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Diagnostic performance of SARS-CoV-2 Real-time Polymerase Chain Reaction testing assays and some platforms available in Ethiopia for the diagnosis of Coronavirus Diseases -2019.

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This is to certify that the thesis prepared by Belete Woldesemayat, entitled: "Diagnostic performance of SARS-CoV-2 Real-time Polymerase Chain Reaction (RT-PCR) testing assays and some platforms available in Ethiopia for the diagnosis of Coronavirus Diseases -2019 (COVID-19)" and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AFTCOR	African Task Force for Coronavirus Preparedness and Response
CT	Cycle Threshold
CRS	Composite Reference Standard
COVID-19	Corona Virus Disease 2019
DNA	Deoxyribose Nucleic Acid
ENAO	Ethiopian National Accreditation Office
EUA	Emergency Use Authorization
IC	Internal Control
MERS-CoV	Middle Eastern coronavirus
NAT	Nucleic Acid Testing
nCoV	Novel Corona Virus
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PPE	Personnel Protective Equipment
RdRP	RNA-dependent RNA polymerase gene
RNA	Ribonucleic Acid
RT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe Acute respiratory Syndrome coronavirus-2
VTM	Viral Transport Medium
WHO	World Health Organization

Abstract

Background: Laboratory has a key role for the management of coronavirus disease-2019 (COVID-19). Since the outbreak reported, many commercial Nucleic Acid Testing (NAT) assays have been developed all over the world, and real-time PCR detection has been the routine and standard method. However, due to a number of NAATs were rapidly developed and quickly applied to clinical testing, diagnostic performance testing for different detection assays and PCR platforms should be considered.

Objective: To assess the diagnostic performance of SARS-CoV-2 real time polymerase chain reaction (RT-PCR) testing assays and some platforms available in Ethiopia for the diagnosis of COVID-19 in Ethiopian public health institute from December 1 to December 30/2020.

Methods: Comparative experimental study was conducted to assess the performance of four PCR testing assays and platforms by using Composite reference standard (CRS) as a reference method in Ethiopian Public Health Institute at National HIV Reference laboratory from December 1 to 30/2020. Sample size was determined based on WHO recommendation for method evaluation, 164 samples were selected by systematic random Sampling technique. Selected samples were extracted manually by using QIAamp® viral RNA mini kit (QIAGEN GmbH, Hilden, Germany) and Abbott DNA sample preparation system (Abbott Molecular Inc. des Plaines, IL, USA) for automated extraction. Amplification and detection was done on Abbott m2000, Roche 4800 and ABI 7500 RT-PCR platforms. Finally, the data was entered, cleared and analyzed by using SPSS version 23. Sensitivity, specificity, positive and negative percent agreement was analyzed and kappa Estimator was employed to determine the strength of agreement of each method with CRS.

Results: A total of 164 samples included in the study, out of these the rate of positive and negative COVID-19 test was 59.1% (97) and 40.9% (67) respectively in the CRS. Rate of positivity/negativity assays with respective platforms were comparatively similar with CRS ($p>0.05$). However, Sansure Biotech result was comparatively different with CRS ($p <0.05$). Sensitivity, specificity, Positive Percent Agreement (PPA), Negative percent agreement (NPA) and overall percent agreement (OPA) for four assays and three platforms lied in ≥93.8%, ≥ 98.5 %, ≥ 93.8%, 98.5 %, ≥96.3 % respectively. The Cohen's Kappa strength of agreement of assays and platforms lied in 0.925 – 1.000. The study also showed N gene sensitivity was more than ORF1a/b sensitivity based on their CT value.

Conclusion: The performance of four SARS-CoV-2 assays and three platforms had almost comparable diagnostic performance. However Sansure Biotech had low rate of positivity compared with CRS. On the other hand N gene sensitivity was better than ORF1a/b gene. Finally, Sansure Biotech assay (RUO) needs further verification on its use in Ethiopia and additional study should be important for the evaluation of respective manufacturers claim.

Key words; SARS-CoV-2; RT-PCR; detection kit; PCR platform; COVID-19; Ethiopia

1. Introduction

1.1. Back ground

The families of Coronaviruses are commonly isolated from human and animals that causes respiratory and intestinal infections. These viruses were not considered as dangerous and uncontrollable, until the outbreak of the severe acute respiratory syndrome (SARS) in 2003 in China, and then the Middle Eastern coronavirus (MERS-CoV (1). In December 2019 testing of samples taking from viral pneumonia presenting patients in Wuhan, China the genus of Beta coronavirus was identified and placing it alongside with SARS and MERS (2). Unfortunately Sever acute respiratory syndrome-2 (SARS-CoV-2) not controlled like as previous families of the virus and three months were enough to be a global pandemic (3).

Coronaviruses (CoVs) are positive sense, enveloped, spherical shape or slightly pleomorphic virions of 60 to 140 nm in diameter and single stranded ribonucleic acid (RNA) viruses belonging to the Coronaviridae family and in the Order of Nidovirales (4). Which are also divided into four genera; alpha, beta, gama and delta coronaviruses. The majority of coronaviruses in humans (HCoVs) like; human coronavirus-OC43 (HCoV-OC43), human coronavirus HKU1 (HCoV-HKU1), SARS-CoV-1, SARS- CoV-2 and MERS-CoV are found in the genus of beta coronavirus (5).

The primary causes of Coronaviruses on mammals and birds from fatal illness to easy self-healing disease. In humans or other species, the disease may affect the respiratory, gastrointestinal (GIT) and central nervous systems. In addition, HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1 viruses could be responsible for more serious illnesses in young, elderly and immune-suppressed individuals (6). On the other hand, Coronaviruses can be mutated, recombined and effectively affect new hosts and tissues easily without the distress of environmental change. Some strain of the virus can generate novel strain from another animal origin during the crossover to human host; this type of novel strain of viruses can be transmitted from human to human and does not have immunity for these viruses (7). These types of viruses can be rapidly leads to the outbreak of a new sort of illness and eventually to a pandemic. In this regard, SARS-CoV-1, MERS-CoV and SARS-CoV-2 affecting the lower respiratory tract and

usually severe respiratory syndrome in humans starting from 2004 are the main indicator of coronavirus mutation and its consequence (8).

As mentioned on the above, SARS-CoV-2 is genetically clusters with the genus Beta-coronavirus, in subgenus Sarbecovirus together with two bat-derived strains. It is 96% identical at the whole genome level to other bat coronavirus samples (Bat-CoV RaTG13). SARS-CoV-2 has four structural proteins: membrane glycoprotein (M), envelope protein (E), nucleo-capsid protein (N), and the spike protein (S) (9).

The virus-host interaction (viral entry to cells) mediated by spike (S) glycoproteins, which is consists of two functional sub-units; S1 is contain the receptor binding domain (RBD) and it is the main protein spike for the attachment of the host cell receptor with S2 sub-unit (10). The SARS-CoV-2 RBD is directly attached to the peptide domain of angiotensin-covering enzyme 2 (ACE2), which is also the cellular receptor of SARS-CoV-2 found in mainly in respiratory epithelial cells (11).

While entering of SARS-CoV-2 viral particle, the immune systems were plays a critical role to defend the pathogen attacks. Harmful tissue damages are commonly a result of uncontrolled or impaired immune response. The main causes of inflammatory responses are the antagonism of interferon with the virus when its replication inside the host cells. Cytokines (Interleukin 6) are found in severe tissue damaged organs and in slow healing lung biopsy, it may indicates cytokines are responsible for severe cases of COVID-19 including hyper coagulation and cytokine storm; these are the primary cause of death (28%) of COVID-19 (12). Evidence showed that the common way of respiratory virus transmission is through droplets, when generated during coughing, sneezing or talking. High viral load of SARS-CoV-2 have been detected in oral swabs of COVID-19 patients, which lead to increase the transmission of the virus. The route of transmission of SARS-CoV-2 are through exhaled oral droplets, aerosol, contamination of surfaces, and possibly through fecal-oral contamination (13). Understanding of the way of transmission of SARS- CoV-2 is an important issue to develop effective infection control measures of this pandemic virus (14).

An African task force for coronavirus preparedness and response (AFTCOR) has been established 6 (six) focusing areas. Out of these laboratory diagnosis and sub- typing is one of the

primary concerns (15). On the other hand rapid and accurate diagnostic testing of novel coronavirus disease has a central role to control and prevention of the global pandemic (16).

Laboratory testing is an integral part of the WHO Strategic Preparedness and Response Plan (SPRP). WHO recommends countries need to increase the laboratory capacity to increase their level of preparedness, proper case management, alert and quick response for the public. The role of laboratory is also a key for case definition, to defining disease characteristics, epidemiology of SARS-CoV-2 and in controlling its spread (17). Since the COVID-19 outbreak reported, many commercial Nucleic Acid Testing (NAT) assays have been developed all over the world, and RT-PCR detection of different genes of SARS-CoV-2 has been the routine and standard method (18) (19).

The main genes used for the laboratory diagnosis of SARS-CoV-2 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 (9). Out of these genes the RdRP and E genes had high analytical sensitivity for detection (technical limit of detection of 3.6 and 3.9 copies per reaction), whereas the N gene provided poorer analytical sensitivity (8.3 copies per reaction) (20).

The analytical performance characteristics of PCR methods can be varied with the quality of extraction reagent, amplification/ detection reagent, method of extraction (manual or automated) the PCR machine and other instrumentation are the main factors for the variability of the final result. 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 (21). However, in developing countries like Ethiopia, because of shortage of standardize testing facility, instrumentations, and because of instruments not previously used, many laboratories are not yet start for PCR working.

1.2. Statement of the problem

As of August 31/2021 the numbers of cases were increase day today profoundly and based on the WHO weekly report the total number was almost nearly 216 million. Out of these the world lost 4.5 million lives (with 2.6% death rate) (22). As of September 7/2021 more than 7.9 million cases and greater than 200,000 deaths (2.5% death rate) were reported in Africa. Which indicated that there are low numbers of cases were reported from Africa (3.6% of all cases of the world). However, the death rate of Africa was almost similar to the world (23).

The disease rate of Africa cannot be grasp the true picture of disease distribution due to limited numbers of testing capacity and absence of testing data. Majority of testing laboratory found in Africa was not experienced PCR testing and the others were couldn't have trained personnel or resource limitation (extraction kits, detection kits, different equipment and materials scarcity) (24).

On the other hand, majority of sub-Saharan countries, including Ethiopia, most of laboratory testing resources are come from developed countries with donations or procurements, it leads to a various type of equipment, materials and PCR testing kits are distributed without or with a limited verification on its work locally. Moreover, testing assay variability, non-standard testing facilities leads to false results. False results (specifically, false negative results had a huge effect on the prevention of the transmission of COVID-19). False negative rate of molecular tests are varies in different time of sample collection and sample type. Missing of true positive cases in this type of pandemic, leads to unintentional transmission, and even unknown results are might be better. Because true positive individuals assumed that, their status are negative, it affect hugely the transmission scenarios (25). A systematic review conducted by Rodriguez etal. Showed that the rate of false negative was estimated 0.13(95% CI; 0.09-0.19). On the other hand, false positive result in RT-PCR testing captured from external quality assessments (EQA) revealed false positive rates of SARS-CoV-2 PCR testing was 0-16.7% (26) (27). These are indicated that, the gaps were increased when the platforms and reagents variability increases.

In Ethiopia, more than fourteen RT- PCR platforms in 39 testing facilities, including ABI 7500, Abbott m2000, Roche 48000, Quant-studio are using for SARS-CoV-2 PCR testing throughout the country. Similarly, different PCR detection kits are available, like; Daan Gene kit, Abbott

SARS-CoV-2 assay, Sansure Biotech kit, and BGI kit. Therefore, for these instrumentations and reagents clinical performance comparisons are very crucial by using the standard methods. Although, RT-PCR assay is a highly sensitive technique, false negative results have been still reported in some COVID-19 patients. These results may occur due to insufficient viral RNA copies in the specimen resulting from improper collection, transportation, storage, and handling, as well as laboratory test conditions and personnel operation (28). On the other hand false positive results can be occurred in various levels due to cross contamination with positive samples, from sample collection to master mix preparation procedures. Moreover, the quality of the examined regents is closely related to the PCR results of SARS-CoV-2.

In response to this outbreak, a number of NAATs for the diagnosis of COVID-19 were rapidly developed and quickly applied to clinical testing. To date, no comparative performance evaluation of SARS-CoV-2 NAATs has been reported in Ethiopia. Even though there are a lot of NAAT reagents and platforms are found in EPHI and as well as in Ethiopia, these platforms and kits performances are not yet comparatively assessed. Therefore, the purpose of this study was assess the diagnostic performance of commercially available SARS-CoV-2 detection kits by using clinical samples and compared the diagnostic performance of different RT-PCR platforms in clinical samples for the qualitative detection of SARS-CoV-2.

1.3. Significance of the study

This study will be used for give important information for different stakeholders for the diagnosis of COVID-19, especially it used as first-hand information on the quality of commercially available detection kits and the compatibility of different detection kits on different RT-PCR platforms. So, to know the performance of different SARS-CoV-2 detection kits on diverse RT-PCR detection instruments helps to identify better detection kits with compatible platforms. That is a crucial tool for better and effective COVID-19 control and prevention program.

2. Literature review

The study conducted in Iran, Kerman University of Medical Sciences, Tropical and Infectious Diseases Research Center, to evaluate the performance of conventional RT-PCR for the detection of SARS-CoV-2 RNA on five conserved genes. The study also used five set of primers for the detection of N gene, ORF1ab gene, RdRP gene, E gene and S gene. The result revealed that N gene and RdRp sensitivity and specificity was equal, which were 96.6% and 100% respectively. This indicates the discovery rate of false negative results on these combined genes were almost zero. In addition to this on the ORF1 gene primer also had a specificity and positive predictive value of 100%. Whereas the false negative rate of this primer was almost 4%. In this study the analytical performance of E gene primer was lower to compare with other gene primers. The sensitivity and specificity of this gene was 66.7% only. The other gene evaluated in the study was surface (S) gene, which had 85% sensitivity and 50% specificity were observed (29).

The other cross sectional study carried out by Medical University of Vienna, Center for Virology, Vienna, Austria, to assess the capability of individual laboratory on the detection of SARS-CoV-2 participating in the EQA scheme. In the EQA four samples, which had different viral concentration were distributed for67 individual laboratories. The best detection performance was reported from the fully automated instruments, and test systems like, GeneXpert and Cobas 6800, which all can detected the low viral samples. Even though this different had not statistical significant (p=0.69) the volume of input (sample for extraction) and volume of nucleic acid for detection of SARS-CoV-2 detection had an impact on the result. Which means high dilution and low volume of sample or and nucleic acid lead to false negative result (30).

The experimental study performed in Erasmus Medical Centre, Department of Viroscience, Rotterdam, Netherlands, to evaluate the analytical performance of different PCR kits, the analytical specificity was examined by triplicating testing by using standardized RNA panel available from the European Virus Archive (EVAg;https://www.european-virus-archive.com/nucleic-acid/coronavirus- rna-specificity-panel). The panel was contained cultured and characterized RNA of hCoV-NL63, hCoV−OC43, hCoV-229E, MERS-CoV, and SARS-CoV-1. The result showed that the sensitivity (lower detection limit) of Sansure Biotech

detection kit ORF1ab gene and N gene were 3.3 RNA per reaction. Whereas the specificity of Sansure Biotech detection kit N gene was not specific for only SARS-CoV-2 but also SARS-CoV-1. The other evaluated kit was DAAN Gene; the ORF1ab gene sensitivity was similar with Sansure Biotech which was 3.3 RNA copies per reaction. However, the DAAN Gene kit N gene assay was only background signal was observed. Generally all kits, except for the Sentinel Diagnostics, Research Use Only (RUO) E gene assay, included the study the PCR efficiencies were above 90%. And the analytical sensitivity of assays in different kit and gene assay was varied between 3.3 to 330 RNA copies per reaction (31).

The other similar validation and verification study conducted in the University of Washington Retrovirology Laboratory at Harborview Medical Center, USA, to verify and validate the analytical and clinical performance of the (Emergency Use Authorized) EUA Abbott RT SARS-CoV-2 assay. Out of 20 replicate of samples 19s were detected at 50 copies/ml and 25 RNA copies/ml were detected in 16 replicates in the Abbott Real-time SARS-CoV-2 assay. It indicated high sensitivity compared to the limit of detection expressed on the EUA document 100 RNA copies/ml. The overall sensitivity and specificity of Abbott Real-time SARS-CoV-2 assay compared to the SARS-CoV-2 CDC-based laboratory developed test assay were 93 % and 100% respectively (32).

The other experimental comparative evaluation study reported from China, Daping Hospital, to compare three extraction methods (magnetic bead extraction method (MB), centrifugation (CF) and one-step (OS) method) on clinical samples which were taken from oropharynges. For All three extraction methods were used Sansure Biotech extraction reagents and consumables. Final amplification and detection was carried out by CFX96 PCR System (Bio-Rad Lab. Inc., USA) and Cobas Z480 (Roche Molecular Diagnostics, Pleasanton, CA) instruments. The result showed that there were no statistical difference between specimen pretreatment methods ($p > 0.05$), but there were statistical significance difference CT values between each extraction methods ($p < 0.05$), especially the CT value different between CF and that of both MB and OS methods ($p < 0.01$), but there were no difference between values of CT of MB and OS ($p > 0.05$). Based on the results CF method had best analytical efficiency with both instruments in different sample pretreatment condition (33).

The experimental study done in Netherlands national institute for Public Health and the Environment, the Netherlands, to evaluate the clinical performance of different SARS-CoV-2 PCR detection kits by using 13 previously confirmed with in-house PCR test panel and 6 clinical panel samples. The result showed that the rate of positive identification from panel sample in different RT-PCR kits were varied from 10 to 13 out of 13 panel specimens. R-Biopharm AG, BGI, CerTest BioTec and Altona Diagnostics test kits were detect 13 out of 13, 12 out of 13, 11 out of 13 and 10 out of 13 respectively. 10 clinical samples out of 13 samples, which were had high concentration of SARS-CoV-2 RNA copies (Ct ≤ 34.50 in in-house E-gene PCR) and can be detected by all kit gene assays and also their pattern of CT values were showed that had similar patterns with the in-house assay CT values (34).

Similar experimental study was conducted in University of Sri Jayawardenapura, Centre for Dengue Research, Faculty of Medical Sciences, Angoda, Sri Lanka to evaluate the performance of two PCR kits and instruments by using Daan gene PCR kit as a reference assay. For comparison 68 clinical samples were used. Out of these samples 30 samples were had the CT value of more than 35, 10 samples had 30 to 35 CT values and the rest 8 samples were had an inconclusive result after testing with reference method (DAAN gene assay). The three RT-PCR kit assay detection was varied highly, the sensitivity for positive results with high CT values (>35) and lower CT values (30-35). The detection rate of Real Star® SARS-Cov-2 RT-PCR Kit 1 and GeneFinderTM were 60% and 3.3% only in low viral load (CT>35) samples. The other key issue observed in the study was the DAAN gene assay was run with non-recommended RT-PCR instruments, which was BioRad CFX96 instead of the ABI Bio systems 7500 (compatible PCR machine), only 86.7% of low SARS-CoV-2 viral RNA contained sample were positive and the rest 13.3% turned to negative (35).

The other similar experimental evaluation study was done in The Second Xiangya Hospital, Central South University, Changsha district, China, to evaluate the diagnostic performance of seven commercially available kits on clinical samples. The selected PCR kits are BGI (BGI Biotech Co., Ltd, Wuhan, China), Outdo (Outdo Biotech Co., Ltd, Shanghai, China), sansure (Sansure Biotech Inc., Changsha, China), Perkin (Perkin Elmer Medical Diagnostic Products, Co., Ltd, Shanghai, China), DAAN gene (Daan Gene Co., Ltd. Of Sun Yat-Sen University, Guangzhou, China), Jiangsu (Jiangsu Bioperfectus Technologies Co., Ltd, Taizhou, China), and

Fosun (Fosun Long March Medical Science Co., Ltd, Shanghai, China).the diagnostic specificity of all kit assays were concordance with the reference assay specificity (100%). The Cohen's Kappa coefficient indicated that all seven kits were highly concordant with reference in-house assay (Kappa coefficient > 0.900, $p<0.001$). however the result of Sansure Biotech assay, Daan Gene kit assay and Fosun Long assay were significantly different which compared to the reference assay result ($p<0.05$). The sensitivity of BGI Biotech, Outdo Biotech and Sansure Biotech were 90.48 %, 92.86 %, and 83.33 % respectively, the difference between each other and from the reference method was not statistically significant ($p> 0.05$). On the other hand the sensitivity of Perkin Elmer, Daan Gene, Jiangsu Bioperfectus, and Fosun Long kit assay were 97.62 %, 78.57%, 90.48%, 76.19% respectively. Sansure Biotech and Fosun Long assay N gene detection rate were very low, which were only 23.81 and 30.95%, respectively. Perkin Elmer and Jiangsu Bioperfectus kit assay N gene detection rate was similar, which was 90.48% (36).

Evidence reported from Anhui University of Science and Technology, Huainan, China, showed that evaluation of analytical performance of five commercial RT-PCR kits, which were Genekey, Daan, BioGerm, Liferiver, and Yaneng. The study indicated that based on the standard of each kit manufacturer performance characteristics, the positive coincidence rate of 20 clinical specimens of three PCR detection kits (Genekey, Daan and BioGerm) was 100% (20/20). The detection ability of each kit gene assay was different each other, for instance out of five level dilution, the sensitivity of kits were substantially different in level 4 and level 5 dilution. Moreover in level four dilution Daan gene kit was the only kit to detect the viral RNA 100%, however the CT values of ORF1ab gene were lied on between 38 and 40. The specificity of all kits in 10 negative samples was 100% and cross reactivity for six human coronaviruses and four other respiratory pathogens for ORF1ab and N gene were not detected. The precision (coefficient of variation) of all test kit genes was less than 5% (37).

The other related study conducted in Liuzhou People's Hospital, Department of Clinical Laboratory, Liuzhou, China to compare the diagnostic efficacy of two different RT-PCR kits for SARS-CoV-2 detection. 18 samples which were taken from infected COVID-19 patients and the other 100 samples were taken from known non-infected patients were involved in the study. Out of 18 known positive samples 3 of were falsely negative in Sansure PCR test kit, and 1 of 18 samples was falsely negative by BioGerm PCR kit. There was no false positive result detected in

both PCR kits, which indicated that the specificity of both detection kits was 100%. Furthermore the sensitivity, positive predictive value (PPV), negative predictive value (NPV) and kappa value of the Sansure PCR kit were 83.3%, 100%, 97.1%, and 0.894, respectively, and 94.4%, 100%, 99.0%, and 0.966 were the sensitivity, positive predictive value (PPV), negative predictive value (NPV) and kappa value of the BioGerm PCR kit (38).

Table 1: Summary of the characteristics of SARS-CoV-2 detection methods, 2020

s/n	Name of PCR detection kit	Company /institute	Target gene	Recommended platform	To be evaluated platform	Cut of CT	Lower detection limit	Sample extraction method	Total CT	Regulatory status	Reference
1	Abbott RT-PCR SARS-CoV-2 assay	Abbott Laboratories Inc. USA.	RdRp and N gene	Abbott m2000 RT and SP instrument	NA	37	100 RNA copies/ml	Magnetic bead extraction method	37	FDA and WHO EUA, CE-IVD marked approved for extraction & detection	(39)
2	Daan Gene assay	Daan Gene Co., Ltd. Of Sun Yat-sen University, China	ORF1ab and N gene	ABI 7500 and Roche light cycler	Abbott m2000, and Roche 4800 RT-PCR	40	500 RNA copies/ml	Spin column extraction method	45	WHO EUA	(40)
3	BGI 2019-nCoV: Real-Time Fluoresce	BGI Genomics Co. Ltd., Shenzhen, China	ORF1ab gene	ABI 7500	NA	37	100 RNA copies/ml	Spin column extraction method	40	NMPA Certified/CE Marked/FDA Approved/PMDA Approved	(41)

	nt RT-PCR kit										
4	Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing	Sansure BioTech Inc. China	ORF1ab and N gene	ABI 7500	NA	40	200 RNA copies/ml	Spin column extraction method	45	RUO	(42)

Abbreviations; ABI; Applied Bio systems, CE; European Conformity, EUA; Emergency Use Authorized, NA; Not Applicable, RUO; Research Use Only.

3. Objectives

3.1. General objective

To determine the diagnostic performance of SARS-CoV-2 RT-PCR testing assays and some platforms available in Ethiopia for the diagnosis COVID-19 at Ethiopian public health institute from December 1 to 30/2020.

3.2. Specific objectives

- To compare the diagnostic performance of Daan Gene assay for the detection of SARS-CoV-2 on Abbott m2000 RT (Open mode), ROCHE 4800 and ABI 7500 Real-time PCR platforms with CRS
- To compare the diagnostic performance of Sansure Biotech nCov-19 assay for the detection of SARS-CoV-2 on ABI 7500 Real-time PCR with CRS
- To compare the diagnostic performance of BGI SARS -COV-2 assay for the detection of SARS-CoV-2 on ABI 7500 Real-time PCR with CRS

4. Hypothesis

- We hypothesized that, the overall diagnostic performance of Dann Gene assay for the detection of SARS-CoV-2 on Abbott m2000 RT-PCR (Open mode), ROCHE 48000 RT-PCR and ABI 7500 RT-PCR are similar with reference standards.
- The diagnostic performance of Sansure Biotech assay for the detection of SARS-CoV-2 on ABI 7500 RT-PCR is similar with reference standard methods.
- The diagnostic performance of BGI SARS-CoV-2 assay for the detection of SARS-CoV-2 on ABI 7500 RT-PCR is similar with reference standard methods.

5. Methods and Materials

5.1. Study design, area and period

Comparative experimental study design was conducted in Ethiopian Public Health Institute at National HIV Reference laboratory from December 1 to 30/2020. The Reference laboratory currently performing HIV viral load, Qualitative EID testing with PCR, hepatitis B and C viral load, PT production for EID and Viral load testing laboratories, COVID-19 testing, HIV drug resistance, CD4 and haematology. The laboratory built by CDC and has well established quality systems and accredited to ISO 15189; 2012 by Ethiopian National Accreditation Office (ENAO). National HIV reference laboratory was selected for this study due to; the laboratory has many RT-PCR platforms, sequencing platforms (Sanger and next generation). Additionally, the laboratory has well established quality system.

5.2. Samples

5.2.1. Sample source

All samples which were under investigation for coronavirus diseases 2019 (COVID-19) and referred to EPHI for SARS COV-2 testing during the study period.

5.2.2. Study samples

The study samples were all samples referred to EPHI National HIV Reference laboratory for SARS COV-2 testing during the study period.

5.3. Inclusion and exclusion criteria

5.3.1. Inclusion criteria

- All positive leftover samples which had sufficient sample volume (approximately 1.6 ml) were included in the study.
- Negative left over samples which were stored in the study period and have sufficient sample volume (approximately 1.6 ml) was included in the study.

5.3.2. Exclusion criteria

- A leftover sample without labeling, invalid or indefinite result and insufficient sample volume was rejected.

5.4. Study variables

5.4.1. Dependent variable

- Diagnostic performance (sensitivity, specificity, PPV,NP, PPA, NPA and OPA) of PCR kits and platforms

5.4.2. Independent variables

- Type of RT-PCR platform
- Type of SARS CoV-2 RT-PCR detection kit

5.5. Sample size determination and Sampling method

5.5.1. Sample size determination

As per WHO recommendation for COVID-19 testing method evaluation, the sample size for sensitivity evaluation was used (43). A total of 164 clinical samples were used for this comparison study.

5.5.2.. Sampling method

Positive and negative samples were selected by systematic random sampling technique from samples which was collected from different COVID-19 sample collection sites and treatment centers during study period. Samples were selected for this study after the regular testing with one of currently using assay and platforms. National HIV reference laboratory is receiving averagely 283 COVID-19 specimens per day. Out of these samples averagely 23(5.8%) were positive and the rest 250 (94.2%) samples are negative with regular testing assays. Thus, the total positive left over samples within the study period was 500, out of these 100 positive samples was selected. Positive samples assigned orderly based on lab ID, was used random probable sampling technique. Then proceeds with the selection of every five (500/100=5=K) sample from then onwards was included in this study. Negative samples were selected similarly from stored leftover sample referred during the study period; it was about 8,000 negative samples.

5.7. Measurement and data collection

5.7.1. Data collection procedure

Naso/Oro pharyngeal sample was collected by trained sample collectors, with 3-4 ml disposable specimen collectors. Then the specimen transported to EPHI by triple packaging. Before

distributing to each laboratory, the sample registered with unique identification number. The tertiary container was opened inside the biosafety cabinet (at least BSL-2).

5.7.2. Laboratory analysis

While tested the sample with regular testing program and assay, we were selected based on the selection criteria and sampling method for this study. The test was done without freeze thaw difference for the first regular testing time. Thus, all selected samples were tested with batch of 92 samples and 2 controls (1 positive, 1 negative), and two no template (NTC) controls were included throughout the procedure (in extraction and detection) of Abbott Real time SARS-COV-2 (EUA). Whereas, the manual extraction was employed by 20 samples and two controls (one negative, one positive), and additionally two no template (NTC) controls were included throughout the procedure (in extraction and detection).

Automated SARS-CoV-2 viral RNA isolation and purification was done with Abbott DNA sample preparation reagents by the principle of magnetic beads. The first sample inactivation and solublization of viral particles done with detergent, which contain guanidine iso-thyocyanate for protein denaturation and for avoid RNase. RNA separated from proteins by means of 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. After washing of remnant protein and debris to find out clear elute solution. The clear RNA separated from silica based micro particles are by using magnetic field of the instrument (39) (44).

On the other hand, manual RNA extraction and purification was performed via spin column method, which is similar principle with magnetic bead, Viral RNA is adsorbed onto the QIAamp silica membrane during centrifugation steps rather using magnetic rack to capture the attached RNA in magnetic bead extraction and purification method. In this study, we used QIAGEN mini RNA kit for manual extraction procedure. Based on that, 140µl sample was used for each sample and the final elute volume was 80 µl (45). (Annex -5)

In this comparative analysis study, we were used QIAGEN mini RNA kit purified RNA (elute) for all assays (Daan Gene assay, BGI assay and Sansure Biotech assay) except, Abbott SARS-CoV-2 assay. All discordant results from primarily reported sample were repeated the test and take as the final result based on the manufacturer recommendations (Fig-1).

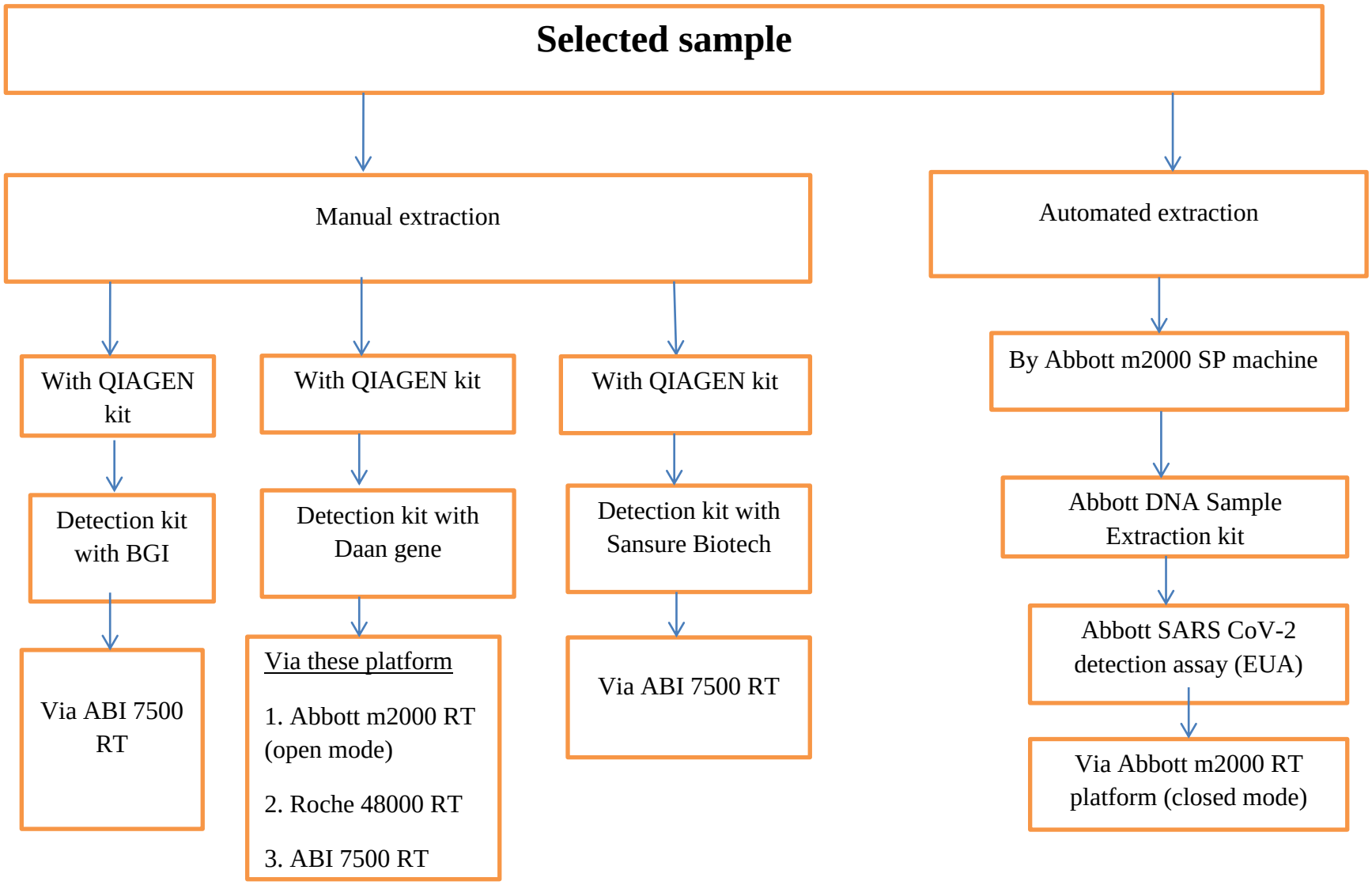

Figure 1: process of specimen testing, 2020

5.7.3. Laboratory biosafety consideration

All specimens were used in this study extraction procedures takes place in an appropriately maintained and validated biological safety cabinet (BSL-2). Appropriate disinfectants with proven activity against enveloped viruses, like 0.5 %, 0.1% bleaches and more than 70% ethanol were used for the recommended contact time (more than 20 sec.) for disinfection. All technical procedures was performed in a way that minimizes the generation of aerosols and droplets, and also appropriate personal protective equipment (PPE), including; powder free glove, N 95 mask, laboratory coat, eye goggle /face shield, head cover and shoe covers were worn when performing extraction procedures. Additionally in the master mixing procedure lab coat (dedicated to master mix room), head cover, eye goggle, and powder free glove were worn by all laboratory personnel handling these specimens (46).

5.7.4. Abbott Real Time SARS-CoV-2 Assay (EUA)

One hundred and sixty four (164) Nasopharyngeal samples was collected with disposable specimen collector containing 3-4 ml VTM (Viral Transport Media) between December 1 to 30 /2020, from patients under investigation for SARS-CoV-2 submitted to the Ethiopian Public health Institute, National HIV reference laboratory for diagnostic testing, was included in this study. The Abbott Real Time SARS-CoV-2 Assay-Abbott Molecular, Inc. test was performed as described in the EUA document (39). Abbott SARS CoV-2 detection kit has got Emergency Use Authorization from WHO and FDA) (39). Additionally, one of the gene using by Abbott SARS-CoV-2 RT detection kit is RdRP gene, which had high analytical sensitivity and currently this gene containing assay using by almost more than 30 laboratories in Europe only (47) (48) (49).

In this protocol pre extraction sample inactivation was performed with water bath at 56 ^{o}C for 30 min, after viral inactivation (46). Nucleic acid extraction was done from 1.3 ml VTM on the Abbott m2000 sample preparation (SP) instrument by using the Abbott m2000 Sample Preparation System DNA according to the manufacturer's recommendations (39). The amplification and detection of SARS-CoV-2 RNA was performed by Abbott m2000rt 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 (FAM™ (Carboxyfluorescein), ROX™ , (Carboxy-X-rhodamine), and VIC® P (Proprietary dye) for target and internal control detection, thus allowing for simultaneous detection of both amplified products (39).

5.7.5. Amplification/ detection by DAAN Gene kit assay SARS COV-2 testing

The Amplification /detection method of this kit is based on one-step RT-PCR technique. The manufacturer was using ORF1ab and N genes as conserved region of Daan Gene technology for amplification and detection. Specific primers and fluorescent probes are designed (N gene probe is labeled with FAM and ORF1ab probe with VIC) for the detection of 2019 Novel Coronavirus RNA in the specimens. The final eluent and master mix preparation was, 5µl of eluate was added to 20 µL of master mix for a final volume of 25 µL. RT-PCR was performed on the Abbott m2000 RT-PCR (open mode), Roche 48000 RT-PCR and ABI7500 RT-PCR instruments. Based on the manufacturer instruction cycling conditions was as follows: hold for 15 minutes at 50°C, hold for 15 minutes at 95°C, then 45 cycles of 94°C for 15 seconds, and 55°C for 45 seconds (40).

5.7.6. Amplification/ detection by Sansure Biotech detection kit for SARS COV-2 testing

The Sansure Biotech novel coronavirus (2019-nCov) nucleic acid diagnostic kit (PCR-florescence probing) was used for the detection of ORF1ab and N gene of SARS-COV-2 in nasopharyngeal swab with VTM. The specific probe for each target gene is prepared, FAM channel is selected for ORF 1ab region and ROX channel is selected for N gene. In this detection kit eluent and master mix addition are as follows; 30ul master mix reagent and 20ul eluted sample was prepared for detection/amplification. ABI7500 RT-PCR was used for Amplification/detection (42). The cycle parameters were set on the RT-PCR as follows based on the manufacturer instruction;

- Reverse transcription step 50 ^{o}C for 30 min
- cDNA pre denaturation step 95^{o}c for 1 min
- denaturation step 95^{o}c for 45 cycle 15 sec
- Annealing, extension and fluorescence collection step for 45 cycle 60 ^{o}c for 30 sec
- Device cooling step 25 ^{o}C for 10 sec

5.7.7. Amplification/ detection by BGI detection kit for SARS COV-2 testing

BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2 is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The assay developed for detecting specific single target gene, which is found on the ORF1a/b region of SARS-CoV-2 genome.

Further, human housekeeping gene β-Actin is the target gene for the 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 in the following order: no template (negative) control, patient specimen(s), and positive control. The PCR configuration protocol is as follows;(41).

Table 2: BGI detection kit PCR configuration protocol, 2020

Step	**cycle**	**Temperature (^{o} c)**	**Duration**	**Fluorescence measured (Y/N)**
1	1 cycle	50	20 minutes	N
2	1 cycle	95	10 minutes	N
3	40 cycles	95	15 seconds	N
		60	30 seconds	Y

5.7.8. Interpretation of the CRS (Composite reference standard)

In this comparative analysis study, we were not used reference standard method to determine sensitivity, specificity, percent agreement (positive, negative and overall) and predictive value (positive and negative) of different assay methods and PCR platforms. The reference result was defined by consensus, 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 (50) (51).

5.8. Quality assurance

The sample collection and processing was performed strictly as per the approved national guideline. To ensure the quality of testing, all positive samples were repeated before issuing the result. Both positive and negative quality control processed with each run simultaneously. Both controls shall pass to accept the result of each sample. All demographic, clinical, and laboratory data collected on hard copy was double entered on SPSS and checked for clerical error. Rigorous data cleaning was done before going to data analysis.

5.8.1. Pre- analytical quality assurance

Nasopharyngeal samples were collected with appropriate sample collection viral transport media (VTM) (Miraclean technology, 3 ml) which were properly labeled with barcode. Sample collection was done by trained professional nurses and general practitioners (GP) with undertaking standard safety precautions considered in COVID-19 sample collection. After collection sample was stored in triple packaging for a maximum of 12 hours with ice (2-8 ^{o}C) until transported to EPHI. All samples were tested within three days after collection and each sample extracted without any thaw freeze difference for automated and manual extraction method.

5.8.2. Analytical quality assurance

In all analytical procedures were performed with great care to avoid the formation of cross contamination and risk of exposure to aerosol containing droplets. Samples were processed with full PPE in class II biosafety cabinet.

In every run internal quality control (IC, assay control (1 positive and 1 negative), which were prepared by the companies was included. In addition to internal control and assay control NTC (molecular grade water) control also included throughout the procedure both in automated and manual procedure. All micropipettes and thermometers were used in this study were calibrated by Ethiopian Standardization Agency (ESA), that minimize the effect coming from volume and temperature variability from sample storage to master mix preparation. To minimize or avoid technical personnel variability in the procedure, Standardized SOP was developed and used for every procedure; and every procedure was performed by competent expertise.

5.8.3. Post –analytical concerns

Results interpreted as positive, negative or invalid based on the manufacturer instructions and positive judgment was done based on the CT value and graph of the result.

5.9. Data processing and analysis

The data was collected using structured data extraction form (Annex VI), data entry and analysis was done using Excel and SPSS version 23.0 statistical software for descriptive statistics. Sensitivity, specificity, positive and negative predictive values were analyzed and kappa Estimator was employed to determine the strength of agreement of each methods with the

combined result. 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 (52).

5.10. Operational definition

- **PCR Platform**: Instruments was used for the amplification and detection of SARS-CoV-2
- **Extraction kit**: Reagents and materials used for extract SARS-CoV-2 RNA
- **Detection kit**: Reagents used for amplify and detect SARS-CoV-2 viral gene
- **Kit and platform performance**: The ability of reagents/platforms to detects and identifies the specific RNA gene of SARS-CoV-2.
- **Composite reference standard**: A combination of at least two EUA assay results with recommended platform by the respective manufacturer.

5.11. Communication and Dissemination of Study findings

The study finding will be communicated to Addis Ababa University, Ethiopian Public health institute, and Ministry of Health will be used for the decision of procurement of SARS CoV-2 PCR detection kits and platforms. And also findings will be presented in different scientific seminars, scientific conferences, workshops, and published in scientific journals.

5.12. Ethical Considerations

Ethical clearance was obtained from Addis Ababa University and Ethiopian Public Health Institute Scientific ethical review committee (reference number 279/20). For this study, all selected samples were obtained from EPHI HIV national reference laboratory. We were not use patient information except, some demographic information. So the client was anonymously unlinked to this study.

6. Results

6.1. Characteristics of study participants

A total of 164 COVID-19 suspected participants were included in this study. Out of these, 15 (9.1%) and 31(18.9%) were clinically suspected, for COVID 19 and has contact with confirmed case respectively. About 92 (56.1%) and 26 (15.9 %) participants were from hospital surveillance and community surveillance respectively. Ninety three (56.7%) of participants were male and the rest 71(43.3%) were female. The mean (±SD) age of the participants was 31.10(±11.82) years.

6.2. Result comparison of different SARS-CoV-2 assays and platforms

In this study the rate of positivity/negativity with different COVID-19 testing assays and platforms were determined. Hence, rate of positivity with Abbott SARS-CoV-2 assay, Daan Gene 2019-nCoV assay via Abbott m2000 RT-PCR (Open mode), Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR, Daan Gene 2019-nCoV assay via ABI 7500 RT-PCR, BGI SARS-CoV-2 assay via ABI 7500 RT-PCR, BGI SARS-CoV-2 assay via ABI 7500 RT-PCR and Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR were 59.1%, 60.4%, 65.2%, 59.8% and 58.5% respectively (Table-3).

Table 3: Results of different SARS-CoV-2 assays and platforms, 2020

Types of assays and platforms	Positive (%)	Negative (%)
Abbott SARS-CoV-2 assay	97 (59.1)	67 (40.9)
Daan Gene 2019-nCoV assay via Abbott m2000 RT-PCR (Open mode)	96 (60.4)	68 (39.6)
Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR	98 (65.2)	66 (34.8)
Daan Gene 2019-nCoV assay via ABI 7500 RT-PCR	96 (59.8)	68 (40.2)
BGI SARS-CoV-2 assay via ABI 7500 RT-PCR	95 (58.5)	69 (41.5)
Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR	91 (56.1)	73 (43.9)
CRS	97 (59.1)	67 (40.9)

In this study, totally 10 (10/164 = 6.1%) discordant test results were recorded. The majority 6 (6/10= 60%) of false negative result are comes from Sansure Biotech assay. On the other hand, there was no discordant result was seen on Abbott SARS-CoV-2 assay (table 4).

Table 4: discordant results with their CT values in different assays and platforms, 2020

Sample ID	**CRS**	**Abbott SARS-CoV-2 assay**		**Daan Gene 2019-nCoV assay via Abbott m2000 RT-PCR**			**Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR**			**Daan Gene 2019-nCoV assay via ABI 7500 RT-PCR**			**BGI SARS-CoV-2 assay via ABI 7500 RT-PCR**		**Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR**		
		Result	CT value	Result	CT value		Result	CT value		Result	CT value		Result	CT value	Result	CT value	
					ORF 1a/b gene	N Gene		ORF 1a/b gene	N Gene		ORF 1a/b gene	N Gene		ORF 1a/b		ORF 1a/b	N gene
E-012	Positive	Positive	16.74	Positive	27.01	25.97	Positive	30.99	28.98	Positive	27.22	26.35	Positive	25.95	Negative	.00	.00
E-019	Positive	Positive	12.47	Positive	22.43	20.94	Positive	26.31	24.06	Positive	22.45	21.39	Positive	22.43	Positive	.00	27.14
E-032	Positive	Positive	15.03	Positive	26.03	23.82	Positive	30.12	27.27	Positive	26.08	24.24	Positive	26.03	Negative	.00	.00
E-054	Positive	Positive	11.52	Positive	22.63	21.95	Positive	26.48	23.94	Positive	22.73	21.45	Positive	25.02	Negative	.00	.00
E-123	Positive	Positive	18.78	Positive	29.80	29.09	Positive	34.75	33.26	Positive	31.49	30.65	Positive	29.80	Negative	.00	.00
E-125	Positive	Positive	12.330	Positive	24.55	20.59	Positive	27.02	22.75	Positive	24.59	21.37	Positive	23.19	Negative	.00	.00
E-150	Positive	Positive	27.40	Positive	.00	34.99	Negative	.00	.00	Positive	.00	36.80	Negative	.00	Positive	38.68	36.76
E-156	Positive	Positive	12.33	Positive	24.55	20.59	Positive	27.02	22.75	Positive	24.59	21.37	Positive	23.19	Negative	.00	.00
E-159	Negative	Negative	.00	Negative	.00	.00	Positive	33.77	.00	Negative	.00	.00	Negative	.00	Negative	.00	.00
E-164	Positive	Positive	25.52	Positive	40.00	34.27	Positive	40.00	38.63	positive	36.10	35.89	Negative	.00	positive	36.72	35.39

Abbreviations: SARS-CoV-2; severe acute respiratory syndrome coronavirus-2, CT; cycle threshold, ORF; open reading frame, nCoV; novel coronavirus, RT-PCR; Real-time polymerase chain reaction.

The sensitivity of each assays with respective platforms are calculated against the Composite reference standard (CRS). Daan Gene assay with Abbott m2000 and ABI7500 platform had similar sensitivity 99 % (94.4-99.8). One discordant result was observed compared to the CRS. Sensitivity of Abbott SARS-CoV-2 assay and Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR was 100 % (96.2-100%). Whereas BGI SARS-CoV-2 assay via ABI 7500 RT-PCR and Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR were 97.9 % (92.8-99.4 %) and 93.8 % (87.2 - 97.1%) respectively. On the other hand all assays with respective platforms specificity were 100%, except Daan Gene assay via Roche 4800 RT-PCR platform which was 98.5 % specificity (one sample was ORF1a/b gene positive twice on this platform, but the result was negative based on the CRS interpretation criteria) (Table-5).

Negative predictive value (NPV) and Positive predictive value (PPV) of Abbott SARS-CoV-2 was 100%, while Sansure biotech NPV and PPV was 91.8% (83.2-96.2) and 100% (95.9-100) respectively. Cohn's Kappa coefficient of agreement between CRS and Abbott SARS-CoV-2 assay result had perfect agreement (K=1.00). Similarly, the Cohn's Kappa value of Daan Gene 2019-nCoV assay via Abbott m2000 RT-PCR (Open mode), Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR and Daan Gene 2019-nCoV assay via ABI 7500 RT-PCR results had also perfect agreement with CRS (K=0.987). Another Cohn's kappa value indicated that BGI SARS-CoV-2 assay via ABI 7500 RT-PCR and Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR results had perfect agreement compared with CRS, 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 via ABI 7500 RT-PCR significantly different compared to CRS (p=0.031) (Table-5).

Table 5: Agreement analysis between the results of different SARS-CoV-2 detection assays and platforms with CRS on nasopharyngeal swabs, 2020

S/n	PCR assay with different PCR platform	Sensitivity N (%) (95% CI)	Specificity N (%) (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Cohen's Kappa value (95% CI)	MacNemar test (p-value)
1	Abbott SARS-CoV-2 assay	97/97 (100%) (96.27-100)	67/67 (100%) (94.6-100)	100% (96.3-100)	100% (94.6-100)	1.00	1.00
2	Daan Gene 2019-nCoV assay via Abbott m2000 RT-PCR (Open mode)	96/97 (99%) (94.4-99.8)	67/67 (100%) (94.6-100)	100% (96.3-100)	98.5% (92.1-99.7)	0.987 (0.95-1.00)	1.00
3	Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR	97/97 (100%) (96.2-100)	66/67 (98.5%) (92-99.7)	99% (94.4-99.8)	100% (94.5-100)	0.987 (0.949-1.00)	1.00
4	Daan Gene 2019-nCoV assay via ABI 7500 RT-PCR	96/97 (99 %) (94.4-99.8)	67/67 (100%) (94.6-100)	100% (96.2-100)	98.5% (92.1-99.7)	0.987 (0.96-1.00)	1.00
5	BGI SARS-CoV-2 assay via ABI 7500 RT-PCR	95/97 (97.9%) (92.8-99.4)	67/67 (100%) (94.6-100)	100% (96.1-100)	97.1% (90-99.2)	0.975 (0.927-1.00)	0.50
6	Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR	91/97 (93.8%) (87.2-97.1)	67/67 (100%) (94.6-100)	100% (95.9-100)	91.8% (83.2-96.2)	0.925 (0.86 - 0.975)	0.031*
7	CRS	97/97 (100%)	67/67 (100%)				

In this study the other important finding was the positive percent agreement (PPA) and negative percent agreement (NPA) of Abbott SARS-CoV-2 assay was 100% compared to the CRS. Daan Gene 2019-nCoV assay via Abbott m2000 RT-PCR (Open mode), Daan Gene 2019-nCoV assay via ABI 7500 RT-PCR, BGI SARS-CoV-2 assay via ABI 7500 RT-PCR and Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR results also had 100 % NPA. On the other hand PPA and NPA of Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR result was 100% and 98.5% respectively. Similarly the PPA of BGI SARS-CoV-2 assay via ABI 7500 RT-PCR and Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR results were 97.9% and 93.8% respectively. The Overall agreement (OAA) between CRS and Abbott SARS-CoV-2 assay was 100 %. While the overall agreement of Daan Gene 2019-nCoV assay via Abbott m2000 RT-PCR (Open mode) (Table 6).

Table 6: Percent agreement of different assay and platform results with CRS, 2020

S/n	PCR assay with different PCR platform	Positive Percent agreement (95% CI)	Negative percent agreement (95% CI)	Overall percent agreement (95% CI)
1	Abbott SARS-CoV-2 assay	100% (96.3-100)	100% (94.6-100)	100% (97.7 -100)
2	Daan Gene 2019-nCoV assay via Abbott m2000 RT-PCR (Open mode)	99 % (94.4-99.8)	100% (94.6- 100)	99.4% (96.6 – 99.9)
3	Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR	100% (96.2-100)	98.5 % (92.0-99.7)	99.4% (96.6-99.9)
4	Daan Gene 2019-nCoV assay via ABI 7500 RT-PCR	99% (94.4-99.8)	100% (94.6- 100)	99.4% (96.6 - 99.9)
5	BGI SARS-CoV-2 assay via ABI 7500 RT-PCR	97.9% (92.8-99.4)	100 % (94.6-100)	98.8% (95.7-99.7)
6	Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR	93.8% (87.2-97.1)	100% (94.6-100)	96.3% (92.2- 98.3)

6.3. Comparative analysis of CT value in different SARS-CoV-2 PCR assay and platforms

In this study the lowest minimum CT value (high viral copies) was observed in the combined gene (RdRp plus N gene CT) Abbott SARS-CoV-2 assay (CT=4.06) and the highest minimum CT value was recorded on Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR result of ORF1a/b gene (CT=17.73). Similarly Maximum CT of Abbott SARS-CoV-2 assay was 27.40, while the maximum CT of Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR result of ORF1a/b gene was 40.0 (Table-6).

Table 7: Statistical analysis of CT values in different assays and platforms, 2020

Descriptive statistics of CT value	PCR assay with different PCR platform									
	Abbott SARS-CoV-2 assay	**Daan Gene 2019-nCoV assay via Abbott m2000 RT-PCR (Open mode)**		**Daan Gene 2019-nCoV assay via Roche 4800 RT-PCR**		**Daan Gene 2019-nCoV assay via ABI 7500 RT-PCR**		**BGI SARS-CoV-2 assay via ABI 7500 RT-PCR**	**Sansure Biotech 2019-nCoV assay via ABI 7500 RT-PCR**	
Type of gene	Compilation of N and RdRp gene	ORF 1a/b gene CT	N gene CT	ORF 1a/b gene CT	N gene CT	ORF 1a/b gene CT	N gene CT	ORF 1a/b gene	ORF 1a/b gene CT	N gene CT
Number of sample	97	96	97	97	96	96	97	95	90	91
Minimum CT value	4.06	12.90	10.00	17.73	13.51	14.10	11.60	15.32	15.79	14.53
Maximum CT value	27.40	40.00	34.99	40.00	38.63	37.00	36.80	37.69	38.68	36.76
Mean	15.29	25.40	23.50	29.26	26.82	25.78	24.34	26.56	27.08	26.16
Median	14.52	25.09	22.97	29.09	26.16	24.82	23.65	25.87	25.92	26.93
St deviation	5.86	5.67	5.78	5.33	5.76	5.69	5.90	5.42	5.21	4.96

The above table (table 7) showed that the highest median value (29.09) was recorded on the Daan Gene assay with Roche 4800 RT-PCR platform of ORF1a/b gene and comparatively the lowest median CT value (14.52) was observed on the Abbott SARS-CoV-2 assay with compiled gene (RdRp and N gene).

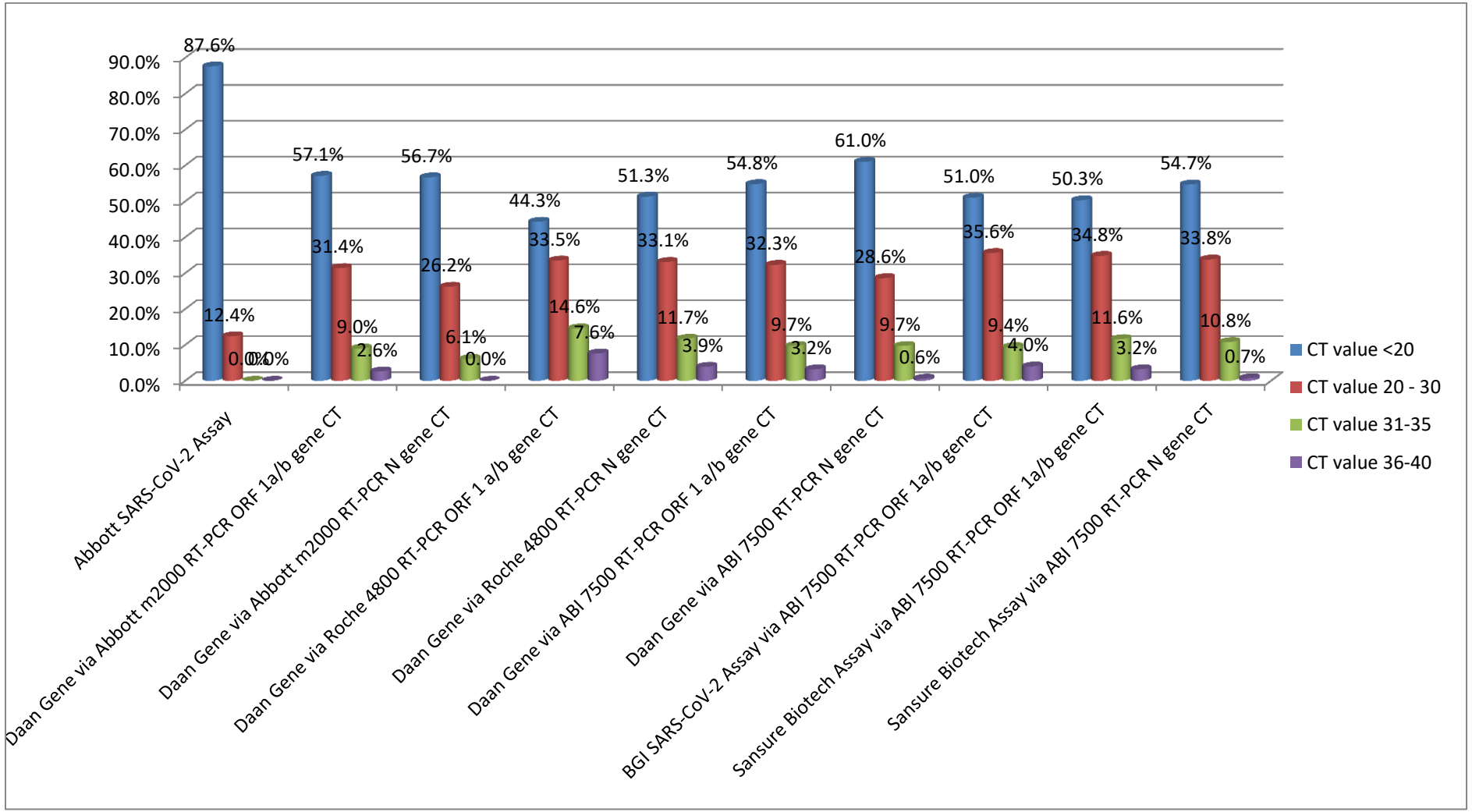

Figure 2: Distribution of CT value in different assay gene and platform, 2020

According to fig-3 the percentage of lowest CT value (<20) of Abbott SARS-CoV-2 assay(combined RdRp and Ngene), Daan Gene assay via Abbott m2000 RT-PCR (Open mode) ORF1a/b and N gene, Daan Gene assay via Roche 4800 RT-PCR ORF1a/b and N gene, Daan Gene assay via ABI 7500 RT-PCR ORF1a/b and N gene, BGI SARS-CoV-2 assay via ABI 7500 RT-PCR ORF1a/b gene and Sansure Biotech assay via ABI 7500 RT-PCR N gene and ORF1a/b gene were 87.6%, 57.1% and 56.7%, 44.3% and 51.3%, 54.8% and 61.0%, 51.0%, 50.3% and 54.7% respectively. The highest CT value (36-40) percentage with each assay and respective platforms were 2.6 % on Daan Gene assay ORF 1a/b gene with Abbott m2000, 7.6% on Daan Gene assay ORF 1a/b gene via Roche 4800 RT-PCR, 3.9% Daan Gene assay N gene with Roche 4800 RT-PCR, 3.2% on Daan Gene assay via ABI7500 RT-PCR, 0.6% on Daan gene assay N gene via ABI 7500 RT-PCR.

7. Discussion

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

This study showed that Abbott SARS-CoV-2 assay had equal detection performance with Composite Reference Standard (CRS), which had 100 % specificity, sensitivity, positive agreement, negative agreement and overall agreement. The Cohn's Kappa agreement was 1.00; it indicates that, it has the perfect agreement with CRS. Similar study reported from Washington University, USA indicated 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) (32). Abbott SARS-CoV-2 assay was using the combined gene of N and RdRP, both genes had more sensitivity and evidence also revealed that, this combination could be minimizing false negative results (29). The study conducted in Vienna, Austria also showed that high amount of sample volume for extraction and volume of elute for detection could be minimize the dilution effect and increase the detection efficacy (30). So the high sensitivity and specificity of Abbott SARS-CoV-2 assay might be due to the assay using the combined gene method, large volume of sample for extraction (1.3ml including 800µl dead volume, the exact extraction volume was 500µl) and using large volume of elute plus master mix reagents (total volume was 50µl).

Our result also showed that, Daan gene assay with different RT-PCR platforms (Abbott m2000 RT-PCR open mode, Roche 4800 RT-PCR, and ABI 7500 RT-PCR) detection performance had almost similar compared to the CRS. Furthermore, sensitivity of Daan gene assay via Abbott m2000 and ABI 7500 was 99% (96/97), whereas specificity in both platforms was 100%. Similarly the study conducted in Anhui University, Huainan, China(37), the positive coincidence rate compared to the claim of the manufacturer Daan gene assay was agreed 100%. Even though concordance result was reported, in our study one sample was falsely negative in both detection platforms after repeat the elute, but the same sample elute was positive in Abbott SARS-CoV-2 assay, BGI SARS-CoV-2 assay and even in Daan Gene assay with Roche 4800 RT-PCR. It indicated that, result variability might be seen in different type of platform due to PCR efficiency difference between platforms to platform.

In the other hand, the study carried out in China (36), the result of Daan gene assay was significantly different ($p > 0.05$) compared to lab defined reference assay. This difference might become due to viral strain difference circulating in the community, hence, at the time of this study SARS-CoV-2 spike (S) gene mutation was reported from different part of the world. This indicated that, to minimize the diagnostic variability of COVID-19 due to high prevalence of new emerging variants (53)(54). The other difference was also due to sensitivity of reference assay to detect SARS-CoV-2 and further study might be important to determine the cause of this differences.

Our study also assesses comparatively the diagnostic performance of BGI SARS-CoV-2 assay with CRS. The sensitivity and specificity of BGI assay was 97.9 % and 100 % respectively. Similarly 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 was showed that excellent agreement. Concordant result was reported from the study done in Netherlands (34) and Changsha district, China, BGI kit sensitivity and specificity were 90.48% and 100% respectively (36). BGI SARS-CoV-2 assay is a single gene (ORF 1a/b) detection assay and using10µl elute for detection. Even though, there is excellent statistical agreement was showed with our reference result, the assay was missed two positive results (1.22%) from the total test. Our study indicated that both missed samples had high CT value (>34) in Daan gene assay. Moreover, one of missed sample was only N gene positive after repeated the test and the other one had high CT value of ORF 1a/b (CT=36.10) and N gene (CT=35.89). Similarly, the study conducted by Wang X et al. the analytical performance of BGI RT-PCR assay was not meet the claimed lower detection limit. So, in higher CT (low viral copies) result the assay might need further consideration for review (22). The other comparator assay included in this study was Sansure Biotech nCoV 2019 RT-PCR assay (RUO), which had good sensitivity (93.8 %), excellent specificity (100 %) and the overall percent agreement was 96.3%. Strength of agreement also determined with Cohn's Kappa value, which was 0.925, it indicates perfect agreement with CRS. Likewise this study, the study performed in The Second Xiangya Hospital, Central South University, Changsha district, China (36), Liuzhou People's Hospital, Department of Clinical Laboratory, Liuzhou, China (38). Even though the above good statistical concordance was recorded, the Chi-square test (MacNemar test) analysis showed that the result of Sansure Biotech assay was had statistically significant difference

compared to CRS($p<0.005$). In our study six (06) samples (3.66%) were turned to falsely negative, this is not small number, especially when we are thinking in terms of the virus transmission dynamics. This low detection rate also supported by the above evidence (36). Sensitivity and positivity rate of detection assays were more dependent on target region selection and primer designing/amplification efficiency of the primers. So, efficient primers are very important for target amplification and minimizing primer dimmer (55).

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 (15.29) and the highest mean (low viral copies) determined on Daan Gene assay via Roche 4800 RT-PCR platform(29.26). The result indicated that the combine gene detection system of Abbott SARS-CoV-2 assay had better efficiency in the detection of COVID-19 even with low viral concentration. Hence, according to fig- 87.6 % of Abbott SARS-CoV-2 result CT value was laid on less than 20. Only few sample results (12.4%) of the result CT was laid between 20 and 30. There was no CT value recorded above 30.

In both assays which were using dual gene detection (Daan Gene and Sansure biotech) the N gene concentration in the sample greater than ORF1a/b gene concentration. Moreover, the mean CT value of N gene in Daan Gene assay via Abbott m2000 RT-PCR, Roche 4800 RT-PCR and ABI 7500 RT-PCR were 23.50, 26.82, and 24.34 respectively, whereas the mean ORF1a/b gene CT within these platforms were 25.40, 29.26, and 25.78 respectively . And in Sansure Biotech via ABI 7500 the mean N gene CT was 26.16, but the ORF1a/b gene CT was 27.08. The result showed that the mean difference of CT between the two genes were almost more than one (1) CT consistently. The overall result showed that the N gene sensitivity in both assay and on indicated platforms was higher than ORF 1a/b gene sensitivity. Similar result was shown on the study conducted by Li, et. al (56).

Limitation of the study

One of the limitations of this study was unable to compare our comparator assays with standard/ reference method (like viral load assay or other LDA assay) due to resource limitation.

8. Conclusion and recommendation

8.1. Conclusion

This study was compared the diagnostic performance of four SARS-CoV-2 qualitative detection assays and three platforms using nasopharyngeal samples. All detection assays and platforms except Sansure biotech assay had almost comparable performance. The chi-square analysis (MacNemar test) also indicated that rate of positivity/negativity was almost similar compared to CRS ($P > 0.05$). However, rate of positivity of Sansure Biotech compared to CRS lower performance ($P<0.05$). Our study also showed that, N gene sensitivity better than ORF 1a/b gene sensitivity in Daan gene and Sansure Biotech assay.

8.2. Recommendation

1. Abbott SARS-CoV-2 assay, Daan Gene assay with Abbott m2000 RT-PCR, Roche 4800 RT-PCR, ABI 7500 RT-PCR and BGI SARS-CoV-2 assay with ABI7500 were almost concordant and excellent diagnostic performance.
2. Sansure Biotech assay (RUO) had low performance detection ability compared to CRS, so selection of assays should be considering further verification on its use in Ethiopia.
3. Detection of SARS-CoV-2 with Abbott m2000 RT-PCR, Roche 4800 RT-PCR, ABI 7500 RT-PCR in Daan gene assay were almost no diagnostic performance difference. So for purchase these assays and platforms considered as no clinical performance difference.
4. Additional study should be done by using better reference method and evaluation of company's claim in terms of lower detection limit and interference will be important.

Reference

1. Raoofi A, Takian A, Akbari Sari A, Olyaeemanesh A, Haghighi H, Aarabi M. COVID-19 Pandemic and Comparative Health Policy Learning in Iran. Arch Iran Med. 2020;23(4):220–34.

2. Peeri NC, Shrestha N, Rahman MS, Zaki R, Tan Z, Bibi S, Baghbanzadeh M, Aghamohammadi N, Zhang W, Haque U. The SARS, MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: what lessons have we learned?. International journal of epidemiology. 2020;49(3):717-26.3.

3. Van Bavel JJ, Baicker K, Boggio PS, Capraro V, Cichocka A, Cikara M, Crockett MJ, Crum AJ, Douglas KM, Druckman JN, Drury J. Using social and behavioural science to support COVID-19 pandemic response. Nature human behaviour. 2020;4(5):460-71.

4. O. Okpara M, Jamabo M. Understanding COVID-19 - A Molecular Perspective. Am J Infect Dis Microbiol. 2020;8(2):64–74.

5. Aboubakr HA, Sharafeldin TA, Goyal SM. Stability of SARS-CoV-2 and other coronaviruses in the environment and on common touch surfaces and the influence of climatic conditions: A review. Transboundary and emerging diseases. 2021;68(2):296-312.

6. Ward S, Lindsley A, Courter J, Assa'ad A. Clinical testing for COVID-19. J Allergy Clin Immunol. 2020;146(1):23–34.

7. Chang L, Yan Y, Wang L. Coronavirus Disease 2019: Coronaviruses and Blood Safety. Transfus Med Rev. 2020;34(2):75–80.

8. Arun Zanke A, R Thenge R, S Adhao V. COVID-19: A pandemic declare by world health organization. IP Int J Compr Adv Pharmacol. 2020;5(2):49–57.

9. World Health Organization. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: interim guidance, 19 March 2020. World Health Organization; 2020. access date September 16/2020.

10. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020;181(2):281-292.e6.

11. Ortiz-Prado E, Simbaña-Rivera K, Gómez- Barreno L, Rubio-Neira M, Guaman LP, Kyriakidis NC, et al. Clinical, molecular, and epidemiological characterization of the SARS-CoV-2 virus and the Coronavirus Disease 2019 (COVID-19), a comprehensive literature review. Diagn Microbiol Infect Dis. 2020;98(1).

12. Shoaib MH, Sikandar M, Yousuf RI, Saleem MT. A Journey From SARS-CoV-2 to COVID-19 and Beyond : A Comprehensive Insight of Epidemiology , Diagnosis , Pathogenesis , and Overview of the Progress into Its Therapeutic Management. Front Pharmacol |. 2021;12(1):1–24.

13. Ferretti L, Wymant C, Kendall M, Zhao L, Nurtay A, Abeler-Dörner L, et al. Quantifying SARS-CoV-2 transmission suggests epidemic control with digital contact tracing. Science (80-). 2020;368(6491):0–8.

15. Gates B. Responding to Covid-19 - A Once-in-a-Century Pandemic? N Engl J Med. 2020;323(16):2019–20.

16. Eloise Williams, Katherine Bond, Bowen Zhang, Mark Putland and DAW. Saliva as a noninvasive specimen for detection of sars-cov-2. J Clin Microbiol. 2020;58(8):1–2.

17. World Health Organization. Laboratory testing strategy recommendations for COVID-19. WHO. 2020;(https://apps.who.int/iris/handle/10665/331509):6. accessed date August 16/2020.

18. Wyllie A, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, et al. Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs. medRxiv. 2020;(https://doi.org/10.1101/2020.04.16.20067835).

19. Mitchell SL, George KS. Evaluation of the COVID19 ID NOW EUA assay. Journal of Clinical Virology. 2020;128:104429.

20. Wood CS, Thomas MR, Budd J, Mashamba-Thompson TP, Herbst K, Pillay D, Peeling RW, Johnson AM, McKendry RA, Stevens MM. Taking connected mobile-health

diagnostics of infectious diseases to the field. Nature. 2019;566(7745):467-74.

21. Paper O, Umaru FA, States U, States U, Umaru F. Scaling up testing for COVID-19 in Africa : Responding to the pandemic in ways that strengthen health systems. Afr J Lab Med. 2020;9(1),:19–20.

22. Wang X, Huang Z, Song J, Xiao Y, Wang H. Analytical sensitivity comparison of 14 conventional and three rapid RT-PCR assays for SARS-CoV-2 detection. Journal of Virological Methods. 2021;293:114144.

23. Africa CDC. Outbreak Brief 50: Coronavirus Disease 2019 (COVID-19) Pandemic – Africa CDC. 2021;(https://au.int/sites/default/files/documents/40785-doc-AfricaCDC_COVIDBrief_7Sep21_EN.pdf):1–7. Accessed date October 10/2020.

24. Kobia F, Gitaka J. COVID-19: Are Africa's diagnostic challenges blunting response effectiveness? AAS Open Res. 2020;3(4):https://doi.org/10.12688/aasopenres.13061.1.

25. Teymouri M, Mollazadeh S, Mortazavi H, Ghale-noie ZN. Recent advances and challenges of RT-PCR tests for the diagnosis of COVID-19. Pathol - Res Pract J. 2021;221(https://doi.org/10.1016/j.prp.2021.153443).

26. Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, Zambrano-Achig P, Campo R Del, Ciapponi A, et al. False-negative results of initial RT-PCR assays for COVID-19: A systematic review. PLoS One. 2020;15(2):1–19.

27. Cohen AN, Kessel B. False positives in reverse transcription PCR testing for SARS-CoV-2. medRxiv. 2020;(https://doi.org/10.1101/2020.04.26.20080911 ;):2020.04.26.20080911.

28. Roland S. Marshall, M. ME. False Negative Tests for SARS-CoV-2 Infection — Challenges and Implications. N Engl J Med. 2020;383(6):1–3.

29. Mollaei HR, Afshar AA, Kalantar-Neyestanaki D, Fazlalipour M, Aflatoonian B. Comparison five primer sets from different genome region of covid-1for detection of virus infection by conventional rt-pcr. Iran J Microbiol. 2020;12(3):185–93.

30. Görzer I, Buchta C, Chiba P, Benka B, Camp JV, Holzmann H, Puchhammer-Stöckl E,

Aberle SW. First results of a national external quality assessment scheme for the detection of SARS-CoV-2 genome sequences. Journal of Clinical Virology. 2020;129:104537.

31. Iglói Z, Leven M, Abdel-Karem Abou-Nouar Z, Weller B, Matheeussen V, Coppens J, et al. Comparison of commercial realtime reverse transcription PCR assays for the detection of SARS-CoV-2. J Clin Virol. 2020;129(104510):4–6.

32. Degli-Angeli E, Dragavon J, Huang ML, Lucic D, Cloherty G, Jerome KR, Greninger AL, Coombs RW. Validation and verification of the Abbott RealTime SARS-CoV-2 assay analytical and clinical performance. Journal of Clinical Virology. 2020;129:104474.

33. Ren X, Tang Q-M, Chen M, Huang Q, Huang H-L, Wang L, et al. An acceptable method to evaluate the analytical performance of real-time fluorescent RT-PCR targeting SARS-CoV-2. medRxiv. 2020;(https://doi.org/10.1101/2020.05.18.20105247):1–19.

34. van Kasteren PB, van der Veer B, van den Brink S, Wijsman L, de Jonge J, van den Brandt A, et al. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. J Clin Virol. 2020;128:104412.

35. Dayarathna S, Jayathilaka D, Jeewandara C, Wijewickrama A, Narangoda E, Idampitiya D, et al. Comparison of the analytical sensitivity of three real time PCR kits used in the detection of the SARS-CoV-2. J Ceylon Coll Physicians. 2020;51(1):53.

36. Wang B, Hu M, Ren Y, Xu X, Wang Z, Lyu X, et al. Evaluation of seven commercial SARS-CoV-2 RNA detection kits based on real-time polymerase chain reaction (PCR) in China. Clin Chem Lab Med. 2020;58(9):149–53.

37. Wang M, Chen D, Wu W, Tang H, Kan L, Zong Z, Dou X, Ji X, Xiong D, Zhang X. Analytical performance evaluation of five RT-PCR kits for severe acute respiratory syndrome coronavirus 2. Journal of Clinical Laboratory Analysis. 2021;35(1):e23643.

38. Lu Y, Li L, Ren S, Liu X, Zhang L, Li W, et al. Comparison of the diagnostic efficacy between two PCR test kits for SARS-CoV-2 nucleic acid detection. J Clin Lab Anal. 2020;34:e23554.(https://doi.org/10.1002/jcla.23554 wileyonlinelibrary.com/journal/jcla):1–5.

39. Abbott. Abbott RealTime SARS-CoV-2 Assay Package Insert [Internet]. Abbott Molecular, Inc. 2020. p. 1–12. Available from: https://www.molecular.abbott/sal/9N77-095_SARS-CoV-2_US_EUA_Amp_PI.pdf. Accessed date August 30/2020.

40. Sun Yat-sen University. Instructions for Use of Detection Kit for Novel Coronavirus (2019-nCoV) RNA (Fluorescent PCR). 2020;(http://www.daangene.com):1–7. Accessed date September 12/2020.

41. BGI FDA. Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2. BGI. 2020;(https://www.fda.gov/media/136472). Accessed date September 10/2020.

42. Sansure BioTech Inc. Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing). 2020;(680):6000. Acessed date September 15/2020.

43. World Health Organization. Risk Based Classification Of Diagnostics For Who Prequalification Prequalification of Diagnostics. 2014:1–18. Accessed date; september 22/2020.

44. Klein S, Müller TG, Khalid D, Sonntag-Buck V, Heuser AM, Glass B, et al. SARS-CoV-2 RNA extraction using magnetic beads for rapid large-scale testing by RT-qPCR and RT-LAMP. Viruses. 2020;12(8).

45. GmbH Q. QIAamp Viral RNA Mini Handbook. 2010. 1–44 p. Accessed date ; September 25/2020.

46. Vanderbilt University and Vanderbilt University Medical Center. Biological Inactivation of COVID-19 / SARS-CoV-2 Specimens for Handling under BSL-2 Containment Conditions Expectations for Use of Inactivation Methods. 2020;9–11. Accessed date ; september 25/2020.

47. Sarlgül F, Doluca O, Akhan S, Sayan M. Investigation of compatibility of severe acute respiratory syndrome coronavirus 2 reverse transcriptase-PCR kits containing different gene targets during coronavirus disease 2019 pandemic. Future Virol. 2020;15(8):515–24.

48. Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, Tjian R. Overcoming the bottleneck to widespread testing: A rapid review of nucleic acid testing approaches for

COVID-19 detection. Rna. 2020;26(7):771–83.

49. Ethiopian Public Health Institute FM off H. Interim National Strategy and Guidance for the Laboratory Diagnosis of COVID-19 in. EPHI. 2020;(https://ephi.gov.et/images/novel_coronavirus/EPHI_PHEOC_COVID-19_Laboratory_Diagnosis_Eng.pdf). Accessed date; December 01/2020.

50. Yujuan Xiong, Zhen-Zhen Li, Qi-Zhen Zhuang, Yan Chao, Fei Li, Yi-Yuan Ge, Yi Wang, Pei-Feng Ke X-ZH. Comparative performance of four nucleic acid amplification tests for SARS-CoV-2 virus. bioRxiv. 2020;(https://doi.org/10.1101/2020.03.26.010975).

51. Lephart PR, Bachman MA, Lebar W, Mcclellan S, Barron K, Schroeder L, et al. Comparative study of four SARS-CoV-2 Nucleic Acid Amplification Test (NAAT) platforms demonstrates that ID NOW performance is impaired substantially by patient and specimen type. Diagn Microbiol Infect Dis. 2020;99(115200).

52. Landis JR, Koch GG. The Measurement of Observer Agreement for Categorical Data. Biometrics. 1977;33(1):159.

53. Gand M, Vanneste K, Thomas I, Gucht S Van, Capron A, Herman P, et al. Deepening of In Silico Evaluation of SARS-CoV-2 Detection RT-qPCR Assays in the Context of New Variants. Genes (Basel). 2021;12(565):1–14.

54. Mlcochova P, Collier D, Ritchie A, Assennato SM, Hosmillo M, Goel N, Meng B, Chatterjee K, Mendoza V, Temperton N, Kiss L. Combined point-of-care nucleic acid and antibody testing for SARS-CoV-2 following emergence of D614G spike variant. Cell Reports Medicine. 2020;1(6):100099.

55. Jing Yang , Yanxi Han , Runling Zhang , Rui Zhang JL. Comparison of analytical sensitivity of SARS-CoV-2 molecular detection kits. Int J Infect Dis. 2021;(https://doi.org/10.1016/j.ijid.2021.08.043).

56. Li M, Zhao Y, Li Y, Chen X, Luo D, Luo M, et al. Development and evaluation of a Novel RT-PCR system for reliable and rapid SARS-CoV-2 screening of blood donations. Transfusion. 2020;60(12):2952–61.

Annex I: procedure for Abbott SARS-COV-2 testing by Abbott m2000 SP/RT (Automation testing)

Part I: Maintenance, Start up and sample extraction procedure

Bio-safety cabinet and vortex Maintenance

- Before and after the work, clean the hood/BSC with 0.1 percent bleach, distilled water, and 70 percent ethanol, and utilize UV light for 15 minutes.
- Clean the centrifuge and vortex it with 0.1 percent bleach, then distilled water and 70% ethanol.

Startup procedure

- Start Abbott m2000System Control Center (SCC) and Abbott m2000 sp instrument
- Enter user ID and password to access the program.
- Click on initialize to initialize the Abbott m2000sp instrument; once initialization is complete, the Abbott sp instrument is ready for operation in ready mode.
- Perform all necessary maintenance including daily, weekly and as needed maintenance

Reagent preparation

- Depending on the amount of samples to be examined, use 1-4 bottles of mLysis buffer.
- Gently invert the Abbott System DNA Preparation bottles to confirm that the solution is homogenous and free of bubbles.
- Make sure no bubbles or foam form; if they do, remove them with a sterile pipette tip, making care to use a new tip for each bottle.
- Wait until the crystals have dissolved before using the reagents.
- Fill the mLysisDNA, mWash1DNA, and mWash2DNA bottles with USP Grade 190-200 Proof Ethanol (95 -100 %) as directed below.
- For each bottle of mLysis add 35 mL ethanol, 23 mL ethanol for each bottle of mWash1, and 70 mL ethanol for each bottle of mWash2 was used.

Pre-extraction procedure

- Prepare sample and generate the work sheet
- Carefully pipette 1.3 ml of liquid to a nunc tube/MMX tube. Avoid transfer of bubbles.
- Inactivate the samples by heat at 56^0c for 30 minutes
- Place maxim of 94 patient specimens including mock into the Abbott *m*2000*sp* sample rack.

- Place the 5 mL Reaction Vessels into the *m*2000*sp* 1 mL subsystem carrier
- Load the Abbott *m*2000 Sample Preparation System reagents and the Abbott 96 Deep-Well Plate on the Abbott *m*2000*sp* worktable as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section
- Select the appropriate application file from the Run Sample Extraction screen that corresponds to the sample volume being tested.
- Initiate the sample extraction protocol as described in the Abbott *m*2000*sp* operations manual, operating instruction section**.**
- Enter sample extraction lot specific values in the Sample Extraction: Worktable Setup and fields. The Master Mix Addition must be initiated within one hour
- Master mix done by ABBOTT M2000 SP
- The *m*2000*rt* protocol must be started immediately after initiation of the Master Mix Addition protocol.
- Switch on and initialize the Abbott *m*2000*rt* instrument in the amplification area which requires 15 min to warm-up.
- Seal the Abbott 96-well optical reaction plate
- Create the new order
- Place the sealed optical reaction plate into the Splash Free Support Base for transfer to the Abbott *m*2000*rt* instrument.
- Make sure the Abbott *m*2000*rt* instrument has been initialized
- Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument.
- From the protocol screen selected the appropriate application file corresponding to the parameter and volume being tested
- Initiate the protocol as described in the Abbott *m*2000*rt* Operations Manual, Operating Instruction section

Analysis of results

After completion of the run, analyze the data following the instrument manufacturers' instructions. Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signal. The procedure chosen for setting the threshold should be used consistently.

Annex II: Procedure for Daan Gene assay detection with Abbott m2000rt (open mode), Roche 48000 rt, and ABI 7500 RT.

Master Mix Addition/PCR reagent preparation

From the kit, remove NC (ORF1ab/N) PCR reaction liquid A and NC (ORF1ab/N) PCR reaction liquid B. Shake and combine after thawing at room temperature. Before using, centrifuge for a few seconds at 8,000 rpm. Fill the PCR reaction tube with N (N = number of specimens to be tested + NC (ORF1ab/N) negative control material + NC (ORF1ab/N) positive control material). Assemble an NC single-reaction amplification system as follows:

NC(ORF1ab/N) PCR reaction liquid A	NC(ORF1ab/N) PCR reaction liquid B	Amplification system
17µl	3µl	20µl

- Centrifuge for a short time after thoroughly mixing the components to spin down all the liquid on the tube wall to the tube bottom.
- 20µl of the amplification system should be aliquoted to the PCR tubes.

Sampling

- Fill the PCR reaction tubes with 5l each of the negative control material, the RNA of the specimens to be tested, and the positive control material processed, cover the tubes tightly, and centrifuge at 8,000 rpm for few seconds before transferring them to the amplification detection area.
- - Follow the directions in the Operating Instructions section of the Abbott m2000sp Operations Manual.
- - The m2000rt protocol must be started as soon as the Master Mix Addition protocol is initiated.
- - In the amplification area, turn on and initialize the Abbott m2000rt instrument, which takes 15 minutes to warm up.
- - Seal the Abbott 96-well optical reaction plate - Create a new order - Transfer the sealed optical reaction plate to the Abbott m2000rt instrument using the Splash Free Support Base.
- - Verify that the Abbott m2000rt instrument has been properly configured.

- In the Abbott m2000rt instrument, place the Abbott 96-Well Optical Reaction Plate.
- Select the relevant application file for the parameter and volume being tested from the protocol screen. - Start the protocol as indicated in the Abbott m2000rt Operations Manual, Operating Instruction section.

Analysis of results

Analyze the data when the run is over according to the instrument manufacturer's recommendations. Manual thresholding should be used to do analyses for each target separately. Thresholds should be adjusted to lie within the fluorescence curves' exponential phase and above any background signal. The threshold-setting technique should be followed consistently.

Determination of results

If the test sample has no amplification curve or Ct value > 40 in the FAM and VIC channels, there is amplification curve in the Cy5 channel, it can be judged that there is no 2019 Novel Corona virus (2019-nCoV) RNA in the sample.

If the test sample has obvious amplification curve in the FAM and VIC channels and Ct value ≤ 40, it can be judged that the sample is positive for 2019 Novel Corona virus (2019-nCoV).

If the test sample only has the Ct value of ≤ 40 in a single channel of FAM or VIC, and there is no amplification curve in the other channel, the results need to be re-tested.

If the re-test results are consistent, the sample can be judged to be positive for 2019 Novel Corona virus (2019-nCoV). If the re-test results are negative, it can be judged that no 2019 Novel Corona viruses (2019-nCoV) RNA has been detected. If the re-test results are negative, it can be judged that no 2019 Novel Corona viruses (2019-nCoV) RNA has been detected.

Positive Judgment Value

Both the kit's reference CT value and the internal control reference value are determined using the Receiver Operating Characteristic (ROC) curve approach.

Interpretation of Test Results

1. Each experiment should include both negative and positive control materials. The test results can only be determined when the control materials meet the quality control requirements.

2. Due to system competition, when the FAM and VIC detection channels are positive, the result from the Cy5 channel (internal control channel) may be negative.

3. When the internal control result is negative, if the FAM and VIC detection channels of the test tube are also negative, it indicates that the system is inhibited or the operation is wrong, the test is invalid. Therefore, the sample needs to be re-tested.

Result Reporting

- The following is the format of a negative result report: no RNA of the 2019 Novel Corona virus (2019-nCoV) was discovered in the specimens, and the concentration was lower than the kit's sensitivity;
- The following is the format of the positive result report: RNA from the 2019 Novel Corona virus (2019-nCoV) was found in the specimens.

Annex III: Procedure for Sansure Biotech detection with ABI 7500 RT-PCR

Detailed procedure

1. Remove each component from the diagnostic kit and set it aside to cool. Allow the reagents to equilibrate at room temperature before vortexing each one for later use.
2. Prepare the 2019-nCoV-PCR Master Mix (26 L 2019-nCoV-PCR Mix + 4 L 2019nCoV-PCR-Enzyme Mix) based on the total number of specimens, 2019-nCoV-PCR-Positive Control, and 2019-nCoV-PCR-Negative Control, and carefully mix them together. The remaining reagent should be kept at -20°C as soon as possible.

Master Mix preparation

	1 sample	10 samples	24 samples	48 samples
2019-nCoV-PCR Mix (μL)	26	260	624	1248
2019-nCoV-PCR-Enzyme Mix (μL)	4	40	96	192
Note: The above configuration is for reference only.				

1. Fill each well with 30 mL of 2019-nCoV-PCR Master Mix. Transfer the wells to the sample processing area after covering them. In the well pre-filled with reagent mix, add 20 L of the extracted RNA in the following order: Patient specimen(s), 2019nCoV-PCR-Negative Control, and 2019nCoV-PCR-Positive Control cover each well, centrifuge for 10 seconds at 2000 rpm, and transfer into an Applied Biosystems ABI 7500 real-time RT-PCR system, noting the precise placement of controls and specimens.
2. PCR amplification on an ABI 7500 with 7500 software version 1.5:
3. Begin using the ABI 7500 real-time PCR system: The computer connected to the system should be turned on first, followed by the ABI 7500 real-time PCR system.
4. Load the instrument: Push the tray door open, then load the prepared plate with samples and controls into the instrument's plate holder. Ensure that the plate in the holder is properly positioned. Close the door of the tray.
5. Begin the experiment.

Interpretation of the result

Prior to interpreting patient results, all test controls should be evaluated. The patient results cannot be interpreted if the controls are invalid. This kit's Ct cutoff value is set to 40, and the end user must evaluate fluorescence curves before making a final interpretation. For weakly positive samples, all positive curves should be standard S-shape amplification curves or without plateau.

Annex IV: BGI SARS-CoV-2 detection procedure

Detailed procedure

The QIAamp Viral RNA Mini Kit (Qiagen) Nucleic Acid Extraction kit should be used to manually extract RNA according to the manufacturer's recommendations. The extracted RNA should be used right away or kept at -70°C for subsequent use.

1. In the preparation area, mix all of the reagents together.
2. To begin, remove all of the ingredients of the package except the Enzyme mix and defrost completely at room temperature.
3. Vortex and centrifuge for a few seconds
4. Keep the Enzyme Mix refrigerated at all times. Next, figure out how many reactions (N) will be included in the test.
5. Make sure to include each specimen as well as the no template (negative) control (1 tube) and the positive control (1 tube).
6. Based on the predicted number of reactions (N), construct 96-well plates for real-time RT-PCR and the PCR-Mix components as stated in Table 1. Fill each well with 20L of PCR-Mix. Cover the plate and place it in the sample processing area.
7. Store the remaining Reaction Mix and Enzyme Mix at -18°C right away.Sample reagent preparation calculation

	SARS-CoV-2 Reaction Mix (µL)	SARS-CoV-2 Enzyme Mix (µL)
PCR-Mix (µL)	18.5 µL × number of specimens and controls (N)	1.5 µL × number of specimens and controls (N)

Sample Addition

- In the well pre-filled with PCR-Mix, add 10L of the extracted sample RNA in the following order: no template (negative) control, patient specimen(s), and positive control
- Seal the plate and centrifuge for 10 seconds at 2000 rpm. Place the plate in a real-time RT-PCR machine and make a note of where the controls and specimens are located.

Annex V: QIAGEN mini RNA kit SARS-CoV-2 extraction procedure

Before you begin, make sure the samples are at room temperature, that Buffer AVE is at room temperature for elution in step, and that Buffer AW1 and Buffer AW2 have been produced according to the bottle's instructions.

Procedure

1. Pipette 560 µl prepared Buffer AVL containing carrier RNA into a 1.5 ml micro centrifuge tube.
2. Add 140 µl sample to the Buffer AVL–carrier RNA in the micro centrifuge tube. Mix by pulse vortexing for 15 seconds.
3. Incubate for 10 minute at room temperature
4. After incubation centrifuge for 10 seconds to remove drops from inside lid.
5. Pour 560µl ethanol (96–100%) into the sample and pulse-vortex for 15 seconds. After mixing, spin the tube briefly to remove any remaining drips from the lid.
6. Carefully pour 630 µl of the solution from step 5 into a 2 ml collection tube and apply to the QIAamp Mini column without soaking the rim. Close the cover and centrifuge for 1 minute at 6000 x g (8000 rpm). Place the QIAamp Mini column in a clean 2 ml collection tube and throw away the filtrate tube.

   Note: To avoid cross-contamination during centrifugation, close each spin column. To reduce micro centrifuge noise, centrifugation is performed at 6000 x g (8000 rpm). The yield or purity of viral RNA will not be affected by centrifugation at full speed. If the solution hasn't completely gone through the membrane, centrifuge at a faster speed until it does.
7. Step 6 is repeated after carefully opening the QIAamp Mini column. Open the QIAamp Mini column with care and add 500 µl Buffer AW1. Close the cover and centrifuge for 1 minute at 6000 x g (8000 rpm). Place the QIAamp Mini column in a clean 2 ml collection tube (supplied) and throw away the filtrate-filled tube.
8. Carefully open the QIAamp Mini column and pour 500 l Buffer AW2 into the column. Close the cap and centrifuge for 3 minutes at full speed (20,000 x g; 14,000 rpm). Step 11 can be

skipped if there is a chance of Buffer AW2 carryover; alternatively, step 10 can be skipped and then step 11 can be followed.

Note; The presence of Residual Buffer AW2 in the eluate may cause issues in downstream applications. On deceleration, some centrifuge rotors vibrate, causing flow through containing Buffer AW2 to contact the QIAamp Mini column. Flow-through may come into contact with the QIAamp Mini column if the QIAamp Mini column and collection tube are removed from the rotor. Optional step 10 should be completed in these circumstances.

9. Place the QIAamp Mini column in a fresh 2 ml collection tube (not provided) and discard the filtrate in the old collection tube. For 1 minute, centrifuge at maximum speed.

10. In a clean 1.5 ml micro centrifuge tube, place the QIAamp Mini column (not provided). Remove the filtrate from the old collection tube and discard it. Carefully open the QIAamp Mini column and add 60 l of room temperature Buffer AVE. Close the cap and set aside for 1 minute at room temperature.

11. For 1 minute, centrifuge at 6000 x g (8000 rpm). At least 90% of the viral RNA is eluted from the QIAamp Mini column after a single elution with 60 l Buffer AVE. Using 2 x 40 l Buffer AVE to perform a twofold elution will boost yield by up to 10%. Elution volumes less than 30 l will result in lower yields and will not increase the RNA concentration in the eluate. When stored at 30 to 15°C or –90 to 65°C, viral RNA can last up to a year.

Annex VI: Data extraction and registry form

Sample ID	Abbott SARS-COV-2 result		DAAN GENE NCOV									SANSURE			BGI
	Result	CT value	Abbott m2000 RT			Roche 48000 RT			ABI 7500 RT			ABI 48000 RT			Abbott m2000 RT
			Result	**Orf Gene CT**	**N gene CT**	**Result**	**Orf Gene CT**	**N gene CT**	**Result**	**Orf Gene CT**	**N gene CT**	**Result**	**Orf Gene CT**	**N gene**	**Orf Gene**

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Belete Woldesemayat (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor:

Kassu Desta (MSc, PhD candidate, Associate Professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Shambel Araya (MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.