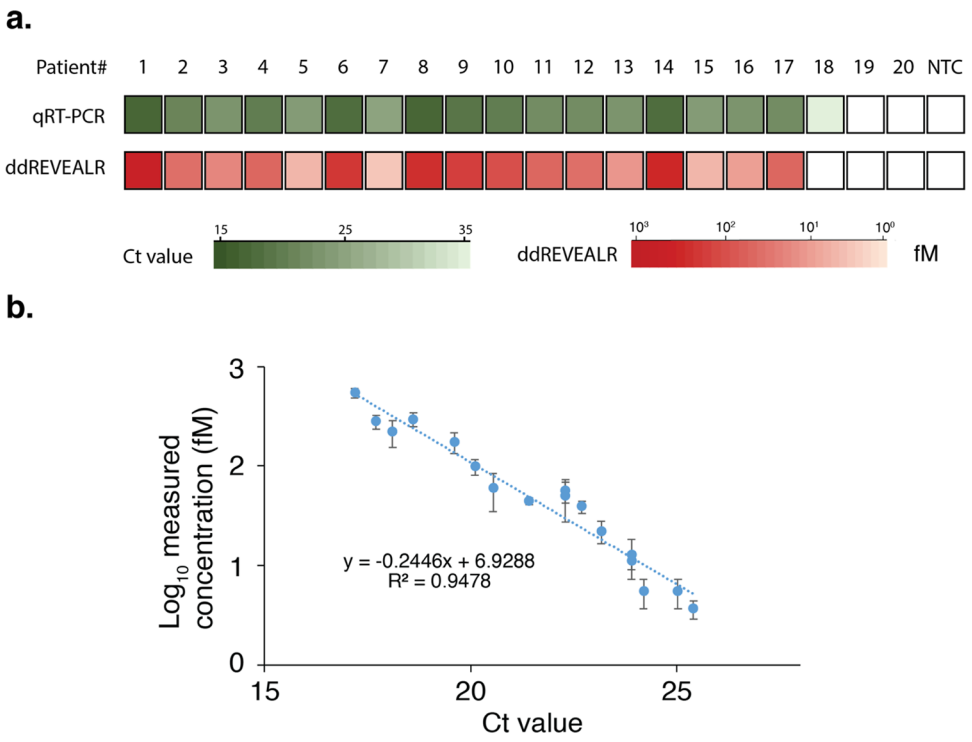

Figure 5. Clinical validation of patient-derived samples. (a) Test results for 20 clinical samples of nasal pharyngeal swabs collected from COVID-19 patients treated at UCI Medical Center in Orange, California. (b) Linear relationship of Ct values obtained from qRT-PCR and viral RNA concentration measured by digital droplet REVEALR. Error bars denote ± standard deviation of the mean for two independent replicates. Reactions were performed in droplets generated from buffer containing 200 mM $MgCl_2$, 50 mM Tris (pH 9.0), 150 mM NaCl, 1 uM FAM-labeled RNA substrate, 500 nM TAMRA labeled reference oligo, 20 nM of each DNAzyme, 1 uL of purified clinical sample, and 1× Bio-Rad ddPCR Supermix. Droplet populations were evaluated after 18 h of incubation at 34 °C by confocal microscopy, with data analysis performed using Biodock.

collected at UCI Medical Center in Orange, California. The samples consisted of 18 PCR-positive patients and 2 PCR-negative patients, which were placed in viral transfer media (VTM) and autoclaved to inactivate the virus prior to receipt. After receipt, the RNA was purified and directly evaluated by ddREVEALR and qRT-PCR. The PCR-positive samples were selected to display a range of viral loads, as measured by qRT-PCR (Ct values of 17−35). ddREVEALR analysis positively confirmed 19 of the 20 samples following overnight incubation at 34 °C. However, sample #18, with a Ct value of 35 (low aM concentration of SARS-CoV-2 RNA), was misread as a COVID negative sample due to the low concentration of the viral RNA present in this sample. Currently, our image-based readout system is evaluating ∼0.05% of the droplets, which limits the testing capability for ultralow concentration of samples. It could be enhanced in the future using a flow-based readout for higher droplet testing fraction. Nevertheless, this false positive value places the current limit of detection at 3.7 fM, which is equivalent to a Ct value of ∼25.4 and within the typical range observed for clinical samples.[15]

DISCUSSION

The COVID-19 pandemic catalyzed unprecedented growth in the development of new analytical techniques for pathogen detection. In the field of nucleic acid recognition, specifically, isothermal amplification strategies have emerged as rapid and inexpensive alternatives to quantitative RT-PCR, which remains the gold standard for SARS-CoV-2 detection. When coupled to sequence-specific detection modalities, such as those found in CRISPR-Cas enzymes, these systems offer sensitive and highly accurate platforms for viral RNA detection.[21] Similar detection routes can also be achieved using DNAzyme constructs that have been engineered into point-of-care diagnostics by introducing an analyte recognition domain into the scaffold.[22] However, despite their many benefits, amplification-based approaches are cumbersome due to the need for a sample preamplification step that places an additional burden of time and cost on the assay. They can also suffer from background problems due to nonspecific DNA amplification.

The current study demonstrates the feasibility of using DNAzymes to directly quantify viral RNA levels in patient-derived clinical samples. The resulting platform, termed digital droplet REVEALR (ddREVEALR), was found to achieve 95% positive predictive agreement from a set of 20 nasal pharyngeal swabs collected at UCI Medical Center in Orange, California. The sensitivity of the assay was greatly improved through a systematic optimization process that involved the use of chemical modifications that increased the catalytic activity of the DNAzyme, reagent multiplexing to improve signal amplification, and volume compression to increase the local concentration of the viral analyte. Additionally, sample analysis was aided using an AI-assisted image-based readout system that allowed for absolute quantitation of patient-derived clinical samples. The software assisted with efficient binary analysis of the droplet population, yielding a strong linear correlation to Ct values measured by qRT-PCR.

ddREVEALR has several advantages relative to other previously reported DNAzyme-based amplification-free nucleic acid strategies. In their original description, DNAzymes were

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shown to achieve picomolar level detection of a nucleic acid target.[11] Subsequent optimizations have improved the sensitivity of the assay to sub-picomolar levels using cascade designs,[23] cationic copolymers,[24] nucleic acids modifications,[25] and microwells.[26] However, to the best of our knowledge, direct viral RNA quantification in a digital droplet format has not been achieved by any nucleic acid enzyme. Since analyte quantification by protein-based digital droplet detection is well established,[16] this work demonstrates the potential for nucleic acid enzymes to compete with protein enzymes in biological assays.[16]

ddREVEALR compares favorably to related CRISPR-based approaches for amplification-free viral RNA detection. Unlike CRISPR, which relies on RNA and protein-based reagents that are temperature-sensitive and must be stored and transported at low temperatures,[21] ddREVEALR is a DNA-based protein-free system (Figure S11) that is stable at room temperature and, as such, avoids the costly expense of the cold storage. The DNA components of ddREVEALR are also easier and cheaper to produce as they can be made by chemical synthesis rather than cellular protein expression and purification. This property allows for higher purity and greater scalability of the reagents, as cellular processes are difficult to scale and can be prone to unwanted viral or bacterial contamination. Finally, the catalytic mechanism of the ddREVEALR system allows for greater targetability of the viral genome, as the targetability of CRISPR-based system is restricted by the protospacer adjacent motif (PAM) for Cas9 and Cas12 or protospacer flanking site (PFS) for Cas13.[21]

Looking ahead to the future, the ddREVEALR system could benefit from further development, such as a new generation of DNAzyme sensors that function with higher activity or a better signal readout modality. An initial step in this direction would likely focus on the development of new chemical modifications that further increase the activity of our current DNAzyme biosensors but could also involve the discovery of entirely new DNAzyme scaffolds. Additionally, assay sensitivity and reaction time could be improved by simply increasing the number of DNAzyme sensors, which may be an easier solution to implement than designing new chemical scaffolds for DNAzyme sensors. The ddREVEALR platform itself could be improved by changing the system from its current image-based readout format to a flow-based system that would allow for greater coverage of low-concentration samples. Finally, it may be possible to incorporate alternative signal generation strategies into the droplet format such as the hybridization chain reaction (HCR) or catalytic hairpin assembly (CHA) methods that boost droplet detection efficiency through enhanced signal amplification.[27,28]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssynbio.3c00105.

Experimental methods; list of oligonucleotides; calculation of percentage of positive droplet (PPD); multicomponent DNAzyme optimization; Poisson distribution calculating the percentage of positive droplet; sensitivity test of multicomponent DNAzyme in a Bio-Rad digital droplet system; chemical modifications evaluated for optimal DNAzyme activity; temperature optimization of DNAzyme sensor; Mg concentration study in droplet system; substrate comparison; contact quenching experiment (PDF)

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Author Contributions

J.C. and K.Y. conceived the project and designed the experiments. K.Y. performed the experiments. J.C. and K.Y. wrote the paper.

Notes

The authors declare no competing financial interest.
The University of California−Irvine, has filed a patent application on REVEALR.

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