International Journal of Biomedicine 13(4) (2023) 364-366
http://dx.doi.org/10.21103/Article13(4)_ShC2

SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF BIOMEDICINE

Analytical Performance of Direct Rapid Nucleic Acid Assay for Detection of SARS-Cov-2

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Abstract

Background: A rapid and accurate test to detect SARS-CoV-2 is essential for controlling the transmission of the COVID-19. Rapid diagnostic tests are currently marketed, although it is uncertain how well they perform in actual clinical settings and with relevant subpopulations. We evaluated the clinical performance of the Direct Detect® SARS-CoV-2 Detection Kit (Coyote Bioscience Co., Ltd., Beijing, China) rapid, molecular-based assay.

Methods and Results: The clinical laboratory received 707 clinical samples for rapid PCR between December 2021 and March 2022, including confirmed or suspected COVID-19 cases. These samples were tested by the Direct Detect® SARS-CoV-2 Detection Kit and by the LabGun® COVID-19 ExoFast RT-PCR Kit. Of 707 specimens tested, 649(91.79%) were negative and 58(8.20%) were positive. The sensitivity and specificity of the rapid RT-PCR test were 79.31% (95% CI: 66.65% to 88.83%) and 99.54% (95% CI: 98.66% to 99.90%), respectively.

Conclusion: The Direct Detect® SARS-CoV-2 Detection Kit evaluated in this study was able to detect SARS-CoV-2 infection with high viral loads but not so for higher loads. To determine strategies for appropriate use, more investigation of the assay's field performance in various conditions is required.**(International Journal of Biomedicine. 2023;13(4):364-366.)**

Keywords: SARS-CoV-2 • molecular-based assay • RT-PCR • rapid tests

For citation: Jagan OA, Vellamkott MMP, Bava A, Anish J, Acharya V, Shakir M, Oommen S. Analytical Performance of Direct Rapid Nucleic Acid Assay for Detection of SARS-Cov-2. International Journal of Biomedicine. 2023;13(4):364-366. doi:10.21103/Article13(4)_ShC2

Introduction

The COVID-19 pandemic evolved to hold a significant influence on human health and life around the globe. The diagnostic standard for testing SARS-CoV-2 is a real-time reverse transcription polymerase chain reaction (RT-PCR) assay.[1] This technique, however, is time-consuming (the result might take up to 24 hours) and requires technically skilled personnel and special laboratories.[2]

The extraction and amplification done in a closed system in molecular testing give a minimal chance for false positives; well-trained personnel, sample type, and quality, or reagent kit quality are some of the many factors that provide a false-negative result despite the high sensitivity of the real-time PCR assay. Hence, analytical sensitivity plays a crucial role in the accuracy of COVID-19 diagnosis in a patient. Currently, a significant number of SARS-CoV-2 RT-PCR diagnostic tests are being widely utilized throughout the world, all of which claim to have different analytical sensitivities. Numerous studies compare the analytical sensitivity of various assays.[3,4]

As a result of the rapidly spreading COVID-19 epidemic, the FDA authorized the use of various molecular assays for in vitro diagnosis.[5] The accuracy of laboratory-based PCR testing combined with the convenience and speed of point-of-care (POC) rapid antigen testing would make for the ideal diagnostic for COVID-19.[6]

Commercially available rapid diagnostic assays for SARS-CoV-2 detection are simple and affordable; however, how well they perform in the real world is unknown. In this study, we tested performance characteristics of the Direct Detect® SARS-CoV-2 Detection Kit (Coyote Bioscience Co., Ltd., Beijing, China) rapid, molecular-based assay.

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Materials and Methods

A retrospective study was conducted at our hospital's Molecular Biology Laboratory between December 2021 and March 2022. The Institutional Review Board (BH/REC/025/22) and the Abu Dhabi Health Research and Technology Ethics Committee - Department of Health (DOH/CVDC/2022/1641) reviewed and approved the project. As per standard hospital procedure, general consent was obtained to collect data for research purposes.

This study included all the participants who underwent rapid PCR testing. The samples were kept at -80°C and retested using the LabGun® COVID-19 ExoFast RT-PCR Kit, the study's reference standard (Lab Genomics, Korea).

Direct Detect® SARS-CoV-2 Detection Kit Assay

Rapid Nucleic Acid Assay for SARS-CoV-2 was performed using a Direct Detect® SARS-CoV-2 Detection Kit. The Coyote Direct Detect® SARS-CoV-2 Detection kit detects the ORF1ab and N genes with an approximate run time of 40 min. The results are interpreted as positive if the cycle threshold (Ct) values of both the ORF1ab and N genes are ≤27; there is no significant amplification curve or if the Ct value is>27, it is considered negative. Repeat testing is recommended if a single gene is positive. The kit has an internal reference gene RNase P to monitor sampling and identify possible RNA transcription and PCR amplification inhibition.

Standard: RT-PCR Assay Kit

The LabGun® Exofast COVID-19 RT-PCR Kit is a real-time assay that detects the N and RdRp genes of the SARS-CoV-2 virus along with human RNase P, which was used as the internal control from human patient samples. This Standard is a CE-IVD, standardized and validated in-house for routine diagnostic of SARS-CoV-2 detection. Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit was used to isolate and purify nucleic acids from nasopharyngeal swabs. The isolated nucleic acid was amplified directly on the Applied Biosystems QuantStudio® 5 Dx Real-Time PCR System using this kit.

We calculated the sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) to determine the diagnostic value of Direct Detect® SARS-CoV-2 Detection Kit Assay.

Statistical analysis was performed using statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). A probability value of $P<0.05$ was considered statistically significant.

Results

A 2×2 table was drawn up based on the test positivity in both assays (Table 1). Reference real-time PCR diagnosed COVID-19 in 58 patients, of which only 46 were detected by the rapid PCR. Compared to the RT-PCR reference kit, the sensitivity and specificity of the Rapid PCR were 79.31% (95% CI: 66.65% to 88.83%) and 99.54% (95% CI: 98.66% to 99.90%), respectively (Table 1).

Table 1 summarizes the Direct Detect® SARS-CoV-2 Detection Kit test performance characteristics. The test result was stratified by the Ct values, and the highest sensitivity was observed for RT-PCR Ct values <30 and reduced substantially at Ct values >30, $P=0.0003$ (Table 2). Figure 1 represents the Ct values of all discordant RT-PCR specimen results in relation to the results of the Direct Detect® SARS-CoV-2 Detection Kit.

Table 1.

Clinical performance evaluation results of the Direct Detect® SARS-CoV-2 Detection test.

Comparison of Direct Detect® SARS-CoV-2 Detection test and reference RT- PCR				
		RT-PCR Results		Total
		Negative	Negative	
Direct Detect™ SARS-CoV-2 Detection Kit	Positive	46	3	49
	Negative	12	646	658
TOTAL		58	649	707

Performance characteristics of the Direct Detect® SARS-CoV-2 Detection test		
STATISTICS	Value	95% CI
Sensitivity	79.31%	66.65% to 88.83%
Specificity	99.54%	98.66% to 99.90%
Positive Likelihood Ratio	171.57	55.06 to 534.66
Negative Likelihood Ratio	0.21	0.13 to 0.34
Disease prevalence (*)	8.20%	
Positive Predictive Value (*)	93.87%	83.10% to 97.95%
Negative Predictive Value (*)	98.18%	97.02% to 98.89%
Accuracy (*)	97.88%	96.53% to 98.81%
(*) These values are dependent on disease prevalence.		

Table 2.

***Sensitivity of the Direct Detect® SARS-CoV-2 Detection test stratified by RT-PCR cycle threshold (Ct)* intervals.**

RT-PCR Ct value	n	Rapid PCR Positive	Sensitivity % (95% CI)	Rapid PCR Negative	False-Negative Rate (%)
≤ 30	34	30	88.24% (72.55% to 96.70%)	4	11.76
30-<40	24	16	66.67% (44.68% to 84.37%)	8	33.33

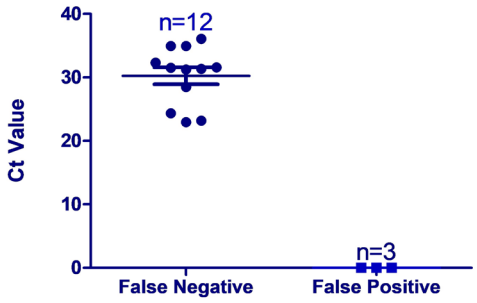

Fig. 1. *Discordant analysis between Direct Detect™ SARS-CoV-2 rapid PCR test and RT-PCR cycle threshold (Ct).*

Discussion

As the SARS-CoV-2 pandemic continues to persist, the disparity between the number of tests required and the testing capacity of laboratories or primary-care settings increases.[7] The ability to detect infected patients in a timely manner has been critical for viral infection control. POC tests have considerably decreased test result lag times, enabling faster clinical intervention and preventative action. There are not enough validation studies to back up the use of these POC tests in various patient scenarios, even though they show potential for usage as a component of a larger strategy for COVID-19 diagnosis and control.[8] This retrospective study comprehensively and systematically evaluated the clinical performance of the Direct Detect® SARS-CoV-2 Detection Test Kit.

As expected, our analysis of the data revealed that false-negative results were seen for high Ct values, whereas we noticed concordance between the POCT and RT-PCR tests at lower Ct, highlighting the potential of the POC test to detect more effectively high viral loads in subjects who were likely to be having symptoms.[9]

Across all 707 tested subjects, there were 12 false-negative results with Ct values between 22 and 40, and three false-positive results for RT-PCR negative results (Figure 1). A single negative test does not rule out infection in individuals because, as indicated for both RT-PCR and rapid PCR testing, the possibility of false-negative results exists due to either sample variability or viral load variation. Repeat testing is recommended if the initial test is negative and if symptoms persist and COVID-19 is suspected. Overall, the rapid PCR test revealed a moderate sensitivity (79.31%) and good specificity (99.54%) in our study compared to the manufacturer-reported sensitivity of 95.02% and a specificity of 99.33%.

However, the data showed a lower sensitivity than the sensitivity of an efficient POC test that the WHO recommended.[8] Data disparity may result from testing samples that were in the late stages of the disease, which may also be a contributing factor. When interpreting the results of POC tests in these samples, caution must be taken because SARS-CoV-2 infection affects a significant section of the population with an asymptomatic presentation.

Since our findings originated from a sizable cohort of participants tested regardless of clinical presentation, the primary limitation of the current investigation is the lack of clinical data. As a result, we are unable to correlate the sensitivity of the POC test with the beginning of symptoms. Recent genetic SARS-CoV-2 virus variants with mutations require close monitoring to assess the potential impact on POC testing.

Acknowledgements

We are thankful to Ms. Shamyla Siddique and Dr. Khaled Musallam for their constant cooperation and support throughout the work. We would like to thank Mr. Mayur Sabhani, Group Director - Laboratory Services, who provided all the facilities for this project in CoLAB, Burjeel Medical City.

Competing Interests

The authors declare that they have no competing interests.

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