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

Running head: High-Throughput SHERLOCK CRISPR SARS-CoV-2 Test

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Keywords

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Nonstandard Abbreviations

CRISPR, SARS-CoV-2, SHERLOCK, FDA, RT-qPCR, DNA, RNA, LAMP, N, ORF, NEB, NO, RP, RT, NTC, CDC, IDT, UTM, VTM, BEI, ATCC, EUA, NP, CLIA, PPA, NPA, HTP, DHMC, Ct

Human Genes

Gene symbol: *POP7,* Gene name: POP7 homolog, ribonuclease P/MRP subunit

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Abstract:

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 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 less than one hour.

RESULTS: Assay performance was evaluated with 105 (55 negative, 50 positive) remnant SARS-CoV-2 specimens previously identified as positive using Food and Drug Administration emergency use authorized assays and re-tested with a modified version of the Centers for Disease Control and Prevention (CDC) 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 percent 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.

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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 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 *L. wadei* (LwaCas13a) combined with isothermal amplification [15,16]. 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-CoV2 [19,20]. LAMP based amplification methods are attractive for diagnostics because LAMP has been reported to be more tolerant than PCR to

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endogenous inhibitors present in biological samples [21,22]. 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 (EUA) 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[17]. 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 [18].

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 two 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

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advancements enable a simpler workflow compatible with liquid handling instrumentation that can enable testing up to 5000 patient samples per day (24 hours) based on a single operator and instrument.

Methods

SARS-CoV-2 Viral Control Material

NATtrol SARS-CoV-2 (NATSARS(COV2)-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 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-CoV2 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 20mg/ml Cat No:P8107S) was added 1µL per 9µL of sample. Samples were

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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 10µL of NEB 2x WarmStart® LAMP kit (E1700L) with 2µL of 10x primer mix for each NO (SARS-CoV-2 N and ORF) and RP (Human RNAse P Pop7). A total of 8µL of extracted RNA was added to 12µL of each LAMP reactions for NO and RP; a total of 2 reactions per sample. For Proteinase K and heat-treated samples 4µL of treated sample plus 4µl of water were added to 12µl of LAMP reaction. Lastly, 20µl 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) and were then heated at 61˚ for 30-40 min and allowed to cool to RT before addition of Cas master mix. While the LAMP reaction was proceeding, the Cas master mix was made. The Cas master mix consists of: 25U T7 RNA Polymerase, 25.2U Murine RNase Inhibitor, 2.5mmol/L rNTP mix, 312nmol/L RNase Alert, 23 mmol/L MgCl2, 63.5 µg/ml LwaCas13 and 22.5nmol/L CRISPER RNA (crRNA) 10µl of Cas master mix was directly added to the 20µL LAMP reaction below the mineral oil layer. After addition of Cas mix, plates were put immediately on a plate reader and pre-heated to 37˚C. Fluorescence was read every 2 minutes over a span of 5 reads for a total of 10 minutes. The Biotek Neo2, Tecan Infinite M Plex, and ThermoFisher Fluoroskan FL were evaluated using an excitation wavelength of 485nm for all three and an emission wavelength of 528nm, 525 nm and 538 nm respectively. A detailed protocol is provided in the online **Supplemental Data**.

SHERLOCK data analysis

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To determine if the negative control is valid the fluorescent ratio of the fluorescent value at 10 minutes divided by the fluorescent value at 0 minutes must be less than 3. If the No Template Control (NTC) is valid calculations for the samples are determined. To determine if a sample is positive the ratio of the fluorescent reading at 10 min for the sample is divided by the fluorescent reading of the NTC at 10 minutes. If the value is above 5 the sample is positive.

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 100µL sample input and 100µL elution volume. CDC primers/probes (IDT 10006770) were used for all assays. For all assays three RT-qPCR reactions were performed per sample for the N1, N2 and RP targets. For RNA extracted samples, 5µL of extracted RNA was combined with 1.5µL primer/probe mix, 5µL of TaqPathTM 1-Step RT-qPCR Master Mix, CG (A15300) and 8.5µL 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 95°C for 3 sec then 55.0°C for 30 sec. 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. 5µL of heat-treated samples were added to 5µL UltraPlex 1-Step ToughMix (4X), 1.5µL CDC primer/probe mix and 8.5µL of water. Samples were put on the QS5 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 sec, then 55.0°C for 30 sec.

Contrived Samples

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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 (Cat# NATSARS(COV2)-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 concentration with a minimum of 20 replicates at a given concentration.

Clinical Samples

Remnant nasopharyngeal swab samples in saline, VTM, 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 and 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 above.

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

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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.

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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 (**Fig. 1**.). Extracted guide 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 less than 30 minutes 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 minutes at 37°C. With this method a single operator can process 190 samples in 70 minutes (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 three

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 three kits had comparable LoDs. With this workflow, our LoD was 2cp/µl (20/20 ,100%) (**Fig. 2A**.). We also compared the sensitivity of three fluorescent plate readers (Biotek Neo2, Tecan MPlex and Fluoroskan Microplate Fluorometer) to expand accessibility of our assay. All three plate readers had similar LoD of viral genomic RNA spiked into the reaction (4cp/µl for the Tecan, 2cp/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 towards 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 labs and confirmed using a modified CDC EUA protocol (online **Supplemental Table S7**), extracted using the MagMAX Viral RNA isolation kit, with a 30-minute LAMP reaction. Our clinical evaluation resulted in a PPA (Positive predicted agreement) of 100% (30/30) and a NPA (Negative predicted agreement) of 100% (30/30) for the SHERLOCK high throughput workflow (**Figure 2 B-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

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issues associated with consistent shortages of RNA extraction materials [23]. 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 minutes at 65ºC followed by 98ºC for 3 minutes to heat-kill the proteinase K enzyme, followed by cooling to 4-10ºC. 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 above, with the amplification time extended to 40 minutes. With an automated implementation parallel processing four 384 well plates, this SHERLOCK Direct method can process 5211 samples per day (online **Supplemental Table S8**). Saline has become a commonly used storage solution for nasal swabs [24] with high levels of accuracy and stability. We tested the compatibility of our high-throughput workflow with NP swabs in saline with the SHERLOCK Direct workflow. First, we established the LoD of our workflow with saline spiked with SARS-CoV-2 inactivated particles from 100cp/ µl to 0.01 cp/µl. Our LoD for contrived saline samples was 10cp/µl (22/23, 95.7%) (**Figure 3A**). This is fivefold higher than the LoD observed when using extraction, **Figure 2A**, this can partially be attributed to the 4x concentration of sample when using the MagMAX Viral extraction kit. We then tested the clinical applicability of our SHERLOCK Direct method on clinically collected NP swabs stored in 0.9% saline, 20 positive and 25 negative samples. All samples were purchased from a biobank after having been tested and confirmed COVID positive or negative by an outside CLIA lab. 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 percent agreement (PPA) for the SHERLOCK Direct high

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throughput method in saline was 100% (25/25) and the negative percent agreement (NPA) was 100% (20/20, **Figure 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, 5cp/µl in pooled saliva (online **Supplemental Table S10**). We then transferred the method to an external clinical laboratory who successfully implemented a partial workflow (excluding sample accessioning) on a Hamilton Microlab STAR system. Using this system, a sensitivity as low at 12.5 cp/µL in pooled saliva has been demonstrated (online **Supplemental Figure 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 Figure S2**).

High-throughput SHERLOCK Direct Clinical Saliva testing

The SHERLOCK Direct HTP method was evaluated in the Laboratory for Clinical Genomics and Advanced Technology at the Dartmouth Hitchcock Medical Center (DHMC). 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 three 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 three different nucleic acid extraction based RT-qPCR platforms, Alinity (Abbott), M2000 (Abbott) and Diasorin RT-PCR (DiaSorin Molecular).

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The SHERLOCK-direct method detected SARS-CoV-2 virus in 15 out of 30 saliva samples. The remaining two samples gave invalid findings. Invalid results for these two 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 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 two 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 (cycle threshold) 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[25]. Saliva samples #27 and 28 were negative by SHERLOCK direct but positive by RT-qPCR for the paired NP swab. The saliva sample was 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 [26]. As outbreaks

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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-CoV2 down to 2 copies/µL using a high throughput magnetic bead-based purification of patient samples, or 10 copies/µL direct from patient samples. We also verified key improvements to the SHERLOCK CRISPR SARS-CoV-2 EUA protocol that resulted in an increased number of samples processed and a decreased time to result. With this high throughput method, up to 5000 samples can be processed with a single liquid handling robot per day. The simple workflow is designed to be automation compatible with the end-to-end process implemented in a single 384 well plate. The inclusion of two independent SARS-CoV-2 viral targets in a single amplification reaction reduces the risk of reduced inclusivity due to viral mutation while maintaining a low cost per test.

Additionally, we demonstrated that this method is compatible with multiple plate readers and magnetic bead RNA isolation kits. The high-throughput SHERLOCK CRISPR test shows 100% specificity and 100% sensitivity with extracted remnant clinical samples in both direct testing (45/45) or with a magnetic bead-based extraction (60/60). When evaluated at an external clinical laboratory the high-throughput SHERLOCK direct method with saliva samples demonstrated high concordance (93%) with extraction-based RT-qPCR methods from nasopharyngeal swab samples.

In this study, the high throughput SHERLOCK direct method demonstrated results in a user friendly, fast, inexpensive, and robust method for detecting SARS-CoV-2 direct from patient samples. This test can be run on common lab equipment, different from what is used

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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.

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	Saliva Sample		NP Sample
Sample #	**Sherlock Result**	**RT-qPCR Result (Ct)**	**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	23.1
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

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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

Table 1. SHERLOCK Direct Saliva Clinical Evaluation. 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

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Figure Captions

Figure 1. High Throughput SHERLOCK Direct SARS-CoV-2 Test Scheme and Workflow using NP swab eluted in Saline

Figure 2. High-throughput SHERLOCK LoD and Clinical evaluation on Extracted NP samples. A) LoD using MagMAX Viral RNA isolation kit of negative NP matrix spiked with Zeptometrix NATtrol SARS-CoV-2 viral particles with 30min LAMP amplification step. B) 30 positive clinical samples were tested by the CDC EUA method or SHERLOCK high-throughput method after MagMAX Viral RNA isolation. 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 minutes after the addition of the Cas detection mix. Dotted line represents a ratio of 5, the cutoff for a positive sample. C) 30 negative clinical NP samples were extracted with the MagMAX Viral RNA Kit with a 30 min LAMP amplification step. 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 Dotted line represents a ratio of 5, the cutoff for a positive sample. D) This depicts the clinical agreement between each assay.

Figure 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.

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4µL of each sample was used for the SARS-CoV2 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 minutes 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 qRT-PCR, and their fluorescence ratios were determined by SHERLOCK Direct. D) The clinical agreement between each assay.

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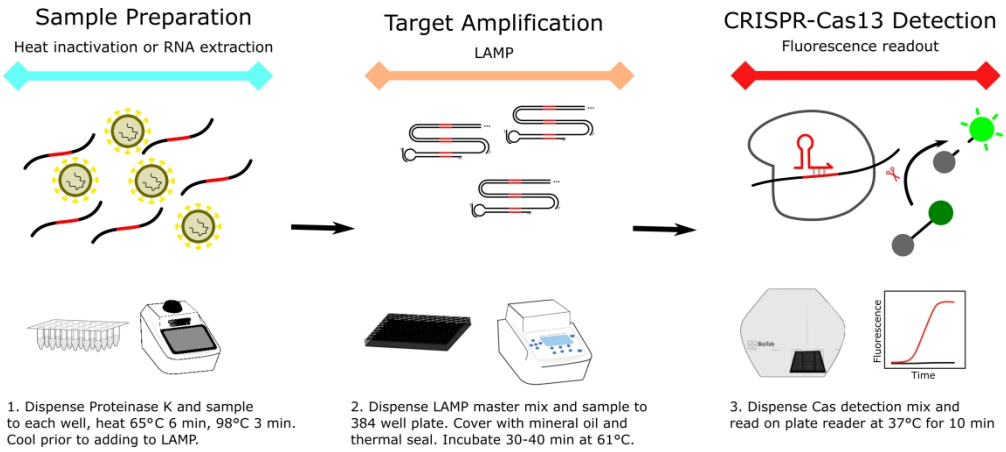


Figure 1. High Throughput SHERLOCK Direct SARS-CoV-2 Test Scheme and Workflow using NP swab eluted in Saline

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A

SARS-CoV-2 Zeptometrix concentration in NP matrix(cp/µL)	Positive NO duplex	Positive RP
100	3/3	3/3
10	3/3	3/3
4	20/20	20/20
2	20/20	20/20
1	20/23	23/23
0.5	15/20	20/20
0.1	0/3	3/3
0	0/20	20/20

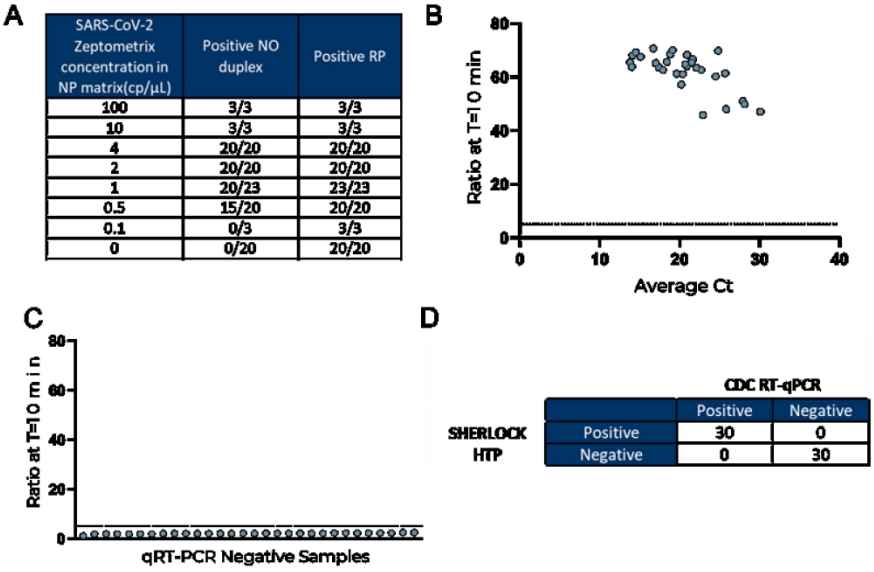


Figure 2. High-throughput SHERLOCK LoD and Clinical evaluation on Extracted NP samples. A) LoD using MagMAX Viral RNA isolation kit of negative NP matrix spiked with Zeptometrix NATtrol SARS-CoV-2 viral particles with 30min LAMP amplification step. B) LoD using negative nasal matrix spiked with heat-inactivated SARS-CoV-2 viral particles (FDA provided control material) with a 40 min LAMP amplification. C) 30 positive samples were tested by the CDC EUA method or SHERLOCK high-throughput method after MagMAX Viral RNA isolation. 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 minutes after the addition of the Cas detection mix. Dotted line represents a ratio of 5, the cutoff for a positive sample. D) 30 negative clinical NP samples were extracted with the MagMAX Viral RNA Kit with a 30 min LAMP amplification step. Signal for each sample is reported as the fluorescence ratio of the sample divided by the florescence of the NTC 10 min after addition of Cas mixture to the LAMP reaction incubated at 37°C Dotted line represents a ratio of 5, the cutoff for a positive sample.

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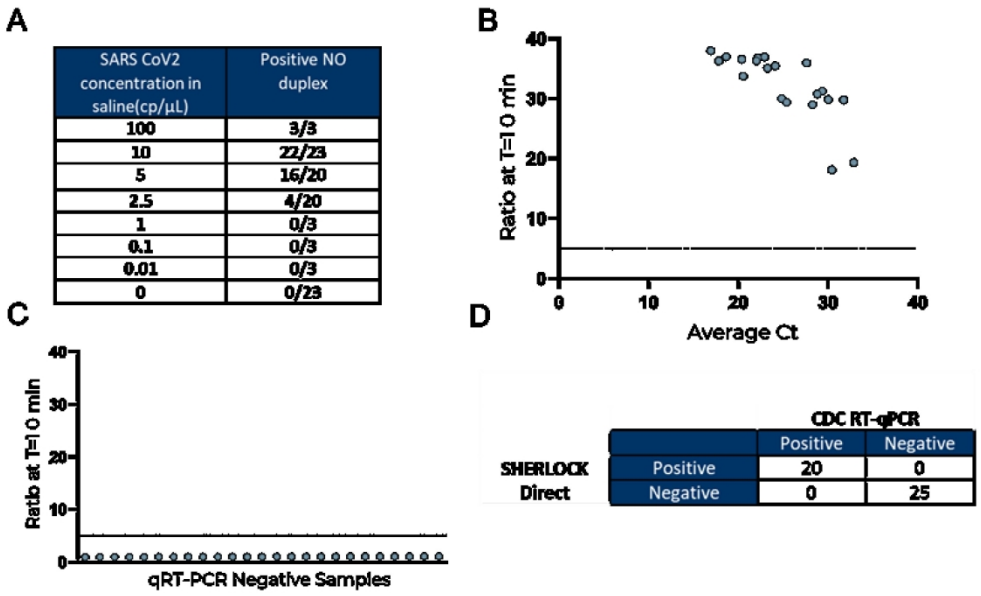


Figure 3. SHERLOCK Direct method on NP saline samples. A) LoD confirmation using NATtrol SARS-CoV-2 viral particles spiked into saline. Zeptometrix NATtrol SARS-CoV-2 viral particles were spiked into saline (0.9% NaCl) at indicated concentrations. Each sample was treated with Proteinase K and heated at 65˚C for 6 min and 98˚C for 3 min. 4µL of each sample was used for the SARS-CoV2 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 minutes 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 qRT-PCR, and their fluorescence ratios were determined by SHERLOCK Direct. D) The clinical agreement between each assay.

137x83mm (600 x 600 DPI)

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