



Evaluation of the Liberty16 Mobile Real Time PCR Device for Use With the SalivaDirect Assay for SARS-CoV-2 Testing

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The COVID-19 pandemic has highlighted the need and benefits for all communities to be permitted timely access to on-demand screening for infectious respiratory diseases. This can be achieved with simplified testing approaches and affordable access to core resources. While RT-qPCR-based tests remain the gold standard for SARS-CoV-2 detection due to their high sensitivity, implementation of testing requires high upfront costs to obtain the necessary instrumentation. This is particularly restrictive in low-resource settings. The Ubiquitome Liberty16 system was developed as an inexpensive, portable, battery-operated single-channel RT-qPCR device with an associated iPhone app to simplify assay set-up and data reporting. When coupled with the SalivaDirect protocol for testing saliva samples for SARS-CoV-2, the Liberty16 device yielded a limit of detection (LOD) of 12 SARS-CoV-2 RNA copies/ μ L, comparable to the upper end of the LOD range for the standard SalivaDirect protocol when performed on larger RT-qPCR instruments. While further optimization may deliver even greater sensitivity and assay speed, findings from this study indicate that small portable devices such as the Liberty16 can deliver reliable results and provide the opportunity to further increase access to gold standard SARS-CoV-2 testing.

Keywords: SARS-CoV-2, COVID-19, diagnostics, saliva, PCR

INTRODUCTION

Timely access to SARS-CoV-2 tests remains a crucial factor in effective clinical and community-wide management of COVID-19 (Larremore et al., 2021). Despite a rapid evolution of alternative clinical diagnostic and surveillance testing approaches, their novelty has perhaps limited their widespread implementation in the field (Mardian et al., 2021). For many, RT-qPCR-based tests remain the gold standard due to their high sensitivity (MacKay et al., 2020). Implementation of RT-qPCR testing can be impeded however, by the high upfront costs required to obtain the necessary instrumentation. This is particularly restrictive in low resource settings. To provide an alternative to some of the limitations presented by traditional RT-PCR testing, the Liberty16 system (Ubiquitome Ltd, Auckland, New Zealand) was developed as an inexpensive, portable, battery-operated single-

channel RT-qPCR device with an associated iPhone app to simplify assay set-up and data reporting. Similarly, the SalivaDirect PCR-based SARS-CoV-2 assay (Vogels et al., 2021) was developed to expand testing capacity by removing specialized reagents, equipment, and time components to decrease the time and cost of nucleic acid extraction. As the SalivaDirect protocol can still be constrained in resource-limited settings by lack of access to RT-qPCR devices, we evaluated the performance of the novel Liberty16 system for detection of SARS-CoV-2 in saliva using a singleplex version of the SalivaDirect workflow.

METHODS

Limit of Detection

A limit of detection (LOD) range finding study was conducted to compare a singleplex version of the SalivaDirect protocol (Vogels et al., 2020) run on the Liberty16 System as compared to the standard dualplex protocol run on the BioRad CFX96 Touch. Briefly, the SalivaDirect protocol takes self-collected, raw saliva samples, to which proteinase K is added before vortexing vigorously for one minute. Samples are then heated at 95°C for 5 minutes to inactivate the proteinase K before proceeding with RT-qPCR testing. While the Liberty16-modified protocol still used the US CDCs FAM-labelled real-time RT-qPCR primer/probe sets for 2019-nCoV_N1 and the human *RNaseP* (RP) as an extraction control, these were tested on saliva lysates in separate reactions due to the single-channel nature of the instrument. Primer and probe concentrations were the same as that used for the SalivaDirect assay (Vogels et al., 2020). In all other respects the assay was performed as described in the SalivaDirect protocol (Vogels et al., 2020) using the Thermo Fisher Proteinase K (A42363) and New England Biolabs Luna Universal Probe One-Step RT-qPCR (2x) kit (E3006).

For the LOD range finding study, a SARS-CoV-2 positive saliva specimen, collected in accordance with the Yale University HIC-approved protocol #2000027690, with a known virus concentration (3.7×10^4 copies/ μ L) was spiked into saliva negative (HIC-approved protocol #2000027690) for SARS-CoV-2 using the CDC assay (Wyllie et al., 2020). The following 2-fold dilution series was tested in triplicate to determine the preliminary LOD: 100, 50, 25, 12.5, 6.25, 3.125, and 1.5 copies/ μ L. The preliminary LOD was then confirmed with 20 additional replicates.

Importantly, the spiked saliva samples used here for the LOD determination of the Liberty16 are the same sample set that has been used to validate the SalivaDirect protocol on numerous other RT-qPCR instruments (USFDA, 2020).

Confirmatory Testing and Protocol Optimization

For the testing of clinical specimens 31 de-identified saliva specimens previously tested by the standard SalivaDirect protocol (Vogels et al., 2020) were selected for assay verification (Yale University HIC-approved protocol #2000029551). The 31 specimens represented an array of 30 positive samples (Ct values

23-39.8) and 1 negative sample (Ct value ND, not detected). The saliva samples were processed by the SalivaDirect protocol, with PCR-testing performed on the Liberty16 device. Each sample was tested for SARS-CoV-2 N1 twice using both the standard SalivaDirect PCR settings used universally across all other validated instruments (95°C, 10s; 55°C, 30s) and an additional fast cycling protocol (95°C, 2s; 55°C, 5s). The same reverse transcriptase activation conditions (52°C, 10min; 95°C, 2 min) were used for both runs. Differences between the two protocols were assessed for statistical significance using the Wilcoxon Signed Rank test for non-parametric data.

RESULTS

The LOD of the Liberty16 SalivaDirect Assay Is Comparable to Other PCR Instruments

A preliminary range-finding study using triplicate samples across a two-fold dilution series indicated an LOD of between 6 copies/ μ L (0/3 samples detected) and 12 copies/ μ L (3/3 detected; mean Ct value = 37.7) (**Supplementary Data**). The same samples run in parallel on the Biorad CFX96 Touch using the standard dualplex assay at 12 copies/ μ L yielded an average Ct value of 36.00. The LOD for the Liberty16 system was confirmed at 12 copies/ μ L with 20/20 samples positive and an average Ct value of 35.18 (standard deviation = 0.71; **Figure 1**). Moreover, by testing

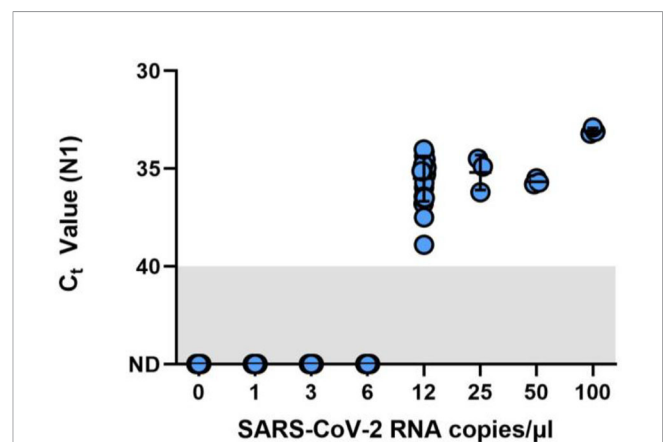


FIGURE 1 | Limit of detection (LOD) finding study for the SalivaDirect protocol adapted for RT-PCR testing by the Liberty16 instrument. A SARS-CoV-2 positive saliva specimen with a known virus concentration (3.7×10^4 copies/ μ L) was spiked into saliva negative for SARS-CoV-2 to create the following 2-fold dilution series: 100, 50, 25, 12.5, 6.25, 3.125, and 1.5 copies/ μ L. Spiked saliva samples were tested in triplicate to determine the preliminary LOD using the SalivaDirect protocol and tested using the NEB Luna 2x PCR mastermix and run on the Liberty16 system using the standard PCR (95°C, 10s; 55°C, 30s) and reverse transcriptase activation conditions (52°C, 10min; 95°C, 2 min). The preliminary LOD was determined as between 6 copies/ μ L (0/3 samples detected) and 12 copies/ μ L (3/3 detected). An additional 20 replicates of 6 copies/ μ L and 12 copies/ μ L were tested, confirming the LOD for the Liberty16 system as 12 copies/ μ L with 20/20 samples positive.

the same sample set as was used to validate the LOD of more complex RT-qPCR instruments (USFDA, 2020), we can verify that the performance of the Liberty16 versus the CFX96 Touch is comparable to the performance as other RT-qPCR instruments which were also directly compared to the CFX96 Touch.

Fast Cycling Protocol Reduces Run Time Without Compromising N1 Detection

As compared to the standard SalivaDirect protocol, the fast PCR cycling protocol completed in under one hour (~57), saving more than 25 minutes. Despite the fast run time, comparative analysis of clinical samples (30 positive and 1 negative) revealed a high level of concordance between N1 values for the fast and regular protocols (median Ct difference = 1.37; Wilcoxon $p > 0.1$; **Figure 2**). Ten samples were not detected (NA) by either protocol. All were previously shown to have Ct value at or below the limit of detection of the Liberty16 device running the SalivaDirect assay.

With the current throughput of samples using the SalivaDirect protocol on the Liberty16 device being 6 samples plus two controls per ~84-minute run, this would allow for 5 complete runs (30 samples) per day. The faster run time (~57 minutes) enables completion of 8 runs per day, increasing the sample throughput by 60% to 48 samples per day.

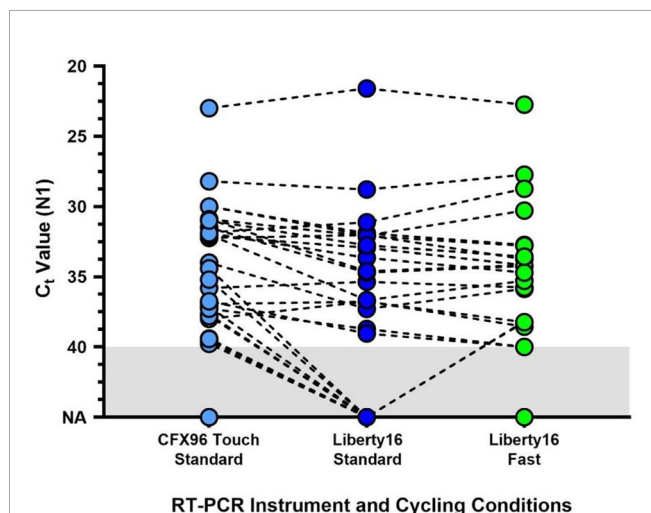


FIGURE 2 | Comparison of the standard SalivaDirect PCR and updated fast cycling protocols on SARS-CoV-2 virus RNA N1 detection. De-identified clinical saliva samples, previously tested using the standard SalivaDirect protocol on the CFX96 Touch, using the NEB Luna 2x PCR mastermix were also run on the Liberty16 system using the standard RT-qPCR (95°C, 10s; 55°C, 30s) or fast RT-qPCR (95°C, 2s; 55°C, 5s) cycling conditions. The same reverse transcriptase activation conditions (52°C, 10min; 95°C, 2 min) were used for both runs. The resulting SARS-CoV-2 N1 (Ct) values did not differ between either Liberty16 protocol (Wilcoxon $p > 0.1$). Two out of 21 sample pairs (10%) yielded not detected (ND) values when run using the standard protocol, while 2 pairs yielded Ct values of 40 and 41 respectively. Ten samples, previously positive when tested on the CFX96 Touch were not detected by either Liberty16 protocol. All were previously shown to have Ct values at or below the limit of detection of the Liberty16 device running the SalivaDirect assay.

CONCLUSIONS

Access to reliable SARS-CoV-2 testing and to vaccines are the two factors currently dividing humanity in the race to minimise COVID-19 mortality and long-term morbidity. Until global vaccination rates reach levels sufficient to provide herd immunity, the first line of defence against widespread disease is timely and accurate screening for infection across all sectors of the community (Ferretti et al., 2020). Current barriers to testing include public dislike of inconvenient and/or uncomfortable sample collection means such as clinician-administered nasopharyngeal swabs as well as availability of specialist testing equipment, reagents and expertise for sample analysis (Ali et al., 2021). Use of saliva as the testing matrix resolves the first issue, being non-invasive and able to be carried out with simple instructions by a non-specialist in the home environment (Allicock et al., 2021).

The SalivaDirect test is highly sensitive and specific for SARS-CoV-2 detection (USFDA, 2020), yet is uniquely situated among US FDA Emergency Use Authorization tests with a protocol that offers a range of RT-qPCR device and reagent options with reported LODs ranging from 3-12 copies/ μ L (USFDA, 2020) with the singleplex protocol performed on the Liberty16 device yielding results comparable to the upper end of that LOD range. While the throughput of the Liberty16 device is modest (6 samples/run) its portability, and comparatively low cost (USD \$5,995), make this an affordable option for standing up new testing capability in remote and/or resource constrained environments. Furthermore, the initial run-time optimization reported here signals an opportunity to increase sample throughput by more than 60% by simply modifying the PCR run protocol. While further optimization may deliver even greater sensitivity and assay speed, this initial study indicates that small portable devices such as the Liberty16 can deliver reliable results and provide the opportunity to further increase access to gold standard testing capability.

Looking to the future, the COVID-19 pandemic has highlighted the need and benefits for all communities to be permitted timely access to on-demand screening for infectious respiratory diseases, including seasonal viruses such as RSV and influenza. This can be achieved with simplified testing approaches and affordable access to core resources.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

ST, PP, and AW conceived the study. DY-C, ST, and AW developed the study protocol. DY-C, DT, and NV performed the experimental work. DY-C, ST, and AW analyzed the data. DY-C, ST, and AW wrote and edited the manuscript. All other co-authors reviewed, edited and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcimb.2021.808773/full#supplementary-material>

Conflict of Interest: ST was an employee of Ubiquitome Limited at the time of this study and PP is the CEO of Ubiquitome Limited.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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