Comparative Performances Of Four Severe Acute Respiratory Syndrome-Coronavirus-2 Real-Time Polymerase Chain Reaction Assays For The Diagnosis Of COVID-19 In Ethiopia

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Abstract

Since coronavirus disease-2019 (COVID-19) outbreak was reported, many commercial Nucleic Acid Amplification Testing (NAAT) assays have been developed all over the world, and it has been the standard method. Though, a number of assays were rapidly developed and applied to clinical testing, performance of these assays were not evaluated in different contexts. Thus, this study was aimed to assess the performance of common assays (Abbott SARS-CoV-2, DaanGene, BGI and Sansure Biotech) available in Ethiopia by using combined reference results (CRR). The study was conducted at Ethiopian Public Health Institute (EPHI) from December 1 to 30/2020. A total of 164 nasopharyngeal samples were extracted by using QIAamp® RNA mini kit and Abbott DNA sample preparation system. Out of 164 samples, 59.1% was positive and 40.9% was negative by CRR. Sansure Biotech positivity was significantly low compared to CRR ($p<0.05$). The overall agreement of the four assays compared to CRR was 96.3% to 100%. The performance of four assays had almost comparable diagnostic performance, except a low positive rate of Sansure Biotech assay. Hence, Sansure Biotech assay (RUO) needs further verification on its use in Ethiopia. Finally, an additional study should be considered for the evaluation of assays with respective manufacturer claims.

Introduction

Laboratory testing is an integral part of the World Health Organization (WHO) Strategic Preparedness and Response Plan (SPRP) of Coronavirus disease-19 (COVID-19).WHO recommends that, countries need to increase the laboratory capacity to boost their level of preparedness, proper case management, alert and quick response for the public health. This indicates that, the role of the laboratory is a key for defining disease characteristics, epidemiology of an emerging infectious pathogen and in controlling its spread [1].

Since the COVID-19 outbreak reported from Wuhan, China, many commercial NAAT assays have been developed all over the world. Still now, real-time PCR (RT-PCR) has been the routine and standard method for the laboratory diagnosis of SARS-CoV-2 infection [2]. The common genes used for the diagnosis of the SARS-CoV-2 viral genome are based on N (Nucleocapsid protein gene), E (envelope protein gene), and RdRP gene (RNA-dependent RNA polymerase gene) in ORF1ab (Open Reading Frame region) considered as the main conserved genes for identification of the virus [3]. Out of these genes, the RdRP and E genes had high analytical sensitivity for detection, whereas, N gene provided poorer analytical sensitivity [4].

The analytical performance characteristics of PCR methods can vary with the quality of extraction reagent, amplification/ detection reagent, method of extraction, the PCR machine and other instrumentation. For the diagnosis of COVID-19 more than 48 different diagnostic devices from 9 (nine) countries received Emergency Use Authorization (EUA) as of April 2020 [5]. In Ethiopia, more than fourteen RT-PCR platforms in 26 governmental public health testing facilities, including ABI 7500, Abbott m2000, Roche 48000, Quant-studio are in use for SARS-CoV-2 PCR testing [6]. Similarly, different PCR detection kits are available, such as; Daan Gene assay, Abbott SARS-CoV-2 assay, Sansure Biotech assay

and BGI SARS-CoV-2 assay. Therefore, clinical performance comparisons are very crucial by using the standard methods. Although RT-PCR assay is a highly sensitive technique, false-negative results have still been reported in some COVID-19 patients due to insufficient viral RNA copies in the specimen resulting from the improper collection, transportation, storage, and handling, as well as laboratory testing conditions and personnel operation [7].

Even though, there are many NAAT reagents available in Ethiopian Public Health Institute (EPHI) and as well as in Ethiopia, to date, no comparative performance evaluation of SARS-CoV-2 NAATs has been reported. Therefore, the purpose of this study was to assess the performance of commercially available SARS-CoV-2 detection kits by using clinical samples.

Results

Characteristics of study participants

A total of 164 COVID-19 suspected participants were included in this study. Out of these, 15 (9.1%) were clinically suspected for COVID 19 and 31(18.9%) had contact with confirmed cases. The majority of samples were taken from treatment center (118/164 = 72%). Ninety-three (56.7%) of participants were male and the mean (± SD) age of the participants was 31.10(± 11.82) years.

Performance comparison of SARS-CoV-2 assays

In this study, the rate of positivity and negativity with different COVID-19 testing assays and platforms were determined. Hence, the positive rate of Abbott SARS-CoV-2 assay, Daan Gene 2019-nCoV assay, BGI SARS-CoV-2 assay and Sansure Biotech 2019-nCoV assay were 59.1%, 58.5% 57.9% and 55.5% respectively. The positive and negative rate of CRR was 97(59.1%) and 67(40.9%) respectively (Table-2).

In this study, we found that the negative percent agreement (NPA) of all assays was 100% (95% CI, 94.6–100) compared to the CRR. The positive percent agreement (PPA) of Abbott SARS-CoV-2 assay was 100% (95% CI; 96.3–100) and the lowest PPA was shown in Sansure biotech assay (93.8% (95% CI, 87.2–97.1)) (Table 3).

Table 1
; summary of PCR cycling and result interpretation of four assays

Type of assay	Cycle/s	Temp °C	Duration	Result interpretation
Abbott SARS-CoV-2 assay				• The result and interpretations were reported automatically by Abbott m2000 RT-PCR workstation. If the target and internal control amplification was detected the result displayed as “positive” and the target was not amplified and internal control amplification was detected the result displayed as “negative”. Error codes and invalid results also displayed based on the error types [15].
Daan Gene nCoV-2019 assay	1 cycle	50	15 min	• If the target gene (N) (labeled with FAM), ORF1a/b (labeled with VIC) and internal control (labeled with Cy5) were detected and Ct values were ≤ 40, the result was interpreted as “positive”. • If the result had no amplification curve or Ct value > 40 in the FAM and VIC channels and there was amplification curve in the Cy5 channel, the result interpreted as “negative”. • If the Ct value of a sample was ≤ 40 in a single channel of FAM or VIC, and there is no amplification curve was detected in the other channel, interpreted as “retested the sample” and retested result was taken as a final result whereas the result was positive or negative [20].
	1 cycle	95	15 min	
	45 cycle	94	15 sec	
		55	45 sec	
BGI SARS-CoV-2 assay	1 cycle	50	20 min	• If there was amplification curve in the FAM channel (ORF1a/b target region) and the Ct value was less than 37, the presence of amplification curve in the VIC channel (internal control) with the Ct value was less than 35,were interpreted as “positive”. • The sample was reported as “negative” when, there was no amplification curve in the FAM channel and there was amplification curve in the VIC channel with the Ct value was less than 35. All samples with their VIC channel amplification curve were not detected or the Ct values greater than 35 were retested and the result of after retesting was taken as final results [22].
	1 cycle	95	10 min	
	40 cycles	95	15 sec	
		60	30 sec	
Sansure Biotech n-CoV assay	1 cycle	50	30 min	• If there was amplification curve in the FAM (target ORF1a/b) channel and or amplification curve was observed in the ROX channel (target N gene) with Ct value was ≤ 40 without consideration of internal control amplification curve (Cy5 channel), interpreted as “positive”. • If there was no amplification curve in both FAM and ROX channel and there was amplification curve in Cy5 channel was interpreted as “negative”. If there was no amplification curve in all channels or the Ct value was greater than 40 was retested the sample [21].
	1 cycle	95	1min	
	45 cycles	95	15 sec	
		60	30 sec	

Table 2
Rates of COVID-19 in different SARS-CoV-2 assays

Types of assays and platforms	Positive (%)	Negative (%)
Abbott SARS-CoV-2 assay	97 (59.1)	67 (40.9)
Daan Gene 2019-nCoV assay	96 (58.5)	68 (41.5)
BGI SARS-CoV-2 assay	95 (57.9)	69 (42.1)
Sansure Biotech 2019-nCoV assay	91 (55.5)	73 (44.5)
Combined reference result (CRR)	97 (59.1)	67 (40.9)

Table 3
Percent agreement of four SARS-CoV-2 PCR testing assays compared to CRR

S/n	PCR assay with different PCR platform	Positive Percent agreement (95% CI)	Negative percent agreement (95% CI)	Overall percent agreement (95% CI)	Cohen's Kappa value (95% CI)	MacNemar test (p-value)
1	Abbott SARS-CoV-2 assay	100% (96.3–100)	100% (94.6–100)	100% (97.7–100)	1.00	1.00
2	Daan Gene 2019-nCoV assay	99% (94.4–99.8)	100% (94.6–100)	99.4% (96.6–99.9)	0.987 (0.96-1.00)	1.00
3	BGI SARS-CoV-2 assay	97.9% (92.8–99.4)	100% (94.6–100)	98.8% (95.7–99.7)	0.975 (0.927-1.00)	0.50
4	Sansure Biotech 2019-nCoV assay	93.8% (87.2–97.1)	100% (94.6–100)	96.3% (92.2–98.3)	0.925 (0.86–0.975)	0.031*

*indicates statistically significant relation

The Overall agreement between CRR and Abbott SARS-CoV-2 assay was 100% (95% CI; 94.6–100). While, the overall agreement of Daan Gene 2019-nCoV assay was 99.4% (95% CI; 96.6–99.9). In contrast, the overall agreement of BGI SARS-CoV-2 assay and Sansure Biotech 2019-nCoV assay were 98.8% and 96.3% respectively (Table-3).

Cohen's Kappa coefficient of agreement between CRR and Abbott SARS-CoV-2 assay result had a perfect agreement (K = 1.00). Similarly, the Cohn's Kappa value of Daan Gene 2019-nCoV assay had also perfect agreement with CRR (K = 0.987). Another Cohn's kappa value indicated that BGI SARS-CoV-2 assay and

Sansure Biotech 2019-nCoV assay had perfect agreement compared with CRR, which were 0.975 and 0.925 respectively. In this comparative analysis, the Chi-square test (MacNemar test) showed that the result of Sansure Biotech 2019-nCoV assay was significantly different compared to CRR (p = 0.031) (Table-3).

Comparative analysis of Ct values in four SARS-CoV-2 PCR assays

As can be seen in Fig. 1, the percentage of lowest Ct value (< 20 Ct) of Abbott SARS-CoV-2 assay (combined RdRp and N gene) was 87.6% and ORF1a/b gene Ct value of Sansure Biotech 2019-nCoV assay showed that, the percentage of low Ct value (< 20 Ct) was 50.3% and the high Ct value (36–40 Ct) was 3.2%. In this study Ct values greater than 30 was not recorded in Abbott SARS-CoV-2 assay. On the other hand, on the BGI SARS-CoV-2 assay ORF1a/b gene high Ct value (> 36 Ct) percentage was 4% (figure-1).

Discussion

In this study, we compared the diagnostic performance of Abbott SARS-CoV-2 assay (EUA), Daan Gene assay (EUA), BGI SARS-CoV-2 assay (EUA) and Sansure Biotech assay (RUO) with CRR by using 164 nasopharyngeal samples.

This study showed that Abbott SARS-CoV-2 assay had equal detection performance with combined reference results (CRR), which had 100% positive, negative and overall agreement. The Cohn's Kappa agreement was 1.00; this indicated that it has a perfect agreement with CRR. A similar study reported from Washington University, USA showed that the overall sensitivity and specificity of Abbott SARS-CoV-2 assay was 93% and 100% respectively compared to CDC based laboratory defined assay (LDA) [8]. Abbott SARS-CoV-2 assay was based on the combined gene of N and RdRP, since both genes had more sensitivity to minimize false negative results [9]. The study conducted in Vienna, Austria also showed that a high amount of sample volume for extraction and volume of eluate for detection could minimize the dilution effect and increase the detection efficacy [10]. So, the perfect agreement of Abbott SARS-CoV-2 assay might be due to the combined gene method, large volume of sample for extraction (0.5ml) and using the large volume of eluate (40µl).

Our result also indicated that, Daan gene assay detection performance was almost similar compared to the CRR. This is in line with the study conducted in Anhui University, Huainan, China [11] and with the claim of the manufacturer, which was the positive coincidence rate was 100%. Even though, concordance result was reported, in our study one sample was falsely negative after retesting on the same eluate, but the same sample eluate was positive in Abbott SARS-CoV-2 assay and BGI SARS-CoV-2 assay. This indicated that result variability might be seen in a different type of assays. Nevertheless, in the study carried out in China [12], the result of the Daan Gene assay was significantly different (p < 0.05) compared to lab defined reference assay. This difference might be due to the sensitivity of reference assay to detect SARS-CoV-2 and further study might be important to determine the reason.

Additionally, our study also assesses the comparative performance of BGI SARS-CoV-2 assay with CRR, our study revealed excellent positive percent agreement (PPA = 97.9%), negative percent agreement (NPA = 100%) and Overall percent agreement (OPA = 98.8%). The Cohn's Kappa value was 0.975, which indicated an excellent agreement. Concordant results were reported from the study done in the Netherlands [13] and China [12]. BGI SARS-CoV-2 assay is a single gene (ORF 1a/b) detection assay and uses 10µl eluate for detection. Even though, there was an excellent statistical agreement compared with our reference results, the assay missed two positive samples (1.22%) from the total samples. It might be has great clinical impact on the patient and as well as on the transmission dynamics at the community level.

The other comparator assay included in this study was Sansure Biotech nCoV- 2019 RT-PCR assay (RUO); the overall percent agreement was 96.3%. The strength of agreement also determined with Cohn's Kappa value, which was 0.925, this indicated a perfect agreement with CRR. Similarly, our result is the same as the study conducted in Central South University, Changsha district, China [12], Liuzhou People's Hospital, Department of Clinical Laboratory, Liuzhou, China [14]. Even though, the above good statistical concordance was recorded, the Chi-square test (MacNemar test) showed that the result of the Sansure Biotech assay has had a statistically significant difference compared to CRR ($p < 0.005$). Six (06) samples (3.66%) were turned to falsely negative compared with CRR **(S-table 1)**; this is critical, especially when we consider the virus transmission dynamics. This low detection rate is also supported by the above evidence [12].

In this study each detection assay with their respective platform Ct values are determined, the lowest mean Ct value (high viral RNA copies) was recorded on the assay of Abbott SARS-CoV-2. The result indicated that the combined gene detection system of the Abbott SARS-CoV-2 assay had better efficiency in the detection of COVID-19 even with low viral concentration. Hence, according to **fig-1**, 87.6% of Abbott SARS-CoV-2 result Ct value was laid on less than 20. Only a few sample results (12.4%) were laid between Ct values of 20–30. There was no Ct value recorded above 30.

Limitation of the study

The limitation of this study was we did not have a standard/reference method (like; viral load assay or other LDA assay) due to resource limitation.

Conclusion

This study compared the performance of four SARS-CoV-2 RT-PCR detection assays by using nasopharyngeal samples. All detection assays except Sansure Biotech assay have almost comparable performance. Besides, the low positivity rate was identified in Sansure Biotech assay compared to the CRR ($P < 0.05$).Sansure Biotech nCov-2019 assay (RUO) PPA, NPA, and overall agreement were greater than 93.5% and Cohn's Kappa agreement strength value was 0.925. Finally, Sansure Biotech assay (RUO)

needs further verification on its use in Ethiopia and additional study should be considered for the evaluation of respective manufacturer claims.

Methods

Study design and setting

A comparative study design was conducted at four health facilities in Addis Ababa, which were Eka kotebe hospital, millennium hall treatment center, Zewuditu memorial hospital and St Peter TB specialized hospital. Data were collected between December 1 to 31/2020. Health facilities for this study were chosen purposively based on their high number of cases and the major treatment centers found in the city.

One hundred sixty-four (164) clinical nasopharyngeal samples were collected by using 3 ml Miraclean Technology (Shenzhen, China) viral transport media (VTM) from patients who were under investigation for COVID-19 and referred to EPHI for SARS COV-2 testing from December 1 to 30/2020. Nasopharyngeal samples were collected by trained sample collectors and transported to EPHI by triple packaging. Each sample was assigned with unique identification number before nucleic acid extraction. The extraction procedure was carried out immediately upon arrival by using manual and automated extraction methods. Thus, 1.3ml (including 0.8ml dead volume and 0.5ml input volume for extraction) sample was taken from each sample and extracted by Abbott DNA sample preparation system (Abbott Molecular Inc. des Plaines, IL, USA) with a batch of 92 samples and 2 controls (1 positive, 1 negative), and two no-template controls (NTC) were included throughout the procedure (in extraction and detection) of Abbott Real-time SARS-COV-2 (EUA). Similarly, from similar specimen used in automated extraction, 140µl samples was aliquated and used for manual extraction by QIAamp® viral RNA mini kit (QIAGEN GmbH, Hilden, Germany) with a batch of 20 samples and two controls (one negative, one positive), and additionally two NTC were included throughout the procedure (in extraction and detection) of BGI SARS-CoV-2 assay, Daan Gene assay and Sansure Biotech assay.

Automated SARS-CoV-2 viral RNA isolation and purification were done with Abbott DNA sample preparation reagents by the principle of magnetic beads. Sample inactivation and solubilization of viral particles were done with detergent, which contains guanidine iso-thyocyanate for protein denaturation and RNase inactivation. Then, RNA separated from proteins through solid-phase separation using silica; *i.e.* nucleic acid bind to negatively charged silica (SiO2) is facilitated by guanidinium salts and the basic pH of the lysis buffer. Washing steps could be removed any remnant protein and debris to produce a clear solution. The clear RNA separated from silica-based micro particles are by using the magnetic field of the instrument [15, 16]. On the other hand, manual RNA extraction and purification were performed via spin column method and separation of micro-particle from eluate, are using centrifugation rather than magnetic rack in spin column method [17].

Abbott Real-time SARS-CoV-2 Assay (EUA)

The Abbott Real-time SARS-CoV-2 assay (Abbott Molecular, Inc.) test was performed as described in the manufacturer instruction. Which has got Emergency Use Authorization from WHO and FDA) [15, 18]. In this protocol pre-extraction sample inactivation was performed with a water bath at 56 ^{O}C for 30 minutes [19], after viral inactivation nucleic acid extraction was done from 0.5 ml VTM on the Abbott m2000 SP instrument and using the Abbott m2000 Sample Preparation System DNA according to the manufacturer's recommendations. The amplification and detection were performed by Abbott m2000 RT-PCR instrument targeted to dual-target assay for the RdRp and N genes. The SARS-CoV-2 and IC-specific probes are each labeled with a different fluorophore, Carboxyfluorescein (FAM™), Carboxy-X-rhodamine (ROX™), and VIC® P (Proprietary dye) for target and internal control detection, thus allowing for simultaneous detection of both amplified products [15].

Daan Gene nCoV-2019 assay (EUA)

The Amplification and detection method of this kit is based on the one-step RT-PCR technique. ORF1ab and N genes were selected as the conserved region of Daan Gene technology for amplification and detection of target regions. Specific primers and fluorescent probes are designed (N gene probe is labeled with FAM and ORF1ab probe with VIC) for the detection of SARS-CoV-2 RNA in the specimens. The final eluent and master mix preparation was 5µl of eluate added to 20 µl of master mix for a final volume of 25 µl. Amplification and detection were performed on ABI 7500 RT-PCR instrument simultaneously [20].

Sansure Biotech detection assay (RUO)

The Sansure Biotech novel coronavirus (2019-nCov) nucleic acid diagnostic kit (PCR-florescence probing) was used for the detection of the ORF1a/b and N gene. The specific probe for each target gene is prepared, the FAM channel is selected for the ORF 1a/b region and the ROX channel is for the N gene. In this detection kit, eluent and master mix addition are as follows; 30µl master mix reagent and 20µl eluted sample were prepared for detection/amplification. ABI 7500 RT-PCR was used for Amplification/detection [21].

BGI SARS-CoV-2 testing assay (EUA)

BGI SARS-CoV-2 assay is the real-time fluorescent RT-PCR Kit for the diagnosis of COVID-19, a specific target region is found in the ORF1ab region of the SARS-CoV-2 genome. It is using single-gene testing assays. Furthermore, the human housekeeping gene β-actin is the target gene for internal control. The master mixing was done by mixing 20µl master mix reagent and 10µl of the extracted sample RNA to the well pre-filled with PCR-Mix [22]. ABI 7500 RT-PCR instrument was used for amplification and detection.

All nucleic acid amplifications, each assay PCR cycling condition and result interpretation were performed based on respective manufacturer instructions (table-1).

Interpretation of the CRR (reference result)

In this comparative analysis study, we have not used the reference standard method to determine percent agreements (positive, negative and overall) and other comparative parameters of four assay methods. The result was defined by consensus, a similar result which was tested at least with two EUA assays (Abbott SARS-CoV-2 assay and or Daan Gene nCoV-2019 assay and or BGI SARS-CoV-2 assay) by compatible platforms recommended by respective manufacturers was taken as reference result [23, 24].

Data processing and analysis

The data was collected using structured data extraction form, data entry and analysis was done using excel and SPSS version 23.0 statistical software for descriptive statistics. Positive, negative and overall percent agreements were analyzed and Kappa Estimator was employed to determine the strength of agreement of each method with the CRR. Kappa values were interpreted as follows from 0.01–0.20 slight agreement; from 0.21– 0.40 fair agreements, 0.41–0.60 moderate agreement, 0.61–0.80 substantial agreement and 0.81–0.99 perfect agreement [25].

Ethical considerations

Ethical clearance was obtained from Addis Ababa University and all experimental protocols of the study were approved by Ethiopian Public Health Institute Scientific ethical review committee. The reference number of EPHI ethical clearance was EPHI/IRB-279-2020. All methods were performed in accordance with the guidelines and regulations of Ethiopian national comprehensive COVID-19 management handbook. Moreover, informed written consent was obtained from all study participants prior to participate in the study.

Declarations

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Author Contributions

B.W.H. conceived, designed, writing the manuscript and editing. K.Z. and G.G.J. curate the data and design the methodology; G.G. and S.T. were made formal analysis; S.A., G.G. and K.Z. performed the RT-PCR laboratory investigation. A.T. was made Resources allocation, B.W.H., K.Z., K.D. and S.A.H. writing the manuscript and editing. All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article. The data that support the findings of this study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no conflict or competing interests.

Consent for publication

Not applicable.

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Figures

Figure 1

Distribution of Ct value in four assays

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