High-Throughput CRISPR–Cas13 SARS-CoV-2 Test

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BACKGROUND: The ability to control the spread of COVID-19 continues to be hampered by a lack of rapid, scalable, and easily deployable diagnostic solutions.

METHODS: We developed a diagnostic method based on CRISPR (clustered regularly interspaced short palindromic repeats) that can deliver sensitive, specific, and high-throughput detection of Sudden Acute Respiratory Syndrome–Coronavirus-2 (SARS-CoV-2). The assay utilizes SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) for the qualitative detection of SARS-CoV-2 RNA and may be performed directly on a swab or saliva sample without nucleic acid extraction. The assay uses a 384-well format and provides results in <1 hour.

RESULTS: Assay performance was evaluated with 105 (55 negative, 50 positive) remnant SARS-CoV-2 specimens previously tested using Food and Drug Administration emergency use authorized assays and retested with a modified version of the Centers for Disease Control and Prevention (CDC) quantitative PCR with reverse transcription (RT–qPCR) assay. When combined with magnetic bead-based extraction, the high-throughput SHERLOCK SARS-CoV-2 assay was 100% concordant (n = 60) with the CDC RT–qPCR. When used with direct sample addition the high-throughput assay was also 100% concordant with the CDC RT–qPCR direct method (n = 45). With direct saliva sample addition, the negative and positive percentage agreements were 100% (15/15, 95% CI: 81.8–100%) and 88% (15/17, 95% CI: 63.6–98.5%), respectively, compared with results from a collaborating clinical laboratory.

CONCLUSIONS: This high-throughput assay identifies SARS-CoV-2 from patient samples with or without nucleic acid extraction with high concordance to RT–qPCR methods. This test enables high complexity laboratories to rapidly increase their testing capacities with simple equipment.

Background

Available SARS-CoV-2 testing has been recognized as a critical requirement to control the global COVID-19 pandemic (1). Most current tests rely on quantitative PCR with reverse transcription (RT–qPCR)-based amplification and detection of viral RNA, and require expensive, complex, and sensitive equipment necessitating highly trained laboratory personnel for operation (2). The ability to quickly scale up the volume of testing required to meet demand has been challenging and has sometimes led to large delays in patient result reporting (3). Isothermal amplification of viral targets has greatly reduced the complexity of equipment required to detect viral targets; however, off-target amplification leading to false positives can occur when using isothermal methods such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) without sequence specific detection (4–9). Methods combining the flexibility and simplicity of LAMP and RPA with a high level of specificity are needed.

In recent years, CRISPR-based diagnostics have emerged as a programmable method for rapid, sensitive, and specific detection of nucleic acids (10–12). SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) was developed as a sensitive CRISPR-based diagnostic that enables detection of DNA or RNA with single-nucleotide specificity using Cas13a from Leptotrichia wadei (LwaCas13a) combined with isothermal amplification (13, 14). We have further enhanced the robustness and performance of this method by incorporating a highly sensitive LAMP-based amplification of the target viral RNA.
LAMP is one example of an isothermal amplification method that has been extensively studied for pathogen detection including SARS-CoV-2 (15, 16). LAMP-based amplification methods are attractive for diagnostics because LAMP has been reported to be more tolerant than PCR to endogenous inhibitors present in biological samples (17, 18). However, traditional LAMP-based detection methods suffer from poor specificity and are challenging to multiplex (5–9). Our SHERLOCK-based methods overcome this limitation with sequence specific Cas13-based amplicon detection.

In May of this year, the US Food and Drug Administration (FDA) issued the first Emergency Use Authorization for a CRISPR diagnostic test to the SHERLOCK CRISPR SARS-CoV-2 kit. The SHERLOCK assay was for the detection of SARS-CoV-2 nucleic acid in upper respiratory tissue samples including nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate, or nasal aspirate and bronchoalveolar lavage specimens collected from individuals suspected of COVID-19 by their healthcare provider (19). It was recently reported in a clinical evaluation that the SHERLOCK assay was 100% concordant to RT-PCR in the detection of SARS-CoV-2 in clinical nasopharyngeal samples (20).

Here we present several advancements that were developed to improve the simplicity and throughput of the CRISPR diagnostic method implemented in the SHERLOCK kit. These include combining 2 independent SARS-CoV-2 targets, Nucleocapsid (N) and open reading frame (ORF), in a single reaction, simplifying the sample preparation, and implementing the assay in a 384-well format with minimal liquid handling steps to increase throughput and improve compatibility with automated processes. Additionally, we evaluated multiple extraction methods and demonstrated that a simple heat and proteinase K treatment is sufficient to allow direct sample (swab in saline, saliva) addition to a SHERLOCK reaction while maintaining high sensitivity (5 copies/μL) and specificity. The method has also been implemented on a Hamilton Microlab STAR system to scale testing of saliva samples in a clinical setting. Together, these advancements enable a simpler workflow compatible with liquid handling instrumentation that can enable testing up to 5000 patient samples per day (24 h) based on a single operator and instrument.

**Methods**

**SARS-COV-2 VIRAL CONTROL MATERIAL**

NATtrol SARS-CoV-2 [NATSARS(COV-2)-ST] viral particles were purchased from ZeptoMetrix. Purified, intact viral particles were inactivated using proprietary treatment methods and quantified by RT–PCR with the quantitation standard determined by digital PCR.

**VIRAL RNA EXTRACTION**

RNA was purified from contrived and clinical samples using the Invitrogen PureLink™ Viral RNA/DNA kit (12280050), Zymo Quick-DNA/RNA viral mag bead kit (R2140), Applied Biosciences MagMax Viral/Pathogen kit (A42352), or Applied Biosciences MagMAX Viral RNA kit (AM1939). RNA purification was performed according to the manufacturer’s protocol except for a 1-min spin step and 15-min air dry step added to the end of the PureLink purification protocol just before elution of RNA.

**PROTEINASE K AND HEAT TREATMENT OF SAMPLES**

Contrived saline samples were made by adding ZeptoMetrix NATtrol SARS-CoV-2 viral particles to 0.9% NaCl. Serial dilutions of the highest concentration sample were used to make the lower concentration samples. Contrived saline samples were transferred to 8-strip PCR tubes and Proteinase K (NEB 20 mg/mL Cat No: P8107S) was added 1 μL per 9 μL of sample. Samples were heated in a thermocycler for 6 min at 65°C, then 3 min at 98°C, and finally cooled to 4°C before addition to LAMP.

**SHERLOCK PROCESS**

LAMP reactions were performed by first combining 10 μL of NEB 2× WarmStart® LAMP kit (E1700L) with 2 μL of 10× primer mix for either NO (SARS-CoV-2 N and ORF) and RP (Human RNase P Pop7) reaction. A total of 8 μL of extracted RNA was added to 12 μL of each LAMP reactions for NO and RP; a total of 2 reactions per sample. For Proteinase K and heat-treated samples 4 μL of treated sample plus 4 μL of water were added to 12 μL of LAMP reaction. Last, 20 μL of mineral oil (Sigma: 69794) was added to top off the LAMP reactions to prevent condensation and cross-contamination during the LAMP reaction. Plates were sealed with a thermal adhesive seal (Fisher Scientific: 08-408-240), were then heated at 61°C for 30 min–40 min, and allowed to cool to room temperature before addition of the Cas master mix. While the LAMP reaction was proceeding, the Cas master mix was made. The Cas master mix consists of: 25 U of T7 RNA Polymerase, 25.2 U of Murine RNase Inhibitor, 2.5 mmol/L rNTP mix, 312 nmol/L RNase Alert, 23 mmol/L MgCl2, 63.5 μg/mL LwaCas13, and 22.5 mmol/L CRISPR RNA. 10 μL of Cas master mix was directly added to the 20 μL LAMP reaction below the mineral oil layer. After addition of Cas mix, plates were put immediately on a plate reader preheated to 37°C. Fluorescence was read every 2 min over a span of 5 reads for a total of 10 min. The Biotek Neo2, Tecan Infinite M Plex, and ThermoFisher Fluoroskan FL were evaluated using an excitation wavelength of 485 nm for
all 3 and an emission wavelength of 528 nm, 525 nm, and 538 nm, respectively. A detailed protocol is provided in the online Data Supplement.

RT–qPCR REACTIONS
RT–qPCR assays were performed according to the CDC protocol with the exception of using the Applied Biosciences QS5 to read the samples instead of a Dx7500. The QIAamp viral RNA kit was used to extract RNA from the clinical samples using 100 μL sample input and 100 μL elution volume. CDC primers/probes (IDT 10006770) were used for all assays. For all assays 3 RT–qPCR reactions were performed per sample for the N1, N2, and RP targets. For RNA extracted samples, 5 μL of extracted RNA was combined with 1.5 μL primer/probe mix, 5 μL of TaqPathTM 1-Step RT–qPCR Master Mix, CG (A153000), and 8.5 μL of water. For the reverse transcription stage, samples were heated at 25 °C for 2 min, then 50 °C for 15 min and 95 °C for 2 min. The PCR stage followed with 45 cycles at 95 °C for 3 s then 55.0 °C for 30 s. For samples tested using the CDC heat inactivation protocol, the samples were heated to 95 °C for 1 min followed by a 4 °C hold. 5 μL of heat-treated samples were added to 5 μL UltraPlex 1-Step ToughMix (4×), 1.5 μL CDC primer/probe mix, and 8.5 μL of water. Samples were put on the Q55 and run using the following steps: reverse transcription stage samples were heated at 50 °C for 10 min and 95 °C for 3 min. The PCR stage followed with 45 cycles at 95 °C for 3 s then 55.0 °C for 30 s.

CONTRIVED SAMPLES
Pooled negative matrices were prepared with SARS-CoV-2 negative nasopharyngeal swab samples in universal transport media (UTM) or viral transport media (VTM) or self-collected anterior nasal swab samples in saline from healthy laboratory personnel. Analytical samples were prepared by serially diluted ZeptoMetrix NATtrol SARS-CoV-2 [catalog no. NATSARS(COV-2)-ST] viral particles in pooled negative matrices. An NTC reaction consisting of water was processed on every plate and a positive control consisting of SARS-CoV-2 viral RNA (BEI NR-52285, or ATCC® VR1986D™) in water at 225 copies/μL was also processed on every plate. Cross-reactivity samples were prepared by spiking high concentrations of common pathogen or nearest neighbor nucleic acid into water as detailed in online Supplemental Table S1. Analytical sensitivities were determined as per FDA EUA guidelines, first by screening at concentrations ranging from 100 copies/μL to 0.01 copies/μL in triplicate. Limit of detection (LoD) confirmations were completed at various concentrations with a minimum of 20 replicates at a given concentration.

CLINICAL SAMPLES
Remnant nasopharyngeal swab samples in saline, VTM, and UTM media were purchased from Boca Biolistics (Boca Raton, Florida) and stored at −80 °C. All samples were purchased with a clinical result from either the Hologic Panther, Roche Cobas, or Perkin Elmer SARS-CoV-2 assays. Sample integrity was confirmed by testing in-house with a modified version of the CDC SARS-CoV-2 RT–qPCR assay as described previously.

EXTERNAL CLINICAL EVALUATION
All sample collection and preparation steps were performed in accordance with CDC interim guidelines (https://www.cdc.gov/coronavirus/2019-ncov/lab/). Saliva samples were processed in parallel with the SHERLOCK assay and tested on the Abbott Alinity or Biocartis Idylla platforms. Nasopharyngeal (NP) swab samples were collected in VTM/UTM and tested on the Abbott M2000/Alinity or the DiaSorin platform.

SHERLOCK DATA ANALYSIS
To determine whether the negative control is valid, the fluorescent ratio of the fluorescent value at 10 min divided by the fluorescent value at 0 min must be <3. If the no-template control (NTC) is valid, calculations for the samples are determined. To determine whether a sample is positive, the ratio of the fluorescent reading for the NO channel at 10 min for the sample is divided by the fluorescent reading of the NTC at 10 min. If the value is above 5 the sample is positive. To determine whether a sample is negative, the ratio of the fluorescent reading for the RP channel at 10 min for the sample is divided by the fluorescent reading of the NTC at 10 min. If the value is above 5 and the NO ratio, as calculated above, is less than 5 the sample is negative. If both the NO channel and RP channel ratios are below 5, the result is invalid.

Results
SHERLOCK HIGH-THROUGHPUT METHOD WITH 96-WELL RNA EXTRACTION
Initially, we developed a workflow based on the SHERLOCK CRISPR SARS-CoV-2 test that increases throughput, simplifies sample preparation, and combines dual target SARS-CoV-2 amplification and detection into a single reaction tube (Fig. 1). Extracted genomic RNA (gRNA) samples are added to a LAMP reaction master mix, with primers specific to the target, in a 384-well deep well, fluorescence-compatible plate. The LAMP reactions are then topped with 20 μL of molecular-biology grade mineral oil to prevent condensation and reduce the risk of contamination. The LAMP reaction occurs on any plate heater capable of maintaining 61 °C.
The time of the LAMP reaction depends on the template and is <30 min for samples extracted with a nucleic acid extraction kit. The Cas detection mix (10 μL) containing the Cas enzyme as well as the guide RNA specific for the amplified target is added to each well. The plate is then read on a fluorescence plate reader over 10 min at 37 °C. With this method a single operator can process 190 samples in 70 min (excluding extraction).

To demonstrate the robustness of the SHERLOCK High-Throughput method, we evaluated several bead-based RNA extraction kits on pooled negative NP swab matrix spiked with ZeptoMetrix NATtrol SARS-CoV-2 viral particles at decreasing concentrations. We tested 3 commercially available kits: MagMAX Viral/Pathogen Isolation kit, MagMAX Viral RNA isolation kit and the Zymo Quick-DNA/RNA Viral MagBead kit (online Supplemental Table S2), all 3 kits had comparable LoDs. With this workflow, our LoD was 2 cp/μL (20/20, 100%) (Fig. 2, A). We also compared the sensitivity of 3 fluorescent plate readers (Biotek Neo2, Tecan MPlex, and Fluoroskan Microplate Fluorometer) to expand accessibility of our assay. All 3 plate readers had similar LoD of viral genomic RNA spiked into the reaction (4 cp/μL for the Tecan, 2 cp/μL for the Biotek and Fluoroskan instruments) (online Supplemental Table S3). To determine cross-reactivity and competitive inhibition, we tested the high-throughput workflow with a panel of genetically related organisms as well as common high-priority organisms circulating in the area. Data shown in online Supplemental Tables S1 and S6 show 100% specificity toward SARS-CoV-2. To test our clinical sensitivity, we tested 30 positive and 30 negative NP samples, previously tested by molecular diagnostic methods in CLIA laboratories and confirmed using a modified CDC EUA protocol (online Supplemental Table S7). These samples were extracted using the MagMAX Viral RNA isolation kit, with a 30-min LAMP reaction using the Sherlock high-throughput workflow. Our clinical evaluation resulted in a PPA (positive percent agreement) of 100% (30/30) and a NPA (negative percent agreement) of 100% (30/30) for the SHERLOCK high-throughput workflow (Fig. 2, B and D). These data support that the SHERLOCK high-throughput workflow for the detection of SARS-CoV-2 in upper respiratory specimens is sensitive, specific, and can improve throughput and turnaround time as compared to standard molecular diagnostics.

**HIGH-THROUGHPUT SHERLOCK DIRECT METHOD**

To further increase the throughput of our assay, we developed a workflow that allowed for use of samples that had not been subjected to a full RNA extraction process. This also addresses issues associated with consistent shortages of RNA extraction materials (21). The SHERLOCK Direct workflow begins with a simplified sample treatment where 2 μL of Proteinase K is added to 18 μL of each sample. The samples are then heated for 6 min at 65 °C followed by 98 °C for 3 min to heat-kill the Proteinase K enzyme, followed by cooling to 4–10 °C. These samples can be heated in a 96- or 384-well PCR plate on a heat block. Samples can then be added directly to the LAMP reaction as detailed before, with the amplification time extended to 40 min. With an automated implementation parallel processing 4
of our SHERLOCK Direct method on 20 positive and 25 negative clinically collected NP swab samples stored in 0.9% saline. All samples were purchased from a biobank after having been tested and confirmed COVID positive or negative by an outside CLIA laboratory. To ensure sample integrity, we also tested the material using an in-house developed protocol modeled on the CDC EUA protocol, i.e., extracted with the Qiagen RNA kit and RT–PCR followed by CDC primers targeting the N gene of SARS-CoV-2 and the internal control of RNaseP (online Supplemental Table S9).

The positive percentage agreement (PPA) for the SHERLOCK Direct high-throughput method in saline was 100% (25/25) and the negative percentage agreement (NPA) was 100% (20/20, Fig. 3, B–D), while reducing the time to result and cost associated with
sample extraction. We also tested saliva with our SHERLOCK Direct method and demonstrated high analytical sensitivity, 5 cp/µL in pooled saliva (online Supplemental Table S10). We then transferred the method to an external clinical laboratory who successfully implemented a partial workflow (exceeding sample accessioning) on a Hamilton Microlab STAR system. Using this system, a sensitivity as low as 12.5 cp/µL in pooled saliva has been demonstrated (online Supplemental Fig. S1). Due to the robust amplification of LAMP and potential concern for cross-contamination in a 384-well plate, we performed a checkerboard study with high copy target and no-template controls and found no false positives across 48 replicates of the NO or RP NTCs (online Supplemental Fig. S2).

**HIGH-THROUGHPUT SHERLOCK DIRECT CLINICAL SALIVA TESTING**

The SHERLOCK Direct high-throughput method was evaluated in the Laboratory for Clinical Genomics and Advanced Technology at the Dartmouth Hitchcock Medical Center. A total of 32 patient saliva samples were collected and matching nasopharyngeal swabs were collected for 30 out of 32 saliva samples (Table 1). Saliva samples were processed across 3 different methods; the Alinity (Abbott), Idylla (Biocartis), and SHERLOCK Direct. The Alinity and Idylla platforms have been validated for clinical testing for nasopharyngeal swabs (NP) and combine automated sample extraction with rRT–PCR. Nasopharyngeal swab samples were processed on 3 different nucleic acid extraction-based RT–qPCR platforms; Alinity

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**Fig. 3. SHERLOCK Direct method on NP saline samples. (A) LoD confirmation using NATtrol SARS-CoV-2 viral particles spiked into saline. ZeptoMetrix NATtrol SARS-CoV-2 viral particles were spiked into saline (0.9% NaCl) at indicated concentrations. Each sample was treated with Proteinase K and heated at 65 °C for 6 min and 98 °C for 3 min. 4 µL of each sample was used for the SARS-CoV-2 SHERLOCK assay using LAMP primers and Cas guides for the N gene and ORF1ab. Signal for each sample is reported as the fluorescence ratio of the sample divided by the fluorescence of the NTC 10 min after addition of Cas mixture to the LAMP reaction incubated at 37 °C. (B) Clinical Evaluation of SHERLOCK Direct with positive NP swabs in saline. 20 positive clinical samples were tested by the CDC extractionless method or SHERLOCK Direct method. Each sample Ct value (average of N1 and N2 Ct values) was plotted against the ratio of the sample fluorescence to the NTC at 10 min after the addition of the Cas detection mix. Dotted line represents a ratio of 5, the cutoff for a positive sample. (C) 25 negative samples were confirmed by RT–qPCR, and their fluorescence ratios were determined by SHERLOCK Direct. (D) The clinical agreement between each assay.**
The SHERLOCK Direct method detected SARS-CoV-2 virus in 15 out of 30 saliva samples. The remaining 2 samples gave invalid findings. Invalid results for these 2 saliva samples were also observed on the Alinity system, suggesting a possible inherent sampling issue at the time collection. Despite this, the NO (SARS-CoV-2 N and ORF) Ratio was below 5 on the SHERLOCK result indicating SARS-CoV-2 was not detected (samples Table 1. SHERLOCK Direct saliva clinical evaluation.

| Sample no. | Saliva sample | SHERLOCK result | RT-qPCR result (Ct) | NP sample | RT-qPCR result (Ct) |
|------------|---------------|-----------------|---------------------|-----------|---------------------|
| 1          | detected      | 31.88           | 32.10               |           |                     |
| 2          | detected      | 27.32           | 13.60               |           |                     |
| 3          | detected      | detected*       | 19.66               |           |                     |
| 4          | detected      | 31.53           | 3.13                |           |                     |
| 5          | detected      | 37.28           | N/A                 |           |                     |
| 6          | detected      | detected*       | 14.72***            |           |                     |
| 7          | detected      | 22.33           | N/A                 |           |                     |
| 8          | detected      | 19.89           |                     |           |                     |
| 9          | invalid IC    | failure         | not detected        |           |                     |
| 10         | detected      | 28.47           | 13.38               |           |                     |
| 11         | not detected  | z axis failure  | not detected        |           |                     |
| 12         | not detected  | not detected    | not detected        |           |                     |
| 13         | not detected  | not detected    | not detected        |           |                     |
| 14         | not detected  | not detected    | not detected        |           |                     |
| 15         | not detected  | not detected    | not detected        |           |                     |
| 16         | not detected  | not detected    | not detected        |           |                     |
| 17         | not detected  | not detected    | not detected        |           |                     |
| 18         | not detected  | IC failure      | not detected        |           |                     |
| 19         | not detected  | not detected    | not detected        |           |                     |
| 20         | detected      | 24.58           | 21.34               |           |                     |
| 21         | not detected  | not detected    | not detected        |           |                     |
| 22         | detected      | 34.51           | 31.69               |           |                     |
| 23         | not detected  | 36.99/not detected* | 23.41             |           |                     |
| 24         | not detected  | 36.99/not detected* | 26.00             |           |                     |
| 25         | not detected  | not detected    | not detected        |           |                     |
| 26         | invalid IC    | failure         | not detected        |           |                     |
| 27         | detected      | 21.2            | 9.22**              |           |                     |
| 28         | not detected  | IC failure      | not detected**      |           |                     |
| 29         | detected      | 24.2            | 12.44**             |           |                     |
| 30         | detected      | 34.31           | 29.1**              |           |                     |
| 31         | not detected  | not detected    | not detected**      |           |                     |
| 32         | detected      | N/A             | 23.26               |           |                     |

External clinical evaluation data. 32 saliva samples were tested with the SHERLOCK Direct method and RT-qPCR, paired nasopharyngeal swab samples were tested by RT-qPCR on the Alinity (Abbott) platform.

*Idylla
**Abbott M2000
***DiaSorin RT-qPCR.
13 and 30) concordant with the paired NP sample. The cross-sample concordance of the SHERLOCK Direct methodology (saliva) with the gold standard RT–qPCR (NP swab) was evaluated for 28 of the 30 samples; the exception being the 2 invalids indicated above. The SHERLOCK Direct method had an NPA of 100% (15/15, 95% CI LB 81.8) and a PPA of 88% (15/17, 95% CI 63.6, 98.5). The Ct (threshold cycle) value for the positive saliva samples ranged from 19.9–34.5 and from 3.13–31.7 for paired NP samples. The low Ct value (3.13) for sample 6 is likely since the Alinity system does not count the first 10 amplification cycles.

SHERLOCK Direct but positive by RT–qPCR for the paired NP swab. These saliva samples were also not detected with the Idylla assay and had a high Ct value with the Alinity assay (Table 1). This suggests these are either low positive samples exhibiting last stages of viral shedding, or are in an emerging infection state. In summary, overall concordance of the SHERLOCK Direct saliva method to the gold standard NP sample with extraction-based rRT–PCR was determined to be 93.33% (n = 28/30, 95% CI 77.96, 99.18).

Discussion

Most of the world’s population will not have access to a COVID-19 vaccine for many months; therefore, testing remains crucial for controlling the spread of the virus (24). As outbreaks throughout the globe continue to stretch testing capacities it remains critical to develop high-throughput and flexible molecular testing methods with differentiated supply chains to RT–qPCR. Here we demonstrated a high-throughput method for detecting SARS-CoV-2 direct from patient samples. This test can be run on common laboratory equipment, different from what is used for RT–qPCR assays, allowing for individual labs that are currently using COVID-19 RT–qPCR tests to increase their testing capacities. Implementation of this test can increase testing capacity that may enable more efficient and reliable contact tracing and decrease the spread of COVID-19.

Supplemental Material

Supplemental material is available at Clinical Chemistry online.

Nonstandard Abbreviations: CRISPR, clustered regularly interspaced short palindromic repeats; SARS-CoV-2, Sudden Acute Respiratory Syndrome-Coronavirus-2; SHERLOCK, Specific High-sensitivity Enzymatic Reporter unLOCKing; CDC, Centers for Disease Control and Prevention; RT–qPCR, quantitative PCR with reverse transcription; LAMP, loop-mediated isothermal amplification; RPA, recombinase polymerase amplification; FDA, Food and Drug Administration; NO, SARS-CoV-2 N; ORF, open reading frame; RP, Human RNase P Pop7; NTC, no-template control; UTM, universal transport media; VTM, viral transport media; LoD, limit of detection; NP, nasopharyngeal; PPA, positive percent agreement; NPA, negative percent agreement; CLIA, Clinical Laboratory Improvement Amendments; Ct, threshold cycle.

Human Genes: POP7, POP7 homolog, ribonuclease P/MRP subunit.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: B.J. Manning, J.M. Peña, E.S. Fiore, H. Boisvert, M.C. Tudino, M.K. Wilson, S. Singh, J.A. Mowatt, H.J. Thompson, and W.J. Blake: Sherlock Biosciences Inc.

Consultant or Advisory Role: None declared.
Stock Ownership: None declared.

Honoraria: None declared.

Research Funding: SHERLOCK has received financial support from the Good Venture Foundation in support of this work. Sherlock Biosciences provided reagents and equipment.

Expert Testimony: None declared.

Patents: Sherlock has submitted patent applications on the technology disclosed in the publication.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of the manuscript, or final approval of the manuscript.

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