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Comparative analysis of Gene FinderTM COVID-19 Plus Real Amp kit, TaqpathTM COVID-19 CE-IVD RT-PCR Kit, and GeneXpert Infinity System for SARS-CoV-2 RNA detection

Edward E. Prestoza, RMT, MLS (ASCPi)CM
College of Allied Medical Professions,
Lyceum of the Philippines University,
Capitol Site, Batangas City
Student Researcher

Abstract

SARS-CoV-2 infection is diagnosed through real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) which can identify the presence of the virus due to its high sensitivity and specificity to SARS-CoV-2 genomic regions. However, there are inadequate data on the performance of commercially available RT-PCR assays for its detection. This study aimed to compare three widely used molecular testing systems: Gene FinderTM COVID-19 Plus Real Amp kit, TaqpathTM COVID-19 CE-IVD RT-PCR Kit and the GeneXpert Infinity System. A total of 80 nasopharyngeal swabs from patient residual samples were tested, including 40 known positive samples and 40 known negative samples. The performance of the three molecular systems was done based on the manufacturer recommendations and evaluated using the results obtained after the amplification. Results revealed a significant difference in N Gene between the three different systems, $X2(2) = 7.181$, $p = 0.028$. On the other hand, E gene Ct values from Gene FinderTM and GeneXpert did not differ significantly (p value = 0.780). Furthermore, the clinical interpretations of the average Ct values across the three systems revealed no significant difference (p value = 0.565). This is a strong evidence that the RT-PCR systems employed in the study are comparable and can be utilized for SARS-CoV-2 RNA detection.

***Keywords**:* COVID-19, Real-time RT-PCR, SARS-CoV-2

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is profoundly infectious and causes a moderately high mortality rate particularly among the elderly and individuals with fundamental conditions (Boccia et al., 2020; Studdert & Hall, 2020; Liu et al., 2020; Koff & Williams, 2020). As indicated by the World Health Organization, as of May 17, 2021, the COVID-19 pandemic has spread to 220 countries and brought about 162,773,940 affirmed cases with 3,375,573 deaths. The Novel Coronavirus was later named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as the causative agent of the COVID- 19 surge across the world (Young et al., 2020; Bernard- Stoecklin et al., 2020; Spiteri et al., 2020; Mousavi et al., 2020; Silverstein et al., 2020; Holshue et al., 2020; Kim et al., 2020; Ghinai et al., 2020) and was first sequenced and recognized by Chinese researchers at the beginning of January 2020 (Tian et al., 2020; ICTV, 2020; Li et al., 2020).

Presently, the clinical diagnosis of COVID-19 is confirmed through the detection of SARS-CoV-2 RNA using real-time RT-PCR assay (Corman et al., 2020; Shirato et al., 2020; Konrad et al., 2020; Chu et al., 2020) which can detect its presence as early as the very first onset of infection (Sethuraman et al., 2020). This was made possible due to the rapid accessibility of genome sequences (Zhou et al., 2020; Wu et al., 2020) and molecular assays were then developed for the identification of the SARS-CoV-2 virus. The primary molecular-based assay depended essentially on the discovery of SARS-CoV- 2 envelope (E), RNA-dependent RNA polymerase (RdRp), and nucleocapsid (N) genes (Corman et al., 2020; WHO, 2020). The more recent development of real-time RT-PCR strategy targets incorporates open reading frame1ab (Orf1ab) and the gene encoding spike (S) protein. These gene sequences were the target of the different primer probes used for SARS-CoV-2 detection.

According to Vogels et al. (2020), all primer-probe sets for RT-PCR assays are highly similar in terms of analytical sensitivities with the exemption of primers targeting the RdRp gene7. Al-Tawfiq & Memis (2020) also claimed that the sensitivity of RT-PCR revolves around 45% to 60%, while Wang et al., 2020 demonstrated that the sensitivity of the molecular assay is around 63% to 72% depending on the samples. The performance sensitivity of the real-time RT-PCR assay can be affected by the RNA purification method, one-step RT- PCR reagent and the primer/probe sets (Ishige et al., 2020; Alcoba-Florez et al., 2020).

The clinical application of average Cycle threshold values in diagnosing COVID-19 relies on the monitoring of viral loads. As the value of the thermal cycle threshold lowers, the higher the quantity of viral genetic material present in the specimen. A recent study conducted by Liu et al. (2020) recommended that the SARS-CoV-2 viral load can be used as a marker in assessing disease severity and prognosis.

This clinical application and patient management concept have been useful in mitigating the mortality rate among the population. Patients with low Ct value (High viral load) are immediately taken to government-owned hospitals for close monitoring and treatment. Medium Ct value (Medium viral load) individuals are placed in the designated quarantine areas. While patients with high Ct value (low viral load) are placed in-home quarantine. This strategy has immensely impacted the total number of deaths in several countries such as in Qatar since the start of the pandemic.

There are many commercially available real-time RT-PCR diagnostic kits (Afzal, 2020; Dayarathna et al., 2020), which are widely used around the world. However, there are inadequate data on the performance of commercially available RT-PCR assays for SARS-CoV-2 RNA detection. This study aimed to compare the three widely used molecular tests, Gene FinderTM COVID-19 Plus Real Amp kit, TaqpathTM COVID-19 CE-IVD RT-PCR Kit in terms of gene-specific and average Ct values with the GeneXpertTM Infinity System used as a reference system as it operates with a one-step, cartridge- based, closed system RT-PCR technology for the detection of SARS-CoV-2 (Ishige et al., 2020; Alcoba-Flores et al., 2020).

MATERIALS AND METHODS

The de-identified residual patient samples that underwent routine COVID-19 testing were utilized to evaluate the Gene FinderTM COVID-19 Plus Real Amp kit (OSANG Healthcare Co., Ltd., Gyeonggi-do, 14040, Korea), TaqpathTM 1-Step Multiplex Master Mix (Thermo Fisher Scientific Inc., Waltham, MA 02451 USA) and GeneXpert Infinity System (Cepheid, Sunnyvale, CA).

Sampling

A total of 80 residual nasopharyngeal swab samples were collected in 3 mL of viral transport media (CTM; Noble Biosciences

Inc., Gyeonggi-do, 18521, Republic of Korea) and sample preservation solution (AnHui WenSheng Medical Materials Co., Ltd, High-tech District, HeFei, AnHui, China) were used. The specimens came from random sample screening locations for testing potential SARS-CoV-2 exposed patients. All nasopharyngeal swabs in transport media were held at 4°C prior to testing, with all testing completed within 48 hours of sample collection.

To evaluate the performance of the three molecular diagnostic systems, 40 known positive specimens were randomly selected to generate Ct values using Gene FinderTM COVID-19 Plus Real Amp kit, TaqpathTM COVID-19 CE-IVD RT-PCR Kit, and the GeneXpert Infinity System. Moreover, additional 40 known negative samples were randomly selected to assess the negative cycle threshold.

Analytical methods

The performance of the three molecular systems was done based on the manufacturer's recommendations and evaluated using the results obtained after the amplification. The residual samples underwent nucleic acid isolation using the automated Bioneer ExiprepTM 96 Lite nucleic acid extractor (Bioneer Corporation, Daejeon 34302, Republic of Korea). The two master mixes (Gene FinderTM COVID-19 Plus Real Amp kit and TaqpathTM COVID-19 CE-IVD RT- PCR Kit) were prepared and pipetted into the 96-well reaction plate. The isolated RNA template was added to master mixes and analyzed using QuantStudio™ 5 real-time RT-PCR System. For the GeneXpert Infinity System, individual samples were placed on cartridges and processed through the automated protocol set by the manufacturer. Samples were all processed in a BioSafety level III COVID-19 facility.

Sample preparation and RNA extraction for Gene FinderTM COVID-19 Plus

Real Amp kit

The sample preparation procedure for Gene FinderTM was based on the manufacturer's recommendation. The powdered proteinase K was dissolved using 1,250 µL of nuclease-free water and mixed vigorously. A 20 µL of dissolved proteinase K was added to each well of cartridge 1 of the Bioneer ExiPrepTM 96 Viral DNA/RNA Kit. All sample tubes (NP swabs) were mixed gently for 1 minute to achieve complete homogeneity of the liquid media. A 200 µL was pipetted from each sample into each well of cartridge 1.

Cartridge 1 was then loaded to Bioneer ExiPrepTM 96 Lite nucleic acid extractor along with the remaining 6 cartridges which contain the binding buffer, washing buffer, elution buffer and magnetic bead solution. After 48 minutes, cartridge 7 containing the pure RNA with elution buffer was used for RT-PCR.

Sample preparation and RNA extraction for TaqpathTM COVID-19 CE-IVD RT-PCR kit)

The sample preparation procedure for TaqpathTM was based on the manufacturer's recommendation. The powdered proteinase K was dissolved using 1,250 µL of nuclease-free water and mixed vigorously. A 20 µL of dissolved proteinase K was added to each well of cartridge 1 of the Bioneer ExiPrepTM 96 Viral DNA/RNA Kit. A 5 µL of MS2 phage control was added as well to each well to verify the efficacy of the sample preparation. All Sample tubes (NP swabs) were mixed gently for 1 minute to achieve complete homogeneity of the liquid media. A 200 µL was pipetted from each sample into each well of cartridge 1. A 200 µL of nuclease-free water will be added to serve as a negative control for the RT-PCR assay. Cartridge 1 was then loaded to Bioneer ExiPrepTM 96 Lite nucleic acid extractor along with the remaining 6 cartridges which contain the binding buffer, washing buffer, elution buffer and magnetic bead solution. After 48 minutes, cartridge 7 containing the pure RNA with elution buffer was used for RT-PCR.

Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) using Gene FinderTM COVID-19 Plus Real Amp kit

The protocol for Gene FinderTM (OSANG Healthcare Co. Ltd. Gyeonggi-do 14040, Korea) was based on the manufacturer's recommendation which generated Ct values for the N gene, E gene and RdRp gene. A 10 uL of COVID-19 Plus Reaction Mixture and 5 µL of COVID-19 Plus Probe Mixture were mixed to prepare a master mixture for each reaction. After mixing well, 15 µL of the master mixture was placed into MicroAmp™ Optical 96-Well Reaction Plate. A 5 µL of extracted RNA sample was added into a 96- well reaction plate, all components were mixed through pipetting the sample up and down. The procedure was repeated in the same way with the rest of the samples together with positive control and negative control. The reaction plate was sealed with MicroAmp™ optical adhesive film, vortexed for 10 seconds to ensure proper mixing, then underwent centrifugation for 1 minute at 2000 rpm. Finally, the 96-well reaction plate was loaded to QuantStudio™ 5 real-time RT-PCR System for amplification.

Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) using TaqpathTM COVID-19 CE-IVD RT-PCR Kit

The protocol for TaqpathTM (Thermo Fisher Scientific Inc., Waltham, MA 02451, USA) was based on the manufacturer's recommendation which generated Ct values for the N gene, ORF1ab and S gene. A 6.25 uL TaqpathTM 1-Step Multiplex Master Mix (No ROXTM) 4X, 1.25 µL of COVID-19 Real-Time PCR Assay Multiplex, and 7.50 µL of nuclease-free water were mixed to prepare master mixture per each reaction. After mixing, a 15 µL of the reaction mixture was pipetted into each well of a MicroAmp™ Optical 96-Well Reaction Plate. The sealed cartridge 1 from the nucleic acid extraction area was gently vortexed, then centrifuged briefly to collect liquid at the bottom of the plate. Cartridge 1 was unsealed and 10 µL of purified RNA samples were added to each well of the reaction plate. The Negative control from cartridge 1 and Positive Control were added to the remaining well of the reaction plate. After each addition, it was mixed by pipetting up and down 10 times. The plate was sealed with MicroAmp™ Optical Adhesive Film, vortexed for 10 seconds to ensure proper mixing, then centrifuged for 1 minute at 2000 rpm. Finally, the 96-Well Reaction Plate was loaded to QuantStudio™ 5 real-time RT-PCR System to start the amplification.

Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) using Gene FinderTM COVID-19 Plus Real Amp kit

The protocol for Gene FinderTM (OSANG Healthcare Co. Ltd. Gyeonggi-do 14040, Korea) was based on the manufacturer's recommendation which generated Ct values for the N gene, E gene and RdRp gene. A 10 uL of COVID-19 Plus Reaction Mixture and 5 µL of COVID-19 Plus Probe Mixture were mixed to prepare a master mixture for each reaction. After mixing well, 15 µL of the master mixture was placed into MicroAmp™ Optical 96-Well Reaction Plate. A 5 µL of extracted RNA sample was added into a 96- well reaction plate, all components were mixed through pipetting the sample up and down. The procedure was repeated in the same way with the rest of the samples together with positive control and negative control. The reaction plate was sealed with MicroAmp™ optical adhesive film, vortexed for 10 seconds to ensure proper mixing, then underwent centrifugation for 1 minute at 2000 rpm. Finally, the 96-well reaction plate was loaded to QuantStudio™ 5 real-time RT-PCR System for amplification.

Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) using TaqpathTM COVID-19 CE-IVD RT-PCR Kit

The protocol for TaqpathTM (Thermo Fisher Scientific Inc., Waltham, MA 02451, USA) was based on the manufacturer's recommendation which generated Ct values for the N gene, ORF1ab and S gene. A 6.25 uL TaqpathTM 1-Step Multiplex Master Mix (No ROXTM) 4X, 1.25 µL of COVID-19 Real-Time PCR Assay Multiplex, and 7.50 µL of nuclease-free water were mixed to prepare master mixture per each reaction. After mixing, a 15 µL of the reaction mixture was pipetted into each well of a MicroAmp™ Optical 96-Well Reaction Plate. The sealed cartridge 1 from the nucleic acid extraction area was gently vortexed, then centrifuged briefly to collect liquid at the bottom of the plate. Cartridge 1 was unsealed and 10 µL of purified RNA samples were added to each well of the reaction plate. The Negative control from cartridge 1 and Positive Control were added to the remaining well of the reaction plate. After each addition, it was mixed by pipetting up and down 10 times. The plate was sealed with MicroAmp™ Optical Adhesive Film, vortexed for 10 seconds to ensure proper mixing, then centrifuged for 1 minute at 2000 rpm. Finally, the 96-Well Reaction Plate was loaded to QuantStudio™ 5 real-time RT-PCR System to start the amplification.

Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) using GeneXpert Infinity System

The protocol for GeneXpert Infinity System (Cepheid, Sunnyvale, CA) was based on manufacturer recommendations which generated Ct values for both N and E genes. All Sample tubes (NP swabs) were mixed gently for 1 minute to achieve complete homogeneity of the liquid media in the biosafety cabinet class II. Each residual specimen was loaded into the cartridge using the disposable pipette included in the testing cartridge package. After the sample was placed, the lid of the cartridge was closed. The cartridge acted as a miniature molecular laboratory that performs sample preparation, amplification, and detection in a closed system. The cartridge containing the sample was placed in the automated system through a conveyor and undergone the full molecular RT-PCR process.

Clinical interpretation of average Ct values

The average Ct values from the known positive samples were obtained by getting the average of the Ct values from each of the target genes across the three RT-PCR systems. The average

Ct values were classified as low (0- 20), medium (20-30) and high (>30) (Smithgall et al., 2020). Results were compared to determine the comparability of the methods for the clinical application and management of COVID-19 positive individuals.

Statistical Analysis

A normality test was performed to determine the appropriate statistical tests. The Kruskal-Wallis H test was used to compare N gene Ct values from Gene FinderTM, TaqpathTM and the GeneXpert. Mann-Whitney U test was also used to compare E gene results from Gene FinderTM and GeneXpert. In addition, the Kruskal-Wallis H test was again utilized to determine if there is a significant difference in the clinical interpretations of the average Ct values across the three RT-PCR systems

RESULTS AND DISCUSSION

To meet the global demand for wide-scale molecular diagnostic testing during the COVID-19 pandemic, numerous molecular tests had been given an emergency use authorization by the US FDA (FDA, 202a). However, there is insufficient data published on the performance of commercially available RT- PCR assays for SARS-CoV-2 RNA detection. The purpose of this study was to compare the three molecular diagnostic assays and to determine differences in the Gene FinderTM, TaqpathTM and the GeneXpert generated gene-specific and average Ct values.

Table 1 shows the comparison of the N gene Ct values obtained from Gene FinderTM COVID-19 Plus Real Amp kit, TaqpathTM COVID-19 CE-IVD RT-PCR Kit and the GeneXpertTM Infinity System.

Table 1

Comparison of N gene Ct values obtained from the three systems

	Group	Mean Rank	A^2c	p-value
N gene	GeneXpert™	68.00	7.181	0.028
	Gene Finder™	64.90		
	Tagapath™	48.60		

p-value significant at less than 0.05 alpha level

The results revealed that there is a significant difference in N Gene between the three different systems, X2(2) = 7.181, p = 0.028, with a mean rank of 68 for GeneXpert Infinity Systems, 64.9 for Gene FinderTM COVID-19 Plus Real Amp kit and 48.6 for

TaqpathTM COVID-19 CE-IVD RT-PCR Kit. Based on the pairwise comparison, GeneXpert and TaqpathTM showed a significant difference and that GeneXpert Infinity Systems showed higher N gene Ct values. Although N gene Ct values obtained from the two systems are found to be statistically significant but their clinical significance and application remain comparable with the other methods. This is in contrast with the study conducted by Krismawati et al. (2021), which found no significant difference with respect to the Ct values obtained from the target genes of positive specimens. According to the study conducted by Ong et al. (2020), the N gene has an increased detection window in comparison to other targets. Thus, molecular tests that yield statistically similar clinical sensitivity is crucial in determining appropriate assay to mitigate misdiagnoses of SARS-CoV-2 infection.

Table 2 shows the comparison of Ct values obtained from the E gene of Gene FinderTM COVID-19 Plus Real Amp kit and the GeneXpert Infinity System. The result demonstrates that the E gene of Gene FinderTM and GeneXpert does not differ significantly with a p-value of 0.780, with a mean rank of 39.78 for GeneXpert, 41.23 for Gene FinderTM. This finding suggests that the Ct values for E gene obtained from both GeneXpert and Gene FinderTM statistically yielded the same results.

Table 2

Comparison of E gene Ct values obtained from Gene FinderTM COVID-19 Plus Real Amp kit and the GeneXpert Infinity System

	group	Mean Rank	Sum of Ranks	U	P-Value
E gene	GeneXpert™	39.780	1591		
	Gene Finder™	41.23	1649	771.00	0.780

p-value significant at less than 0.05 alpha level

The CDC recommended the N1 and N2 targets within the N gene (Zhuet al., 2020; Holshue et al., 2020) and WHO recommends an initial test with the E gene superseded by confirmation with RNA-dependent RNA-polymerase(RdRp) (Corman et al., 2020). A recent study conducted by Nalla et al. (2020) concurred with the recommendations given by CDC and WHO which states a higher sensitivity for primers that target the N2 or E gene. However, other published studies demonstrate that the performance of an assay is not dictated by the selected gene for SARS-CoV-2 detection. The

approach of targeting three genes seems to increase the sensitivity and specificity of an assay. In comparison with GeneXpertTM which only has two targets (N2 and E gene), the Gene Finder and Taqpath hold the advantage of having multiple targets (three genes), which enhances the sensitivity in detecting SARS-CoV-2 RNA. The difference in sensitivity among the two molecular diagnostic tests is primarily due to their different components (primers, buffer, enzymes, and contents of the reagent). These data can help determine the impact of the solution and to choose an assay that meets the urgent need for extensive diagnostic testing during the pandemic

Table 3. Sensitivity and Specificity of GeneFinder™ COVID-19 Plus Real Amp kit, Taqpath™ COVID-19 CE-IVD RT-PCR Kit

	Sensitivity	Specificity
Gene Finder™	100%	97.5%
Taqpath™	100%	100%

Tables 3 indicate the sensitivity and specificity of Gene Finder™ COVID-19 Plus RealAmp kit, Taqpath™ COVID-19 CE-IVD RT-PCR kit. In this study, the GeneXpert Infinity System was used as a reference system as it operates with a one-step, cartridge-based, closed system RT-PCR technology for the detection of SARS- CoV-2 (Ishige et al., 2020; Alcoba-Flores et al., 2020) and was compared with Gene Finder™ and Taqpath™ for COVID-19 positivity detection. Both systems showed a 100% sensitivity with respect to the measurement of the true positive rate.

The 40 residual samples that resulted positive in varying Ct values from GeneXpertTM Taqpath™ were correctly identified by Gene Finder and Taqpath. However, the obtained specificity was 97.5% and 100% for Gene FinderTM and TaqpathTM respectively. One sample tested negative by GeneXpertTM in both N2 and E gene targets but presumed positive by Gene Finder based on the detection of the N gene (ct value: 38) with no amplification on E gene and RdRp targets. The results showed that the TaqpathTM is more specific in terms of correctly identifying patients without the disease. These findings are in contrast with the conclusion of Ong et al. (2020) that suggests that Gene Finder has a high specificity in respect to detecting SARS-CoV-2 RNA.

Table 4. PPV and NPV of Gene FinderTM COVID- 19 Plus Real Amp kit, TaqpathTM COVID-19 CE-IVD RT-PCR Kit

	PPV	NPV
Gene Finder™	97.6%	100%
Taqpath™	100%	100%

The positive predictive value (PPV) and negative predictive value (NPV) of the two molecular systems were shown in table 4. The two molecular systems yielded a 100% negative predictive value (NPV) suggesting that the proportion of negative results are truly negative. This means that all negative samples in this study were correctly identified by both molecular systems. The positive predictive value (PPV) on the other hand, revealed 97.6% for Gene Finder and 100% for Taqpath which indicates that the Gene Finder identified 2.6% false positive and 97.6% true positive. One sample that was tested in Gene Finder yielded a positive result while in GeneXpert and Taqpath did not generate any amplification in all the target genes. These findings highlighted an important limitation of Gene FinderTM for positive specimens. While Ong, Claas, Breijer, and Norbert (2020) and Farfour et al. (2020) concluded high sensitivity of Gene FinderTM, the specificity of an assay should be considered as well in determining diagnostic RT-PCR tests. Although the performance of these systems is not identical, they both fit for purpose in the clinical setting where a high prevalence of COVID-19 prevails.

Table 5. Difference in clinical interpretations of the average Ct values across the three systems

	N	Mean Rank	Chi-Square	ds	Sig
GeneXpert™	40	62.75			
Gene Finder™	40	62.75	1.42	2	0.565
Taqpath™	40	56.00			
Total	120				

p-value significant at less than 0.05 alpha level

To determine the difference in the clinical interpretations of the average Ct values across the three methods, the Kruskal-Wallis H Test wasused and results are presented in table 5. The test revealed no significant difference in the clinical interpretations of the average Ct values between the three methods (p-value = 0.565).

The data suggest that regardless of the RT-PCRsystem used in the study, with consideration to the number of targets for SARS-CoV-2 detection,the average Ct values of the three systems are similar when used as a basis for clinicalinterpretation and reflect similar management and approach to COVID-19 positive individuals.

These findings are in concurrence with the study conducted by Smithgall et al. (2020) comparing three assays with different target genes for samples with low, medium, and high viral concentrations. It was concluded that the methods compared showed a high positive and negative agreement with the reference method. In addition, the sensitivity of the tests included inthe study detected a 100% positive rate in both low (Ct value <20) and medium Ct values (Ct value 20-30) in comparison to the in-house method. Another similar study performed by Tastanova et al. (2021), showed high sensitivity to SARS-CoV-2 RNA, despite the real-time RT- PCR based tests compared, which were composed of singleplex and multiplex assays.

CONCLUSION

Based on the findings, the Gene Finder™ COVID-19 Plus Real Amp kit, Taqpath™ COVID- 19 CE-IVD RT-PCR Kit and the GeneXpert Infinity System are comparable and can be utilized for SARS-CoV-2 RNA detection. These three RT-PCR systems hold a promising deliveryof efficiency and valuable clinical information. Other factors should also be considered in deciding appropriate tests, such as short turnaround time, availability of reagents and cost- effectiveness. These components are crucial during this pandemic for timely and appropriate patient management decisions.

RECOMMENDATIONS

Limitations of the study include the relativelyfew numbers of residual samples from three different viral concentrations. Furthermore, the sample collection did not take into consideration the stage of infection or the time difference between sample collection and the onset of symptoms. Another consideration for comparing or validating RT-PCR systems is that GeneXpert Infinity System has only two target genes (N2 gene and E gene) used for SARS-CoV-2 RNA detection, whereas the Gene Finder (N gene, E gene, and RdRp) and Taqpath (N gene, ORF1ab, and S gene) use three target genes. To further evaluate the performance of these

three assays, there is a need for systematic research and careful analysis with a larger sample size to find conclusive evidence of its efficiency.

RT-PCR assays must be appropriately validated before use especially when there is no declaration of sensitivity and specificity. Further comparisons of commercially available molecular diagnostic tests are recommended in order to identify other diagnostic errors and to determine the usefulness of each assay. This information helps medical providers to recognize and determine the molecular diagnostic tests that are appropriate for their patients.

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