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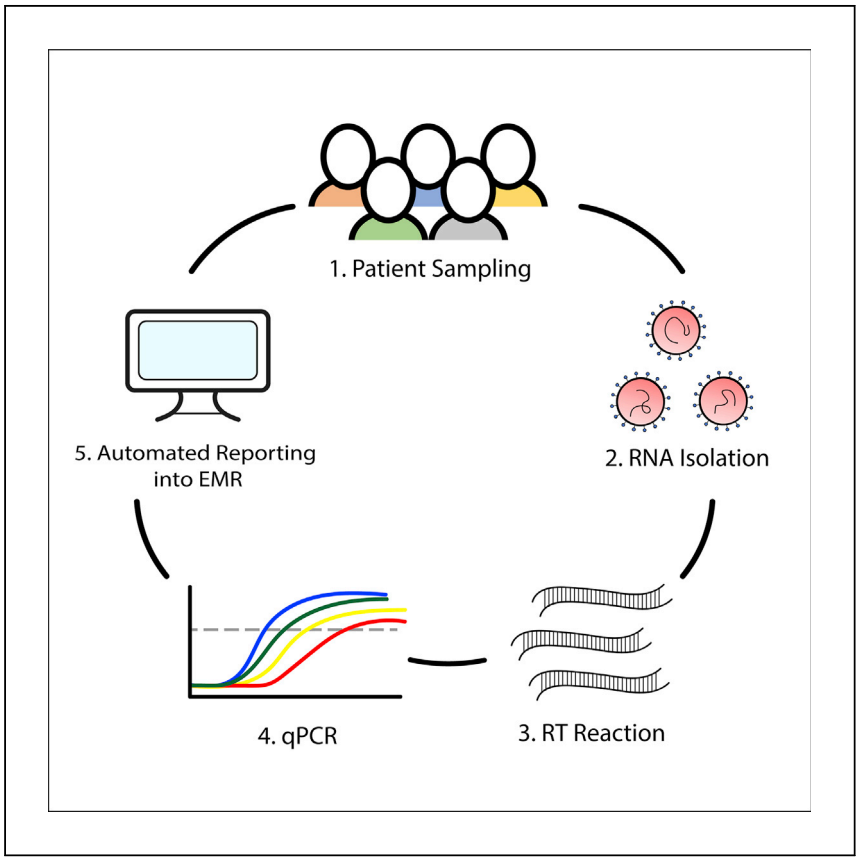
Rapid Implementation of a SARS-CoV-2 Diagnostic Quantitative Real-Time PCR Test with Emergency Use Authorization at a Large Academic Safety Net Hospital



In this study, we provide a blueprint to quickly "stand-up" an in-house SARS-CoV-2 (COVID-19) RT-PCR-based diagnostic assay at a large academic safety net hospital that predominantly serves at-risk and underserved populations, resulting in greatly improved turnaround times and conservation of PPE.

Foundational Research

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HIGHLIGHTS

Basic research laboratories can be repurposed into COVID-19 testing sites

Widespread testing is essential in underserved communities to contain viral spread

This study offers a blueprint to navigate challenges in implementing in-house testing

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Rapid Implementation of a SARS-CoV-2 Diagnostic Quantitative Real-Time PCR Test with Emergency Use Authorization at a Large Academic Safety Net Hospital

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SUMMARY

Background: Significant delays in the rapid development and distribution of diagnostic testing for SARS-CoV-2 (COVID-19) infection have prevented adequate public health management of the disease, impacting the timely mapping of viral spread and the conservation of personal protective equipment. Furthermore, vulnerable populations, such as those served by the Boston Medical Center (BMC), the largest safety net hospital in New England, represent a high-risk group across multiple dimensions, including a higher prevalence of pre-existing conditions and substance use disorders, lower health maintenance, unstable housing, and a propensity for rapid community spread, highlighting the urgent need for expedient and reliable in-house testing.

Methods: We developed a SARS-CoV-2 diagnostic medium-throughput qRT-PCR assay with rapid turnaround time and utilized this Clinical Laboratory Improvement Amendments (CLIA)-certified assay for testing nasopharyngeal swab samples from BMC patients, with emergency authorization from the Food and Drug Administration (FDA) and the Massachusetts Department of Public Health.

Findings: The in-house testing platform displayed robust accuracy and reliability in validation studies and reduced institutional sample turnaround time from 5–7 days to less than 24 h. Of over 1,000 unique patient samples tested, 44.1% were positive for SARS-CoV-2 infection.

Conclusions: This work provides a blueprint for academic centers and community hospitals lacking automated laboratory machinery to implement rapid in-house testing.

Funding: This study was supported by funding from the Boston University School of Medicine, the National Institutes of Health, Boston Medical Center, and the Massachusetts Consortium on Pathogen Readiness (MASS CPR).

Context and Significance

In a setting of widespread community transmission of SARS-CoV-2 (COVID-19), it is vital to rapidly and reliably identify and isolate cases to mitigate and ultimately eradicate viral spread. National failures to launch widespread testing in the United States have necessitated academic and non-governmental institutions to fill this critical unmet need. Furthermore, it is particularly important for testing to be widely available to at-risk communities, which are greatly impacted by the current pandemic. This study describes the rapid implementation of a COVID-19 diagnostic test at a large safety net hospital that primarily treats an underserved population and aims to provide a blueprint for other institutions to navigate the logistical and regulatory challenges to provide equitable access to COVID-19 testing for all.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is now a major global public health crisis. In the United States, significant delays in the rapid development and distribution of diagnostic testing for SARS-CoV-2 infection have prevented adequate COVID-19 patient care and public health management of the pandemic,[1] impacting the timely mapping of the dynamics of viral spread in the general population, and more topically, the conservation of personal protective equipment.

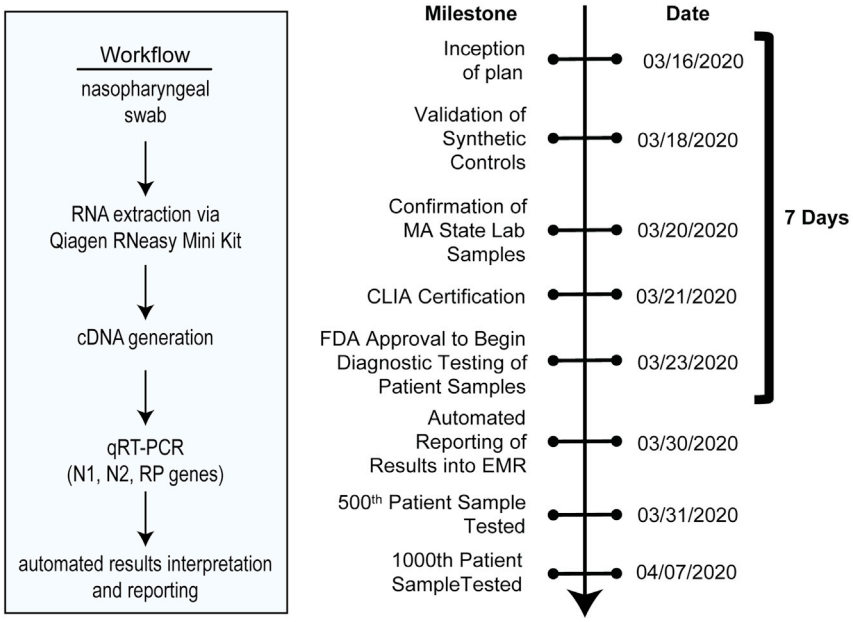

Figure 1. Workflow of a Quantitative Real-Time PCR-Based SARS-CoV-2 Diagnostic Assay
Schematic of our in-house SARS-CoV-2 diagnostic assay workflow, depicting the steps (left) and overall time (right) from inception of platform to approvals and active testing.

Furthermore, vulnerable populations, such as those served by the Boston Medical Center (BMC), the largest safety net hospital in New England, represent a high-risk group across multiple dimensions, including a higher prevalence of pre-existing conditions and substance use disorders, lower general health maintenance, unstable housing, and a propensity for rapid community spread, highlighting the urgent need for rapid and reliable in-house testing infrastructure.

RESULTS

We requested and received emergency permission from the Food and Drug Administration (FDA) and the Commonwealth of Massachusetts Department of Public Health to repurpose a BMC-affiliated biosafety level 2 plus (BSL-2+) basic science research facility into a clinical diagnostic laboratory for high complexity testing. It now functions as an approved site of the BMC Department of Pathology & Laboratory Medicine, as an extension of their Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists (CAP) accredited space. Subsequently, we implemented a quantitative real-time PCR-based assay to detect viral SARS-CoV-2 RNA from nasopharyngeal swab, based on guidelines from the Centers for Disease Control and Prevention (CDC) and the FDA for use with in-house testing of BMC patient samples (Figure 1).[2,3] RNA extraction from patient swabs was performed via the QIAGEN RNeasy Minikit (catalog [Cat] #74106) due to its relative abundance in the context of a limited supply of similar reagents. cDNA was prepared from patient sample RNA, followed by quantitative real-time PCR to interrogate expression of the SARS-CoV-2 nucleocapsid protein RNA (N1, N2) as well as human ribonuclease P (RNase P; internal housekeeping control) (IDT Cat #10006606). The assay has been submitted for approval under an FDA Emergency Use Authorization (EUA).

Our medium-throughput assay enables the simultaneous testing of over 50 patient samples on a single 384-well quantitative real-time PCR plate (including requisite

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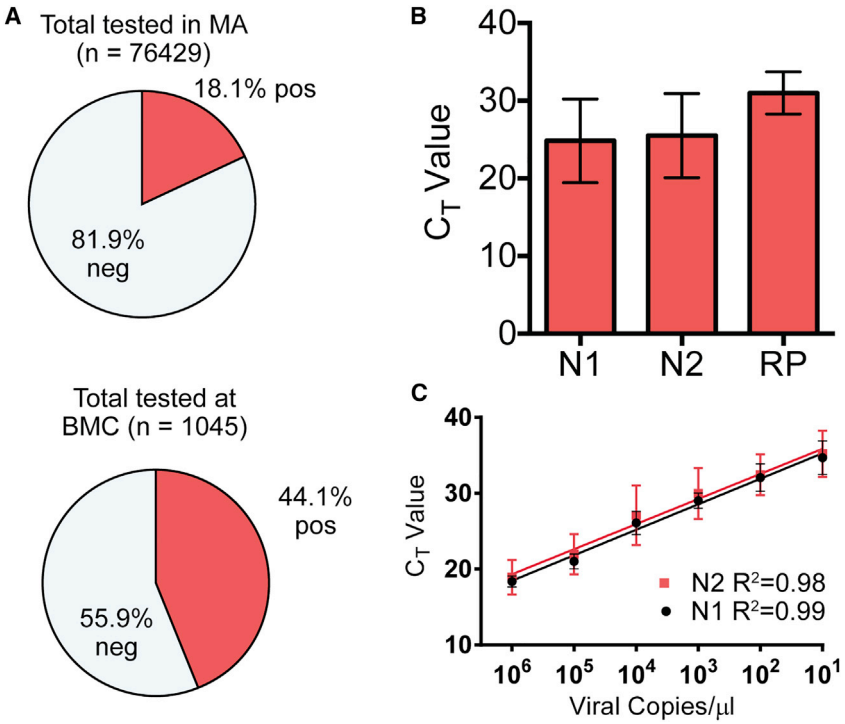

Figure 2. SARS-CoV-2 Testing and Quantitative Real-Time PCR Validation Metrics

(A) Visualization of testing across Massachusetts and at Boston Medical Center, including total tests performed and percentage of positive results (based on publicly available data as of April 7, 2020). (B) Average cycle threshold (C_T) values for each gene probed across 96 SARS-CoV-2-positive patients (error bars represent standard deviation). (C) Visualization of standard curves across multiple real-time PCR runs, with linear regression and R^2 values displayed for each probe (n = 6, error bars represent standard deviation).

controls). In order to minimize errors associated with manual interpretation and data transfer, we generated an automated data analysis and reporting pipeline capable of importing unmanipulated quantitative real-time PCR data (including cycle threshold values for each gene per patient sample). All team members completed expedited BMC onboarding, including protected health information (PHI) training. This automated pipeline subsequently interfaces with our institution's laboratory information system (SunQuest), allowing for direct upload into BMC's electronic medical record (Epic), further reducing overall turnaround time from sample intake to result reporting. This script is publicly available (https://github.com/TaylorMatte/Quant6-Covid_Analysis).

Following FDA EUA submission requirements, our in-house SARS-CoV-2 quantitative real-time PCR laboratory-derived test (LDT) was launched March 23, 2020. We are currently actively testing both BMC patients and healthcare workers. Notably, the development of the described in-house testing platform greatly reduced our institutional sample turnaround time (TAT) to under 24 h, which had previously been 5–7 days when specimens were sent out to commercial entities and state public health agencies.

As of April 6, 2020, we successfully completed testing on >1,000 unique patient samples, of which 44.1% were positive for SARS-CoV-2 infection, a higher ratio than other Massachusetts testing facilities per publicly available datasets

(Figure 2A). Our assay has displayed robust accuracy and reliability in validation studies performed to fulfill FDA requirements, with a limit of detection (LOD) of 10^1 viral copies/μL (based on serial dilutions of commercially available positive control from ATCC (Cat #VR-3276T) and an average cycle threshold value (in positive samples) for the N1 probe of 24.83 ± 5.35 (n = 94, mean ± SD) (Figure 2B). Additionally, our standard curve shows minimal run-to-run variability (n = 6) (Figure 2C).

DISCUSSION

In response to a critical testing shortage in our large urban safety net academic medical center, we converted our research laboratory to a CLIA-certified SARS-CoV-2 diagnostic facility in 7 days. Notably, our ability to extend CLIA certification from a BMC pathology clinical facility to include basic research laboratory space was unprecedented and instrumental in our ability to test patient samples in an expedient manner, particularly at a point during the pandemic when the commercial laboratory TAT for samples sent from our hospital ranged from 5–10 days. Although our hospital does possess a high throughput Roche Cobas system for molecular testing, a nationwide shortage in the supply and distribution of SARS-CoV-2-specific kits led us to adapt and implement an in-house quantitative real-time PCR-based FDA EUA diagnostic using readily sourced reagents. Our test offers a more rapid TAT in comparison to currently available commercial or state laboratory facilities and enables clinicians and patients to make more informed decisions with significant personal and public health ramifications.

Following the initial inception of this assay, and to increase the utility of our platform, bridging studies have now been performed to add additional flexibility and throughput. Notably, we have the ability to run a one-step PCR reaction (omitting a separate reverse transcription cDNA step) to multiplex samples using multiple fluorophores and validate multiple swab methodologies to overcome potential bottlenecks in the supply chain. Our ability to perform several variations of our core assay ensures that, if any single element of our supply chain becomes unstable, we can seamlessly pivot to an alternate approved strategy under our EUA. Furthermore, in contrast to commercial automated sample-to-answer platforms, the inherent granularity of our system is also amenable to experimental validation studies and COVID-19-related scientific inquiry.

Our 44.1% rate of positivity is higher than the overall state average of 18.1%, a potential indicator that we are sampling from a higher risk population. Given that our hospital takes care of a large portion of Boston's homeless and housing unstable patients, rapid testing is vital to mitigate community spread in an already vulnerable population living in low resource settings in which social distancing is not possible. Based on publicly available data, 46% (roughly 4 in 10 patients) of BMC's inpatient census on April 6 and April 7 of 2020 (approaching the peak of the pandemic in Massachusetts) was comprised of COVID-19 patients.[4] This point is further highlighted by our urban location, as zip codes in socioeconomically disadvantaged neighborhoods in Boston have higher than average levels of COVID-19 when compared to city-wide data.[5] Taken together, these data reflect emerging preliminary numbers of COVID-19 outcomes from several states and cities across the country that suggest that ethnic minorities and the underserved face higher risk of significant novel coronavirus-related disease.

Finally, we have been able to implement comprehensive testing before larger more well-resourced institutions in our community, suggesting that the template and

protocol we have generated and validated could be useful for other smaller community hospitals lacking capital intensive automated clinical laboratory machinery to run molecular biology assays.

Limitations of Study

It is important to note that LDTs, such as the one described herein, must be validated for reagents, hardware, and software by individual sites that implement their own in-house testing assays. Unlike sample to answer assays, which are primarily run on commercially available clinical diagnostic hardware, our LDT enables customization at various stages of the assay in response to reagent supply chain availability. However, the manual nature of our assay does potentially increase the risk of human error. Furthermore, there are a variety of limitations regarding the specificity and sensitivity of the nasopharyngeal swab as a reliable procedure for COVID-19 diagnostic testing.[6,7] Finally, real-time PCR-based COVID-19 testing represents a single time point in the course of an individual patient's disease, and a negative test result may not preclude infection.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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AUTHOR CONTRIBUTIONS

K.V., A.M., R.M.G., A.K.Y., T.M.M., T.W.D., R.B.W., and G.J.M. designed and performed validation of the in-house real-time PCR assay. They also ran patient samples following EUA submission and CLIA certification. A.M. and R.M.G. wrote the manuscript and prepared the figures. N.S.M. and C.D.A. provided invaluable logistical, regulatory, and technical guidance and leadership. K.V. and G.J.M. revised the manuscript and led the technical team.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Sharfstein, J.M., Becker, S.J., and Mello, M.M. (2020). Diagnostic Testing for the Novel Coronavirus. JAMA. Published online March 9, 2020. https://doi.org/10.1001/jama.2020.3864.

2. CDC (2020). CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. https://www.fda.gov/media/134922/download.

3. Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., et al. (2020). Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan. *323*, 1061–1069.

4. Wen, P. (2020). Coronavirus cases surge at Boston Medical Center; nearly half of all beds taken up by such patients. The Boston Globe, April 7, 2020. https://www.bostonglobe.com/2020/04/07/metro/coronavirus-cases-surge-citys-safety-net-hospital-four-out-10-cases-are-now-related-pandemic/.

5. BPHC (2020). Boston COVID19 Report-For the Week Ending 4/2/2020 (Boston, MA: Communicable Disease Control Division).

6. Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., and Tan, W. (2020). Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA *323*, 1843–1844.

7. Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., Yu, J., Kang, M., Song, Y., Xia, J., et al. (2020). SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. N. Engl. J. Med. *382*, 1177–1179.

STAR★METHODS

KEY RESOURCE TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Patient swabs	Boston Medical Center	N.A.
Critical Commercial Assays		
MicroAmp Optical 384-Well Reaction Plate with Barcode	Fisher Scientific	43-098-49
High Capacity cDNA Reverse Transcription Kit 1000 rxn	Fisher Scientific	43-688-13
Synthetic SARS-CoV-2 RNA	ATCC	VR-3276
RNeasy Mini Kit (250)	QIAGEN	74106
TaqMan Fast Advanced Master Mix	ThermoFisher	4444557
QuantStudio 6 Flex Real-Time PCR System, 384-well, desktop	ThermoFisher	4485701
High Capacity cDNA Reverse Transcription Kit	ThermoFisher	43-688-13
BD UVT 3-mL collection kitwith flexible minitip flocked swab	BD	220531
2019-nCoV CDC Emergency Use Authorization Kits	IDT	110006606
Software and Algorithms		
GraphPad Prism version 7	GraphPad Software, La Jolla, CA	https://www.graphpad.com/
RStudio	RStudio, Inc., Boston, MA	https://www.rstudio.com/
Script for automated analysis, reporting and uploading of qRT-PCR data	This paper	https://github.com/TaylorMatte/Quant6-Covid_Analysis
Other		
Resource website for pre-EUA submission and protocol	This paper	http://www.bu.edu/dbin/stemcells/covid-19.php

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, George J. Murphy (gjmurphy@bu.edu)

Material Availability

This study did not generate new unique reagents.

Data and Code Availability

The script generated during this study to analyze raw qRT-PCR data and interface with the SunQuest Laboratory Information System is available at GitHub [(https://github.com/TaylorMatte/Quant6-Covid_Analysis)].

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Samples were collected from patients at Boston Medical Center who had been designated for COVID-19 testing by their attending physician. These included

patients from ambulatory settings, the emergency department, as well as inpatients including those in intensive care units.

METHOD DETAILS

RNA Isolation and Generation of Viral Standards

Patient samples were collected at the point of care via nasopharyngeal swab, and inactivated/lysed in the clinical microbiology laboratory. 300μL of patient sample was mixed with 300uL buffer RLT (from the QIAGEN RNeasy Mini Kit), and 600μL 100% molecular grade EtOH. Prior to inactivation and RNA extraction, patient samples can be stored at 4°C for short-term or −20°C for long-term storage. RNA was then extracted via the QIAGEN RNeasy Mini Kit as per the manufacturer's protocol. RNA was then stored on ice for same day use or at −80°C for long term storage. To generate synthetic SARS-CoV-2 RNA, commercially available stock solutions from ATCC (Cat No. VR-3276) were used as per the manufacturer's specification. Serial dilutions of the stock viral RNA were performed using 8 μL of dsDNA solution in 72 μL H_2O for each step to generate dilutions of 10^6, 10^5, 10^4, 10^3, 10^2, 10^1, 10° genome copies/μL.

Generation of cDNA and qRT-PCR for SARS-CoV-2

cDNA was generated from patient sample-derived RNA using the High Capacity cDNA reverse transcription kit, as per the manufacturer's instructions. cDNA RT master-mix was prepared as follows: 10X RT Buffer (2.0μl/reaction), 25X dNTP Mix (0.8μl/reaction), 10X RT Random Primers (2.0μl/reaction), and RT enzyme (1.0μl/reaction). 14.2μL of clinical sample RNA was added to 5.8μL cDNA RT master-mix. The RT reaction was carried out in a thermocycler with the following incubation times: 25°C for 5 minutes, 37°C for 120 minutes, and 85°C for 5 minutes.

The 2019-nCoV kit from Integrated DNA Technologies (IDT: Cat No 10006606) containing qRT-PCR primers for viral genes N1 and N2, as well as human housekeeping gene RP, was thawed and aliquoted as per the manufacturer's recommended instructions. Master mixes were prepared using the following recommended dilutions: Nuclease Free Water (2.33μl/reaction), PCR Primer/Probe (1μl/reaction), and 2X TaqMan Fast Advanced Master Mix (6.66μl/reaction).

10μL of each master mix along with 5μL of sample cDNA or standard curve controls were loaded into individual wells of a 384-well PCR plate (See Additional Resources for Plate Maps and Master Mix Template). The plate was then sealed, centrifuged briefly, and loaded into the QuantStudio 6 Flex Real Time PCR System. The PCR reaction was carried out with the following incubation times: Preamplification (UNG Inactivation), 1x, 120 s 25°C; Preamplification (Polymerase Activation), 1x 120 s 95°C; Amplification, 45x 3 s 95°C and 30 s 55°C. Full instrument settings and parameters can be found in the Additional Resources Section. Data was analyzed using an automated script described above. Individual plates were checked for adequate amplification of the control standard curve, and samples were called positive or negative based on the following guidelines from the CDC (Note: C_T values above 40 were considered negative): N1+, N2+, RP ± indicates positive for SARS-CoV-2, either N1 or N2+, RP ± indicates an inconclusive result, N1-, N2-, RP+ indicates negative for SARS-CoV-2, and N1-, N2-, RP- indicates an invalid result.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification of RT-PCR C_T Values and standard curves was performed using GraphPad Prism 7. The automated analysis and reporting was performed using R Studio.

ADDITIONAL RESOURCES

Our full pre-EUA submission and detailed protocol (including a comprehensive list of all reagents and controls) are publicly available at http://www.bu.edu/dbin/stemcells/covid-19.php.