EVALUATION OF THE SARS-COV-2 DETECTION PROCESS ON THE ABBOTT AUTOMATIC SYSTEM M2000RT

Vu Duy Dung[1], Tran Hong Tram[1,2], Vu Thi Lan Anh[2], Do Thi Huong[2], Dang Anh Ngoc[2], Vuong Thi Mai Linh[2], Doan Huu Xuyen[2], Nguyen Duy Thai[1,2*]

[1] National Institute for Control of Vaccine and Biologicals, Ministry of Health

[2] Vietnam University of Traditional Medicine, Ministry of Health

Received 20 June 2023

Accepted 25 August 2023

Abstract: Amidst the complex progression of the COVID-19 pandemic in Vietnam, in response to the need for diagnosing suspected COVID-19 patients, we conducted research to validate the procedure for detecting the SARS-CoV-2 virus on the Abbott m2000 system using real-time PCR. We used a positive control sample (Abbott Real Time SARS-CoV-2 Positive control provided by the manufacturer) to assess the accuracy and Limit of Detection (LOD) of the procedure. The results indicated that the detection limit of the method met the manufacturer's recommendation with a test sample concentration of 100 copies/ml and a 100% positivity rate. The accuracy, encompassing both specificity and sensitivity, was 100% with community-collected test samples having a concentration ≥100 copies/ml. Our study has proven that the detection procedure for the SARS-CoV-2 coronavirus on the Real-Time PCR m2000 system meets the accuracy and detection limit criteria set by the manufacturer. Based on these research findings, there's a scientific basis to apply this procedure for diagnostic testing of the SARS-CoV-2 coronavirus in samples at the National Institute for Control of Vaccine and Biologicals (NICVB).

Keywords: SARS-CoV-2, antigens, nasopharyngeal, Real-time PCR, Abbott m2000.

1. Introduction

SARS-CoV-2 is a novel strain of the Coronavirus responsible for Severe Acute Respiratory Syndrome, known as COVID-19. It was first reported in late 2019 in Wuhan, China, and subsequently spread rapidly worldwide [1]. People with COVID-19 face serious health challenges, leading to acute respiratory failure and death [2], especially among older adults, those with compromised immunity, or those with underlying conditions such as cardiovascular diseases, diabetes, chronic kidney, respiratory illnesses, etc [3]. Additionally, SARS-CoV-2 continuously evolves with more dangerous variants rapidly replacing previously circulating strains, creating challenges for treatment [4,5]. Dominant variants that have emerged globally include Alpha, Beta, Delta, and Omicron [6]. Particularly, the Omicron variant became predominant globally from March 2022, with characteristics making it more transmissible and potentially reducing the vaccine's efficacy [7,8]. From an initial outbreak of 27 severe pneumonia cases in Wuhan, the SARS-CoV-2 virus has spread swiftly, culminating in a global pandemic. As of April 2022, there have

* Corresponding author
E-mail address: thainguyenduy@hotmail.com
https://doi.org/10.56086/jcvb.v3i3.102

been over 649 million COVID-19 cases globally with 6.6 million fatalities [9].

The spread of COVID-19 highlights an urgent need for accurate and swift diagnostic tests for timely public health and clinical interventions [10]. The Realtime RT-PCR method is regarded as the gold standard for the precise detection of the SARS-CoV-2 virus since the onset of the COVID-19 outbreak [11]. It can determine the presence or absence of the SARS-CoV-2 virus in test samples swiftly, even in samples with very low viral concentrations, helping detect mild or asymptomatic cases [12]. This method offers the highest sensitivity and specificity compared to rapid diagnostic methods detecting SARS-CoV-2 antigens [13]. Therefore, it has been approved by the World Health Organization (WHO) for widespread use in qualitative testing for SARS-CoV-2 by detecting the virus‘s nucleic acid in nasal swabs and throat swab samples from suspected COVID-19 patients. This technique can identify the RNA of the SARS-CoV-2 virus in specimens during the acute phase of the disease by amplifying selected target gene segments [14, 15].

Abbott Molecular (USA) has developed and received Emergency Use Authorization (EUA) from the EU Food and Drug Administration for a Real-time PCR test to detect the RNA of SARS-CoV-2 in samples taken from the nasal and throat of individuals suspected of having COVID-19. The test employs two sets of primers to amplify regions in the RNA-dependent RNA polymerase gene and the highly conserved N gene. Fluorescent probes targeting the amplified virus sequences indicate a positive test result [16].

The Abbott m2000 system is an automated testing platform manufactured by Abbott, used for the detection and diagnosis of infectious diseases, including COVID-19. This system determines the presence of the SARS-CoV-2 virus in test samples using the Real-time PCR (RT-PCR) method. The RT-PCR method combines the process of converting RNA to DNA (reverse transcription) and the DNA replication process (PCR) to produce a large quantity of the target DNA of the SARS-CoV-2 virus [17].

In light of the unpredictable developments of the COVID-19 pandemic in Hanoi and other provinces and cities nationwide, the Ministry of Health of Vietnam has identified diagnostic testing as an essential strategy to curb infections with the aim of slowing the spread of COVID-19, reducing strain on health care resources, and guiding public health policies. Therefore, the Government and the Ministry of Health have focused many resources on enhancing the testing capabilities of healthcare facilities. The National Institute for Control of Vaccine and Biologicals is among the units supplied by the Ministry of Health with the Abbott m2000 system to implement COVID-19 testing for epidemic control and meet the testing needs of the public. Although the Abbott m2000 system has been applied for SARS-CoV-2 testing in several molecular biology labs, it was first introduced at the Institute, so the evaluation of the procedure absolutely essential. Therefore, we conducted this study with the objectives of assessing accuracy, including sensitivity, specificity, and the detection limit of the

SARS-CoV-2 virus on the Abbott m2000 system.

2. Method

This study was carried out using the experimental description method in the in vitro laboratory.

2.1. Subjects

The procedure for detecting SARS-CoV-2 on the automated Abbott m2000 system using the Real-time PCR method at the National Institute for Control of Vaccine and Biologicals, from June 2022 to December 2022.

2.2. Materials

Standard Sample: Abbott Realtime SARS-CoV-2 Negative control with product code 522567, containing 1.0% ammonium sulfate and 7.9% detergent in a buffer solution; Abbott Realtime SARS-CoV-2 Positive control containing 1.000 copies/ml of non-infectious recombinant Sindbis virus, with sequences of SARS-CoV-2 RNA, 1.0% ammonium sulfate, and 7.9% detergent in a buffer solution.

Test Samples: 5 dilution levels of Abbott Realtime SARS-CoV-2 Positive control: 300 copies/ml; 120 copies/ml; 100 copies/ml; 80 copies/ml, and the negative samples from patients.

Equipment and chemicals: Abbott m2000 system, refrigerator, incubator, micropipette, Class II biological safety cabinet, all calibrated annually according to ISO/IEC 17025. The test kit for extracting SARS-CoV-2 RNA includes Lysis Buffer, Wash 1, Wash 2, Microparticles, and Elution Buffer. DNA and RNA are captured by magnetic μ-particles.

2.3. Design

Realtime SARS-CoV-2 testing was run on the automated m2000 system at NICVB according to the EUA product usage guide [18]. A total of 5 test samples were conducted in 20 repetitions over 5 different days to ensure repeatability and intermediate precision requirements.

Intermediate precision standard: At least 5 samples, repeated at least 5 times in the same laboratory on different days under the same conditions in terms of reagents and equipment, but with a change in the operators. Conducted over 5 - 20 days.

Repeatability standard: Testing is performed by the same group, under the same equipment conditions, chemicals, and materials. At least 5 samples, repeated at least 5 times, under the same conditions in terms of operators, reagents, and equipment. Conducted consecutively over a short period of 1-10 days.

2.3. Accuracy

The accuracy of the procedure is determined based on the following criteria:

Specificity = d/(c+d)

Sensitivity = a/(a+b)

Results at the laboratory	**Results of the standard method**	
	Positive	*Negative*
Positive	a	b
Negative	c	d

Evaluation criteria: As per the manufacturer's guidelines (NSX) and recommendations

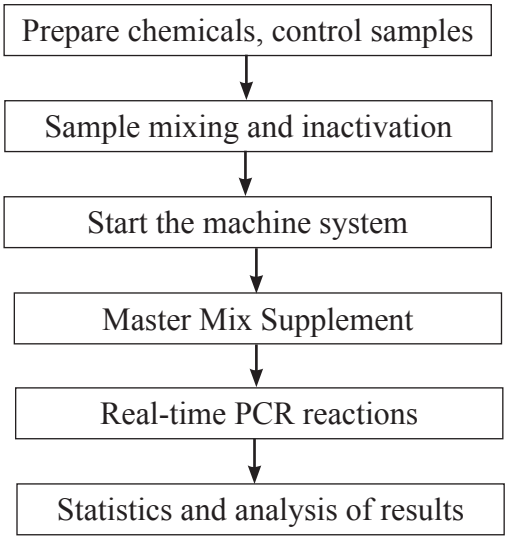
from the World Health Organization (WHO), and the U.S. Centers for Disease Control and Prevention (CDC). The recommended accuracy is a minimum sensitivity of 95% and a minimum specificity of 98%.

2.4. Limit of Detection

Conducted on 5 dilution levels of the Abbott Realtime SARS-CoV-2 Positive control sample: 300 copies/ml; 120 copies/ml; 100 copies/ml; 80 copies/ml. Calculate the number of positive reactions for each concentration level, input the data into the PODLOD software to construct a probit regression model, thereby determining the limit of detection (LOD) for the procedure within a 95% confidence interval.

Evaluation criteria: Based on the manufacturer's guidelines and recommendations from the WHO and CDC. The LOD has the lowest detectable concentration of 100 copies/ml with a positive rate of ≥ 95%. If it's ≤ the LOD of the manufacturer, then it is reported according to the manufacturer's LOD.

2.5. Research procedure



2.6. Data Analysis

The results of the Realtime RT-PCR tests are analyzed using the maxRatio algorithm available in the software of the Abbott system. Data are compiled in Excel software (Office 2019 version). Statistical analyses are conducted on SPSS software version 20 (SPSS Inc., Chicago, IL) to calculate the mean value of the test sample (M: Mean), standard deviation (SD), relative standard deviation percentage (RSD), and the coefficient of variation (CV). Acceptance criterion: CV ≤ 3.3 (19).

3. RESULTS

3.1. Diagnosis of SARS-CoV-2 on Test Samples

Test samples at different concentrations tested positive for the SARS-CoV-2 virus, except for the test sample at a concentration of 80 copies/ml which had 2 repeats that tested negative (Table 1). Both the positive and negative control samples produced the expected results as recommended by the manufacturer.

Table 1: Results of Real-time PCR SARS-CoV-2 on the Abbott m2000 system.

Day	Repeat	Test Samples					Positive control	Negative control
		300 copies/ml	120 copies/ml	100 copies/ml	80 copies/ml	Negative		
1	1	33.57	34.38	36.12	37.23	ND	31.61	ND
	2	32.72	34.17	37.00	37.84	ND		
	3	33.07	33.79	35.94	39.46	ND		
	4	32.89	35.24	35.93	39.51	ND		
2	5	32.19	33.67	35.78	38.25	ND	31.32	ND
	6	32.74	33.77	35.68	36.99	ND		
	7	32.01	33.75	37.63	38.47	ND		
	8	32.29	34.52	36.16	37.46	ND		
3	9	32.69	34.58	38.98	39.57	ND	31.25	ND
	10	33.13	34.21	35.71	37.66	ND		
	11	32.61	34.15	36.82	37.50	ND		
	12	32.19	33.89	36.51	39.22	ND		
4	13	33.07	33.86	36.03	37.61	ND	31.61	ND
	14	32.63	33.48	36.79	38.05	ND		
	15	33.02	35.02	37.01	37.71	ND		
	16	32.72	35.23	37.40	37.33	ND		
5	17	32.71	34.02	37.57	37.44	ND	31.65	ND
	18	32.20	33.93	37.04	36.76	ND		
	19	32.73	34.47	36.23	KPH	ND		
	20	32.41	35.15	36.03	KPH	ND		

ND: Not detected

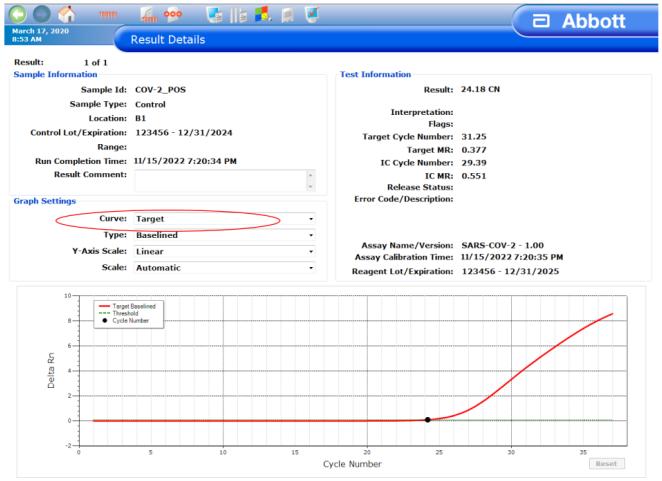


Figure 1: Realtime PCR SARS-CoV-2 results with positive control sample

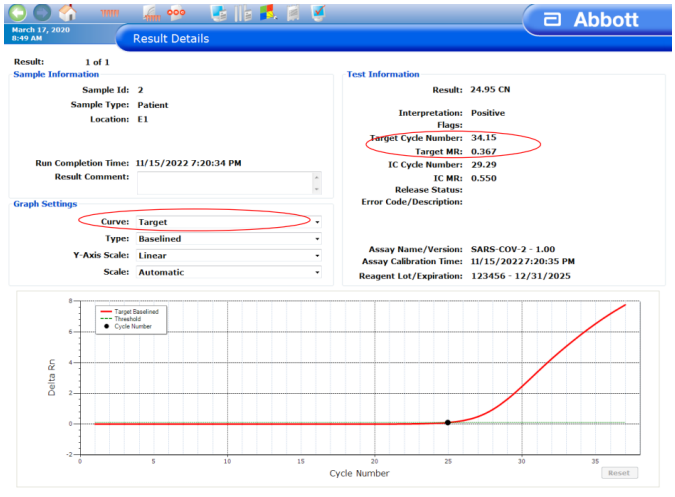


Figure 2: Realtime PCR SARS-CoV-2 results with sample concentration of 120 copies/ml

3.2. Accuracy of the Procedure (Specificity, Sensitivity, LOD)

The test samples were repeated 20 times over 5 different days at 5 different concentrations with a CV<3.3 (Table 2), ensuring the standards for intermediate precision and repeatability as recommended by the manufacturer.

Table 2: Evaluation of repeatability and intermediate precision of the procedure.

Day / Sample	1	2	3	4	5	Mean	SD	CV
300 copies/ml	33.06	34.40	32.66	32.86	32.51	33.10	0.75	2.27
120 copies/ml	34.40	33.93	34.21	34.40	34.39	34.26	0.20	0.58
100 copies/ml	36.24	36.31	37.01	36.81	36.72	36.62	0.33	0.90

80 copies/ml	38.51	37.79	38.49	37.67	37.10	37.91	0.60	1.58
Negative	-	-	-	-	-	-	-	-
PC	31.61	31.32	31.25	31.61	31.65	31.49	0.19	0.60
NC	-	-	-	-	-	-	-	-

(-): Unspecified; PC: Positive control; NC: Negative control

Table 3: Evaluation of procedure specificity and sensitivity.

Results at the laboratory	**Results of the standard method**	
	Positive (≥100 copies/ml)	**Negative (Negative control)**
Positive	60	0
Negative	0	5

The results in Table 3 show that the specificity of the procedure is 100%, and the sensitivity of the procedure is 100%. The detection limit of the procedure for a sample at a concentration of 100 copies/ml is 100%.

4. Discussion

Rapid and accurate diagnosis of the SARS-CoV-2 virus is essential for managing the global COVID-19 pandemic [20]. PCR testing methods are considered the gold standard for diagnosing suspected COVID-19 cases, patient care, contact and outbreak tracing, and detecting new variants [21]. The Abbott m2000 is an advanced system capable of detecting the SARS-CoV-2 virus with high accuracy. Beyond validating test results through positive and negative controls, the system has an internal control mechanism (IC: Internal Control) to demonstrate that the process is accurate for each sample through an RNA sequence unrelated to the SARS-CoV-2 sequence introduced into each specimen at the onset of sample preparation. This unrelated RNA sequence is amplified simultaneously and acts as an internal control component [22]. The presence of the target SARS-CoV-2 sequence is represented by fluorescent signals on the Abbott m2000 device, probes for both target sequences are labeled with the same fluorescent color (FAM). Firstly, a constant amount of background fluorescence reference (ROX) is added to each reaction to allow uniform target and IC signals and is simultaneously used for validity checking. After that, target and IC signals for each test are assessed for the presence or absence of the target nucleic acid sequence (PCR reaction) using the maxRatio (MR) algorithm for the SARS-CoV-2 test. Finally, the target and IC signals for each test are evaluated based on criteria set for their threshold cycle numbers (Ct or Cn), using the foundational algorithm for the SARS-CoV-2 test [18; 23].

In this study, we validated the analytical performance of the Realtime PCR SARS-CoV-2 test on the Abbott m2000 system. The limit of detection results exceeded the 95% positive target at a test sample concentration of 100 copies/ml as recommended by the manufacturer and WHO (17), with 20 samples detected at 300 copies/ml, 20 samples at 120 copies/ml, and 20 samples at 100 copies/ml. This LOD is significantly

higher than the LODs of the EUA CDC and TaqPath tests, which are 1,000 and 2,000 copies/ml, respectively [24]. Differences in LODs among reference tests have been discussed in many prior reports, and many factors influence the LOD of procedures, such as sample inactivation treatment, virus transport environment, or concentration, it showed the need to standardize methods to ensure transparency in test performance (24). Furthermore, we found that the Ct values in positive test samples on the Abbott m2000 system are higher than in tests on other systems, the reason being the Abbott m2000 design where the m2000 instrument does not read the first 10 cycles [18; 23].

With an LOD recommended by the manufacturer of 100 copies/ml of test sample, our study results show that the sensitivity of the Realtime PCR SARS-CoV-2 test on the Abbott m2000 system is 100% across all 60 samples. The high sensitivity of this test is extremely significant and aligns with prior studies of commercial SARS-CoV-2 test kits [25; 26]. It helps in quickly diagnosing and preventing infection waves in the COVID-19 pandemic, especially for cases with low viral loads [27; 28]. The specificity of the test on the Abbott m2000 system is also 100%; all negative samples did not detect the SARS-CoV-2 virus, in line with the In silico analysis reported in the manufacturer's EUA Realtime SARS-CoV-2 test instructions [17; 18; 29].

There are some limitations in this study. Using SARS-CoV-2 positive test samples from Abbott to determine the LOD may limit the comparison of LODs between different SARS-CoV-2 test kits. The process of handling this test sample may also contribute to inconsistent results. However, this is not significant as our research focus is to evaluate the method, the procedure of the SARS-CoV-2 test on the Abbott m2000 system.

5. Conclusion

Our research has demonstrated that the detection limit of the Abbott m2000 system for SARS-CoV-2 testing exceeds the manufacturer's reported standard of 100 copies/ml sample, with a positive sample detection rate of 100%. The sensitivity and specificity of the procedure are 100% for samples with concentrations ≥100 copies/ml, as recommended by the manufacturer. Consequently, this study provides a scientific foundation for deploying SARS-CoV-2 testing on the Abbott m2000 system, aiding in enhancing and supplementing the laboratory capacity of NICVB in efforts to accelerate testing with the goal of reducing the spread of COVID-19.

References

[1] Liu Y-C, Kuo R-L, Shih S-R. COVID-19: The first documented coronavirus pandemic in history. Biomedical journal 2020;43:328-333.

[2] Baj J, Karakuła-Juchnowicz H, Teresiński G, et al. COVID-19: specific and non-specific clinical manifestations and symptoms: the current state of knowledge. Journal of clinical medicine 2020;9:1753.

[3] Gao Yd, Ding M, Dong X, et al. Risk factors for severe and critically ill COVID-19 patients: a review. Allergy 2021;76:428-455.

[4] Guo S, Liu K, Zheng J. The genetic variant of SARS-CoV-2: would it matter for controlling the devastating pandemic? International journal of biological sciences 2021;17:1476.

[5] Krishnamoorthy S, Swain B, Verma R, et al. SARS-CoV, MERS-CoV, and 2019-nCoV viruses: an overview of origin, evolution, and genetic variations. VirusDisease 2020;31:411-423.

[6] Liu Y, Liu J, Shi P-Y. SARS-CoV-2 variants and vaccination. Zoonoses (Burlington, Mass) 2022;2.

[7] Guo Y, Han J, Zhang Y, et al. SARS-CoV-2 Omicron variant: epidemiological features, biological characteristics, and clinical significance. Frontiers in immunology 2022:2010.

[8] Chen J, Wang R, Gilby NB, et al. Omicron variant (B. 1.1. 529): infectivity, vaccine breakthrough, and antibody resistance. Journal of chemical information and modeling 2022;62:412-422.

[9] Huang AC-C, Lin S-M, Chiu T-H, et al. Comparison of Clinical Characteristics and Outcomes of Hospitalized Patients Infected with the D614G Strain or Alpha Variant of COVID-19 in Taiwan: A Multi-Center Cohort Study. International Journal of Medical Sciences 2022;19:1912-1919.

[10] Vogels CBF, Brito AF, Wyllie AL, et al. Analytical sensitivity and efficiency comparisons of SARS-COV-2 qRT-PCR primer-probe sets. medRxiv 2020:2020.2003.2030.20048108.

[11] Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA 2020;323:1843-1844.

[12] Ren X, Liu Y, Chen H, et al. Application and optimization of RT-PCR in diagnosis of SARS-CoV-2 infection. medRxiv 2020:2020.2002.2025.20027755.

[13] Caruana G, Croxatto A, Coste A, et al. Diagnostic strategies for SARS-CoV-2 infection and interpretation of microbiological results. Clinical Microbiology and Infection 2020;26:1178-1182.

[14] Bwire GM, Majigo MV, Njiro BJ, et al. Detection profile of SARS-CoV-2 using RT-PCR in different types of clinical specimens: a systematic review and meta-analysis. Journal of medical virology 2021;93:719-725.

[15] Torretta S, Zuccotti G, Cristofaro V, et al. Diagnosis of SARS-CoV-2 by RT-PCR using different sample sources: review of the literature. Ear, Nose & Throat Journal 2021;100:131S-138S.

[16] Berg MG, Zhen W, Lucic D, et al. Development of the RealTime SARS-CoV-2 quantitative Laboratory Developed Test and correlation with viral culture as a measure of infectivity. Journal of Clinical Virology 2021;143:104945.

[17] Degli-Angeli E, Dragavon J, Huang M-L, et al. Validation and verification of the Abbott RealTime SARS-CoV-2 assay analytical and clinical performance. Journal of Clinical Virology 2020;129:104474.

[18] Abbott. Abbott RealTime SARS-CoV-2 assay (EUA). Instructions for use: package insert. Abbott Molecular, Abbott Park, IL., 2019.

[19] Trang NLH. Quy trình chuẩn Thẩm định Quy trình. Hà Nội: Viện Kiểm định Quốc gia Vắc xin và Sinh phẩm Y tế, 2017.

[20] al. WCe. A National Plan to Enable Comprehensive COVID-19 Case Finding and Contact Tracing in the US. Available at: https://www.centerforhealthsecurity.org/our-work/pubs_archive/pubs-pdfs/2020/200410-national-plan-to-contact-tracing.pdf Accessed 23 2023.

[21] European Centre for Disease Prevention and Control. Diagnostic Testing and

Screening for SARS-CoV-2. Available at: https://www.ecdc.europa.eu/en/covid-19/latest-evidence/diagnostic-testing. Accessed 23 2023.

[22] Abbott. WHO Emergency Use Assessment SARS-CoV-2 IVDs: WHO; 2022.

[23] Abbott. Abbott RealTime SARS-CoV-2 Instructions for Use 2020.

[24] Hirschhorn JW, Kegl A, Dickerson T, et al. Verification and Validation of SARS-CoV-2 Assay Performance on the Abbott m2000 and Alinity m Systems. Journal of Clinical Microbiology 2021;59:10.1128/jcm.03119-03120.

[25] Mostafa HH, Hardick J, Morehead E, et al. Comparison of the analytical sensitivity of seven commonly used commercial SARS-CoV-2 automated molecular assays. Journal of Clinical Virology 2020;130:104578.

[26] Fung B, Gopez A, Servellita V, et al. Direct comparison of SARS-CoV-2 analytical limits of detection across seven molecular assays. Journal of clinical microbiology 2020;58:e01535-01520.

[27] Sieker JT, Horowitz C, Hu C-TK, et al. Analytic sensitivity of 3 nucleic acid detection assays in diagnosis of SARS-CoV-2 infection. The Journal of Applied Laboratory Medicine 2021;6:421-428.

[28] Arnaout R, Lee RA, Lee GR, et al. The limit of detection matters: the case for benchmarking severe acute respiratory syndrome coronavirus 2 testing. Clinical Infectious Diseases 2021;73:e3042-e3046.

[29] Ehret R, Breuer S, Dhein J, et al. Clinical evaluation of the automated Abbott RealTime SARS-CoV-2, Alinity m SARS-CoV-2, and Alinity m Resp-4-Plex assays. J Virol Methods 2022;299:114338.