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Journal of Clinical Virology 129 (2020) 104474

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Validation and verification of the Abbott RealTime SARS-CoV-2 assay analytical and clinical performance

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ARTICLE INFO

Keywords:
SARS-CoV-2
Coronavirus
COVID-19
EUA
PCR
High-throughput assay

ABSTRACT

Background: High-throughput assays for the SARS-CoV-2 virus are critical to increasing test capacity and slowing the spread of COVID-19. Abbott Molecular developed and received emergency use authorization (EUA) to deploy the new RealTime SARS-CoV-2 assay, run on the automated *m*2000*sp/rt* system.

Objective: To evaluate analytical and clinical performance of the RealTime SARS-CoV-2 assay compared to the SARS-CoV-2 CDC-based laboratory developed test (LDT) in clinical use by the University of Washington Clinical Virology Laboratory (UW Virology).

Methods: RealTime SARS-CoV-2 assay limit of detection (LOD) was evaluated by testing two dilution panels of 60 replicates each. Cross-reactivity was evaluated by testing 24 clinical samples positive for various non–SARS-CoV-2 respiratory viruses. Clinical performance was evaluated using 30 positive and 30 negative SARS-CoV-2 clinical samples previously tested using the UW Virology SARS-CoV-2 LDT.

Results: Exceeding the 100 copies/mL LOD reported in the RealTime SARS-CoV-2 assay EUA product insert, 19 of 20 replicates were detected at 50 copies/mL and 16 of 20 replicates were detected at 25 copies/mL. All clinical samples positive for 24 non–SARS-CoV-2 respiratory viruses were SARS-CoV-2 negative on the RealTime SARS-CoV-2 assay. The assay had high sensitivity (93%) and specificity (100%) for detecting SARS-CoV-2 in clinical samples. Two positive samples that tested negative with the RealTime SARS-CoV-2 assay had cycle numbers of 35.94 or greater and required dilution prior to testing. One of these samples was also inconclusive on the SARS-CoV-2 LDT.

Conclusion: The RealTime SARS-CoV-2 assay is acceptable for clinical use. With the high-throughput, fully automated *m*2000 system, this assay will accelerate the pace of SARS-CoV-2 testing.

1. Introduction

Since the SARS-CoV-2 virus was first detected by a polymerase chain reaction (PCR)-based assay in December of 2019 in Wuhan City, China, the disease caused by the virus, COVID-19 has spread rapidly to nearly every country around the globe [1]. The first case of COVID-19 in the United States was reported in mid-January 2020 [2], and the number of confirmed cases and infection-related deaths have continued to rise exponentially. Efforts have focused on slowing the spread of infection through social distancing and access to fast and accurate SARS-CoV-2 testing to track and contain the disease [3]. Delays in test availability have heightened the urgency for the development of high-throughput SARS-CoV-2 assays for use under emergency use authorization (EUA) from the Food and Drug Administration.

Abbott Molecular (Des Plaines, IL) recently developed and received EUA approval for a real-time PCR test to detect SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swabs from individuals with suspected COVID-19 [4]. The RealTime SARS-CoV-2 assay amplifies target

Abbreviations: EUA, emergency use authorization; LTD, laboratory developed test; LOD, limit of detection; NPA, negative percent agreement; PCR, polymerase chain reaction; PPA, positive percent agreement; RdRp, RNA-dependent RNA polymerase

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https://doi.org/10.1016/j.jcv.2020.104474
Received 28 April 2020; Received in revised form 18 May 2020; Accepted 25 May 2020

regions of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) gene and the N gene with the same fluorophore within a single well. The assay is run on the automated *m*2000 system currently in use nationally by reference laboratories and university hospitals; in addition, the automated *m*2000 platform is available internationally. The *m*2000 system can process up to 96 samples simultaneously, with 470 test results reported in approximately 24 h.

We validated the analytical performance of the RealTi*m*e SARS-CoV-2 assay and verified the clinical performance compared to the SARS-CoV-2 CDC-based laboratory developed test (LDT), which is currently in use at the University of Washington Clinical Virology Laboratory (UW Virology). The SARS-CoV-2 LDT is a real-time PCR assay that targets and amplifies two regions within the SARS-CoV-2 N-gene in separate reaction wells to detect nucleic acid in nasopharyngeal/oropharyngeal swabs, nasal swabs, sputum, and bronchoalveolar fluid samples [5,6].

2. Methods

2.1. Study site

Validation and verification of the analytical and clinical performance of the Abbott RealTi*m*e SARS-CoV-2 assay was conducted using the automated Abbott *m*2000 *sp/rt* System located in the University of Washington Retrovirology Laboratory at Harborview Medical Center. Use of de-identified excess clinical material for assay validation was deemed to be non-human subjects by the University of Washington Institutional Review Board.

2.2. Assay procedures

To validate assay performance specifications the assay was performed per the RealTi*m*e SARS-CoV-2 assay EUA product insert [4]. An optional step of vortexing samples followed by centrifugation at 2000 x g for 5 min was performed prior to loading samples onto the instrument. A sample volume of 0.5 mL was extracted and an eluate volume of 40 µL was utilized in the PCR reaction.

2.3. Validation of analytical performance

The RealTi*m*e SARS-CoV-2 assay limit of detection (LOD) was evaluated by testing two panels of 60 replicates each with the following target concentrations: 100 copies/mL (n = 20), 50 copies/mL (n = 20), 25 copies/mL (n = 20), 10 copies/mL (n = 40), and 5 copies/mL (n = 20). The panel was prepared by serially diluting assay positive controls (with a target concentration of 1000 copies/mL) with RPMI in lieu of viral transport medium, which was in limited supply at the time of the study. The performance of the RealT*ime* SARS-CoV-2 assay was compared to the UW Virology SARS-CoV-2 CDC-based LDT using the same diluted positive controls [6].

RealTi*m*e SARS-CoV-2 assay cross-reactivity was evaluated by testing 24 clinical samples from the UW Virology Laboratory that were positive for various non–SARS-CoV-2 respiratory viruses (Table 1). Samples with insufficient volume were diluted with RPMI to obtain the required minimum volume for the assay (0.8 mL).

2.4. Verification of clinical performance

RealTi*m*e SARS-CoV-2 assay clinical performance was evaluated with 30 known SARS-CoV-2 positive clinical samples and 30 known SARS-CoV-2 negative clinical samples (nasal and nasopharyngeal) in viral transport media. Frozen remnants of samples previously tested for SARS-CoV-2 at the UW Virology Laboratory using the SARS-CoV-2 LDT were used for the evaluation. Remnant samples with insufficient volume were diluted with RPMI to obtain the required minimum volume for the assay (0.8 mL). Remnant SARS-CoV-2 negative clinical samples had sufficient volume for testing.

Table 1
Clinical Samples used for Cross-reactivity Testing of the Abbott RealTi*me* SARS-CoV-2 Assay.

Virus	Number of Samples Tested
Coronavirus (non–SARS-CoV-2)*	7
Rhinovirus	2
Parainfluenza virus 1	1
Parainfluenza virus 3	2
Parainfluenza virus 4	2
Respiratory syncytial virus	2
Adenovirus	2
Metapneumovirus	3
Influenza A	2
Influenza B	1

* Respiratory specimens were obtained during the past calendar year and were RT-PCR-positive using a set of pancoronavirus primers and probes.

3. Results

3.1. Analytical performance

Per the product insert, the RealTi*m*e SARS-CoV-2 assay 95% LOD is 100 copies/mL determined using a recombinant virus containing SARS-CoV-2 RNA serially diluted in simulated nasal matrix. [4]. The number of replicates that were detected for each level of the LOD panel are shown in Table 2 for the Abbott and UW Virology SARS-CoV-2 assays. All 24 clinical samples positive for non-SARS-CoV-2 respiratory viruses yielded SARS-CoV-2 negative results (Supplementary Table 1).

3.2. Clinical performance

Twenty-eight out of 30 SARS-CoV-2 positive clinical samples on the SARS-CoV-2 LDT were also positive by the RealTi*m*e SARS-CoV-2 assay (Supplementary Table 2). One of the two potentially discordant samples that was negative with the RealTi*m*e SARS-CoV-2 assay had a cycle number of 35.97 for target N1 and 35.94 for target N2 with the SARS-CoV-2 LDT. The second discordant sample was inconclusive with the SARS-CoV-2 LDT, with only one of two targets (target N1) detected with a cycle number of 36.14. Additionally, both of the discordant samples required a dilution of 1:2 or more prior to testing with the RealTi*m*e SARS-CoV-2 assay (Supplementary Table 2).

4. Discussion

This study validated the analytical performance of the EUA RealTi*m*e SARS-CoV-2 assay. Limit of detection results exceeded the 100 copies/mL 95% LOD target, with 19 of 20 replicates detected at 50 copies/mL and 16 of 20 replicates detected at 25 copies/mL. Specificity of the assay was consistent with the in silico analysis reported in the EUA RealTi*m*e SARS-CoV-2 assay product insert [4]. We found that all clinical samples positive for 24 non–SARS-CoV-2 respiratory viruses were SARS-CoV-2 negative on the RealTi*m*e SARS-CoV-2 assay.

We also found high sensitivity (93%) and specificity (100%) of the RealTi*m*e SARS-CoV-2 assay in our clinical performance analysis. As reported in the product insert [4], initial clinical performance of the EUA RealTi*m*e SARS-CoV-2 assay with 60 known positive samples (at 1X to 20X LOD) and 30 known negative samples found 100% positive percent agreement (PPA: CI, 94.0, 100.0) and 100% negative percent agreement (NPA: CI, 88.8, 100.0). We did not expect 100% agreement due to differences between the RealTi*m*e SARS-CoV-2 assay and the SARS-CoV-2 CDC-based laboratory developed test. Two samples that were discordant between the two methods had a late cycle number with the SARS-CoV-2 LDT and required dilution prior to testing with the RealTi*m*e SARS-CoV-2 assay. Previous handling of the remnant samples also could have contributed to the discordant results.

In conclusion, we validated the analytical performance and

Table 2
Limit of Detection Verification Panel Results for the Abbott RealTi*me* SARS-CoV-2 Assay Compared to the UW Virology SARS-CoV-2 CDC-based Laboratory Developed Test.

Target Concentration (copies/mL)	Number of Replicates Tested	Number of Replicates Detected Abbott Assay	Number of Replicates Detected or Inconclusive UW Virology LDT
100	20	20	16
50	20	19	8
25	20	16	3
10	40	14	4
5	20	4	0

evaluated the comparative clinical performance of the RealTi*me* SARS-CoV-2 assay and found the assay to be acceptable for clinical use. Based on the limit of detection determined here, we expect this assay has the highest analytical sensitivity of any qRT-PCR currently on the market [7]. Running the assay on the high-throughput, fully automated *m*2000*sp/rt* system will make it possible to accelerate the pace of SARS-CoV-2 testing both domestically and internationally, with the ultimate goal of tracking the disease and targeting interventions that will eventually slow the spread of COVID-19.

Funding

This work was supported in part by NIAID grants AI-106791; AI-068618.

CRediT authorship contribution statement

Emily Degli-Angeli: Validation, Resources, Data curation, Writing - review & editing. **Joan Dragavon:** Validation, Resources, Data curation, Writing - review & editing. **Meei-Li Huang:** Resources, Data curation, Writing - review & editing. **Danijela Lucic:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Gavin Cloherty:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Keith R. Jerome:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Alexander L. Greninger:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Robert W. Coombs:** Conceptualization, Methodology, Data curation, Writing - review & editing.

Declaration of Competing Interest

Alex L Greninger declares personal fees from Abbott Molecular, outside the scope of the submitted work.

Danijela Lucic is an employee of Abbott Molecular. Gavin Cloherty is an employee of Abbott Diagnostics. Abbott Molecular provided the RealTi*me* SARS-CoV-2 assays for use in the study but was not involved in data collection. The other authors have no declarations of interest.

Acknowledgement

We thank Stacey Tobin for manuscript preparation.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104474.

References

[1] Johns Hopkins University of Medicine. Coronavirus Resource Center. https://coronavirus.jhu.edu/map.html. Accessed April 6, 2020.
[2] M.L. Holshue, C. DeBolt, S. Lindquist, K.H. Lofy, J. Wiesman, H. Bruce, et al., First case of 2019 novel coronavirus in the United States, N. Engl. J. Med. 382 (2020) 929–936, https://doi.org/10.1056/NEJMoa2001191.
[3] Centers for Disease Control and Prevention, Interim Pre-pandemic Planning Guidance: Community Strategy for Pandemic Influenza Mitigation in the United States—early, Targeted, Layered Use of Nonpharmaceutical Interventions, US Department of Health and Human Services, CDC, Atlanta, GA, 2007.
[4] Abbott RealTime SARS-CoV-2 Assay. [EUA Package Insert], Abbott Molecular, 2020.
[5] University of Washington, Department of Laboratory Medicine. SARS-CoV-2 (COVID-19) Qualitative PCR. https://testguide.labmed.uw.edu/public/view/NCVQLT. Accessed April 6 2020.
[6] A.K. Nalla, A.M. Casto, M.-L.W. Huang, G.A. Perchetti, R. Sampoleo, L. Shrestha, Y. Wei, et al., Comparative performance of SARS-CoV-2 detection assays using seven different Primer/Probe sets and one assay kit, J. Clin. Microbiol. (2020), https://doi.org/10.1128/JCM.00557-20.
[7] J.L. Lieberman, G. Pepper, S.N. Naccache, M.-L. Huang, K.R. Jerome, A.L. Greninger, Comparison of Commercially Available and Laboratory Developed Assays for in vitro Detection of SARS-CoV-2 in Clinical Laboratories, J. Clin. Microbiol. (2020), https://doi.org/10.1128/JCM.00821-20.