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The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia.

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A thesis submitted to the Department of Microbiology, Immunology and Parasitology, college of health sciences of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Medical Microbiology

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Acronyms/Abbreviations

ACE	Angiotensin-Converting Enzyme
bp	Base Pair
CCHE	CURE Children's Hospital of Ethiopia
cDNA	Complementary Deoxy-Ribonucleic Acid
CDC	Center for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
CI	Confidence Interval
COR	Crude Odds Ratio
CoV	Coronavirus
CT	Cycle Threshold
DMIP	Department of Microbiology, Immunology And Parasitology
E	Envelope
EBI	European Bioinformatics Institute
EMBL	European Molecular Biology Laboratory
EPHI	Ethiopian Public Health Institute
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
GISAID	Global Initiative on Sharing All Influenza Data
HIV	Human Immuno-Deficiency Virus
HR	Heptad-Repeat
IL-1	Interleukin 1
LDT	Laboratory Developed Tests
LoD	Limit of Detection
M	Membrane
MERS	Middle East Respiratory Syndrome
mRNA	Messenger Ribonucleic Acid
N	Nucleocapsid
NCBI	National Center for Biotechnology Information
NGS	Next-Generation Sequencing
NPA	Negative Percent Agreement

NSP	Non-structural protein
NTD	N-terminal domain
ORF	Open Reading Frame
PCC	Probe Check Control
PPA	Positive Percent Agreement
QC	Quality Control
RBD	Receptor Binding Domain
RdRp	RNA-Dependent RNA Polymerase
RNA	Ribonucleic Acid
RNP	ribonucleocapsid
RT-PCR	Real Time Reverse Transcription Polymerase Chain Reaction
SARS-COV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOPS	Standard Operating Procedures
SPC	Sample Processing Control
TAT	Turnaround Time
UPIC	Unique Participant Identification Code
VOC	variants of concern
VOI	Variants of interest
VTM	Viral Transport Media
WHO	World Health Organization
αCoV	Alphacoronavirus
βCoV	Betacoronavirus
γCoV	Gammacoronavirus
δCoV	Deltacoronavirus

Abstract

Background: Xpert® Xpress and Cobas® are assays offering rapid test for the detection of SARS-CoV-2. Pooled testing allows for continued testing even when supplies are relatively scarce. Whole genome sequencing of SARS-CoV-2 refers to the process of determining the complete genetic sequence of the virus, providing a detailed and comprehensive analysis of its entire genome. The agreement between assays for SARS-CoV-2 detection from individual and pooled samples is uncertain in Ethiopia. Moreover, there is limited availability of whole genome sequencing data for SARS-CoV-2, leading to insufficient information regarding the circulating variants, lineages, and mutations.

Objective: The study aimed to compare the performance of Xpert® Xpress with Cobas® SARS-CoV-2 assays for SARS-CoV-2 detection from individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 to identify lineages, sub-lineages and mutations for nucleocapsid and envelop gene, in Ethiopia.

Methods: A cross-sectional study design was utilized on individuals suspected of having COVID-19 in seven selected hospitals in Addis Ababa, Ethiopia, between June 25, and July 20, 2022. The samples were tested for SARS-CoV-2 using the GeneXpert® and Cobas 8800®. A total of fifty samples that tested positive with the Xpert® were chosen and divided into four groups according to their CT-value and then pooled with negative samples and subjected to testing using both assays. Positive samples with a CT-value of ≤30 were selected for sequencing. The CovidSeq Assay was used to prepare sequencing libraries, which were then sequenced on the Illumina Miseq. Data were entered into Epi data version 3.1 and exported to SPSS version 28 for statistical analysis. Analysis was performed by using descriptive statistics, pearson correlation, and cohen's kappa. Nextflow work flow manager and different bioinformatics tools were used for the analysis of variants, lineages, and mutations.

Results: In this study, a total of 440 nasopharyngeal swabs were collected, and the overall rate of positivity for SARS-CoV-2 was found to be 100 (22.73%). The overall agreement between the assays from individual samples was 91.73(95% CI, 89.99% to 92.66 %, κ=0.715) indicating a substantial level of agreement. The Xpert® assay showed a high positive percent agreement (98.25%) and negative percent agreement (90.71%). The overall agreement between the assays from pooled samples was 89%, (95% CI, 86.31% to 91.52% κ=0.552). All 16 sequenced samples were identified as the Omicron variant, with ten samples assigned to clade 22A. The consensus

sequence length ranged from 29,578 to 29,903, with a GC% ranging from 33.1% to 39.0%. The average length of amino acids in the sequences was 9519. The pairwise identity between the samples and the reference genome ranged from 98.6% to 99.9%. Several amino acid changes were observed in the sequenced samples, including P13L, P151S, N E31del, N R32del, N S33del, N P151S, N R203K, N G204R, and N S413R on the N genes. Likewise, there was one common mutation at position 9 in the E gene, E31del.

Conclusion: The Xpert® performed better than the Cobas® assay in detecting positive results due to its ability to detect low viral load cases, utilizing the more sensitive nucleocapsid gene instead of the ORF1a gene used in the Cobas® assay. Confirming presumptive positive results by different methods and using multiple molecular assays targeting different genes is important to ensure high sensitivity in detecting RNA. Pooling up to six samples was possible regardless of viral load and pool size for Xpert®. But viral load effect was significant in all pool size of Cobas® assay. The agreement on individual samples indicated that both assays can be used interchangeably for diagnostic purposes. Furthermore, there were variations in amino acid length, the presence of both unique and existing mutations, and variations in mutation frequencies among the analyzed sequences.

Key words: Agreement, Cobas® SARS-CoV-2 assay, COVID-19, Xpert® Xpress SARS-CoV-2 assay, Pooling, SARS-COV-2, WGS

1. Introduction

1.1 Background

On January 30, 2020, the World Health Organization (WHO) declared the SARS-CoV-2 virus, which originated in Wuhan, China in December 2019, as a public health emergency of global concern (1). The coronaviruses (CoVs), among which SARS-CoV-2 is a member, are enveloped, positive-sense, non-segmented, single-stranded RNA viruses that belong to the subfamily Coronavirinae, family Coronavirdiae, order Nidovirales, and they have a genome that is approximately 30,000 nucleotides long (2). The CoVs genome consists of several open reading frames (ORFs), with the first ORF (ORF1a/b) making up more than half of the entire genome length and encodes 16 non-structural proteins. The remaining ORFs, encode the primary structural proteins, such as the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (1). The envelope protein coded by the E gene acts as a viroporin and triggers excessive IL-1beta production (1). The nucleocapsid protein, encoded by the N gene which contains two overlapping ORF9a and ORF9b, and this protein is crucial for virus assembly and enhances subgenomic viral RNA transcription and replication (1).

Five significant lineages of SARS-CoV-2 have been identified as variants of concern, which are αCoV (B.1.1.7), first detected in the United Kingdom; βCoV (B.1.351), first identified in South Africa; δCoV (B.1.617.2), first observed in India; γCoV (P.1), first seen in Japan/Brazil; and Omicron (B.1.1.529), first reported in South Africa (3).

Mutations of SARS-COV-2 can occur naturally as the virus replicates and spreads, and associated with the search for adaptation and the gain of function, such as increased transmissibility, immune evasion, or enhanced binding to host cells (4,5). The emergence of the Omicron variant was unexpected event, because it likely did not evolve from other known circulating SARS-CoV-2 variants. It is a variant that has undergone significant changes in several different proteins, especially the spike protein, which plays a crucial role in viral entry into host cells (6). The accumulation of non-synonymous mutations, which result in amino acid changes, can contribute to the variant's ability to evade immune responses and potentially increase its transmissibility (5,7).

The availability of accurate and fast testing procedures plays a crucial role in controlling the spread of SARS-CoV-2 infection. Companies in worldwide have been working relentlessly to create and distribute essential test kits (8).

The gold standard for diagnosing SARS-CoV-2 is the detection of its RNA. There are currently multiple nucleic acid amplification test (NAAT) platforms available (9), each with varying performance characteristics. Various RT-PCR technologies have been developed to detect the presence of SARS-CoV-2, including the Xpert® and Cobas® SARS-COV-2 assays, which can produce results in very short Turn Around Time (TAT) (10). The Xpert® test, which was granted Emergency Use Authorization status in March 2020, uses a cartridge-based assay to process, extract, amplify, and detect SARS-CoV-2 RNA in a single cartridge, offering flexibility in testing individual specimens (11). The test assay detects the presence of SARS-CoV-2 by targeting the N2 region of the N gene that is specific to SARS-CoV-2 and the E gene at different cycle threshold (Ct-values), depending on the viral load in the sample (12–15). The Cobas 8800® is a fully automated system that can perform SARS-CoV-2 testing by extracting RNA directly from primary and secondary tubes, setting up PCR amplification, detecting signals, and reporting results (16,17). This system has the shortest Turn Around Time (TAT) and offers improved efficiency and flexibility, with a throughput results (18). The assay targets the conserved E gene shared by the Sarbecovirus subgenus and the unique ORF1/a gene of SARS-CoV-2 (19).

Pooling samples is a technique which multiple patient samples are combined and tested in a single reaction (20). Pooled testing is commonly considered a more important approach, whereby a negative result implies that all individuals in the group are negative, and a positive result suggests that at least one person is infected with SARS-CoV-2 (21). Pooling strategies are widely recognized as practical and efficient techniques for analyzing large sample volumes while maintaining performance levels (22).

Whole genome sequencing (WGS) can offer the most precise information available about the genome of an organism, and has the capability to revolutionize the management of infectious diseases (7). The WGS of SARS-CoV-2 can determine the alteration of nucleotides and amino acid sequences, which can provide insights into the evolution of the virus and its variants. WGS of the virus involves extracting RNA from a sample of an

infected individual and then converting it into complementary DNA (cDNA) through a process called reverse transcription. The cDNA is then sequenced using high-throughput sequencing technologies, such as Illumina. The application of WGS in the investigation of SARS-CoV-2 has gained more acceptance, and the Illumina COVIDSeq library prep assay has been commonly used Next Generation Sequencing (NGS) assay with high throughput capability to detect and monitor novel variants and lineages of the virus (23) . Different data bases and SARS-CoV-2 sequence analysis tools are available publicly, including GISAID (24).

Although resources for analyzing the sequence of SARS-CoV-2 are becoming more accessible, there is limited genomic information available for the virus circulating in Ethiopia. Currently, as of May 5, 2023, only 628 sequences have been deposited in the GISAID database from Ethiopia, and the genomic features of SARS-CoV-2 in Ethiopia are still largely unidentified (24).

Comparing the performance of different COVID-19 testing platforms is crucial for ensuring accurate results. To this end, this study evaluated the diagnostic accuracy of two PCR assays using individual and pooled nasopharyngeal samples. Additionally, in this study whole genome sequencing of SARS-CoV-2 viruses was also conducted with the aim of analyzing genetic variations and mutations in the genomes of SARS-CoV-2 isolates from Addis Ababa. The goal of the latter part of this study was identifying virus variants and mutation patterns of the N gene and E gene of SARS-CoV-2 virus genome collected from Addis Ababa. The research findings provided essential information for controlling the spread of COVID-19 in Ethiopia and contributed to the global efforts towards understanding the evolution and spread of the SARS-CoV-2 virus.

1.2 Statement of the Problem

At the time when COVID-19 started spreading outside of Wuhan, Ethiopia did not have the necessary infrastructure to deal with the pandemic. There were only a few molecular laboratories, and most diagnostic centers lacked the proper equipment to handle the increasing number of samples (25). However, by the beginning of March 2020, the Ethiopian Public Health Institute (EPHI) made remarkable efforts to establish the required laboratory capacity and initiated COVID-19 testing (26,27).

In Ethiopia, the primary method for laboratory testing of SARS CoV-2 was using nasopharyngeal/oropharyngeal swabs to detect viral RNA by a laboratory-developed test (LDT) based on RT-PCR equipment (26). However, later on, EPHI began using Cobas 8800® test, and Xpert® Xpress assay additionally based on a modified CDC protocol. However, none of these tests utilized a standardized laboratory test protocol. Even currently, the level of Xpert® and Cobas® assays to detect SARS-C-2 viruses under the Ethiopian setting is uncertain, which may lead to the exclusion of positive samples that are close to the assays' detection limit (28).

Although Cobas 8800® testing is limited to national reference laboratories and specialized hospitals, the GeneXpert® is widely available and was originally approved for tuberculosis diagnosis in low-resource settings and easy to use, especially for pool testing. However, the single-use cartridge tests for GeneXpert® are considerably more expensive per sample than Cobas® testing. Hence, the Xpert® assay was suggested for cases where there is a lack of readily available expertise in SARS-CoV-2 testing and inadequate laboratory facilities (73). The high demand for test kits globally often results in insufficient supply of cartridges, making pooling a justifiable strategy for this assay (23).

Studies on the performance of the two platforms used for closed polymerase chain reaction (PCR) tests has been comparatively limited in Africa, especially when compared to the extensive amount of studies done in the United States (29,30). Given the potential impact of various factors, such as strain variability, lifestyle differences, and geographic variations, it is believed that validation studies of these assays within the Ethiopian context would provide valuable insights. Evaluating the performance of these closed PCR SARS-COV-2 assays, would provide important baseline data on the level of agreement

among these assays, which could potentially leading to more effective testing practices and improved public health outcomes.

Inadequate testing infrastructure has posed a challenge in meeting the rising demand for SARS-COV-2 testing, leading countries to explore specimen pooling as a potential solution (31). Pooling has been shown in various studies to be a feasible and cost-effective approach to expand testing capacity, especially during severe shortages of test reagents and trained personnel (32). Most studies have focused on screening large asymptomatic populations with low prevalence, primarily detecting highly infectious samples with the lowest Ct-values from the pooled samples. This approach aims to identify individuals with a higher viral load who are more likely to transmit the infection (20,33,34). In low-resource countries like Ethiopia, where individuals commonly seek care at health centers when experiencing COVID-19 symptoms, studying the performance of pooling techniques among symptomatic individuals would be particularly meaningful. This approach can provide valuable insights into the feasibility and effectiveness of pooling strategies in real-world healthcare settings. For laboratories utilizing pooling, it was recommended to implement a tested and validated protocol (33). While some studies have shown that pooling up to 32 samples has a minimal effect on SARS-CoV-2 detection rates (35), others have raised concerns that using large pool sizes in pooling can make it difficult to identify specific positives (59). Optimal pooling size can be determined by considering important factors such as the viral load in positive samples, and the number of samples pooled together (20).

During the initial SARS-COV-2 testing campaign, government facilities, including EPHI, used the one-way pool testing method to pool 4 or 5 samples (37). However, private diagnostic facilities still employ this method to save resources and reagents. Despite the potential cost savings achieved by pooling samples, government diagnostic institutes continue to test individual samples. While pooling has been effectively used with other LDTs in previous studies, there was not enough information available on its performance when used with the Xpert® SARS-CoV-2 assay and Cobas® assay. In Ethiopia, a study was conducted to validate the pooling of biological samples, specifically using a 4 in 1 pooling method, as well as the pooling of RNA samples up to 8 in 1 (37). This study on pooling for SARS-CoV-2 detection evaluated the pool performance of various testing

components rather than one entire PCR platform. They followed the manufacturer's instructions (Da An Gene Co., Ltd.) for all laboratory processes, including NA extraction and purification, master mix preparation, nucleic acid and reagent mixing but for the amplification/detection, and analysis the study used an open or automated system, the BioRad CFX96 RT-PCR system (37).

In previous studies, the Xpert® and Cobas assays were compared to evaluate the overall PCR system since they are fully automated closed systems that include pre-analytic, analytic, and post-analytic components. The use of automated systems in these platforms reduces the risk of contamination and increases the sensitivity of PCR assays for testing sample pools, which is not always the case with other LDT-based PCR systems (31,38). Taking the aforementioned advantages of pooling in to consideration, one of the aims of this study was also to assess the efficiency of Cobas® and Xpert® SARS-CoV-2 assay in detecting SARS-CoV-2 in pooled clinical samples in comparison to individual samples. In order to determine the optimal number of pooled samples for avoiding the loss of weak positive samples, pool sizes of 4, 6, 8, and 10 samples were used.

The utilization of WGS is critical in understanding the development and transmission of SARS-CoV-2, recognizing new concerning variants, and providing guidance for public health responses. WGS provides the most detailed pathogen characterization possible and has been advantageous in managing previous outbreaks such as Ebola, yellow fever, and the current COVID-19 pandemic. Currently, there is a vast collection of over 15 million whole genome sequences (WGS) of SARS-CoV-2 available in the publicly accessible Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV database (24). Ethiopia is collaborating with organizations such as Africa CDC and Biohub to optimize the use of genomics data in the efforts to control the pandemic. However, the lack of genomic data on SARS-CoV-2 in Ethiopia has been a hindering issue to this effort, which was a prompting factor to conduct WGS of SARS-CoV-2 isolates in this study. Consequently, one of the main aims of this study was to examine the genetic variation and mutation of SARS-CoV-2 in Ethiopia by analyzing the whole genome sequences of positive patients. Moreover, the study's focus was to detect mutations that could impact N and E gene of the genome.

1.3 Significance of the study

The significance of this study lies in its contribution to improving the accuracy and efficiency of COVID-19 testing in Ethiopia. By comparing the performance of two different PCR platforms, the study will provide valuable information to the Federal Ministry of Health and EPHI on the diagnostic performance of the Xpert® Xpress and Cobas 8800® systems for COVID-19 testing. The study also highlights the importance of sample pooling as a strategy for maximizing testing efficiency without compromising accuracy.

Along with other studies the findings of this study can guide decision-making in the selection and implementation of diagnostic assays and testing strategies in Ethiopia. Additionally, the study serves as a baseline for future related studies, contributing to the country's ongoing efforts to combat the COVID-19 pandemic.

The study can provide information for future research on the genetic epidemiology of SARS-CoV-2 in Ethiopia and the African continent as a whole. The genetic data generated in the study can be used for comparative studies with other global SARS-CoV-2 sequences to understand the evolution and spread of the virus worldwide.

Overall, the study has important implications for public health in Ethiopia and can inform broader efforts to improve COVID-19 testing accuracy and efficiency in other settings.

2. Literature review

2.1. Overview of SARS-COV-2

2.1.1 History and Evolution of SARS-COV-2

The first study into coronaviruses was documented in 1931, whereas the identification of the first coronaviruses affecting humans took place in the 1960s (39). There have been seven different types of coronaviruses identified in humans, which can result in respiratory tract infections with varying levels of severity. Four of these CoVs, namely HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1, are generally considered to be low-risk and are commonly associated with causing common colds. On the other hand, there are three highly concerning CoVs: Middle East respiratory syndrome coronavirus (MERS-CoV), Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), and the recently discovered SARS-CoV-2, also known as 2019-nCoV (1,39,40). Both MERS-CoV and SARS-CoV had higher case fatality rates compared to other CoVs, characterized by flu- like symptoms, and can possibily transmit from animals to humans (39). Bats and rodents are identified as the main contributors of genetic material for the majority of αCoVs and βCoVs, according to evolutionary studies. On the other hand, avian species are the gene sources for most δCoVs and γCoVs. CoVs have repeatedly shown the ability to cross species boundaries throughout their evolution, leading to the emergence of certain strains as significant human pathogens (41).

On January 9, 2020, a newly discovered coronavirus, initially referred to as 2019-nCoV, was officially identified as the primary cause of a viral pneumonia outbreak in Wuhan, China. Although the precise origin was uncertain initially, there were suspicions of a potential link to a seafood wholesale market. The virus rapidly spread within China, resulting in an epidemic, and later escalated into a global pandemic, impacting multiple countries worldwide (42). Subsequent investigations and research unveiled that the virus responsible for the pneumonia cases had the capacity for human-to-human transmission (1). Through genetic sequencing, scientists determined the causative agent to be SARS-CoV-2, a member of the coronavirus family (43). This breakthrough discovery supplied vital information regarding the precise virus behind the outbreak, enabling a deeper comprehension of its characteristics, transmission patterns, and facilitating vaccine development (44).

2.1.2 Genome Organization of SARS-COV-2

In the beginning of 2020, the entire genetic makeup of a new coronavirus belonging to the β genus was identified. SARS-CoV-2's genome was found in samples of bronchoalveolar lavage fluid taken from a patient in Wuhan (41). The viral genome sequence Wuhan-Hu1, with GenBank accession number MN908947, was shared on virological.org on January 10th (41). The phylogenetic analysis indicated that the 2019-nCoV belongs to the betacoronavirus genus, which includes CoVs found in humans, bats, and other wild animals such as SARS-CoV and bat SARS-like CoV (1,40). Further studies, using subsequent virus isolates, have confirmed that the 2019-nCoV is most closely related to bat SARS-like CoV, specifically SL-ZC45 and SLCoVZXC21 (1,40,45). The presence of intact ORF 8 in SARS-CoV-2 isolates indicates a potential involvement in host-virus interactions and strengthens the hypothesis of a bat origin for the coronavirus (41).

The CoVs are enveloped, non-segmented, positive-sense, single-stranded RNA viruses belong to the subfamily Coronavirinae, family Coronavirdiae, order Nidovirales (2). There are four genera of CoVs, namely, Alphacoronavirus (αCoV), Betacoronavirus (βCoV), Deltacoronavirus (δCoV), and Gammacoronavirus (γCoV) (3).

The whole genome of SARS-COV-2 encodes different protein with different function. The initial open reading frames (ORF1a/b), which make up around two-thirds of the genome, encode numerous non-structural proteins (1). Proteins such as RNA-directed RNA polymerase (RdRp), helicase (Hel), proofreading exoribonuclease/Guanine-N7 methyltransferase (ExoN), and others have been identified in the protein database (https://www.rcsb.org/). Other ORFs on the one-third of the genome near the 3'-terminus encodes the main structural proteins: S, M, E, and N proteins (1). The spike glycoprotein of SARS-CoV-2 plays a vital role in determining which hosts the virus can infect and the resulting disease pathology. This protein is a primary target for diagnosis and treatment strategies, given its importance in viral infection (6). The spike glycoprotein is a transmembrane protein that exists as a trimer and consists of two subunits: S1 and S2. The S1 subunit contains the N-terminal domain (NTD) and the receptor-binding domain (RBD). The RBD specifically facilitates the attachment of the virus to host cells during infection (43). On the other hand, S2 subunit mediates viral entry and contains a fusion

peptide domain, internal fusion peptide, two heptad-repeat domains (HR1 and HR2), transmembrane domain, and a C-terminal domain (6). The nucleocapsid protein plays a key role in packaging the viral genome into a helical ribonucleocapsid (RNP) and is essential for virion assembly by interacting with the viral genome and membrane protein M. The nucleocapsid protein is crucial in enhancing the efficiency of subgenomic viral RNA transcription and viral replication. The envelope protein plays a central role in virus morphogenesis and assembly, acting as a viroporin and self-assembling in host membranes to form pentameric protein-lipid pores. These pores allow ion transport and also contribute to the induction of apoptosis. Furthermore, the envelope protein activates the host NLRP3 inflammasome, leading to the overproduction of IL-1beta (https://zhanggroup.org/COVID-19/).

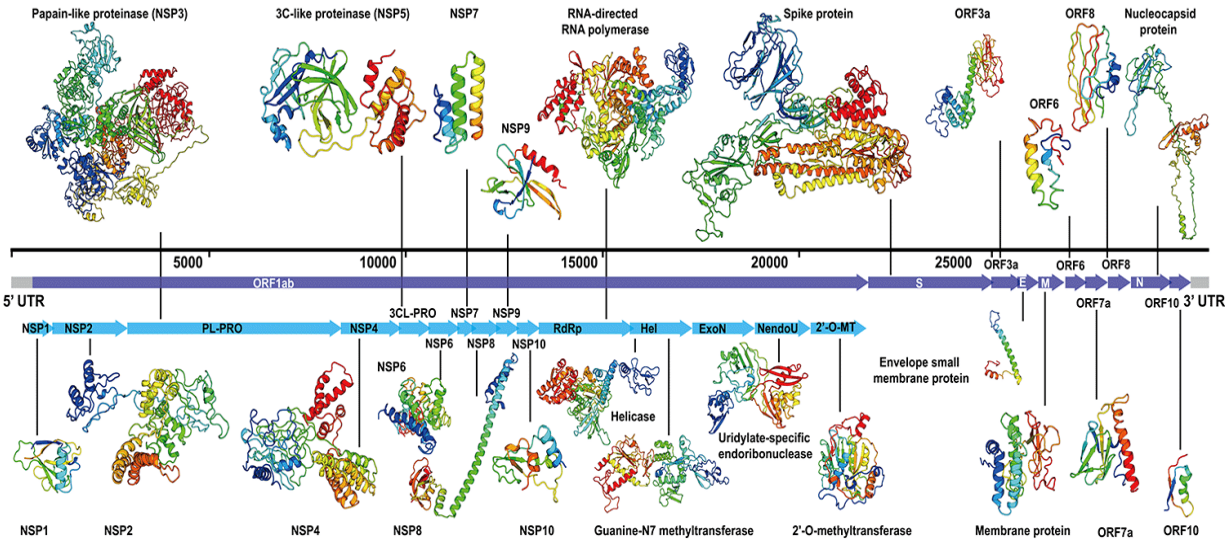


Figure 1: Genome-wide structure and function modeling of SARS-CoV-2 Virus

Source: (https://zhanggroup.org/COVID-19/).

2.1.3 Epidemiology of SARS-COV-2

As of May 31, 2023, 6:00 pm EST, globally, there have been over 767 million reported cases of SARS-CoV-2, resulting in approximately 6.9 million deaths. Worldwide, over 13.3 billion vaccine doses have been administered. In Africa, there have been around 9.5 million confirmed cases and 175,000 deaths. Specifically in Ethiopia, there have been 500,893 confirmed cases and 7,574 reported deaths as of May 31, 2023. Ethiopia has

administered approximately 54 million vaccine doses as of May 7, 2023. These figures are based on data from the WHO (https://covid19.who.int/).

Phylogenetic and likelihood-mapping analyses of 12 virus genome sequences, sampled primarily from Wuhan city, Hubei Province, China, and with known sampling dates, have indicated the presence of a potentially significant human-to-human transmission of the virus. Inhalation of respiratory droplets produced by infected patient's causes human infection (46). COVID-19 has an estimated incubation period of up to 14 days following exposure. Patients infected with SARS-CoV-2 can exhibit a wide range of clinical presentations, varying from asymptomatic infection to severe pneumonia leading to acute respiratory distress syndrome and even death (1).

2.1.4 The immunology of SARS-CoV-2 infections and vaccines

The virus, SARS-CoV-2, has the capability to infect any organ in the human body that possesses angiotensin-converting enzyme 2 (ACE2) receptors on its cells (47). The spike proteins of SARS-CoV-2, which protrude from the surface of the viral envelope, recognize the host's receptor ACE2. Following recognition, the spike proteins attach to ACE2 and undergo membrane fusion with the host cell's membrane (48). Following infection with SARS-CoV-2, the immune system produces antibodies that target crucial viral antigens, including the nucleoprotein and spike protein. Typically, these antibody responses peak approximately 14 to 25 days after the symptoms first appear (47).

Both vaccines and natural infection can trigger the production of antibodies, which serve as a crucial defense against viral infection. These antibodies contribute to humoral immunity by signaling effector cells to identify and neutralize pathogens, thereby interrupting the viral life cycle, especially at the entry stage (48). Consequently, antibodies produced in response to SARS-CoV-2 infection will bind to the receptor-binding domain (RBD) of the spike protein, inhibiting the virus from attaching to the host cell and neutralizing its infectivity (49). The production of antibodies reduces the risk of re-infection and helps to lessen the severity of the disease in the event of a re-infection (47).To date, after the effortful investigation the researchers develops effective vaccines and authorized it for emergency use.

The vaccine platform is categorized into inactivated, live-attenuated, recombinant protein and vectored vaccines, mRNA and DNA vaccines (49). The first approved vaccines were

the Pfizer–BioNTech (vaccine: BNT162b) and Moderna (mRNA-1273) mRNA vaccines which were developed at pandemic Speed (44). Several vaccines have been approved in different parts of the world, such as CoronaVac, BBIBP-CorV, CoviVac, Covaxin, Oxford–AstraZeneca vaccine (ChAdOx1 nCoV-19), Sputnik V, the Johnson & Johnson vaccine, and EpiVacCorona (44). To date, four types of vaccines approved by the World Health Organization (WHO) have been used in Ethiopia. These vaccines include BNT162b2 (BioNTech, Pfizer vaccine), ChAdOx1 nCoV-19 (Oxford, AstraZeneca vaccine), Ad26.COV2.S (Johnson & Johnson vaccine), and BBIBP-CorV (Sinopharm BBIBP vaccine).

2.2. Laboratory Diagnostics of SARS-COV-2

Given the swift transmission of COVID-19, there was a crucial requirement for a dependable diagnostic approach to detect and monitor patients globally, particularly during the initial phases of the illness (39). Methods employed for the laboratory detection of human coronaviruses, can be categorized into various classes. These include cell culture and electron microscopy, RNA amplification-based detection methods, viral RNA biosensors, as well as whole virus or viral protein detection assays (44,45) .

The identification of a new virus typically involves traditional methods like cell culture and electron microscopy (46). Viral culture, following Koch's postulate, is considered the gold standard for virus diagnosis. In this approach, the specimen is cultured with primary monkey cells and cell lines such as Vero and LLC-MK2, and the appearance of characteristic cytopathic effects is observed (50). However electron microscopy is another technique used to visualize the virus and examine ultrastructural details of virus-infected cells (46). However, due to the biosafety level-3 classification of SARS-CoV-2, specimen processing and result interpretation for cell culture and electron microscopy require skilled and experienced laboratory staff, making them challenging to implement in standard clinical institutions (46).

Real-time RT-PCR, a nucleic acid amplification test (NAAT), is widely used as the gold standard for diagnosing current COVID-19 infections (8,14,39,51). This method amplifies the viral RNA for subsequent sequence analysis and offers high sensitivity, specificity, and the ability to quantify the RNA (46). It is commonly employed for detecting SARS-CoV-2 RNA in respiratory samples. However, certain technical factors

can impact result accuracy, including mutations in target regions, PCR inhibition, and sample cross-contamination (46). Since the full genome of SARS-CoV-2 was released on January 10, 2020, numerous in-house and commercial assays have been developed to target different gene regions (46). These targets include structural proteins (e.g., S, E, and N genes) (52) that are more sensitive for coronavirus detection, as well as species-specific accessory genes (e.g., RNA-dependent RNA polymerase (RdRp), ORF1a, and ORF1b genes) that are specific to SARS-CoV-2 (1). However, many available assays target only short fragments of SARS-CoV-2, which may not indicate active viral replication within host cells (46).

Immunoassays are used to detect SARS-CoV-2 antibodies or antigens in blood, serum, or plasma. Lateral flow immune-chromatography is a commonly employed method for point-of-care testing of COVID-19 due to its rapid, simple, safe, and cost-effective detection (53). The production of antibodies varies depending on disease progression and the patient's health condition, including age, nutritional status, and immune status. However, the presence of cross-reactivity with antigens from other coronaviruses poses a challenge in this type of assay (46). Plasma samples from COVID-19 patients have shown a high frequency of cross-reactivity with the S protein of both SARS-CoV and SARS-CoV-2 (46). Moreover, the Nucleocapsid protein, which is highly expressed, may exhibit cross-antigenicity among SARS-CoV, SARS-CoV-2, and OC43-CoV, leading to possible false positive results in antigen detection assays (46). Therefore, the precise formulation of reagents in the kits plays a crucial role in assay performance. Furthermore, compared to nucleic acid amplification tests (NAATs), immunoassays have lower sensitivity and specificity in detecting SARS-CoV-2 (54).

2.3. Diagnostic performance of each Xpert® and Cobas® for SARS-CoV-2 detection on individual samples.

Nucleic acid amplification tests (NAATs), specifically RT-PCR, are widely regarded as the gold standard for diagnosing current SARS-CoV-2 infections. The cobas® 6800/8800 and GeneXpert® systems are rapid real-time PCR platforms that provide quick detection of SARS-CoV-2, with results available in 20 minutes and 50 minutes, respectively (55). The Cobas® assay uses RT-PCR to amplify and detect two viral targets: ORF1 a, a specific non-structural region of SARS-CoV-2, and a conserved region in the E-gene,

which is a structural protein envelope used for detecting pan-Sarbecoviruses. The Xpert assay is an automated RT-PCR assay that amplifies and identifies two specific viral targets: N2, a distinct nucleocapsid recombinant protein of SARS-CoV-2, and a region within the structural envelope E-gene (15,56).

Based on the available evidence, it appears that both Xpert® Xpress and Cobas® have good diagnostic performance for SARS-CoV-2 testing. According a multi-center evaluation conducted by Loeffelholz MJ et al., found that Xpert® Xpress SARS-CoV-2 had a 100% agreement compared to all in-house RT-PCRs (14).

Similarly, a study was conducted to assess the diagnostic performance of Cobas® SARS-CoV-2 with prospectively collected nasopharyngeal swab clinical samples, and the findings showed that the performance against the expected results were 100% for both positive percent agreement (PPA) and negative percent agreement (NPA) (18).

The Xpert® Xpress assay did not frequently show presumptive positive results, which could indicate the presence of sarbecovirus. In contrast, the Cobas® assay more commonly reported such results. In a study by Smithgall et al.(13), one sample was identified as a presumptive positive using the Xpert® assay based on the detection of the E-gene target but not the N2 target. Pujadas et al. observed a presumptive positive rate of 7 (0.70%) out of 1006 samples using the Cobas® assay (19). Similarly, Poon et al. reviewed the detection of presumptive positive cases for 24 samples (57).When the presumptive positive samples were repeated using the Xpert® Xpress assay, Poon et al. observed the following outcomes: 21 samples tested positive for SARS-CoV-2, 2 samples yielded presumptive positive results again, and 1 sample tested negative for SARS-CoV-2 (57). In this study the retesting of presumptive positive samples in the study revealed that the assay targeting the N2 gene exhibited higher sensitivity in detecting cases with a low viral load compared to the assay utilizing the ORF1a region (57).

In several studies, the Xpert® Xpress assay encountered errors as an unsuccessful result due to various reasons, whereas such errors were not observed in the Cobas® assay (58). All most all error results were resulted either positive or negative when repeated using the same assay. Noble et al, in which recorded an error rate of 6/157 tests (3.8%) (58). The result shows that the 5007 error code was the most common error code encountered in the study, which may be caused by either of or multiple of factors such as inadequate sample

volume, presence of inhibitors in the sample, or issues with the testing process. These errors occurred in both positive and negative results (58). Consequently, repeating unsuccessful results becomes crucial to ensure the validity and reliability of the findings. Most Error result get result when repeated with same assay. Overall, these studies suggest that Xpert® Xpress and Cobas® are highly accurate and reliable tests for SARS-CoV-2 detection (73), which could be useful in diagnosing COVID-19 in both clinical and community settings. In this study, the detailed performance of both the Xpert® Xpress assay and the Cobas® assay was addressed to address the objectives of the study.

2.4. Ct-value of Xpert® and Cobas® for SARS-CoV-2 assay

The Ct-values obtained from both the Cobas® and GeneXpert® platforms provide valuable information about viral load and can be utilized to monitor the spread of SARS-CoV-2 in the population. It is important to consider these Ct-values when interpreting SARS-CoV-2 test results and implementing appropriate public health measures to control the transmission of the virus.

There were differences in Ct-values between studies can be attributed to several factors, highlighting the complexity involved in interpreting SARS-CoV-2 test results. One crucial factor contributing to these variations is the diversity of patient populations and the variability in sample collection and handling techniques used.

The Ct-values also vary based on the target gene, as reported in a study by Moran A et al., which showed that for positive samples, Ct-values for the E gene ranged from 15.05 to 39.75 for the Cobas® assay and 13.6 to 38.2 for the Xpert® Xpress assay (15) . Broder K et al, 2020, reported that the Ct-values ranged from 30.1 to 37.9 on the Cobas® assay and 24.6 to 42.4 on the Xpert® Xpress SARS-CoV-2 assay (30). Tham et al., reported higher Ct-values than two studies other studies which reported median Ct-values of 31.6 and 29.7 for the N2 and E gene targets, respectively and reported median Ct-values of 21.8 and 22.2 for the ORF1a and E gene targets, respectively (29).

These difference in Ct-values suggest that Ct-values should be considered when interpreting SARS-CoV-2 test results and that further studies are needed to evaluate the agreement between different tests in different settings and populations.

2.5. Test agreement between Xpert® Xpress and Cobas® SARS-CoV-2 assay on direct individual samples.

Previously conducted studies revealed that there is high test agreement between Xpert® compared to Cobas 8800®. Several studies conducted in the USA and Singapore have shown high agreement between Xpert® and Cobas 8800® in SARS-CoV-2 testing. In a study conducted by Smithgall MC et al., the testing agreement between Xpert® and Cobas® was very high, with an overall positive agreement of 98.9% and negative agreement of 92.0% (13). Another study conducted in the USA found that of 35 samples, 34 were tested positive on both instruments, with one sample being tested positive on the Cobas® and negative by the Xpert® Xpress SARS-CoV-2 assay, which then became negative on the LDT (30). Moreover, a study conducted by Moran A et al., showed that Xpert® and Cobas® SARS-CoV-2 assays showed almost perfect agreement (99%), and their combined usage can be tailored to maximize SARS-CoV-2 testing (15). Similarly, a study conducted in Singapore on 241 fresh clinical nasopharyngeal swab samples revealed an agreement of 95% between both methods (29).

In the some studies, discordant between the two assays were observed, Smithgall et al., reported two samples tested negative on Cobas® but positive on Xpert® (13). Among these 34 samples that showed discordant results, 6 samples were positive for both the N2 and E gene targets, while 28 samples were positive for the N2 gene but negative for the E gene. This observation aligns with the findings of Smithgall et al., who reported two samples that were positive for the N2 gene but negative for the E gene (13).

The agreement between Xpert® and Cobas® in SARS-CoV-2 testing depends on the CT-value, with higher agreement observed at lower Ct-values. In a study by Smithgall MC et al., Xpert® showed 100% positive perfect agreement with Cobas® for medium and high viral concentrations (CT-value<30), but the agreement decreased to 97.1% for Ct-values >30 (13). Overall, these studies suggest that Xpert® and Cobas® have high agreement in SARS-CoV-2 testing. The combined usage of these tests could be tailored to maximize SARS-CoV-2 testing, which could be useful in diagnosing COVID-19 in different settings. However, it's important to note that additional studies may be needed to further evaluate the agreement between these tests in different populations and settings.

2.6. Pooling performance of Xpert® for detection of SARS-CoV-2.

Several studies have been conducted to determine the effectiveness of pooled testing for SARS-CoV-2 detection on Xpert® assay. Most studies results indicate that pooling samples for SARS-CoV-2 testing can be an effective strategy by Xpert® assay, with a high percentage of pooled samples testing positive compared to individual testing for different pool size (31,59). In Vibol Iem et al.'s study, pooling samples into four resulted in a 94% agreement with individual testing (60). Graham et al. reported a 100% perfect agreement for both pool four and pool six (31). The FDA's report on October 11, 2022, also reported a detection rate of 95% for pool-five and 90% for pool eight (11). At the pool-10 level, Procop et al. reported 85% positive percentage agreement samples (59). All these studies suggested that pooled testing can be an effective method for SARS-CoV-2 detection, but the accuracy of SARS-CoV-2 test results can be influenced by the viral load present in the samples (31,59).

Studies also revealed that the change in Ct-values occurred after the sample was pooled, and when the low viral load samples become negative after pooling (31,59). The median Ct-values, which indicate the viral load in the samples, showed a slight increase with increasing pool size (31,59). Studies also show that, the detection of the N2 gene was found to be more sensitive than the reaction for the E gene (57,59). In Procop et al.'s study, it was found that the detection rate for the N2 gene decreased from 95% to 80%, while the detection rate for the E gene decreased from 85% to 45% (59). The overall Ct differences between the individual and 10:1 pool test were 2.9 cycles for the N2 gene target and 3 cycles for the E gene target and were mathematically consistent with the expected changes from a 10:1 dilution (59).

There were no studies conducted which asses the comparison of the assays on the agreement of pooled samples. However, a study conducted in Ethiopia using pooling up to 8 in 1 pooling strategy was able to detect SARS CoV-2 positive samples which tested positive in individual RT-PCR tests, which were neither Xpert® Xpress SARS-CoV-2 assay nor Cobas® (35). Despite the promising results of pooled testing, there is still a lack of published studies on both assays.

2.7. Pooling performance of by Cobas® assay for detection of SARS-CoV-2.

Studies conducted using the Cobas® PCR method has shown high test agreement between pooled samples and individual samples (37,67). A study by McMillen et al., found that pooling nasopharyngeal samples using the Cobas® method had a sensitivity of 100%, indicating that this method has the potential to significantly increase testing capacity without significant loss in sensitivity (38). Similarly, a study by Bidisha Barat et al., showed that the Cobas® method had a high sensitivity of 94% when detecting positive specimens in a pool compared to testing individually, with decreased detection of samples with lower viral loads (61).

However, pooled samples have higher Ct-values than individual samples and less effective in detecting samples with lower viral loads the same as Xpert® (37,67). McMillen et al., found that mean Ct-values shifted from 25.23 and 25.81 for ORF1a and E gene, respectively, in individual nasopharyngeal samples to 26.9 and 27.95 for ORF1a and E gene, respectively, in nasopharyngeal pools (38). Insufficient studies have been conducted on the pool performance of the Cobas® assay compared to the Xpert® assay and other laboratory-developed tests (LDTs). This study aims to bridge the information gap by investigating the pool performance of the Cobas® assay using direct nasopharyngeal swabs.

2.8. Whole Genome sequencing of SARS-COV-2

On January 5, 2020, the first whole- genome sequence of SARS-CoV2 was published (62). Prior to mid-January, six viral genomes were made publicly available, enabling the swift development of diagnostic assays and strategies for comprehensive genomic sequencing of the virus (63). Subsequently, numerous countries contributed the complete genome sequence of SARS-CoV-2 to the Global Initiative on Sharing All Influenza Data (GISAID) database (24). Sequencing efforts have continued as the virus has spread across the world, resulting in a constantly growing data set of more than 60 000 near-complete viral genomes within the 6 months following the identification of SARS-CoV-2. Frequently, genomes have been generated within days of case identification, and used to understand virus spread during the pandemic (63). In addition to GISAID, other resources became available for SARS-CoV-2 sequence analysis. The Nextstrain team analyzes virus sequence data on a global and continental level, and the Nextclade tool has

been designed to compare new sequences to the SARS-CoV-2 reference sequence, assign them to clades, and see their phylogenetic relationships (24).

Variants and mutations of SARS-CoV-2 are detected through whole genome sequencing (WGS) of specific samples. Global surveillance of SARS-CoV-2 genomes has identified several clinically and/or public health significant variants known as variants of concern (VOCs). Examples of VOCs include Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) (3). Some of these variants have seen a decrease in prevalence globally and have been de-escalated (5). WGS has also identified a number of variants of interest (VOI), which may share one or more mutations in common with a VOC, but do not have sufficient evidence at this time to be categorized as a VOC (5).

Virus isolates from five patients with severe pneumonia at Jin Yin-tan hospital in Wuhan (hospitalized from Dec 18 to Dec 29, 2019) showed 99.8-99.9% nucleotide identity in the initial outbreak data(45). A study on 32 genomes of strains sampled from China, Thailand, and USA between Dec 24, 2019, and Jan 23, 2020, suggested an increase in genetic diversity of SARS-CoV-2 over time, providing early evidence of this trend in human hosts(64).

Between June and July 2022, more than 95% of variants circulating globally including Africa were omicron (65). Based on the Sisay et al., B.1/B.1.1 was prevalent in Ethiopia from Nov 2020 to Mar 2021. B.1.1 circulated with low prevalence in Ethiopia until May 2021. B.1.480 lineage was detected in Ethiopia six months after its initial detection, but the last reported case attributed to the B.1.480 lineage was in Ethiopia (66). In Jan 2021, the Alpha variant was detected but short-lived as the Delta variant emerged. The Delta variant was detected in Feb 2021 and circulated with low prevalence until May 2021. No sequence data were available from Sep to Dec 2021, but the majority of sampled and sequenced cases from Jan 2022 were attributed to the Omicron lineage (66). The genomic data generated from this study also will help the scientific community to better understand the virus which is a critical role in the control of the virus transmission and in the vaccine or treatment development.

3. Objectives

3.1. General objective

- The general objective of this study was to compare the performance of the Xpert® Xpress and Cobas® SARS-CoV-2 assays on SARS-CoV-2 detection from individual and pooled nasopharyngeal swab samples; to determine the variant, lineage, and sub-lineage; as well as to analyze the mutation patterns of the N and E genes in the genome of SARS-CoV-2 from samples collected in Addis Ababa, Ethiopia during the times between June and July 2022.

3.2. Specific objectives

- To determine the level of agreement between Xpert® Xpress and Cobas® SARS-CoV-2 assays for detecting SARS-CoV-2 from individual nasopharyngeal samples.
- To determine the level of agreement between Xpert® Xpress and Cobas® SARS-CoV-2 assays for detecting SARS-CoV-2 from pooled nasopharyngeal samples.
- To validate the number of pooled samples that are effective under Xpert® Xpress and Cobas® SARS-CoV-2 assays by comparing the gene targets and Ct-values effectively detected by each assay.
- To determine the variants, lineages, and sub-lineages those were prevalent during June and July 2022.
- To determine the mutation patterns of the N and E genes in the genome of SARS-CoV-2 from samples collected in this study.

4. Materials and methods

4.1. Study area

The study took place by recruiting participants from various hospitals in Addis Ababa, Ethiopia, including St. Paul's Hospital Millennium Medical College, CURE Ethiopia Children's Hospital, Zewditu Memorial Hospital, Gandhi Memorial Hospital, Menelik II Comprehensive Specialized Hospital, Ras Desta Damtew Memorial Hospital, and Yekatit 12 Hospital Medical. These hospitals sent samples for SARS-CoV-2 PCR testing to the Ethiopian Public Health Institute (EPHI). EPHI has played a crucial role as a national public health institute in the COVID-19 management by leading the country's response to the pandemic. Additionally, EPHI has conducted SARS-COV-2 laboratory testing, quality assurance activities, and trainings, which have increased the country's testing capacity. The institute has been conducting research on the epidemiology and transmission of COVID-19 in Ethiopia, which has helped to in pandemic response efforts. In April 2021, EPHI established a pathogen genomic sequencing laboratory in collaboration with the Africa CDC and the Ethiopia ministry of health for COVID-19 surveillance. The laboratories in EPHI are equipped with Sanger, Nanopore MinION, Illumina Next-seq 550 and MiSeq, sequencing machines. The pathogen genomics laboratory aims to generate data on the genomic diversity of the SARS-CoV-2 virus in Ethiopia, track the transmission dynamics of the virus, and identify any emerging variants of concern (https://ephi.gov.et/).

4.2. Study design

A cross-sectional study was used on nasopharyngeal swabs collected from participants who met the case definition for individuals suspected of having COVID-19.

4.3. Study period

Data for the study was collected between June 25, and July 20, 2022.

4.4. Population

4.4.1. Source population:

The source population of the study includes individuals with a variety of disease symptoms who visited the selected study sites in order to seek medical assistance.

4.4.2. Study Population:

Individuals who experienced symptoms associated with COVID-19 and had visited the selected hospitals included in the study.

4.5. Inclusion and exclusion criteria

4.5.1. Inclusion criteria

- All individuals with symptoms (at least one of the following symptoms history of fever, cough, sore throat, runny nose, shortness of breath, loss of smell, loss of taste, headache, arthralgia, chest pain, myalgia, fatigue, abdominal pain, vomiting, conjunctivitis, skin rash, or lymphadenopathy) of COVID-19 at the selected health facility, agreed to participate in the study, and signed informed consent were included.
- Positive samples by Xpert® assay were selected based on their CT-value, while all negative samples were used for pooling.
- Xpert® positive samples with CT-value of ≤30 were selected for sequencing.

4.5.2. Exclusion criteria

- Study participants who were unable to give the required sample and informed consent were excluded from the study.
- SARS-CoV-2 positive samples with Ct-values above 30 were not included for whole genome sequencing.

4.6. Study variables

4.6.1. Dependent variables:

The dependent variables in the study were SARS-CoV-2 positivity, testing assays, pooled results, variants, lineages, and mutations.

4.6.2. Independent variables:

The independent variables in the study were demographic characteristics (age and sex), geographic location, CT-value, error rate, viral load group and number of samples pool**.**

4.7. Sample size calculation and Sampling method

4.7.1. Sample size calculation

The sample size was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for comparing assays at lower levels, where the candidate

method can only be evaluated by comparing it to a well-described method under conditions in the absence of diagnostic accuracy criteria (Gold standard) (67). In this study, the Cobas® assay was considered the comparative method because it has been being used to test for COVID-19 in suspected individuals in Ethiopia, while the Xpert® Xpress SARS-CoV-2 assay was considered the candidate method.

According to CLSI guidelines, a minimum sample size of 50 positive samples with the comparative method (Cobas 8800®) is required for comprison (67). During the time of sample collection for this study, the mean prevalence of COVID-19 by PCR in Addis Ababa was approximately 18%, which was calculated using data from the EPHI Public Health Emergency Management. Therefore, Based on the prevalence of COVID-19 in Ethiopia, it was estimated that 278 (→ 50= 18/100* total initial sample size → 50*100/18 ≈ 278) negative samples were anticipated to get at least 50 positive samples. However, in order to account for potential issues such fluctuations in the prevalence of COVID-19, variations in adherence to health facility visits, variations in testing availability and criteria, demographic factors, changes in vaccination rates, nonresponse rates, and issues with sample transportation or storage a contingency of 15% (42 participants) has been added. This means that the final sample size to be collected from participants was 320 (278 + 42). The sample collection was continued to get all the positive samples that used for pooling and sequencing.

For pooling purposes, 50 positive samples were selected based on their viral load, which was determined by the candidate method's CT-value. Subsequently, all 50 individual positive samples were pooled with other negative samples to form sample pools of four, six, eight, and ten, and total pool sample size was 200 (Figure 2).

4.7.2. Sampling Method

All individuals who met the eligibility criteria, arrived consecutively at the selected health facilities and volunteered to take part in the study were enrolled. To determine if participants were eligible to participate, the questions outlined in Annex VII were asked for each individual visiting the study site during the study period.

4.8. Data collection procedure

4.8.1. Study flow

Prior to enrolling participants, information about the study was provided, eligibility was confirmed, and written informed consent was obtained. A clinician then conducted screening using a structured form that included information on demographic data such as age group, sex, and region. All willing participants were asked to provide a nasopharyngeal sample to the laboratory for testing. Pooling and sequencing of selected samples were conducted after individually samples were tested by both assays (figure 2).

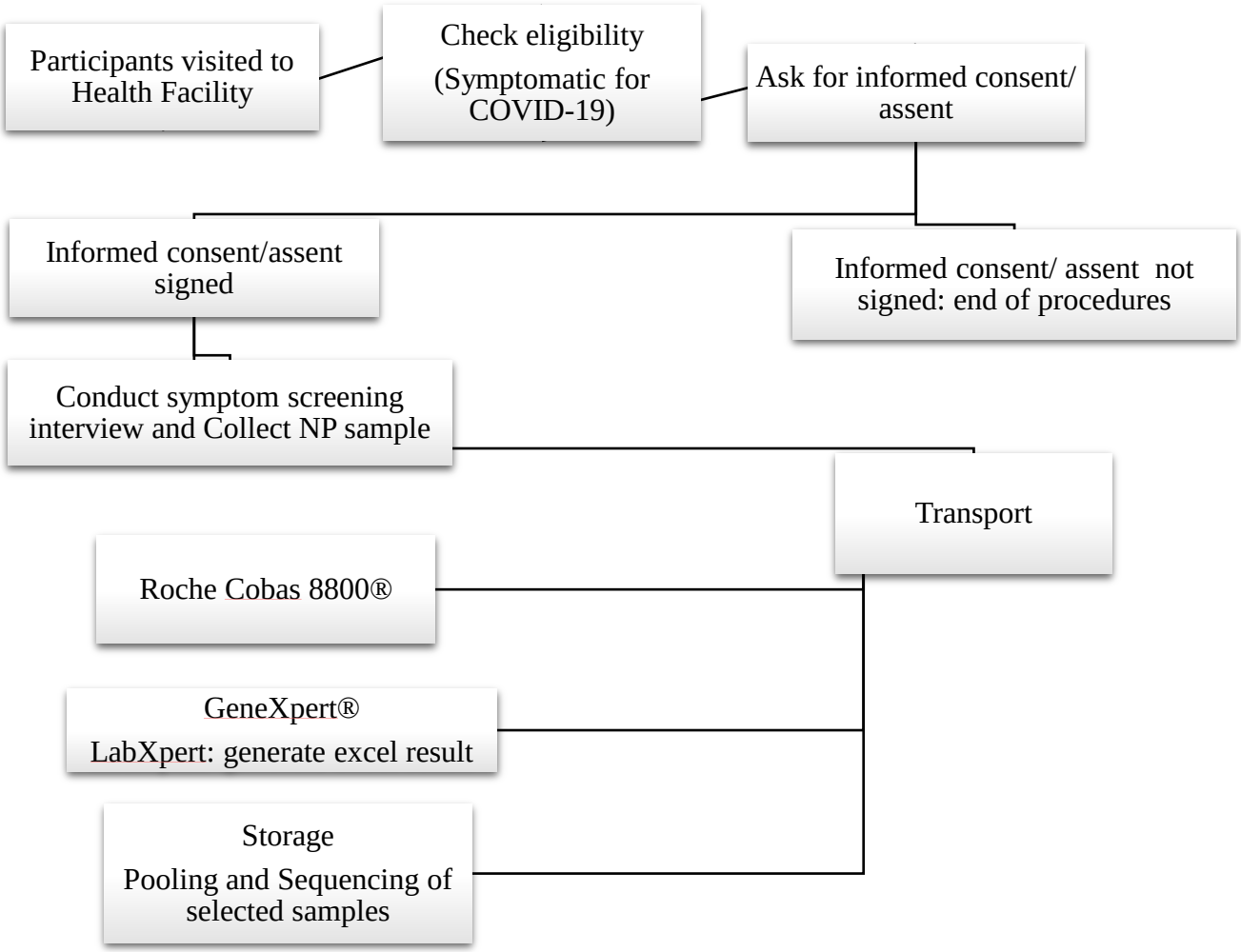


Figure 2: The study flow procedures

Unique participant identification code (UPIC)

All participants were assigned a UPIC for identification purposes. Identifiable participant information was linked to the UPIC only on the informed consent form. The UPIC was

composed of a prefix “CO-GX-” followed by a three-digit number for each participant. The UPICs were pre-printed on barcode stickers that were affixed to all study forms and used to identify samples during laboratory testing, data entry, and data analysis. No identifiable participant information was stored in electronic files.

4.8.2. Laboratory diagnosis for SARS-COV-2 by Cobas 8800 ® and GeneXpert®

4.8.2.1 Sample collection

Nasopharyngeal swabs were collected from the participants using 3 ml of a Viral Transport Medium (VTM) (Liofilchem, Italy). This particular VTM can be used with swabs and helps to keep the virus viable for up to 48 hours at room temperature.

4.8.2.2 Transportation and Storage:

Following collection, samples in VTM were transported at room temperature and processed within 48 hours in accordance with WHO guidelines (68). All the remaining samples were frozen at -80^{o}c for future use in pooling and sequencing.

4.8.2.3 Sample analysis by Cobas 8800®

All individual and pooled samples were analyzed using the Cobas 8800® machine, which is located at the National Immunohematology Laboratory of the EPHI. The Cobas 8800® can analyze samples with a minimum required volume of 0.6 ml, using the Cobas® Omni secondary tube. The system is fully automated that utilizes RT- PCR technology for the detection of SARS-CoV-2. It works by extracting and purifying the RNA from a specimen, and then amplifying and detecting specific sequences of the virus's genetic material using PCR. The PCR reaction is performed in a series of cycles, each of which exponentially increases the amount of target RNA present in the sample (18), and The interpretation of the test result for the study relied on the Qualitative assay for use on the Cobas 6800/8800® Systems of ©2021 Roche Molecular Systems, Inc. (https://www.fda.gov/media/136049/download) (18).

4.8.2.4. Sample analysis by GeneXpert®

All individual and pooled samples were also tested for Xpert® Xpress SARS-CoV-2 assay, using XVI Module GeneXpert® PCR machine which is available at the National Tuberculosis Reference Laboratory of EPHI. To obtain the results from the machine, the

Medx relay connectivity was utilized and an excel spreadsheet was generated. The sample volume of 300 µl was used to test the GeneXpert® system. The GenXpert® system is a fully automated molecular diagnostic platform that utilizes RT-PCR technology for the detection of SARS-CoV-2. It operates the entire process from sample extraction to detection within a single compartment. What sets the GeneXpert® system apart from other PCR-based platforms is its cartridge-based design. Every cartridge comprises the complete master mix required for the PCR reaction, along with the essential controls to ensure accurate results. This eliminates the need for manual preparation of reagents and reduces the risk of contamination and human error during the testing process. The interpretation of the test result for the study relied on the summary of the emergency use authorization (EUA) for the Xpert® Xpress SARS-COV-2 test (https://www.fda.gov/media/136314/download) (11).

4.8.2.5 Pooling procedure and testing.

This study aimed to create 200 pooled samples by combining 340 negative samples and 50 selected positive samples with different Ct-values. The Ct-values were determined using the Gx software, and these 50 samples were selected from four viral load groups determined on their Ct-values (Figure 3).

To begin the process, a random positive sample selection was made of 15 positive nasopharyngeal samples from the very low group with Ct-values 35.0 to 39.9. Similarly, another random selection was made of 15 positive samples from the low group with Ct-values 30.0 to 34.9. Additionally, 10 positive nasopharyngeal samples were chosen at random from the medium group with Ct-values 25.0 to 29.9. Lastly, 10 positive nasopharyngeal samples were taken from the high group with Ct-values below 24.9. The number of positive samples in the very low and low viral load group was increased in order to simulate natural positive samples and examine the precision of the assay's performance on pooled samples. Each of these positive nasopharyngeal samples was then individually placed in separate racks for further processing. In addition to the positive samples, a separate rack was prepared to collect a total of 340 both Cobas® and Xpert® negative samples.

For each very low and low viral load group, 60 pooled samples were created by combining 15 selected samples into four separate pools (pool-4, pool-6, pool-8, and pool-

10). Furthermore, for the medium and high viral load groups, 40 pooled samples were prepared by combining 10 selected samples into pool sizes. A total of 200 pooled samples were prepared, each with its own unique identifier. To pool the samples, a sterile 7 mL plain tube was used for each pool. Each tube was affixed with a unique identifier that included the initial UPIC number of the specific positive sample, along with a prefix indicating the viral load group (V for very low, L for low, M for medium and H for high). Example V-087: indicated that the original positive sample was CO-GX-087 and it's from very low viral load group.

During the pooling procedures, each selected positive sample from all viral load groups, was subjected to vortexing for 20 seconds. This step was taken to ensure the even distribution of the nucleic acid (NA) within each sample. To create a pool-n, a sample was transferred from n-1 negative samples into a labeled plain tube. The positive sample was then added to the same tube. To make pool-4, a volume of 300 µl was transferred from three different negative samples into a labeled plain tube. Following that, 300 µl of the vortexed positive sample was added to the same tube. For pool-6, a volume of 200 µl was transferred from five different negative samples, and an additional 200 µl of the positive nasopharyngeal sample was added into the same tube. Likewise, for pool-8, and pool-10 the same formula was used. In the case of pool-4, a unique sample volume of 300 µl was utilized to ensure that there were enough samples available for testing by Xpert® and Cobas® methods.

To ensure the prevention of cross-contamination, all necessary precautions were taken throughout the procedure. These precautions included performing all steps within a class 2 A2 biosafety cabinet, which provides a controlled and sterile environment. The transfer of samples was carried out using a calibrated micro-pipette with sterile pipette tips. To minimize the risk of contamination, only after completing the pooling of the negative samples, the positive samples were dispensed into the designated containers. Caution was maintained to avoid creating bubbles, foam or aerosols while mixing. The pooled sample volumes in the plain tubes were visually compared to ensure and confirm the desired pool volume. Additionally, the process began with the very low viral load group then to other groups sequentially, ensuring that all necessary steps and transfers were completed for

this group before moving on to the next group, and also avoid contamination from high viral load samples to low.

A total of 200 pooled samples were vortexed for 20 seconds before testing to ensure proper mixing. Then, 300µl of this mixture was added into the Xpert® cartridge and tested within 30 minutes of specimen addition. Simultaneously, 800µl of the same mixture was tested on the Cobas 8800®. Result interpretation was the same as the individual samples but the presumptive positive on Cobas® was taken as positive since dilution effect on positive was obvious.

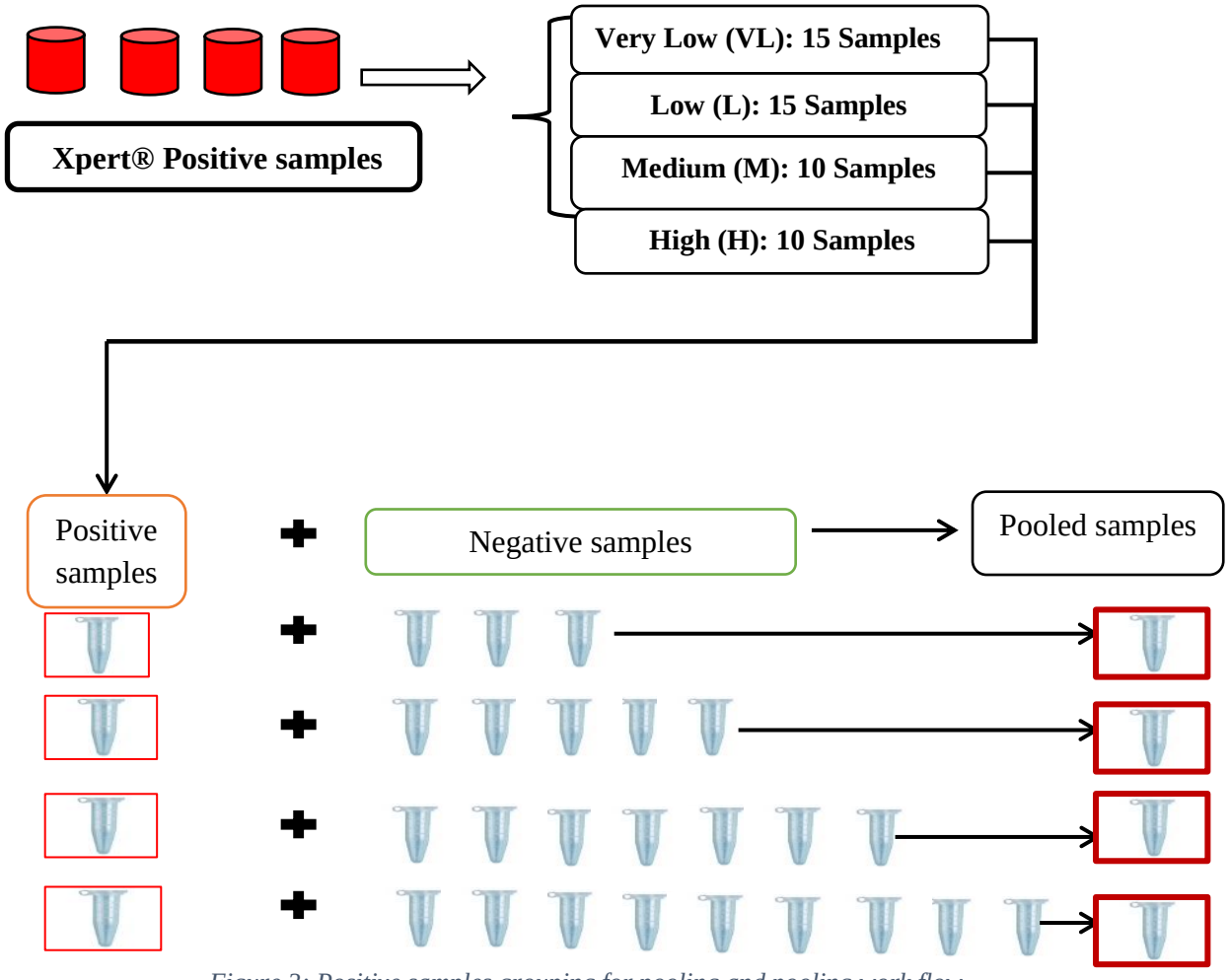


Figure 3: Positive samples grouping for pooling and pooling work flow.

4.9. Genome Sequencing

WGS was performed in the Pathogen Genomic Center of EPHI after selecting appropriate samples. Samples were tested for the presence of SARS CoV-2 RNA using PCR, and

only those samples that tested positive with a CT-value of ≤30, indicating a high viral copy number, were chosen for sequencing.

4.9.1. SARS-CoV-2 RNA Isolation

RNA samples found to be positive for SARS-CoV2 RNA with Ct-values ≤30 by Xpert® assay were identified and selected for sequencing. Nucleic acid was extracted from nasopharyngeal samples using the GenePure Pro Automated Nucleic acid Purification System (Bioer Technology, China) utilizing MagaBio plus Virus RNA Purification Kit II (Bioer Technology, China). The sample volume of 300µl used in the procedure underwent lysis, binding, and washing steps and finally 60µl was eluted to be used in the downstream protocols.

4.9.2. SARS-CoV-2 library preparation and sequencing

The SARS-CoV-2 genomes were sequenced using the more targeted ARTIC amplicon-based method which allows more samples to be tested at once. The cDNA was separated into two pools for amplification using Artic v4 primers covering the whole SARS-CoV-2 genome. PCR reactions were performed on both pools as follows: 98 °C 3min sec × 1 cycle; 98 °C: 15 sec; 63 °C: 5 min × 35 cycles. Amplicon libraries were pooled after normalization and SARS-CoV-2 sequencing libraries were prepared using CovidSeq Assay (Illumina, Cat. No. 20049393) following the manufacturer's protocol with volumes of reagents and inputs reduced by half. The Illumina COVIDSeq Assay (96 samples) package has all the materials required for converting cDNA, amplifying, and preparing libraries. It includes the ARTIC v3 primer pool which uses the ARTIC multiplex PCR protocol to identify and study SARS-CoV-2 RNA. The prepared libraries were pooled, quantified using Qubit HS DNA kit (ThermoFisher, Cat. No Q32851), fragment analyzed using Bioanalyzer 2100 (Agilent, Germany), and loaded onto either MiSeq platforms for a paired end 600 cycles sequencing.

4.10. Bioinformatics analysis

The base call (BCL) files from MiSeq were demultiplexed and converted to FASTQ file using Illumina bcl2fastq2 conversion software V 2.20. FASTQ files were used to generate quality check using FASTQC Software. The paired end FASTQ files were then trimmed using TRIMMOMATIC V0.36) with parameters of average threshold quality and minimum read length 20. Taxonomic classification was done using Kraken2. Ivar

was utilized to generate the consensus sequences. The consensus sequences were then aligned to the complete genome of SARS-CoV-2 Wuhan-Hu-1(NCBI reference number of NC_045512.2) using BWA; and samtools was used for intermediate file conversion and summary. Variants were called using Snippy and Nextclade. Local Nextstrain/nextclade v 0.13.0 (24) was also implemented for clade assignment and variant annotation to reveal the amino acid substitutions and deletions and/or insertion. Lineage assignments were made using the Phylogenetic Assignment of Named Global Outbreak Lineages tool PANGOLIN [(Phylogenetic Assignment of Named Global Outbreak Lineages] v1.07 and clades from GISAID (24).

4.11. Data Quality Assurance

To ensure data quality assurance in the study, all involved clinicians and laboratory technicians received on-site orientation about the study's objectives, procedures, and significance. Laboratory technicians were oriented on accurately completing test results in the data collection form prepared for the study. Existing Standard Operating Procedures (SOPs) for nasopharyngeal sample collection, Xpert® Xpress SARS-CoV-2 assay, Cobas® SARS-CoV-2 assay, and sequencing platforms were followed. To maintain quality assurance, laboratory personnel working on Cobas® machines, GeneXpert® machines, and sequencing laboratory were blinded to each other's results. Additionally laboratory personnel running pooled samples were blinded to individual result, as 10 negative pooled samples were run along with 200 pooled samples as a control.

Nasopharyngeal samples were collected using a sterile technique, labeled with unique barcode labels, and associated with a record on a specimen information format. Before sequencing the quality and quantity of the viral RNA extracted from the patient sample were assessed by Qubit 1X dsDNA HS (Thermo Fisher Scientific, Waltham, MA, USA). As part of the SOP, the laboratory conducted quality control activities at various stages of the process, monitored RNA quality, and processed one negative and one positive control using Cobas® buffer and RNA isolation for sequencing in each batch of tests. The GeneXpert® system has an in-built control system that monitors the proper functioning of the system, including a Sample Processing Control (SPC) and Probe Check Control (PCC). The PCC measures the fluorescence signal from the probes before starting the

PCR reaction to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. During sequencing, data quality control measures such as raw data assessment, base calling quality scores, read quality filtering, and adapter trimming were performed to ensure the accuracy and reliability of the data. All laboratory findings were checked by PI and documented properly. Leftover samples were stored appropriately for further use, and after sequencing, the data was processed and analyzed using the bioinformatics tools mentioned earlier.

4.12. Data Management and interpretation

4.12.1. Data entry

The data were entered into the EpiData version 3.1 database, and the unique personal identification code (UPIC) was used to link the data from interviews and laboratory results. To ensure the accuracy of the data, 15% of the data was entered a second time by one data clerk in EPHI.

4.12.2. Data storage and transfer

To ensure long-term storage and preservation of the data collected during the study, two repositories were utilized: the EPHI National Data Management Center and the Research Data Repository of Addis Ababa University. All the generated consensus sequences used for the study were deposited in the GISAID sequence database and are publicly available.

4.12.3. Data validation

Daily assessments of completed forms were conducted to ensure the completeness and internal consistency. If any missing information or inconsistencies were identified, the participants were contacted to obtain or correct the data.

4.12.4. Data analysis and Interpretation

The data entered into Epi Data version 3.1 were exported to Statistical Product and Service Solutions (SPSS) version 28 (IBM Inc., Chicago, USA). Descriptive statistics were used to summarize the study variables, and the overall agreement between positive and negative test results was calculated. The data was then presented in the form of tables, graphs and figures. Cross-tabulation was used to compare the individual final results with the pooled final results. To compare the individual mean CT-value with the

pooled mean CT-value, a paired T-test was utilized. Pearson correlation was applied to determine the extent of linear association between the individual final result and the final result after pooling the samples. The Chi-square (χ^2) test was used to evaluate the relationship between the final pooled result and the number of pools, as well as the viral load group for both Xpert® Xpress and Cobas® SARS-CoV-2 assays. Crude odds ratio was used to assess the association between the number of pools and the final pooled result for both Xpert® Xpress and Cobas® SARS-CoV-2 assays. Cohen's Kappa (κ) was used to evaluate the level of agreement between the assays for both individual and pooled results. For COVID-19 diagnosis, PCR are commonly used as the primary diagnostic method but not gold standard; therefore, sensitivity and specificity could not be calculated (67). The Xpert® Xpress SARS-CoV-2 assay was evaluated by comparing it to the comparative assay, which is the Cobas® SARS-CoV-2 assay. The comparison was done to estimate the overall percent agreement (OPA) positive percent agreement (PPA) and negative percent agreement (NPA) by putting the results as displayed on table 1 (67).

Table 1: 2 x 2 Contingency table when using a comparative method without diagnostic accuracy criteria

Candidate method (Xpert® Xpress SARS-CoV-2 assay)	Comparative method (Cobas® SARS-CoV-2 assay)		
	Positive	Negative	Total
Positive	a	b	a + b
Negative	c	d	c + d
Total	a + c	b + d	n

A simplistic approach was used to report the overall percent agreement, which is given below.

Overall percent agreement = 100× (a+d)/n

Pair of agreement measures, PPA and NPA were used to report the agreement between two methods (67).

PPA of Xpert® Xpress SARS-CoV-2 assay = 100×a/ (a+c)

NPA of Xpert® Xpress SARS-CoV-2 assay = 100×d/ (b+d)

Confidence Intervals for Agreement Measures

An exact confidence interval for percent agreement was computed as indicated in a previous publication (67). A 95% score confidence interval for agreement is calculated as:

$$100\%\frac{Q1-Q2}{Q3}$$

$$100\%\frac{Q1+Q2}{Q3}$$

Where the quantities Q1, Q2, and Q3 are computed from the data using the formulae below (67).

$$Q1 = 2(a+d) + 1.96^2 = 2(a+d) + 3.84$$

$$Q2 = 1.96\sqrt{1.96^2 + \frac{4(a+d)(b+c)}{n}} = 1.96\sqrt{3.84 + \frac{4(a+d)(b+c)}{n}}$$

$$Q3 = 2(n + 1.96^2) = 2n + 7.68$$

4.13. Operational definitions

Diagnostic Accuracy Criteria: CLSI defines diagnostic accuracy criteria as the best currently available criteria for establishing the presence or absence of the condition, event, or characteristic of interest using a single method or combination of methods, including laboratory tests, imaging tests, pathology, and clinical information including follow-up (67).

Test agreement: the degree of concordance between two assays.

CT-value: In PCR (Polymerase Chain Reaction), it is the number of cycles required for the fluorescent signal emitted by amplified DNA to cross a set threshold above the background fluorescence

Presumptive Positive: The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management (11).

Pooling: is approach that increases the number of individuals that can be tested using the same amount of resources by mixing several samples together in a batch or pooled sample.

Pool Size: total number of samples (positive + negatives) used to pool.

Pool-4: Nasopharengeal samples pooled by mixing one positive and three negatives.

Pool-6: Nasopharengeal samples pooled by mixing one positive and five negatives.

Pool-8: Nasopharengeal samples pooled by mixing one positive and seven negatives.

Pool-10: Nasopharengeal samples pooled by mixing one positive and nine negatives.

4.14. Ethical considerations

The research obtained ethical approval from the Departmental Research Ethics Review Committee (DRERC) of DMIP, College of Health Sciences, Addis Ababa University. For adult participants, informed consent was acquired, while for children below the age of 12 and adolescents, guardians were asked for informed consent. For children aged between 12 to 18 years both informed assent from the children and consent from parents or guardians was used. The confidentiality of all participants was strictly kept during and after the study period. Unique study identifier numbers were used instead of individual names. A written letter was prepared and permission was obtained from EPHI to conduct laboratory tests (Cobas®, GeneXpert®, and Sequencing). The laboratory test results of each specimen were reported to the clinician for treatment and further management.

4.15. Dissemination of the result

The results of the study were shared with DMIP, Addis Ababa University as part of the requirements for the completion of the MSc in Medical Microbiology program. Additionally, the findings were communicated to EPHI, and Addis Ababa city administration health bureau. The study will also be presented at scientific workshops and published in peer-reviewed local or international journals.

5. Results

5.1. Demographic characteristics of the study participants.

Nasopharyngeal samples were collected from 440 COVID-19 suspected individuals who visited seven hospitals in Addis Ababa. The mean age of the participants was 36.28 years (SD=17.00), with a range from 1 to 93 years. The majorities (365/440, (83.0%)) were adults aged between 18 and 64, followed by children younger than 18 years old (43/440, (9.8%)). More than half were females (257/440, 58.4%). The majority of participants were residents of Addis Ababa, with a proportion of 279/440 (63.4%). St. Paul Hospital Millennium Medical College (SPHMMC) had a high representation in the study, with 229 participants (52.1%). The detail demographic characteristics of the participants are presented in Table 2.

5.2. Prevalence of COVID -19 among suspected individuals by both assays.

5.2.1. Overall prevalence of COVID -19

In total, among the 440 individuals who were suspected of having SARS-CoV-2, 100 (22.73%) of them were found to be positive by an of Xpert® or Cobas® assays. Females had a higher number of positive cases, with 61 (23.74%) among all the suspect females; and 61% among confirmed positives) testing positive, compared to males, with 39 (21.31% among all the suspect males; and 39% among confirmed positives) testing positive. Among children less than 12 years old, 9(34.6%) became positive. Suspected individuals from Addis Ababa (AA) had the highest number of positive cases at 73/279 (26.16% among all suspects from AA; and 73% of all positives). From the 229 participants referred from SPHMMC, 30 (13.10%) became positive (Table 2).

Table 2: Distribution of sars-cov-2 test results by demographic, address and referring health facility.

Variables	**Cases Number (%)**	**Over-all SARS-COV-2 result (by any of Xpert® or Cobas®)**	
		Negative (%)	Positive (%)
Age :			
Mean	36.28		
SD	17.002		
Min	1		
Max	93		
Age category in *years* (%):			
1-12	26 (5.9)	17(65.4)	9(34.6)

13-24	78 (17.7)	64(82.1)	14(17.9)
25-39	173 (39.3)	139(80.3)	34(19.7)
40-54	87 (19.8)	62(71.3)	25(28.7)
55-64	44 (10.0)	33(75.0)	11(25.0)
> 65	32 (7.3)	25 (78.1)	7(21.9)
Total	440(100)	340(77.27)	100(22.73)
Gender (%):			
Male	183 (41.6)	144(78.69)	39(21.31)
Female	257 (58.4)	196(76.26)	61(23.74)
Total	440(100)	340(77.27)	100(22.73)
Address (Region):			
AA	279 (63.4)	206(73.84)	73(26.16)
Afar	1 (0.2)	0(0.00)	1(100.00)
Amhara	10 (2.3)	8(80.00)	2(20.00)
B. Gumuz	2 (0.5)	2(100.00)	0(0.00)
Harari	1 (0.2)	1(100.00)	0(0.00)
Oromia	138 (31.4)	116(84.06)	22(15.94)
SNNPR	9 (2.0)	7(77.78)	2(22.22)
Total	440(100)	340(77.27)	100(22.73)
Hospital:			
SPHMMC	229(52.1)	199(86.90)	30(13.10)
CCHE	55(12.5)	34(61.82)	21(38.18)
ZMH	60(13.5)	40(66.67)	20(33.33)
GMH	44(10.0)	33(75.00)	11(25.00)
MCSH	6(1.4)	2(33.33)	4(66.67)
RDDMH	17 (3.9)	11(64.71)	6(35.29)
Y12HMC	29(6.6)	21(72.41)	8(27.59)
Total	440(100)	340(77.27)	100(22.73)

** AA*: *Addis Ababa * SPHMMC: Saint Paul's Millennium Medical College * ZMH: Zewditu Memorial Hospital * MCSH: Menelik II Comprehensive Specialized Hospital * Y12HMC: Yekatit 12 Hospital Medical College * CCHE: CURE Children's Hospital of Ethiopia* * GMH: Gandhi *Memorial Hospital * RDDMH: Ras Desta Damtew Memorial Hospital * SNNPR: South National and Nationalities Region * B. Gumuz: Benishangul Gumuz*

5.2.2. Performance of Xpert® Xpress SARS-CoV-2 assay

As shown in Figure 4, out of the 440 samples tested using GeneXpert®; the positivity rate was 21.6% (95/440). Small percentage of samples (3.6%) resulted in errors, and two samples (0.5%) resulted as one of unsuccessful result which was displayed as no-result in the software.

Figure 4: Prevalence of SARS-COV-2 by Xpert® Xpress SARS-CoV-2 assay

Based on the information provided in Figure 4, in Xpert® Xpress assay, 18 results were found to be unsuccessful on the first run. Among these, 16 cases were identified with errors, while 2 cases yielded no-result. The predominant error code observed in the Xpert® Xpress SARS-CoV-2 assay was error code 5007. This error occurred in 10 out of 18 cases (55.6%) and was primarily associated with probe check failures (Figure 5).

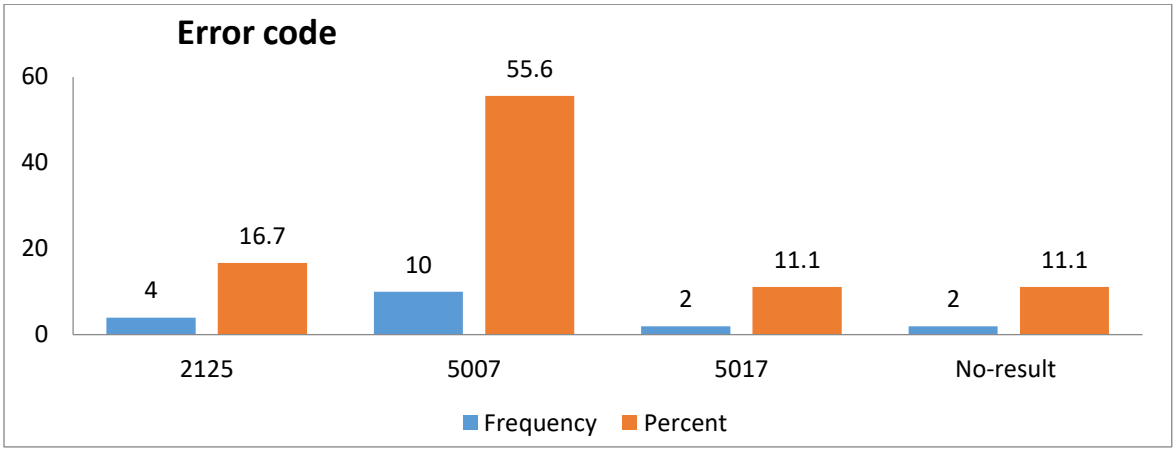


Figure 5: Frequency and percentage distribution of error codes in GeneXpert® by first run

** **2125**: Termination error due to insufficient sample volume * **5007** and **5017**:The probe check was unsuccessful due to either one or combination of the following reasons: an incorrect quantity of reagent was added to the cartridge, the reagent's quality was compromised, there was a failure in fluid transfer, and the sample was mishandled during processing within the cartridge* (69). ** **No-result:** which shows that test could not be completed and insufficient data was collected*

After the initial unsuccessful Xpert® results, all 18 cases were retested using the same assays. Out of these, 4 (22.2%) (95% CI, 5.6 to 44.4) cases were found to be positive for SARS-CoV-2, while 14 (77.8%) (95% CI, 55.6 to 94.4) cases tested negative when repeated. Following the addition of retested outcomes from the unsuccessful samples (18 samples); a subsequent analysis was conducted on the findings of the Xpert® target genes, encompassing a total of 440 samples. The analysis revealed that 99 (22.6%) (95% CI, 18.6 to 26.4) samples were found to be positive for SARS-CoV-2 by Xpert® assay. Among positive samples in both targets, 68 (68.69%) were positive for both probes, 31 (31.31%) were positive only for N2 gene probes, and but none were positive for E gene probes only.

5.2.3. Performance of Cobas® SARS-COV-2 assay

Among the 440 samples that underwent Cobas® testing, 57 (13.0%) (95% CI, 10.0 to 16.2) had positive results, 17 (3.9%) (95% CI, 0.9 to 3.5) samples were presumptive positive and the remaining 366 (83.2%) (95% CI, 81.3 to 88.0) samples were negative for SARS-CoV-2 RNA (Figure 6).

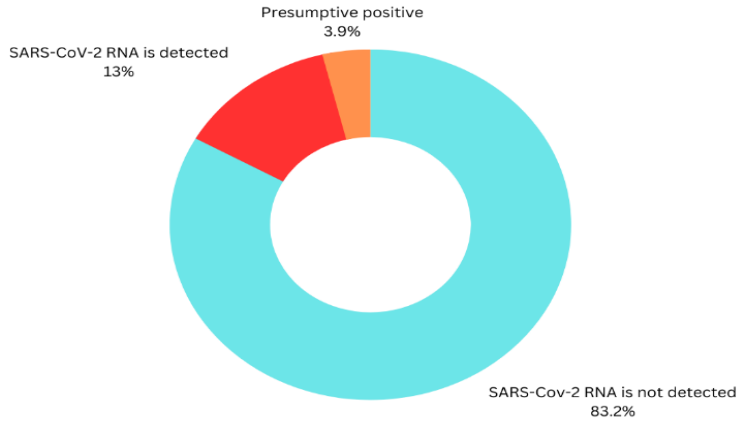


Figure 6: Prevalence of SARS-COV-2 by Cobas 8800®

Out of presumptive positive results, 9 (52.95%) (95% CI, 29.4 to 76.5) becomes positive when repeated using a Xpert® assay. After including the retested results of the presumptive positive cases, further analysis was performed on the final Cobas® ORF1a gene and E gene for a total of 440 samples. The analysis revealed that 56 (12.7%) tested positive for both targets, while 366 (83.2%) samples tested negative for both targets.

Additionally, 17 (3.9%) samples tested negative for ORF1a gene but positive for the E gene, and only one sample (0.2%) tested positive for the ORF1a gene but negative for the E gene.

5.2.4. Individual samples Ct-values

The Cobas® ORF1a gene had a median CT-value of 30.54 (IQR: 26.25-32.42), while the Cobas® E gene had a median CT-value of 31.73 (IQR: 26.56-34.06). Likewise, the Xpert® N2 probe had a median CT-value of 37.20 (IQR: 30.80-39.80), and the Xpert® E probe had a median CT-value of 31.10 (IQR: 26.28-35.10) (Table 3).

Table 3: Median Ct-values and endpoints for Cobas® and Gene Xpert® assays

SARS-COV-2 Assay			Ct-values				
	Target genes	N	min	max	Median	Q1	Q3
Cobas®	ORF1a:	57	19.74	35.49	30.54	26.25	32.42
	E:	57	19.85	35.93	31.73	26.56	34.06
	control:	57	33.08	36.74	34.73	34.12	35.45
Xpert Xpress	N2:	99	17.80	44.10	37.20	30.80	39.80
	E:	68	16.20	41.30	31.10	26.28	35.10
	SPC:	99	.00	30.80	28.30	28.10	28.70

5.3. Test agreement between Xpert® Xpress and Cobas® SARS-COV-2 assay on individual Samples.

For test agreement 423 samples was selected by exclusion of the 17 presumptive positive cases of Cobas® but considering the retested result of Gene Xpert® for the unsuccessful result from the first run. There were 56 (13.24%) samples tested positive and 332(78.54%) samples that tested negative using both methods. A single sample was positive using the Cobas® assay but negative from Xpert® assay, whereas 34 (8.04%) samples that were positive from Xpert® assay only. The overall percent agreement (OPA) between the two methods was 91.73% (95% CI, 89.99% to 92.66 %,) with κ=0.715 (95% CI, 0.628 to 0.802). The PPA and NPA of the Xpert® assay was 98.25% and 90.71%, respectively.

Table 4: Comparison of Xpert® Xpress and Cobas® assay for SARS-CoV-2 detection from individual nasopharyngeal samples

		Result of Comparative method (Cobas®)		
		Positive n **(%)**	Negative n **(%)**	Total n **(%)**
Result of Candidate method (Xpert® Xpress)	Positive n **(%)**	56(13.24)	34(8.04)	90(21.28)
	Negative n **(%)**	1(0.24)	332(78.54)	333(78.80)
	Total n **(%)**	57(13.48)	366(86.52)	423 (100)
	Agreement (%)			
	OPA			91.73
	PPA			98.25
	NPA			90.71
	κ-value			0.715
	κ-value 95% CI:			0.628 to 0.802

Following the exclusion of presumptive positive samples identified by the Cobas® assay, a cross-tabulation analysis was conducted on a subset of 91 positive samples (excluding 9 positive samples from the retested presumptive group). This analysis compared the results of the Xpert® N2 and Cobas® ORF1a gene, among these samples, 56(62.2%) tested positive for both the N2 and ORF1a gene. Additionally, there were 34(37.8%) samples that tested positive only for the N2 gene, while only one sample negative for the N2 but positive for the ORF1a gene (Table 5).

Table 5: Cross-tabulation analysis of Cobas® ORF1a gene and Xpert® Xpress N2 gene results

		Cobas® ORF1a gene		
		Positive n **(%)**	Negative n **(%)**	Total n **(%)**
Xpert® Xpress N2 gene	Positive n **(%)**	56(62.2)	34(37.8)	90(100)
	Negative n **(%)**	1(100)	0(0)	1(100)
	Total n **(%)**	57(62.6)	34(37.4)	91 (100)

A cross-tabulation analysis was also conducted between the Xpert® E and Cobas® E gene, and 55(90.2%) were positive for both E genes. There were 6(9.8%) positive for the Xpert® E but negative for the Cobas® E gene but only 1(3.3%) sample tested negative for the Xpert® E but positive for the Cobas® E gene (Table 6).

Table 6: Cross-tabulation analysis of Cobas® E gene and Xpert® Xpress E gene results

		Cobas® E gene		
		Positive n **(%)**	Negative n **(%)**	Total n **(%)**
Xpert® Xpress E gene	Positive n **(%)**	55(90.2)	6(9.8)	61(100)
	Negative n **(%)**	1(3.3)	29(96.7)	30(100)
	Total n **(%)**	56(61.5)	35(38.5)	91 (100)

5.4. Pool testing of nasopharyngeal swabs by Xpert® Xpress SARS-COV-2 assay

5.4.1. Positive percentage of average pooling Versus Number of pools and viral load groups

As shown in Table 7, out of the total 200 pooled samples, an average of 182 (91.0%) samples tested positive when tested using the Xpert® Xpress assay. Within each pool size consisting of 50 pooled samples, the highest detection rates were found in Pool 4 and Pool 6, with 47(94.0%) of positive samples detected in each. Pool 8 had a detection rate of 45 (90.0%), while Pool 10 had a detection rate of 43 (86.0%). The observed differences in the proportion of positive samples between the different pools were not statistically significant (p=0.463).

Table 7: Comparison of the average final pooled Xpert® results by number of pools

Variables		Number of pools				
		Four	**Six**	**Eight**	**Ten**	**Total**
Final pooled Xpert® result	Positive	47(94.0%)	47 (94.0%)	45 (90.0%)	43 (86.0%)	182 (91.0%)
	Negative	3 (6.0%)	3 (6.0%)	5 (10.0%)	7 (14.0%)	18 (9.0%)
	COR	1.000	1.000	1.741	2.550	
	95% CI		0.192-5.210	0.393-7.713	0.620-10.492	
	P-value	0.463				

When the positive individual samples with medium and high viral load groups (Ct-values <30) were pooled together, all of them showed positive results, indicating a 100% detection rate for all pool size. The difference in the average pool positivity rate among the viral load groups was statistically significant (χ^2=32.728, p<0.001) (Figure 7).

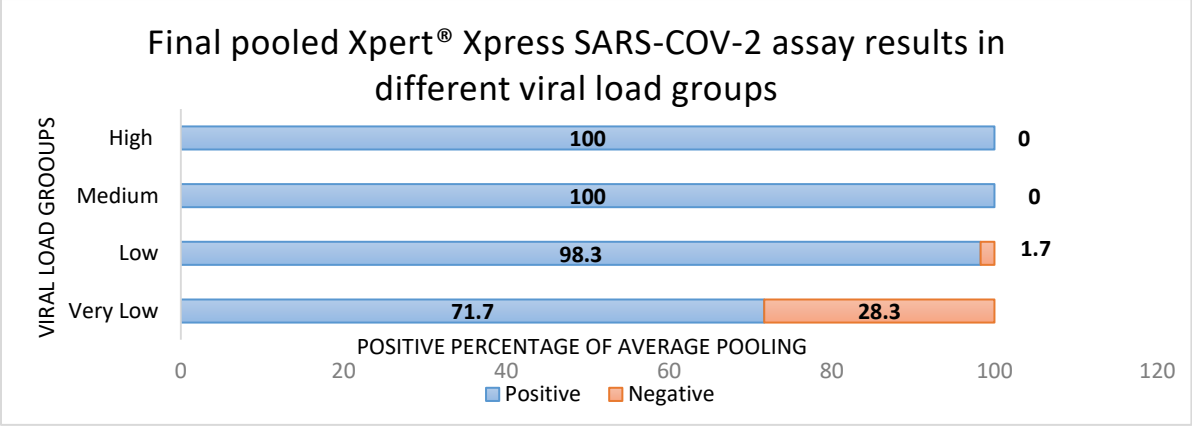


Figure 7: Final pooled Xpert® Xpress SARS-COV-2 assay results in different viral load groups

5.4.2. Viral load group Versus Number of pools on Xpert® Xpress

Within each high and medium viral load groups consisting of 40 pooled samples, the highest detection rates were found with all pools detected positive results (100%). In the low viral load group, which consisted of 60 pooled samples, 1 (1.67%) negative result was observed in pool ten. The viral load group showed significant differences when initially positive individual samples were pooled into 8 and 10 (p=0.105), but not in pool 4 and 6 (p=0.005 and 0.008, respectively).

Table 8: Viral load group distribution for Xpert® Xpress SARS-COV-2 assay results across different pools

Variables		Viral load group for Xpert® Xpress SARS-COV-2 assay				
		Very Low (%)	Low (%)	Medium (%)	High (%)	Total (%)
Pool-4	Positive	12 (80.0)	15 (100)	10 (100)	10 (100)	47 (94)
	Negative	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6)
	Total	15 (100)	15 (100)	10 (100)	10 (100)	50 (100)
	P-value	0.105				
Pool-6	Positive	12 (80.0)	15 (100)	10 (100)	10 (100)	47 (94)
	Negative	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6)
	Total	15 (100)	15 (100)	10 (100)	10 (100)	50 (100)
	P-value	0.105				
Pool-8	Positive	10 (66.7)	15 (100)	10 (100)	10 (100)	45 (90)
	Negative	5 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	5 (10)
	Total	15 (100)	15 (100)	10 (100)	10 (100)	50 (100)
	P-value	0.005				
Pool-10	Positive	9 (60.0)	14 (93.3)	10 (100)	10 (100)	43 (86.0)
	Negative	6 (40.0)	1 (6.7)	0 (0.0)	0 (0.0)	7 (14.0)
	Total	15 (100)	15 (100)	10 (100)	10 (100)	50 (100)
	P-value	0.008				
Total	Positive	43 (72.0%)	59 (98.3%)	40 (100%)	40 (100)	182 (91.0)
	Negative	17 (28%)	1 (1.67%)	0 (0.0%)	0 (0.0)	17 (9.0)
	Total	60 (100%)	60 (100%)	40 (100%)	40 (100)	200 (100)

5.4.3. Pooled samples median CT-value by Xpert® Xpress SARS-COV-2 assay

The N2 gene probe resulted median Ct-values ranging from 33.40 to 34.00 while the E gene, resulted from 30.15 to 31.10 across the pools. For the Xpert® E gene, Pool-10 had the highest pool median CT value of 31.10, while Pool 6 had the lowest pool median CT value of 30.15. The correlation analysis between CT-value of individual samples of all viral load groups and pooled samples showed a strong positive correlation (ranging from 0.93 to 0.99) for both N2 and E gene targets across all pools (Figure 8 and table 9). The

median difference in Ct-values between pooled and individual samples for the N2 and E genes ranged from 2.25 to 3.6 respectively.

Table 9: Median Ct-values and correlation for N2 and E targets in pools, irrespective of viral laod by Xpert® Xpress SARS-COV-2 assay

Target	Individual median CT	Pool median CT	IQR OF Pool	correlation	median difference	No	Pool -no
N2 gene	31.10	33.40	(27.90-38.10)	0.99	2.3	47	pool-4
	31.10	33.60	(28.00-37.70)	0.98	2.5	47	pool-6
	30.40	34.00	(28.60-36.75)	0.98	3.6	42	pool-8
	30.40	33.90	(28.50-36.80)	0.54	3.5	43	pool-10
E gene	27.90	30.25	(25.10-33.15)	0.98	2.35	40	pool-4
	27.90	30.15	(25.45-33.17)	0.95	2.25	40	pool-6
	27.70	31.00	(26.08-34.35)	0.93	3.3	38	pool-8
	27.90	31.10	(26.80-33.90)	0.96	3.2	39	pool-10

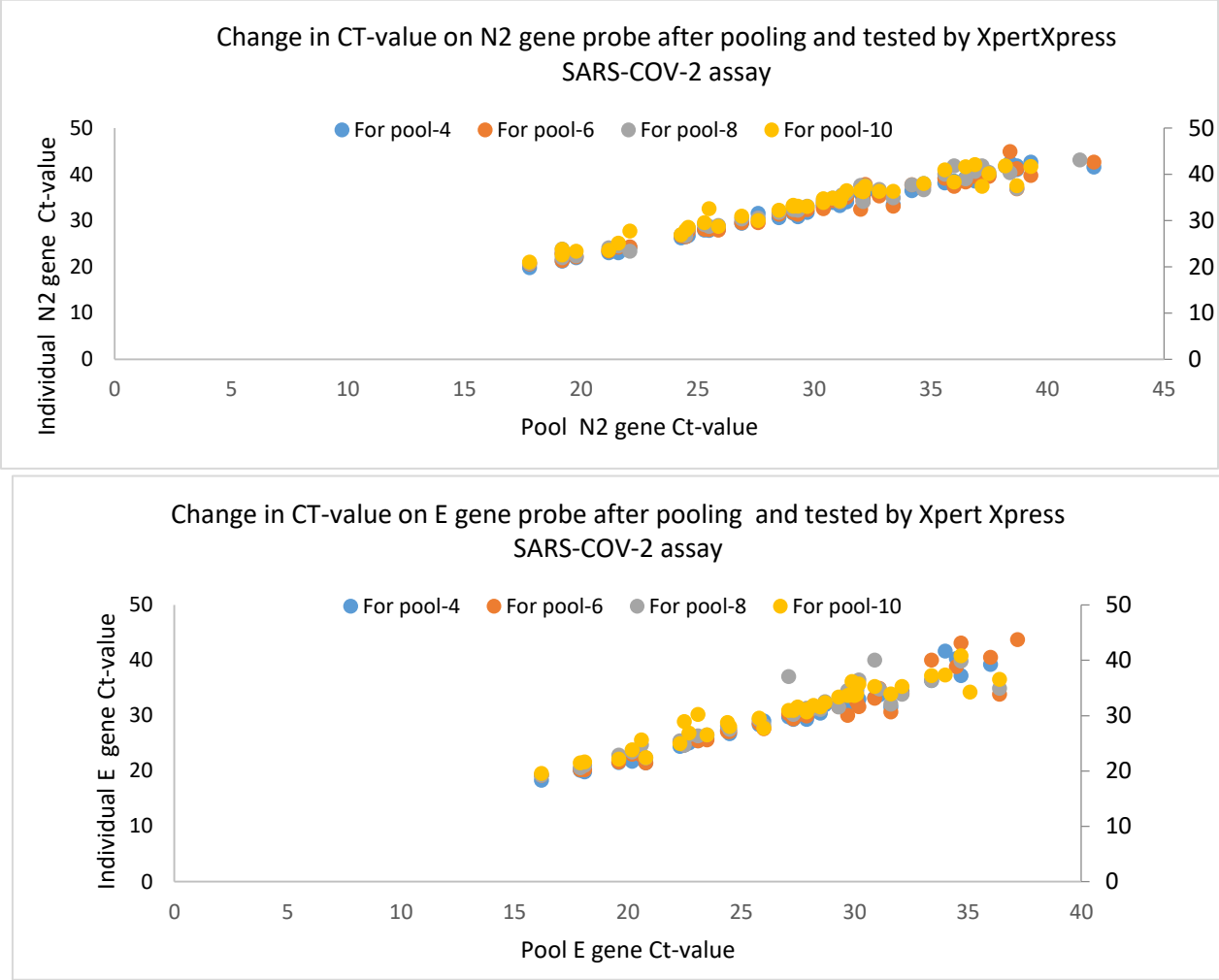


Figure 8: The change in CT-value of N2 and E probe resulting from pooling and testing by GeneXpert®

5.5 Pool testing of nasopharyngeal swabs by Cobas® for SARS-CoV-2 detection

5.5.1. Final pooled Cobas® result Versus Number of pools and viral load groups

Among the pooled positive individual samples with negative results, 41/50 (82.0%) of the positive samples were detected in pool four, six, and eight irrespective of viral load. In pool ten, 78% (39/50) of the positive samples were detected. There was no statistically significant difference among pool numbers (pool four, pool six, pool eight, and pool ten) when tested using Cobas® (p-value = 0.943). (Table 10)

Table 10: Comparison of final pooled Cobas® results by number of pools regardless of viral load group

Variables		Number of pools				
		Four (%)	**Six (%)**	**Eight (%)**	**Ten (%)**	**Total (%)**
Final pooled Cobas® result	Positive	41 (82.0)	41 (82.0)	41 (82.0)	39 (78.0)	162 (81.0)
	Negative	9 (18.0)	9 (18.0)	9 (18.0)	11 (22.0)	38 (19.0)
	COR	1	1	1	1.285	
	95% CI		0.360-2.744	0.360-2.745	0.620-10.492	
	P-value	0.943				

The very low viral group had the lowest percentage (40.0%) of positive samples, while the medium and high groups had 100% positive samples. The difference in the percentage of positive samples between the viral load groups was statistically significant (χ^2=87.239 P=<0.001) (Figure 9).

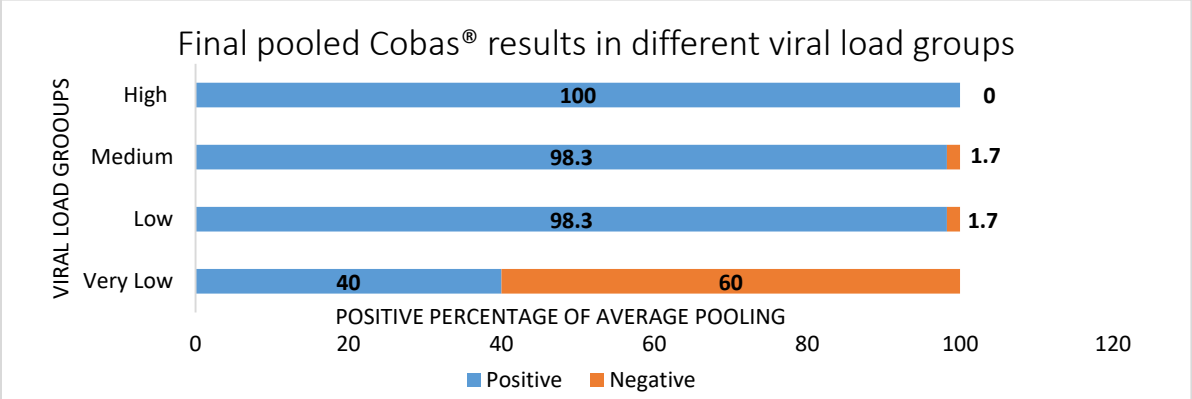


Figure 9: Final proportion of average SARS-CoV-2 positivity results in pooled Cobas® assay from different viral load groups in their respective pool number

5.5.2. Viral load group Versus Number of pools on Cobas®

Out of the 200 pooled samples, 162 (81%) samples tested positive, while 38 (19%) samples tested negative. All the high viral load groups had detected positive in all pool size. But medium and low viral load groups detected one negative in pool ten. While in

very low viral load group 9 (60.0%) of initially positive individual samples becomes negative when it pooled into ten (Table 11). There was a significant association between the viral load group and the pool number (p-value <0.001).

Table 11: Viral load group distribution for Cobas® testing results across different pools

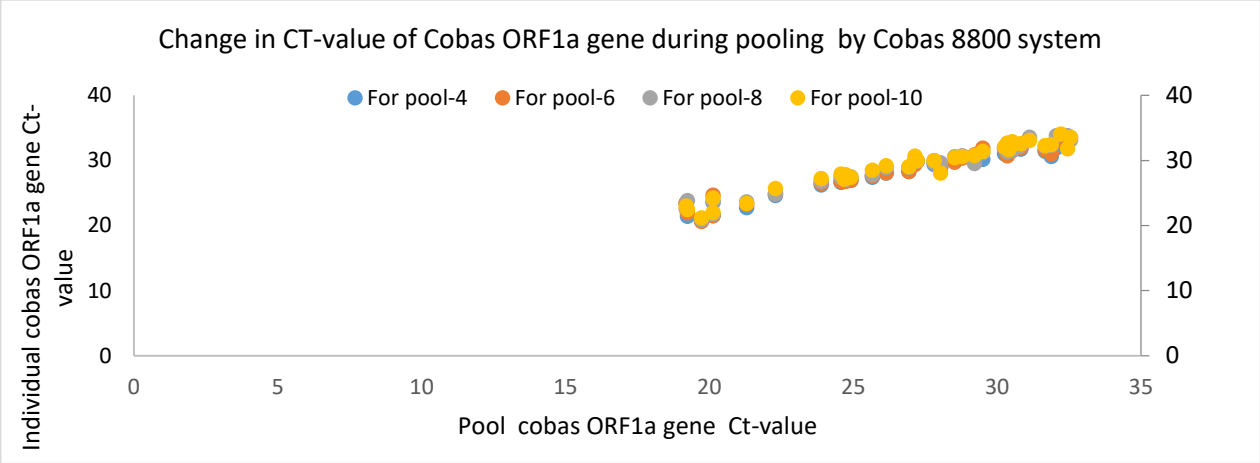
Pool number		Viral load group				
		Very Low (%)	**Low (%)**	**Medium (%)**	**High (%)**	**Total (%)**
Pool-4	Positive	6 (40.0)	15 (100)	10 (100)	10 (100)	41(82)
	Negative	9 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (18.0)
	Total	15 (100)	15 (100)	10 (100)	10 (100)	50 (100)
	P-value	<0.001				
Pool-6	Positive	6 (40.0)	15 (100)	10 (100)	10 (100)	41(82)
	Negative	9 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (18.0)
	Total	15 (100)	15 (100)	10 (100)	10 (100)	50 (100)
	P-value	<0.001				
Pool-8	Positive	6 (40.0)	15 (100)	10 (100)	10 (100)	41(82)
	Negative	9 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (18.0)
	Total	15 (100)	15 (100)	10 (100)	10 (100)	50 (100)
	P-value	<0.001				
Pool-10	Positive	6 (40.0)	14 (93.3)	9 (90)	10 (100)	39(78)
	Negative	9 (60.0)	1 (6.7)	1 (10.0)	0 (0.0)	11 (12.0)
	Total	15 (100)	15 (100)	10 (100)	10 (100)	50 (100)
	P-value	<0.001				
Total	Positive	24 (40.0)	59 (98.3)	39 (97.5)	40 (100)	162(81)
	Negative	36 (60.0)	1 (1.67)	1 (2.5)	0 (0.0)	38(19)
	Total	60 (100)	60 (100)	40 (100)	40 (100)	200(100)

5.5.3. Pooled samples median CT-value by Cobas® SARS-COV-2 assay

The ORF1a gene consistently had median Ct-values between 29.73 and 29.97 across the pools, while the E gene resulted ranging from 30.61 to 31.24. The correlation analysis between CT-value of individual samples and pooled samples showed a strong positive correlation (ranging from 0.94 to 0.98) between the ORF1a and E gene targets across all pools (Figure 10). The median difference between pooled and individual samples for the N2 and E genes varied from 1.78 to 2.34 (Table 12).

Table 12: Median Ct-values and correlation for N2 and E targets in pools, irrespective of viral load by Cobas® SARS-COV-2 assay

Target	Individual median CT	Pool median CT	IQR of Pool	correlation	Median difference	No	Pool - no
ORF1a gene	27.95	29.73	(26.66-31.53)	0.98	1.78	36	pool-4
	27.93	29.80	(26.75-31.81)	0.98	1.87	36	pool-6
	28.04	29.90	(25.08-31.96)	0.98	1.86	37	pool-8
	27.82	29.97	(27.22-32.25)	0.97	2.15	35	pool-10
E gene	28.74	31.08	(27.17-32.89)	0.98	2.34	41	pool-4
	28.74	31.01	(27.28-3346)	0.98	2.27	40	pool-6
	28.95	31.24	(27.88-33.77)	0.98	2.29	41	pool-8
	28.53	30.61	(27.53-33.61)	0.94	2.08	40	pool-10



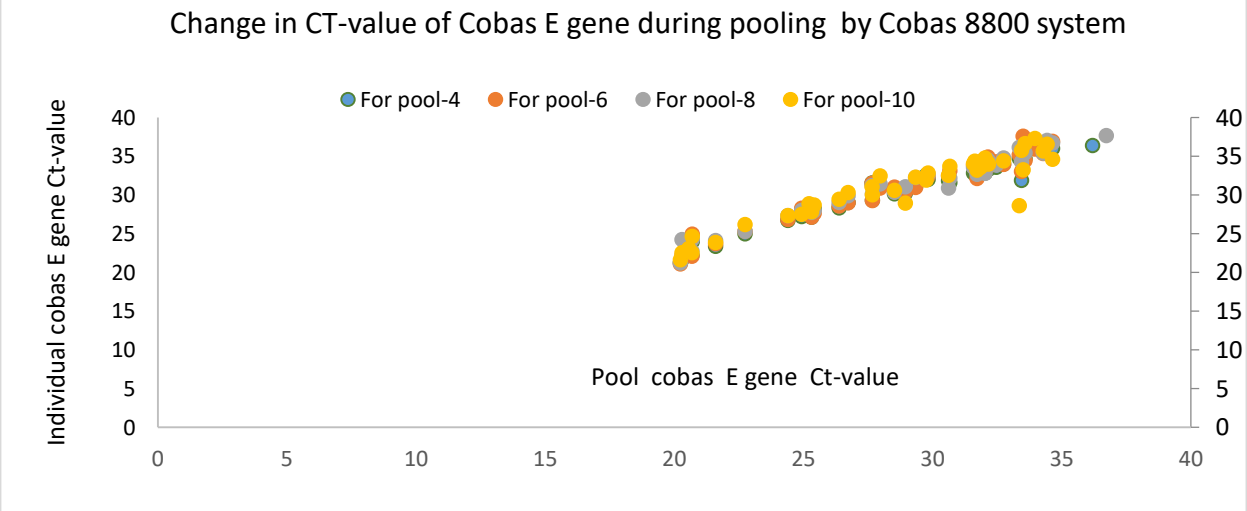


Figure 10: The change in CT-value of ORF1a and E gene resulting from pooling by Cobas® 8800®

5.6. Agreement between Xpert® Xpress and Cobas® SARS-COV-2 assay on pooled samples

According to figure 11, out of all pooled samples tested by both assays, 161 (80.5%) samples had a positive result, while 17 (8.5%) samples were found to be negative by both assays. The Overall Percent Agreement (OPA) for all pooled samples was found to be 89% (95% CI, 86.31% to 91.52%). The OPA for pool-4 was 84% (95% CI, 75.93% to 90.46%), pool-6 was 88% (95% CI, 80.90% to 92.12%), pool-8 was 90% (95% CI, 87.45% to 94.41%), and pool-10 was 86% (95% CI, 78.33% to 93.17%) with κ=0.552 regardless of viral load group.

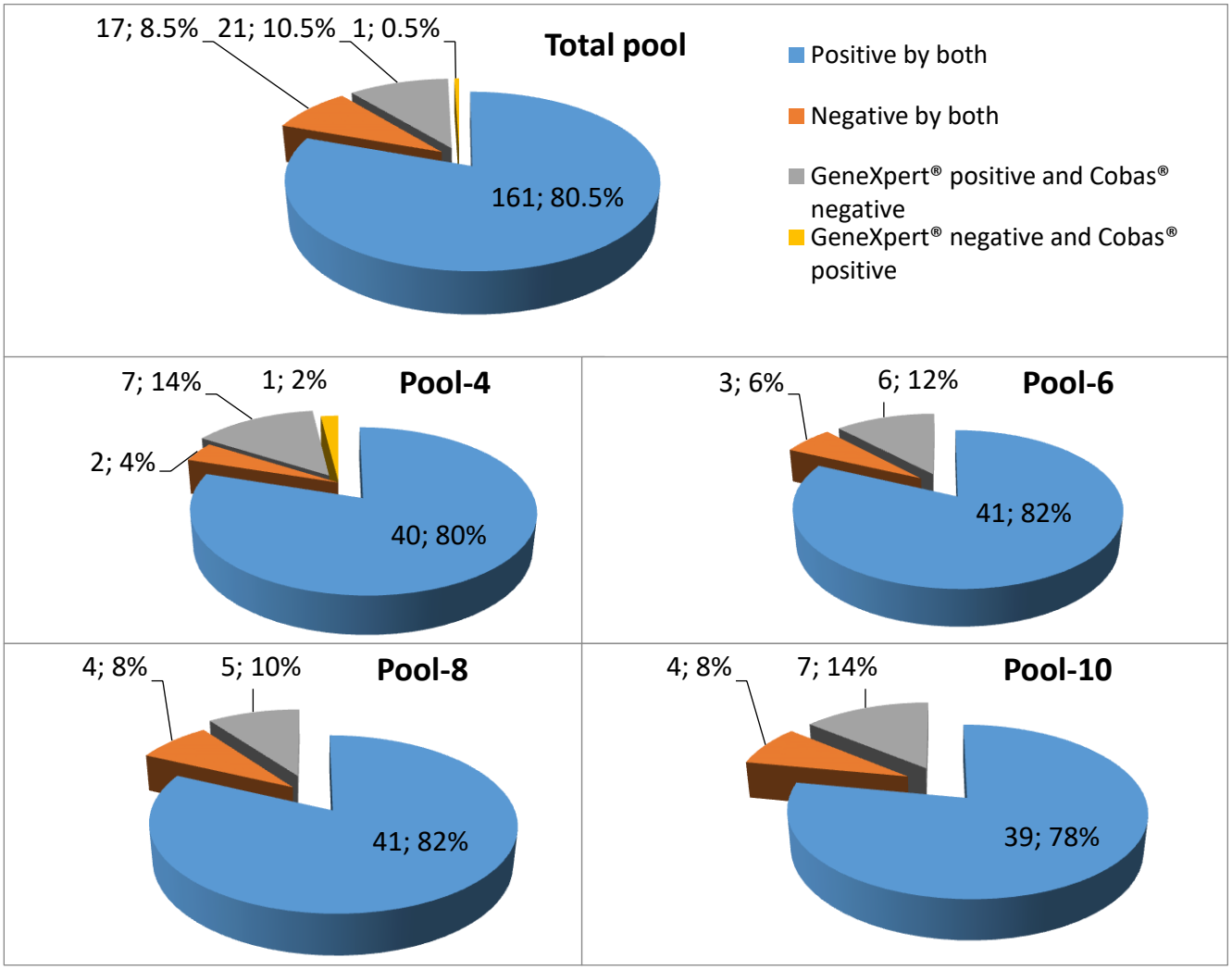


Figure 11: Comparison of Xpert® Xpress and Cobas® SARS-COV-2 assay for different pool size irrespective of viral load.

5.7. Whole Genome sequencing

To conduct Whole Genome Sequencing (WGS), a total of 16 nasopharyngeal samples that tested positive for SARS-CoV-2 by Xpert® were selected with Ct-value ≤ 30. These samples were subsequently sequenced. Most of the individuals from whom the viral sequences were obtained were male (12, 75%). The age of the patients ranged from 3 to 93 years. The Xpert® and Cobas® assay testing revealed that the highest CT-value for nucleocapsid gene and envelope gene was 27.70 and 31.10, respectively. The Qubit quality analysis of a SARS-CoV-2 genome-positive sample revealed a combined DNA concentration of 16.1 ng/µl.

Overall, the analysis of these samples using Pangolin and Nextclade confirms all of them fall under the classification of Omicron variant. Specifically, 10 (62.5%) samples belonged to clade 22A, 4 (25%) belonged to clade 22B, 1 (6.25%) belonged to clade 22B, and 1 (6.25%) belonged to clade 22K (table 11). The consensus sequence length of the samples ranged from 29,578 to 29,903 nucleotides, with a GC% ranging from 33.1% to 39.0% (table 13). The pairwise identity between the samples and the reference genome, which is the SARS-CoV-2 Wuhan strain (GenBank accession number NC_045512.2); ranged from 98.0% to 99.9%, and the gaps range from 80 to 2524 nucleotides. Fig 11 shows the consensus sequence length coverage of all our sequenced genomes.

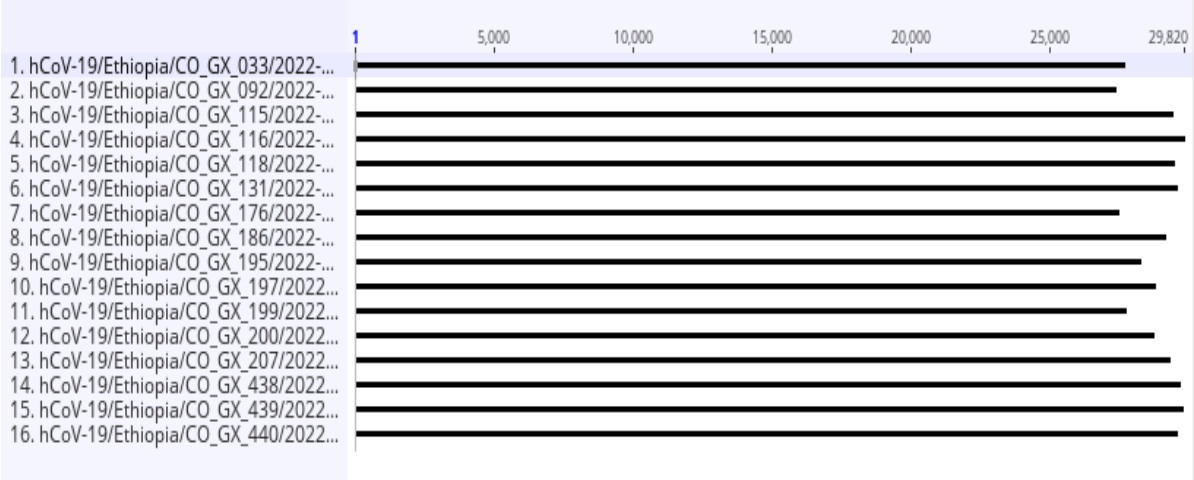


Figure 12: Consensus sequence length

The amino acid and mutational analysis of the SARS-CoV-2 genome using CoVsurver revealed that the average length of amino acids in the query sequences was 9519. Among the sequences, EPI_ISL_17615494 had the highest number of amino acids (9699).

Regarding the number of mutations it varied among the query sequences, ranging from 62 (0.64%) for samples with highest number of amino acids to 276 (2.99%) for samples with lowest number of amino acids. EPI_ISL_17616422 showed the highest number of unique mutations, with 85 (0.91%) observed. Conversely, no unique mutations were identified in EPI_ISL_17615494, EPI_ISL_17615495, EPI_ISL_17616423, and EPI_ISL_17615497. Existing mutations were detected in the sequences, with EPI_ISL_17616417 having the highest number of mutations at 206 (2.23%).

Several other AA changes on N and E gene were identified in the sequenced samples, including P13L P151S, N E31del, N R32del, N S33del, N P151S, N R203K, N G204R, and N S413R on the N genes and E T9I on E genes (Table 14). In this study, the AA changes P13L, P151S, N R203K and N G204R occurred in all samples that were sequenced, whereas the N P151S substitution was present in only 10 samples (figure 12). There is also a common mutation at position 9 in the E gene in all samples that were sequenced.

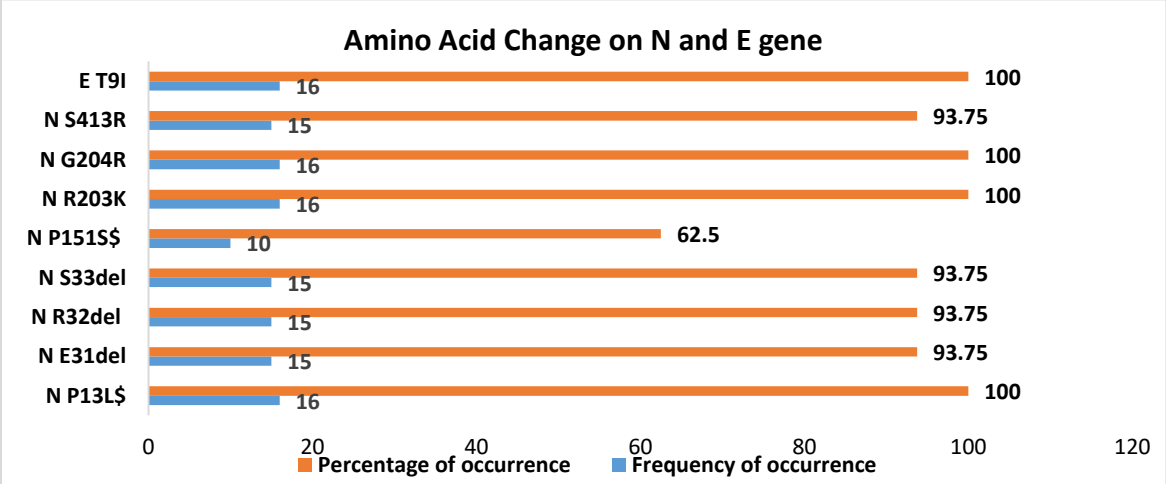


Figure 13: Type of amino acid change on N and E gene

**N refers to N gene * E refers to E gene*

Table 13: Nextclade tool, clade assignment, mutation calling, and sequence quality checks

Lab sample ID given	GISAID Accession ID	Consensus seq length	GC %	clade	Pango lin	WHO name	Cov %	gaps	% PI	No AA (%)	No M (%)	No UM (%)	No EM(%)
CO-GX-033	EPI_ISL_17616416	29,756	37.9	22A	BA.4.1	Omicron	98.9	1900	98.9	9404	199(2.12)	52(0.55)	147(1.56)
CO-GX-092	EPI_ISL_17616417	29,578	38.1	22A	BA.4.1	Omicron	99.8	2524	98.6	9236	276(2.99)	70(0.76)	206(2.23)
CO-GX-115	EPI_ISL_17616418	29,766	38.1	22B	BA.5	Omicron	99.9	498	99.2	9651	97(1.01)	27(0.28)	70(0.73)
CO-GX-116	EPI_ISL_17615494	29,903	37.6	22B	BA.5.2.1	Omicron	100.0	80	99.9	9699	63(0.65)	0(0.00)	63(0.65)
CO-GX-118	EPI_ISL_17616419	29,743	37.8	22B	BA.5.2.1	Omicron	99.9	411	99.0	9601	74(0.77)	12(0.12)	62(0.65)
CO-GX-131	EPI_ISL_17615495	29,850	38.1	22A	BA.4.1	Omicron	99.9	308	99.4	9621	62(0.64)	0(0.00)	62(0.64)
CO-GX-176	EPI_ISL_17616420	29,765	37.8	22A	BA.4	Omicron	99.2	2212	99.2	9352	145(1.55)	66(0.71)	79(0.84)
CO-GX-186	EPI_ISL_17616421	29,811	39.0	22A	BA.4.1.1	Omicron	99.9	756	99.2	9473	146(1.54)	2(0.02)	144(1.52)
CO-GX-195	EPI_ISL_17616422	29,724	37.9	22A	BA.4.1	Omicron	99.9	1613	99.0	9362	267(2.85)	85(0.91)	182(1.94)
CO-GX-197	EPI_ISL_17616423	29,858	38.5	22A	BA.4.1.1	Omicron	99.0	863	99.5	9509	94(0.99)	0(0.00)	94(0.99)
CO-GX-199	EPI_ISL_17616424	29,810	38.0	22A	BA.4.1.1	Omicron	99.9	2141	99.3	9362	225(2.40)	79(0.84)	146(1.56)
CO-GX-200	EPI_ISL_17616425	29,628	38.6	22A	BA.4.1	Omicron	99.9	1167	98.6	9531	141(1.48)	47(0.49)	94(0.99)
CO-GX-207	EPI_ISL_17616426	29,860	38.5	22A	BA.4	Omicron	99.9	580	99.3	9551	113(1.18)	15(0.16)	98(1.03)
CO-GX-438	EPI_ISL_17616427	29,839	33.1	22B	BA.5	Omicron	100.0	218	99.4	9653	64(0.66)	1(0.01)	63(0.65)
CO-GX-439	EPI_ISL_17615496	29,886	37.9	22K	BA.1.1	Omicron	100.0	137	99.7	9673	94(0.97)	24(0.25)	70(0.72)
CO-GX-440	EPI_ISL_17615497	29,848	38.1	22B	BA.5	Omicron	99.9	301	99.5	9625	57(0.59)	0(0.00)	57(0.59)

* *Pango lin is Pango lineage (Nextclade) which is inferred by Nextclade from the nearest neighbour in the reference tree.*
* *mut* reperesents mutations which displays number of mutations in the sequence.
* *Non-ACGTN* displays number of non-ACGTN characters in the sequence.
* *Ns* displays number of N characters (missing data) in the sequence.
* *Cov* displays coverage of the sequence.
* % PI (%Pairwise identity): refers to the percentage of identical nucleotide or amino acid residues when comparing to Wuhan strain.
* EM: Existing Mutations * UM: Unique Mutations * No M: Number of Mutation * No AA: Number of amino acids

Table 14: Amino acid changes and their frequencies in N and E Genes

GISAID ID	N gene				E gene			
	% Idn	% cov	#Δs	List of aa changes	% Idn	%cov	#Δs	aa change
EPI_ISL_17616416	98.80	99.30	8	P13L$, E31del, R32del, S33del, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616417	98.80	99.30	8	P13L$, E31del, R32del, S33del, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616418	99.00	99.30	7	P13L$, E31del, R32del, S33del, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17615494	99.00	99.30	7	P13L$, E31del, R32del, S33del, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616419	99.00	99.30	7	P13L$, E31del, R32del, S33del, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17615495	98.80	99.30	8	P13L$, E31del, R32del, S33del, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616420	98.80	99.30	8	P13L$, E31del, R32del, S33del, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616421	98.80	99.30	8	P13L$, E31del, R32del, S33del, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616422	98.80	99.30	8	P13L$, E31del, R32del, S33del, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616423	98.80	99.30	8	P13L$, E31del, R32del, S33del, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616424	98.80	99.30	8	P13L$, E31del, R32del, S33del, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616425	98.80	99.30	8	P13L$, E31del, R32del, S33del, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616426	98.80	100	5	P13L$, P151S$, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17616427	99.00	99.30	7	P13L$, E31del, R32del, S33del, R203K, G204R, S413R	98.70	100	1	T9I
EPI_ISL_17615496	99.30	99.30	6	P13L$, E31del, R32del, S33del, R203K, G204R	98.70	100	1	T9I
EPI_ISL_17615497	99.00	99.30	7	P13L$, E31del, R32del, S33del, R203K, G204R, S413R	98.70	100	1	T9I

**% Iden: percent identity *% cov: percent coverage *#Δs: number of deletions*

** List of aa changes: list of amino acid changes*

6. Discussion

The objective of the study was to assess the performance of the Xpert® Xpress and Cobas® SARS-CoV-2 assays in detecting the presence of SARS-CoV-2 in both individual and pooled nasopharyngeal swab samples. Additionally, to sequence the genomes of selected SARS-CoV-2 samples that tested positive to identify variants, lineages, sub lineages and mutations present in Addis Ababa, Ethiopia.

6.1. Diagnostic performance of Xpert® Xpress SARS-COV-2 assay.

This study observed variations in the prevalence of SARS-CoV-2 between the diagnostic assays tested. The Xpert® Xpress assay detected a higher number of positive results compared to the Cobas® SARS-COV-2 assay. In this study, 21.6% of the samples tested positive for SARS-CoV-2 using Xpert®, whereas Cobas® assay detected 13.0% definite positive results along with 3.9% presumptive positive that needed additional confirmatory tests. The 21.6% positivity result is consistent with the finding from a cross-sectional study conducted in Oman by Al-Kindi et al., which reported a positivity rate of 25.16% (70). Even though there was a significant time gap between the two studies, the similarity in the study design and sample collection methods may have contributed to the concordance in the prevalence rates observed (70). Both studies collected nasopharyngeal swabs not from the general population but from targeted individuals who met the case definition of being suspected to have COVID-19, according to their respective national guidelines. On the contrary, a different study from Hong Kong Adventist Hospital in Hong Kong yielded a higher positivity rate of 43.0% from 79 tested specimens, including 9 nasopharyngeal swabs and 70 saliva samples between August 2020 and March 2021 (54). Various factors could be attributed to such big prevalence gap between the rates in the former two studies and the 43% reported in the latter study from Hong Kong: differences in sample size, demographics, source of samples, testing methodologies, or actual differences in COVID-19 prevalence at the time of the studies (e.g., where Hong Kong's proximity to the epicenter of COVID-19 [Wuhan, China] may have contributed to larger prevalence rate).

Interestingly, in the current study, no presumptive positives were detected from Xpert® Xpress SARS-COV-2 assay. However, in a study conducted elsewhere (13) , a

presumptive positive result was sample detected by Xpert® from one sample at the E-gene target but not at the N2 target. This latter observation indicates the importance of considering multiple targets for accurate and reliable detection of SARS-CoV-2. In regards to effectiveness of the Xpert® Xpress SARS-COV-2 assay, this study identified 3.6% errors associated with this assay, which were largely post-run analysis errors caused by probe check failure. The result showed that the 5007 error code was the most common error code, which may be caused by various factors such as inadequate sample volume, presence of PCR inhibitors in the sample such as food or other particles, or issues with the testing process. This same error rate was reported from a study conducted by Noble et al, in which the authors recorded an error rate of 3.8% from the same assay (58). Out of the 18 unsuccessful Xpert® results, four were found to be positive when retested using the same assay. This highlights the significance of repeating unsuccessful tests for confirmation of their true positivity.

In contrast to Xpert® Xpress SARS-COV-2 assay which identified no presumptive positive result, the Cobas® test identified a 17(3.9%) samples as presumptive positive for SARS-CoV-2, which made additional confirmational re-testing necessary to determine their true positivity, which was done using the Xpert® assay. Up on confirmation more than half of the samples (9, 52.9%) were confirmed positive for SARS-CoV-2. Among the confirmed positive samples, seven were positive for both the N2 and E gene targets, while two samples were positive for the N2 gene target only. Similar to the finding in this study, other investigators also documented presumptive positive results from Cobas® assay. For example, study by Pujadas et al., observed a 0.79% presumptive positivity, which is lower than the 3.9% observed in the current study (19).

Reports on confirmation of Cobas® assay-produced presumptive positive results by re-testing the samples with Xpert® assay were common in other studies. For example, Poon et al., reported that they found 17 out of 24 initially presumptive positive samples to be positive on both E and N2 target genes with Xpert® assay (57). Moreover, they also observed that while two samples was the E gene target only, 4 samples were positive for the N2 gene target only, and 1 sample tested negative for both gene targets when assessed using the Xpert® assay (57). This suggests that presumptive positive results could indicate the presence of SARS-CoV-2, negative samples, or other sarbecoviruses.

Therefore, it is very important to repeat the testing using other PCR assays, such as Xpert® to further investigate and confirm the results, rather than dismissing them as one of the aforementioned result possibilities. Furthermore, the results support the recommendation forwarded by researchers (e.g., Poon et al; (57)) regarding the importance of using multiple molecular kits and assays with more than one viral gene targets particularly in situations where the initial test results are presumptive positive or unsuccessful.

6.2. Ct-values for Individual sample

The Ct-values obtained from both the Cobas and GeneXpert® platforms provide valuable information about viral load and can be utilized to monitor the spread of SARS-CoV-2 in the population. It is important to consider these Ct-values when interpreting SARS-CoV-2 test results and implementing appropriate public health measures to control the transmission of the virus.

The Ct-values obtained from both the Cobas® and GeneXpert® platforms provide valuable information about viral load and can be used to monitor the spread of SARS-CoV-2. In this study, significant variation in the Ct-values was observed obtained for different targets and the two assays. The Xpert® Xpress SARS-CoV-2 assay exhibited a wide range of Ct-values for the N2 and E gene targets (median Ct-values of 37.20 vs 31.10, respectively), which were consistent with the findings of Al-Kindi et al., (median Ct-values) (70), and Angelica Moran et al (median Ct-values) (15). Contrary, the Cobas® SARS-CoV-2 assay demonstrated a narrower range of Ct-values for the ORF1a and E gene targets (median values of 30.54 vs. 31.73, respectively), as compared to Xpert® Xpress SARS-CoV-2 assay. Median Ct-values for E target gene in both assays were very close, 31.73 in Cobas® assay vs. 31.10 in Xpert® assay, showing this target gene is equally detected by both assays. Compared to Ct-values for N2 and E target genes of Xpert® assay reported from other similar studies, the Ct-values from this study are comparable with some (e.g., 38.30 and 35.80 for the N2 and E, respectively (29)), but higher than others (e.g., 31.6 and 29.7 for the N2 and E gene targets, respectively (51)). Overall, however, this shows that the median Ct-values generated from Xpert® assay across different studies were more-or-less consistent, providing further evidence on the reliability and reproducibility of these measurements from Xpert® assay.

In this study, in contrast to Xpert® assay where difference in median Ct-values between the two target genes (N2 and E) was larger, median Ct-values for the ORF1a and E gene targets in Cobas® assay were close to each other (30.54 and 31.73, respectively). As was the case for Xpert® assay and for the same reasons discussed above for Xpert® assay, median Ct-values lower (21.8 and 22.2, respectively (71)) and higher (31.29 and 36.90, respectively (29)) than these values were reported from other studies also.

Nevertheless, it should be kept in mind that differences in median Ct-values obtained from different studies for a given target gene does not necessarily indicate inconsistency of the test assay, as several other factors may have their impacts on results of the tests such as sample collection and handling techniques employed, prevalence of COVID-19 in the given area and diversity of investigated population (e.g., geographic areas where the pandemic hit hardest vs. areas where it has been moderate), source of sample (e.g., sample from nasopharyngeal vs. nasopharyngeal plus throat vs. saliva), stage of the infection (e.g., early infection vs. established infection), level of pathogenicity of the infecting virus (e.g., low or moderately virulent causing milder disease vs. highly virulent variant causing severe infection with concomitant high viral shading), immune status of the patients, etc.

6.3. Test agreement between Xpert® Xpress and Cobas® for SARS-CoV-2 testing and their targets for detection.

Based on the findings of this study, there is a high degree of agreement between the Xpert® Xpress and Cobas® for SARS-CoV-2 assay for the detection of SARS-CoV-2. The overall 91.73% agreement and a 0.715 Kappa value indicate substantial agreement, between the two methods (72). However, these results are slightly lower than concordance rate reported in some previous studies such as those conducted by Tham et al., Moran et al., and Broder et al., which reported higher agreement rates of 95%, 99%, and 97.14%, respectively (15,29,30). Variations in study design, patient populations, sample types, and study procedures can influence the observed differences in concordance rate between different studies. In the study by Tham et al., (29) they used comparable sample size and similar sample type to the current study, which resulted in agreement rates closer to the current study compared to the other studies mentioned here. Moran et al., collected eight nasal and 95 nasopharyngeal specimens from inpatients and

ambulatory patients, which could potentially increase the positivity rate (15). The current study included participants who were symptomatic, irrespective of the severity of their symptoms. The observed agreement differences may have been influenced by variations in sample selection criteria between these studies. On the other hand, Broder et al., used 35 positive nasopharyngeal samples with an E target CT value of ≥30 on the Cobas® assay, retested with Xpert® Xpress assay and the agreement was high between the two assays (30).

The 98.25% positive percent agreement (PPA) and 90.71% negative percent agreement (NPA) between Xpert® Xpress and Cobas® SARS-COV-2 assays in this study indicate a strong performance agreement between the two assays. These results align with previous studies where it was reported 98.9% PPA and 92.0% NPA by Smithgall et al. (13); and 100% and NPA of 98.0% NPA by Tham et al. (29). Such high agreement between the Xpert® Xpress and Cobas® SARS-COV-2 assay is encouraging, as it validates the reliability and accuracy of both assays for SARS-CoV-2 detection

In this study, discordant between the two assays were observed, with one sample tested positive on Cobas® but negative on Xpert®, and 34 samples testing positive on Xpert® only. Similar findings were reported by Tham et al., where 11 samples were positive only by Xpert® and one sample was detected positive by Cobas® only (29). Additionally Smithgall et al., reported two samples tested positive by Xpert® only (13). These findings indicate that the Xpert® assay have higher sensitivity compared to the Cobas® assay.

Among the 34 discordant samples in the current study (17 of which were presumptive positive for Cobas®), six were positive for both N2 and E gene targets, while twenty eight samples were positive for N2 gene but negative for the E gene of Xpert® assay. This retesting Cobas® presumptive positive samples using the Xpert® assay demonstrated that the Xpert® N2 gene showed greater sensitivity in detecting cases with low viral load compared to the Cobas® ORF1a gene. Supporting this finding, a study conducted by Tham et al., reported that the N2 gene was detected in 98% of samples (57 out of 58), whereas the ORF1a gene was only detected in 16% of samples (9/58) (29). These findings support the idea put by Poon et al. that the N2 gene persists for a longer duration compared to fragments from the ORF1a gene. It also suggests the importance of

utilizing multiple molecular assays that target more than one viral gene (57). Despite differences in test principles and PCR cycling steps between the Xpert® and Cobas® assays, our data agrees with previous studies that demonstrate similar performance of the pan-sarbecovirus E gene targets in both assays (15,29,30).

Based on the findings, both assays can be used interchangeably for routine COVID-19 diagnostics and especially Xpert® Xpress assay can be used for the detection of low-positive SARS-CoV-2 samples. The similarity in performance and consistent results between the two assays support their suitability for accurate detection in various testing scenarios.

6.4. Pool testing of nasopharyngeal swabs by Xpert® for detection of SARS-CoV-2.

The results of this study indicate that pooling samples for SARS-CoV-2 testing can be an effective strategy, as a high proportion (a total of 182 /200; 91.0%) of pooled samples tested positive regardless of their vial load groups and pool numbers. In the current study, each pool size consisted of 50 pooled samples. When initially positive samples were pooled with negative samples in pool four and six, there was a 47(94%) agreement between pooled and individual testing, demonstrating a high level of concordance. In contrast to our study, Vibol Iem et al., utilized a different sample type (nasopharyngeal and oropharyngeal) and methodology, where individual samples within pool four tested simultaneously (60). Despite these differences, both studies yielded the same results, demonstrating the reliability of pooling samples to pool four with the Xpert® assay. Similarly, Graham et al., reported a 100% agreement for both pool four and pool six (31). (31). In the study, similar sample types and methodology were used, but the sample size was smaller (7 positive and 24 negative samples). In contrast, the current study increased the sample size, which led to a higher probability of detecting negative results. When the effect size (i.e., the agreement of pooled and individual sample) is larger, it requires a smaller sample size (36). Both studies suggested that testing samples in pools of four or six using Xpert®, was expected to retain accuracy of the test irrespective of the CT-value (relative RNA copy number) of the individual sample spiked and tested. Additionally, the FDA's report on October 11, 2022, which employed the same sample type and pre-selection of positive samples for pooling, revealed a 95% positive for pool five (11).

This suggests that pooling positive samples into both four and five may yield comparable results for Xpert®.

Looking into effectiveness of pooling at each pool level, this study also indicate a high level of concordance for pool eight and ten, with an agreement of 45(90%) and 43(86%) respectively. The results are consistent with the FDA's findings, which indicated a 90% agreement for pool eight (11) and a study conducted Procop et al., which reported an 85% concurrence for pool ten (59). This suggests that pooling positive samples into eight and ten may yield comparable results with other studies.

An interesting observation regarding effectiveness of pooling is that there was no statistical significant difference in the correlation between pool four, pool six, pool eight, and pool ten, indicating that the accuracy of the test was not significantly affected by the number of samples used for pooling. The findings of this study were consistent with previous researches that has demonstrated the utility of pooling samples to increase the efficiency and cost-effectiveness of testing (33,65).

However, initial individual sample viral load had impact on the level of positivity when pooled. For example, after pooling the initial positive individual samples into pool four to ten pool size, both the medium and high viral load groups had a 40/40 (100%) proportion of positive samples. While, the low viral load group showed positive results in 59/60 (98.3%) cases, while the very low viral load group had positive results in 36/60 (72.0%) cases. These differences in the proportion of positive samples between the viral load groups were found to be statistically significant, suggesting that the accuracy of SARS-CoV-2 test results can be influenced by the viral load present in the samples. Samples with higher viral loads are more likely to yield positive results, while samples with lower viral loads may have a higher chance of producing false-negative results, during pooling.

The correlation analysis between the Ct-values of individual and pooled samples revealed strong correlations for both probes. However there was a slight increase in the median Ct-values, as the pool size increased. The observed difference was particularly pronounced for probe E compared to probe N2, suggesting that the non-specific E gene was more affected by dilution than N2. These results also support previous studies that have reported a higher detection rate for the N gene compared to the E gene on Xpert® Xpress SARS-COV-2 assay (57,59).

The analysis of the viral load group revealed significant differences when initially positive individual samples were pooled into groups of eight and ten, whereas no significant differences were observed in pools of four and six. These results support the suggestion by Vibol Iem et al., that accuracy of pooling results can be influenced by the viral load, indicating the potential for missed samples with viral loads near the limit of detection of the assay when testing samples in pools (60). These findings suggest that using sample pooling up to six samples can be an effective strategy to increase testing capacity. The results indicate that the pooling outcomes were not affected by the pool size or the initial viral load group. Therefore, implementing sample pooling in low-resource settings, where testing resources are limited, it is possible to increase testing capacity (60).

6.5. Effectiveness of pooled testing for SARS-CoV-2 detection using the Cobas® assay

The results of this study indicate that pooling samples for SARS-CoV-2 testing can be an effective strategy, as a high proportion (a total of 162 /200; 81.0%) of pooled samples tested positive regardless of their vial load groups and pool numbers. The results of indicate that pooling samples for SARS-CoV-2 testing using the Cobas® assay can also be an effective strategy for detection of the infection, as the proportion of positive samples did not significantly differ between different pool sizes, suggesting that the accuracy of the test results from pooling may not be affected by the number of samples used for pooling.

When the initially positive samples were pooled with negative samples in pools four and six, we observed a 41 (82.0%) agreement between the pooled testing and individual testing methods. This level of concordance indicates a reasonably good agreement between the two approaches. However, it is important to note that the concordance rate is relatively weak compared to the Xpert® assay. In a study conducted by Barat et al., they reported a sensitivity of 94% for detecting positive specimens in a pool of five saliva samples when compared with individual testing (61). This suggests that their pooled testing approach demonstrated a higher sensitivity than what we observed in our study. The higher sensitivity reported by Barat et al., (61) could be attributed to several factors,

including differences in the sample types (saliva vs. our samples), the pooling strategy used, the testing methodology employed, or variations in the population characteristics.

The Ct-values of the pools were found to be more than the individual positive samples in this study and on other study conducted elsewhere but detected one positive sample exception (74). In this study, we calculated the mean difference or average loss of signal, which was found to be 1.78 to 2.34 CT values compared to the individual samples for each target. This indicates that there was a slight decrease in the signal intensity or amplification efficiency in the pooled samples compared to the individual samples. These findings are comparable to the results reported by Barat et al., who also observed a 2 to 3 CT value difference between pooled and individual testing (61). In line with the findings reported by Barat et al. (61), we also observed a decrease in the level of positivity as the viral load levels decreased. This means that samples with lower viral loads were less likely to be detected using our pooled testing approach.

The evaluation conducted by McMillen et al. using the 6-pool approach and the Cobas® assay demonstrated a sensitivity of 100% when pooling nasopharyngeal samples (38). This indicates that all positive samples were successfully detected using their pooling strategy and testing methodology. However, in the current study, a lower positivity rate of 41 (82%) was observed in pool-6. It is important to consider that the differences in the observed positivity rates could be attributed to various factors, including the sample characteristics and the selection criteria used in each study.

In McMillen et al.'s study, the majority of the samples selected for pooling had CT values less than 34 for both the ORF1a and E gene (38). Lower CT values generally indicate higher viral loads and higher concentrations of viral genetic material in the samples. Therefore, the selection of samples with relatively low CT values in their study may have contributed to the higher sensitivity observed in their pooled testing approach. In contrast, in the current study, more than half of the samples (120 out of 200) were obtained from individuals with CT values less than 30 for both targets. It is worth noting that the selection of samples in the current study was aimed at mimicking real-life situations, where the distribution of CT values in the population may vary. This approach allows for a more comprehensive evaluation of the performance of pooled testing in diverse

scenarios, capturing the challenges and limitations that may arise when testing samples with different viral load levels.

These findings highlight the importance of considering the viral load dynamics and the distribution of CT values when designing and interpreting the results of pooled testing studies. The selection criteria for samples and the choice of testing methodology should be carefully considered based on the specific goals and context of the study. Further studies and optimization of pooled testing strategies are necessary to enhance the sensitivity and accuracy of pooled testing approaches in various settings and population groups.

The study findings demonstrate the usefulness and effectiveness of pooled sampling, particularly when implemented on a high-throughput machine like the Cobas 8800. The simplicity of the method, its alignment with approved practices, and the absence of specific sample handling requirements or additional information make it easily implementable on a larger scale. By adopting a pooling strategy, testing time, workload, and reagent consumption can be significantly reduced, leading to a substantial increase in productivity for clinical diagnostic laboratories. Additionally, the ability to screen large populations for the presence of SARS-CoV-2 infection becomes feasible and productive.

However, it is important to consider the potential impact of viral load variation on the accuracy of the assay across different pool sizes. As the viral load of positive samples can affect the assay's performance, careful consideration should be given to determining the optimal pool size and setting appropriate thresholds for detecting positive results. This ensures that the benefits of pooling, such as increased efficiency and productivity, are balanced with maintaining the accuracy and sensitivity of the assay.

Overall, the study underscores the potential of pooling strategies to revolutionize COVID-19 testing and screening efforts. By implementing pooling effectively, clinical diagnostic laboratories can streamline their operations, conserve resources, and contribute to the timely detection of SARS-CoV-2 infections in larger populations (31,37).

6.6. Agreement between Xpert® Xpress and Cobas® SARS-COV-2 assay on pooled samples

Based on the findings of this study, it can be suggested that pooling samples can be an effective method for detecting SARS-CoV-2 using both Cobas® and GeneXpert®

systems. The study revealed an Overall Percent Agreement (OPA) of 89% for all pooled samples, indicating moderate agreement between the two assays Mary L. McHugh Interrater reliability: the kappa statistic interpretation (72). However, it is worth noting that the previous studies did not specifically assess the agreement of the assays on pooled samples, which could be an area for further study. The findings support the potential of sample pooling as a viable strategy for SARS-CoV-2 detection, offering benefits in terms of testing capacity and resource utilization.

6.7. Whole Genome sequencing

The Whole Genome Sequencing result from this study suggests that Omicron was the dominant SARS-CoV-2 variant in Ethiopia during the months of June and July. This is consistent with the global spread of the Omicron variant during that period (75). Specifically, the BA.4.1 subvariant of the Omicron variant was found to be very common in Ethiopia, while the BA.5 subvariant was more prevalent in Macau and the US during the same period (66). Another study conducted in Ethiopia supports these findings, indicating that the majority of cases sampled and sequenced after January 2022 were attributed to the Omicron lineage (66). The samples analyzed showed some variations in terms of mutations, insertions, and pairwise identity, suggesting some level of diversity within the variant in the country. The phylogenetic tree analysis revealed close relatedness among sequences assigned to the same lineages. This implies that the sequences analyzed in the study shared a common ancestry and were relatively similar to each other, without substantial levels of divergence.

Amino acid change and Mutations of N gene and E gene

The analysis compared the nucleocapsid gene of 16 different strains with the reference genome N WIV04 on the GISAID covsurver mutations application (https://gisaid.org/database-features/covsurver-mutations-app/). This analysis identified two protein substitutions that cause antigenic drift within the nucleocapsid protein of the SARS-CoV-2 virus in all samples analyzed in this study. The first substitution, known as P13L, replaces proline with leucine at position 13. This substitution has been observed globally about 1.5 million times (45.10% of all global samples with N sequence) in 184 countries. The initial occurrence of this substitution was detected in South Korea in

February 2020, with the most recent observation in India in April 2023 (24). This substitutions of the proline (additionally P13S and P13T) at position 13 of the N gene may affect the B*27:05-restricted CD8+ nucleocapsid epitope recognition which was shown to result in complete loss of responsiveness to the T-cell lines evaluated (65). The second substitution, P151S, involves the replacement of proline at position 8151 with serine within the N protein. This substitution has been observed in 10 samples in this study and occurred 47,086 times (1.46% of all samples with N sequence) in 125 countries (24). It has been reported in the literature that this substitution impacts a specific T cell epitope within the nucleocapsid protein, further contributing to antigenic drift (65). Additional substitution mutation R203K and G204R was identified from N gene in all samples analyzed in this study. These findings agreed with other study mentioned R203K (Arginine→Lysine) + G204R (Glycine→Arginine) substitutions that occur most frequently during N protein evolution in only omicron (24). Further studies are important on N gene of SARS-CoV-2 because the impact of mutations in the N-gene on the accuracy of commercially available PCR assays for SARS-CoV-2 diagnosis has not been thoroughly investigated (76). But in a study conducted by Alkhatib et al. (77), the authors observed N gene target failure for the first time, as far as they were aware, when using the Seegene Allplex SARS-CoV-2 assay.

The E T9I mutation refers to the substitution of the amino acid glutamic acid (E) at position 9 with isoleucine (I) within the Envelope protein. This mutation was found in all samples sequenced in this study. Additionally, based on the search on GISAID, the E T9I mutation has occurred globally for 1.5 million times, accounting for 45.00% of all samples with E sequence, across 180 countries as of May 15, 2023 (24). These findings indicate that the E T9I mutation is widespread and has been observed in a significant number of samples globally (24). The non-spike proteins have multiple significant roles in immune regulation, transcriptional regulation, and viral pathogenesis. But, there reside gaps in knowledge regarding their specific mode of action and roles, especially in terms of unique mutations of Omicron (24). The emergence of new variants has a direct correlation and a significantly positive association with the increase in the number of cases and prevalence in Ethiopia (66).

7. Strength and Limitation of the study

The study had several strengths. Firstly, the tests were performed in three different laboratories in EPHI, with each test performed in a different laboratory. The laboratory personnel who conducted the tests were blinded to each other's results. To increase the statistical power and improve the accuracy of the results, the study used a large sample size. Secondly, the samples were analyzed immediately to prevent RNA degradation. They were stored in a calibrated and daily monitored -80°C freezer until they were required for pooling and sequencing. In order to simulate real-life situations, positive samples with low viral loads were intentionally included in the pooling process, rather than solely relying on high viral load samples.

There are several important limitations to this study. Firstly, the study was unable to sequence the discordant results due to the lack of primers for target sequencing for those specific genes, and the whole genome sequencing of these results was not possible because the Ct-values for these genes were above 35, which made them unsuitable for WGS. The study also only focused on nasopharyngeal samples and used the Xpert® Xpress CoV-2 assay, which was an older version that only used N gene and E gene, whereas a newer version was approved by FDA in September 2022 that included RNA-dependent RNA polymerase (RdRp) gene as a third gene target for SARS-CoV-2 detection. Additionally the study did not include a comparative analysis with other bioinformatics pipelines. Therefore, the effectiveness and performance of the chosen pipeline could not be evaluated in relation to alternative methods. The mutational analysis focused solely on the N gene and E gene. As a result, the bioinformatics analysis did not address the amino acid changes and mutations in other genes that might be relevant to the research question.

8. Conclusion

This study, which involved 440 individuals suspected of having SARS-CoV-2, found that the Xpert® Xpress SARS-COV-2 assay detected more positive results than the Cobas® assay. Using more than one molecular assay with more than one viral gene targets can maintain high sensitivity and reproducibility of the test results. The Xpert® assay targeting the neuclocapsid gene was more sensitive in detecting cases with low viral load than the assay that utilizes the ORF1a gene of Cobas®. Confirming Cobas® presumptive positive results by different methods is important to prevent misdiagnosis and improve patient care. Both the Xpert® E gene and Cobas® E gene targets exhibited similar performance in this study.

The comparison study indicates that Xpert® Xpress and Cobas® SARS-COV-2 assay can be used interchangeably for diagnostic purposes as they had strong concordance in both PPA and NPA, providing assurance to laboratories who may utilize different testing assays. The consistent findings across multiple studies, including the present study, strengthen the evidence for the reliability of these assays in detecting SARS-CoV-2.

Pooling samples up to six has been found to be an effective strategy on Xpert® Xpress assay, irrespective of the viral load and dilutional effect. Nevertheless, samples with lower viral loads have shown a tendency to yield negative results when pooled with other negative samples, particularly in the case of pool-8 and pool-10. The effect of lower viral load samples was observed in all pool sizes when utilizing the Cobas® assay, regardless of the number of samples combined in each pool. There was moderate agreement on pooling samples between the two assays, indicating that either one can be used for pooled testing, but the pool size or assay being used should be considered.

Omicron variant of SARS-CoV-2 was dominant in Ethiopia during the months of June and July, 2022. The phylogenetic tree analysis revealed close relatedness among sequences assigned to the same lineages. The amino acid and mutational analysis of the SARS-CoV-2 genome reveals variations in amino acid length, and the frequency of unique and existing mutations among the analyzed sequences. These findings provide insights into the genetic diversity and potential evolutionary changes within the SARS-CoV-2 virus in Ethiopia. These variations may have implications for the virus's antigenicity, infectivity, and response to immune recognition.

9. Recommendations

- Employ diverse molecular assays and viral gene targets in the lab for enhanced RNA virus detection, including SARS-CoV-2.
- Clinicians must recognize the test limitations, including assay and gene target variations, as they greatly impact the accuracy of SARS-CoV-2 detection.
- Implementation of sample pooling can provide significant benefits in low-resource settings with limited testing resources, effectively increasing overall testing capacity, especially when utilizing the Xpert® Xpress assay.
    - ✓ To increase testing capacity and regardless of viral load, it is recommended to implement sample pooling of up to six samples using Xpert® assay.
    - ✓ It is important to note that the pooling results were affected by the initial viral load group for all pool sizes of the Cobas® assay.
- Further studies are needed to examine and explore
    - ✓ The underlying causes of discordant results and identify the factors that contribute to variations in agreement rates across different studies.
    - ✓ The factors that influence variations in CT values and understand their clinical implications.
    - ✓ The functional implications of observed amino acid changes and their effects on the behaviour, transmissibility, and virulence of the virus.
    - ✓ The impact of different bioinformatics pipelines on the results, taking into consideration the availability of the consensus FASTA file from this study in the GISAID database.

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11. Annex

Annex I: Participant information and Consent form for adult >18 years [English version].

Good morning/afternoon/evening.

My name is Betselot Zerihun Ayano. I'm studying for my Master's degree in Medical Microbiology at Addis Ababa University. Now, I'm conducting research on "The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia.'' This information will help to improve the laboratory diagnosis of SARS-COV-2 across the country. The study recruits eligible people to take part on voluntary basis. Therefore, we would like to invite you to participate in this study.

Procedures: The procedure followed in this study includes a brief questionnaire that will seek to gather socio-demographic and general clinical condition-related information from the attending physician. If you are willing to participate in the study, we would like you to give us a nasopharyngeal sample for laboratory analysis.

Risks and benefits of participating in the study

An estimated 5 minutes interview will be conducted for you. There is no risk you face due to the interview. Even though there is little discomfort during sample collection, there is no risk of serious invasive procedures at the beginning as well as at the end of the study. There is no financial or other benefit you will get from this study. The results of this study will be used to improve SARS-CoV-2 diagnosis in the country. Your COVID-19 testing result will be communicated by phone or mobile text to you and also will be reported to the attending clinician within 24 hours.

Confidentiality: If you are not willing to participate in the study, no information will be collected from you. However, if you intend to take part in the study, the information you give me and your laboratory analysis results will be kept confidential and will not be shared with anyone outside of this study. Personal identifying information will not be shared outside of the study. Your name and another personal identifier will not appear on

any reports if we present this study or publish the results. We will not use your name and details for the analysis of the data.

Voluntary participation and withdrawal: As stated above, participation in this study is solely based on your willingness. If you are not willing to participate in this study you can withdraw at any time. You will be allowed to read or else be read to you the information sheets of the study. If you are willing to participate in the study, you will sign this consent form. The routine services are provided to you as usual even if you are not willing to participate in this study.

Contact person: If you have any questions related to this study, you can call and contact using the following contact information.

Principal Investigator: Mr. Betselot Zerihun

Mobile Number: +251911567355,

E-mail betselotzerihun3@gmail.com

Statement

Your signature below indicates that you have read or listened to and understood the information provided to you about the study entitled "The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia.''

I have read and/or listened to the description of the study and I understand what the procedures are and what is expected from me as a participant in the study. I agree to participate in it and I know that I can quit at any time.

Are you willing to participate in the study? *(Interviewer: circle correct answer)*

1- Yes 2 – No

Name and signature of Participant :-_____

Date ……/……/……

I, the data collector clinician, sign hereunder ascertain that I have introduced the participant about the study.

Name and signature :-_____

Annex II: Information and Ascent form for Participants between 12-17 years of age) [English version].

Good morning/afternoon/evening.

My name is Betselot Zerihun Ayano. I'm studying my Master's degree in Medical Microbiology at Addis Ababa University. Now, I'm conducting research on Title: "The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia." This information will help to improve the laboratory diagnosis of SARS-COV-2 across the country. The study recruits eligible people to take part on voluntary basis. Therefore, we would like to invite you to participate in this study.

Procedures: The procedure followed in this study include a brief questionnaire that will seek to gather socio-demographic and general clinical condition related information from attendant physician. If you are willing to participate in the study, we would like you to give us nasopharyngeal sample for laboratory analysis.

Risks and benefits of participating in the study

An estimated 5 minutes' interview will be conducted for you. There is no risk you face due to the interview. Even though there is little discomfort during sample collection, there is no risk and serious invasive procedure at the beginning as well as at the end of the study. There is no financial or other benefit you will get from this study. The results of this study will be used to improve SARS-COV-2 diagnosis in the country. Your COVID-19 testing result will be communicated by phone or mobile text to you and also will be reported to the attending clinician in within 24 hrs.

Confidentiality: If you are not willing to participate in the study no information will be collected from you. However, if you intend to take part in the study, the information you give me and your laboratory analysis results will be kept confidential and your laboratory analysis results will not be shared for anyone outside of this study. Personal identifying information will not be shared outside of the study. Your name and another personal identifier's will not appear on any reports when we present this study or publish the results. We will not use your name and details for the analysis of the data.

Voluntary participation and withdrawal: As stated above participation in this study is solely based on your willingness. If you are not willing to participate in this study you can withdraw at any time. You will be allowed to read or else be read to you the information sheets of the study. If you are willing to participate in the study, you will sign this consent form. The routine services are provided to you as usual even if you are not willing to participate in this study.

Contact person: If you have any questions about the study, I will be happy to answer now. Please let me know if anything I have stated is not clear and I will be happy to explain it further to ensure you understand. If you have further questions related to this study, you can call and contact using the following contact information.

Principal Investigator: Mr. Betselot Zerihun

Mobile Number: +251911567355, e-mail betselotzerihun3@gmail.com

Statement

I have read and/or listened to the description of the study entitled "The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia." and I understood what the procedures are and what will happen to me in the study. I have received permission from my parent(s)/guardian(s) to participate in the study and I agree to participate in it. I know that I can quit at any time.

Name of Son/Daughter/Child/Adolescent youth _____

Date ……/……/……

Name and signature of Parent(s) or Legal Guardian;_____

Date ……/……/……

I the data collector clinician sign here under ascertain that I have introduced the participant about the study.

Name and signature :-_____

Date ……/……/……

Annex III: Parental Information and consent form for children less than 12 years of age [English version].

Good morning/afternoon/evening.

My name is Betselot Zerihun Ayano. I'm studying for my Master's degree in Medical Microbiology at Addis Ababa University. Now, I'm conducting research on "The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia." This information will help to improve the laboratory diagnosis of SARS-COV-2 across the country. The study recruits eligible people to take part on voluntary basis. Therefore, we would like to invite you to participate in this study.

Procedures: The procedure followed in this study include a brief questionnaire that will seek to gather socio-demographic and general clinical condition related information from attendant physician. If you are willing on your child's participation in the study, we like your child to give us nasopharyngeal sample for laboratory analysis.

Risks and benefits of participating in the study

An estimated 5 minutes' interview will be conducted for you. There is no risk your child will face due to the interview. There is no financial or other benefit you or your child will get by participation in this study. Your child COVID-19 testing result will be communicated by phone or mobile text to you and also will be reported to the attending clinician in within 24 hrs.

Confidentiality: If you are not willing on your child's participation in the study no information will be collected from you. However, if you intend to take part in the study the information you give me about your child and your child laboratory analysis results will be kept confidential and will not be shared for anyone outside of this study. Personal identifying information will not be shared outside of the study. Your child's name and another personal identifier's will not appear on any reports if we present this study or publish the results. We will not use your/your child's name and details for the analysis of the data.

Voluntary participation and withdrawal: As stated above if you are not willing to participate in this study you can withdraw at any time. You will be allowed to read or else be read to you the information sheets of the study. If you are willing on your child's participation in the study, you will sign this consent form. The routine services are provided to your child's as usual even if you are not willing on your child's participation in this study.

Contact person: If you have further questions related to this study, you can call and contact using the following contact information.

Principal Investigator: Mr. Betselot Zerihun

Mobile Number: +251911567355, e-mail betselotzerihun3@gmail.com

Statement

Your signature below indicates that you have read or listened to and understood the information provided to you about the study entitled "The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia." By signing you are making a decision to allow your child (son/daughter/child/adolescent youth) to participate in the study. If you decide that you wish your child to withdraw or discontinue participation in the study, you any do so at any time.

Are you willing for your child to participate in this study?

1- Yes 2 - No

I have read and/or listened to the description of the study and I understand what the procedures are and what expected from to my child as a participant in the study. I agree to allow him/her to participate in it and I know that my child can quit at any time.

Name of Son/Daughter/Child/Adolescent youth _____

Name and signature of Parent(s) or Legal Guardian;_____

Date ……/……/……

I the data collector clinician sign here under ascertain that I have introduced the participant about the study.

Name and signature :-_____

Annex IV: Participant information and Consent form for adult >18 years [Amharic Version].

ጤና ይስጥልኝ

በፀሎት ዘሪሁን አያኖ እባላለው በአዲስአበባ ዩኒቨርሲቲ የሜዲካል ማይክሮባዩሎጂ ሁለተኛ ድግሪ ተማሪ ነኝ:: ኮቪድ የሚመረምሩ ማሽኖች ለይ ጥናት እያካሄድኩ እገኛለሁ:: ይህ ጥናት የማሽኖች የመመርመር አቅማቸው እና ቫይረሱን የመለየት ሁኔታቸው ምን ይመስላል የሚለውን ለማወቅ ይረዳናል:: ከጥናቱ የሚገኘው መረጃ በሀገራችን የኮቪድ ላቦራቶሪ ምርመራ አቅምን ለማሳደግ ይረዳል።

የጥናቱ ሂደት፤ ስለ ዕርሶ አንድ አንድ መረጃዎች ለምሳሌ እንደ ፆታ፤ እድሜ፤ አጠቃላይ የጤና ሁኔታ እና የመሳሰሉትን ለጥናቱ አጋዥ የሆኑ ጥያቄዋችን ከታካሚው የቅርብ ሃኪም ወይም ነርስ ጋር በመሆን መረጃ ከተሰበሰበ በኋላ ለላብራቶሪ ምርመራ የሚሆን ናሙና ከ አፍንጫ የምንወስድ ይሆናል።

በጥናቱ መካተት ጥቅሙና ጉዳቱ ፤ የ5 ደቂቃ ያክል ቃለ መጠይቅ ይኖረናል። ምንም እንኳ ለጥናቱ የሚያስፈልገው ናሙና ሲወሰድ ምቾት የሌለው ቢሆንም በእርሶ ላይ ምንም የጎንዮሽ ጉዳት አይኖረውም። በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገርግን ከጥናቱ የሚገኘው ውጤት ለእርስዎ ደውለን የምናሳውቅ አልያም በሞባይል መልክት የምናሳውቅ ይሆናል ። በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም። በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜም አይኖርም።

የመረጃ ሚስጥራዊ አጠባበቅ፤ በጥናቱ የመካፈል ፍላጎት ከሌሎ ምንም አይነት መረጃ ከእርሶ የማንወስድ ይሆናል ። የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚይዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል። ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል።በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል።

ፈቃደኝነት በጥናቱ መካፈል በፋቀደኝነቶ ላይ ብቻ የተመሰረተ ይሆናል።በጥናቱ መካፈል ፍላጎት ከሌሎ በፈለጉት ጊዜ ከጥናቱ መውጣት ይችላሉ። የጥናቱ አላማ፣ ሂደት ፣ጉዳት፣ጥቅም እና የመረጃው ሚስጥራዊነት በግልጽ የምናስረዳዎት ይሆናል። ይህን መጠይቅ ማንበብ ይችላሉ አልይም ሌላ ባለሙያ ሊያነብሎት ይችላል።በጥናቱ ለመካተት ፈቃደኛ ሲሆኑ የስምምነት ወረቀት ላይ ይፈርማሉ። በጥናቱ በመካፈሎ እና ባለመካፈሎ የሚቋረጥቦ ምንም አይነት አገልግሎት አይኖርም።

የዋና ተመራማሪ አድራሻ፤ ስለዚህ ጥናት ማንኛዉንም መረጃ ለመጠየቅ ከፈለጉ ከታች በተቀመጠው ስምና አድራሻ መጠቀም ይችላሉ፡-

በፀሎት ዘሪሁን ስልክ ቁጥር፡- 0911567355 ኢሜይል betselotzerihun3@gmail.com

መግለጫ ከዚህ በታች የሰፈረው ፊርማዎ "The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia." በተሰኘው ጥናት ላይ የተቀመጡትን መረጃዎች በሚገባ መስማትዎንና መረዳትዎን ያረጋግጣል። ከመፈረምዎ በፊት ስለጥናቱ

- ✓ ዓላማ
- ✓ ሂደት
- ✓ ጉዳት እና ጥቅም
- ✓ በጥናቱ ያለመካተት እና የማቋረጥ መብትዎ
- ✓ የጥናቱ መረጃ ሚስጥራዊነት እና
- ✓ በጥናቱ ወቅት ስለሚያገኙት ሰው

ከላይ የተቀሱትን መግለጫዎች በሚገባ ተረድቼ በጥናቱ ለመካፈል ፈቃደኛ ነኝ።

በጥናቱ ለመካፈል ፈቃደኛ ኖት?

1- አዎ 2 - አይደለሁም

የጥናቱ ተሳታፊ ስም እና ፊርማ፡-_____

ቀን ……/……/……

እኔ መረጃ ሰብሳቢው የጤና ባለሙያ ስለጥናቱ ሙሉ ገለጻ ማድረጌን በፊርማዬ አረጋግጣለሁ።

ስም እና ፊርማ ፡-_____

Annex V: Information and Ascent form for Participants between 12-17 years of age) [Amharic Version].

ጤና ይስጥልኝ

በፀሎት ዘሪሁን አያኖ እባላለው በአዲስአበባ ዩኒቨርሲቲ የሜዲካል ማይክሮባዩሎጂ ሁለተኛ ድግሪ ተማሪ ነኝ:: ኮቪድ የሚመረምሩ ማሽኖች ለይ ጥናት እያካሄድኩ እገኛለሁ:: ይህ ጥናት የማሽኖች የመመርመር አቅማቸው እና ቫይረሱን የመለየት ሁኔታቸው ምን ይመስላል የሚለውን ለማወቅ ይረዳናል:: ከጥናቱ የሚገኘው መረጃ በሀገራችን የኮቪድ ላቦራቶሪ ምርመራ አቅምን ለማሳደግ ይረዳል።

የጥናቱ ሂደት፤ ስለ ዕርሶ አንድ አንድ መረጃዎች ለምሳሌ እንደ ፆታ፤ እድሜ፤ አጠቃላይ የጤና ሁኔታ እና የመሳሰሉትን ለጥናቱ አጋዥ የሆኑ ጥያቄዋችን ከታካሚው የቅርብ ሃኪም ወይም ነርስ ጋር በመሆን መረጃ ከተሰበሰበ በኋላ ለላብራቶሪ ምርመራ የሚሆን ናሙና ከ አፍንጫ የምንወስድ ይሆናል።

በጥናቱ መካተት ጥቅሙና ጉዳቱ የ5 ደቂቃ ያክል ቃለ መጠይቅ ይኖረናል። ምንም እንኳ ለጥናቱ የሚያስፈልገው ናሙና ሲወሰድ ምቾት የሌለው ቢሆንም በእርሶ ላይ ምንም የጎንዮሽ ጉዳት አይኖረውም። በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገርግን ከጥናቱ የሚገኘው ውጤት ለእርስዎ ደውለን የምናሳውቅ አልያም በሞባይል መልክት የምናሳውቅ ይሆናል ። በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም። በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜም አይኖርም።

የመረጃ ሚስጥራዊ አጠባበቅ፤ በጥናቱ የመካፈል ፍላጎት ከሌሎ ምንም አይነት መረጃ ከእርሶ የማንወስድ ይሆናል ። የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚይዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል። ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል።በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል።

ፈቃደኝነት በጥናቱ መካፈል በፋቀደኝነቶ ላይ ብቻ የተመሰረተ ይሆናል።በጥናቱ መካፈል ፍላጎት ከሌሎ በፈለጉት ጊዜ ከጥናቱ መውጣት ይችላሉ። የጥናቱ አላማ፣ ሂደት ፣ጉዳት፣ጥቅም እና የመረጃው ሚስጥራዊነት በግልጽ የምናስረዳዎት ይሆናል። ይህን መጠይቅ ማንበብ ይችላሉ አልይም ሌላ ባለሙያ ሊያነብሎት ይችላል።በጥናቱ ለመካተት ፈቃደኛ ሲሆኑ የስምምነት ወረቀት ላይ ይፈርማሉ። በጥናቱ በመካፈሎ እና ባለመካፈሎ የሚቋረጥቦ ምንም አይነት አገልግሎት አይኖርም።

የዋና ተመራማሪ አድራሻ፤ ስለዚህ ጥናት ማንኛዉንም መረጃ ለመጠየቅ ከፈለጉ ከታች በተቀመጠው ስምና አድራሻ መጠቀም ይችላሉ፡-

በፀሎት ዘሪሁን ስልክ ቁጥር፡- 0911567355 ኢሜይል betselotzerihun3@gmail.com

መግለጫ ከዚህ በታች የሰፈረው ፊርማዬ "The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia.'' በተሰኘው ጥናት ላይ የተቀመጡትን መረጃዎች በሚገባ መስማቴን እና መረዳቴን ያረጋግጣል። ከመፈረሜ በፊት ስለጥናቱ ከቤተሰቦቼ ወይም ከአሳዳጊዎቼ ፈቃድ አግኝቻለሁ። እናም በፈለኩበት ሰዓት ማቋረጥ እንደምችል ተረድቻለሁ።

የልጅ ስም _____

ቀን ……/……/……

የቤተሰብ ወይም የአሳዳጊ ስም እና ፊርማ;_____

ቀን ……/……/……

እኔ መረጃ ሰብሳቢው የጤና ባለሙያ ስለጥናቱ ሙሉ ገለጻ ማድረጌን በፊርማዬ አረጋግጣለሁ።

ስም እና ፊርማ ፡-_____

Annex VI: Parental Information and conscent form for children less than 12 years of age [Amharic Version].

ጤና ይስጥልኝ

በፀሎት ዘሪሁን እባላለው በአዲስአበባ ዩኒቨርሲቲ የሜዲካል ማይክሮባዩሎጂ ሁለተኛ ድግሪ ተማሪ ነኝ:: ከቪድ የሚመረምሩ ማሽኖች ለይ ጥናት እያካሄድኩ እገኛለሁ:: ይህ ጥናት የማሽኖች የመመርመር አቅማቸው እና ቫይረሱን የመለየት ሁኔታቸው ምን ይመስላል የሚለውን ለማወቅ ይረዳናል:: ከጥናቱ የሚገኘው መረጃ በሀገራችን የኮቪድ ላቦራቶሪ ምርመራ አቅምን ለማሳደግ ይረዳል።

የጥናቱ ሂደት፤ ልጅዎ በጥናቱ ተሳታፊ እንዲሆን ከፈቀዱ ስለ ልጅዎ አንድ አንድ መረጃዎች ለምሳሌ እንደ ፆታ፤ እድሜ፤ አጠቃላይ የጤና ሁኔታ እና የመሳሰሉትን ለጥናቱ አጋዥ የሆኑ ጥያቄዎችን በቅርብ ሃኪም ወይም ነርስ ጋር በመሆን መረጃ ከተሰበሰበ በኋላ ለላብራቶሪ ምርመራ የሚሆን ናሙና ከልጅዎ አፍንጫ የምንወስድ ይሆናል።

በጥናቱ መካተት ጥቅሙና ጉዳቱ የ5 ደቂቃ ያክል ቃለ መጠይቅ እርስዎ ጋር ይኖረናል። ምንም እንኳ ለጥናቱ የሚያስፈልገው ናሙና ሲወሰድ ምቾት የሌለው ቢሆንም በልጅዎ ላይ ምንም አይነት የከፋ የጎንዮሽ ጉዳት አይኖረውም። ልጅዎ በጥናቱ በመካተቱ ምንም አይነት ልዩ ጥቅም ወይም የገንዘብ ክፍያ አይኖረውም ነገርግን ከጥናቱ የሚገኘው ውጤት ለእርስዎ ደውለን የምናሳውቅ አልያም በሞባይል መልክት የምናሳውቅ ይሆናል ። በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ ልጅዎ በመሳተፍዎ ሊደርስበት/ባት የሚችል አንድም ጉዳት አይኖርም። በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜም አይኖርም።

የመረጃ ሚስጥራዊ አጠባበቅ፤ ልጅዎ በጥናቱ እንዲካፈል ፍላጎት ከሌለዎ ምንም አይነት መረጃ ከእርሶ የማንወስድ ይሆናል ። የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚይዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል። ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል።በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል።

ፈቃደኝነት የልጅዎ በጥናቱ መካፈል በፋቀደኝነቶ ላይ ብቻ የተመሰረተ ብቻ ይሆናል።ልጅዎ በጥናቱ እንዲካፈል ፍላጎት ከሌለዎ በፈለጉት ጊዜ ከጥናቱ ማስወጣት ይችላሉ። የጥናቱ አላማ፣ ሂደት ፣ጉዳት፣ጥቅም እና የመረጃው ሚስጥራዊነት በግልጽ የምናስረዳዎት ይሆናል። ይህን መጠይቅ ማንበብ ይችላሉ አልያም ሌላ ባለሙያ ሊያነብሎት ይችላል።ልጅዎ በጥናቱ እንዲካተት ፈቃደኛ ሲሆኑ የስምምነት ወረቀት ላይ ይፈርማሉ። ልጅዎ በጥናቱ በመካፈሉ/ሏ እና ባለመካፈሉ/ሏ የሚቀርበት/ባት ምንም አይነት አገልግሎት አይኖርም።

የዋና ተመራማሪ አድራሻ፤ ስለዚህ ጥናት ማንኛዉንም መረጃ ለመጠየቅ ከፈለጉ ከታች በተቀመጠው ስምና አድራሻ መጠቀም ይችላሉ፡-

በፀሎት ዘሪሁን ስልክ ቁጥር፡- 0911567355 ኢሜይል betselotzerihun3@gmail.com

መግለጫ ከዚህ በታች የሰፈረው ፊርማዎ “The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia.” በተሰኘው ጥናት ላይ የተቀመጡትን መረጃዎች በሚገባ መስማትዎንና መረዳትዎን ያረጋግጣል። ከመፈረምዎ በፊት ስለጥናቱ

- ✓ ዓላማ
- ✓ ሂደት
- ✓ ጉዳት እና ጥቅም
- ✓ በጥናቱ ያለመካተት እና የማቋረጥ መብትዎ
- ✓ የጥናቱ መረጃ ሚስጥራዊነት እና
- ✓ በጥናቱ ወቅት ስለሚያገኙት ሰው

ከላይ የተቀሱትን መግለጫዎች በሚገባ ተረድቼ ልጄ በጥናቱ ለመካፈል ፈቃደኛ ነኝ።

ልጅዎ በጥናቱ ለመካፈል ፈቃደኛ ኖት?

1- አዎ 2 - አይደለሁም

የቤተሰብ ወይም የአሳዳጊ ስም እና ፊርማ; _____

ቀን ……/……/……

እኔ መረጃ ሰብሳቢው የጤና ባለሙያ ስለጥናቱ ሙሉ ገለጻ ማድረጌን በፊርማዬ አረጋግጣለሁ።

ስም እና ፊርማ ፡-_____

Annex VII: Study eligibility form

UPIC: *paste barcode sticker here*

Q	Question	Answer	Action
1.	Known COVID-19 patient?	Yes ☐	→ Q2
		No ☐	→ Q4
2.	Taking antiviral treatment	Yes ☐	STOP
		No ☐	→ Q3
3.	Diagnosed with COVID-19 within the past month?	Yes ☐	→ Q4
		No ☐	→ Q4
4.	Coming for COVID-19 testing/suspicion of SARS-COV-2 infection?	Yes ☐	→**INCLUDE**
		No ☐	STOP
5.	Has COVID-19 symptoms or otherwise feels not well?	Yes ☐	**INCLUDE**
		No ☐	STOP

Annex VIII: Demographic data collection form/Questionnaire

UPIC: *paste barcode sticker here*

Name of data collector _____Signature_____Date _____

	Part I: Demographic data	
No.	Question and/or Characteristics	Possible Response
1.	Sample Barcode	
2.	Participants location: Hospital name	[_____]
3.	Gender	01 Female ○ 02 Male ○
4.	Age In Years	[_____]
5.	Nationality	[_____]
6.	Address	Country [_____] Region [_____] Sub-city/Zone[_____] Woreda [_____] Kebele[_____]
7.	Does the client have any symptom compatible with COVID-19?	1 Yes ☐ 2 No ☐
8.	Does the patient have underlying conditions?	1 Yes ☐ 2 No ☐
9.	Has the patient/Client had contact with a confirmed case?	1 Yes ☐ 2 No ☐
10.	Have you Vaccinated for COVID -19?	1 Yes ☐ 2 No ☐
11.	If Yes, how many of the vaccine dose you have received?	[_____]

Annex IX: Laboratory data collection form for individual sample

UPIC: *paste barcode sticker here*

General information		
1.	Data completed by:	
2.	Sample Barcode	
Test results: Cobas 8800®		
3a	Cobas 8800® test done on nasopharyngeal sample?	○ Yes, date: _ _ / _ _ / _ _ _ _ *dd mm yyyy* ○ No, reason: ○ sample lost ○ leaking container ○other, specify: _____
	Target 1 result	○ Positive ○ Negative ○ Invalid
	CT-value	
	Target 2 result	○ Positive ○ Negative ○ Invalid
	CT-value	
3b	Cobas 8800® test result:	○ SARS-CoV-2 RNA is Detected ○ Presumptive Positive ○ SARS-CoV-2 RNA is Not Detected ○ Not all Target Results were valid. ○ All Target Results were invalid.
3c	If result is invalid Invalid Code Description	[_ _]
3d	If no test result was obtained, or presumptive Positive was the test repeated?	○ Yes, date: _ _ / _ _ / _ _ _ _ *dd mm yyyy* ○ No, reason: ○ sample lost ○ not enough sample left ○ other, specify: _____
	Target 1	○ Positive

		○ Negative ○ Invalid
	CT-value	
	Target 2	○ Positive ○ Negative ○ Invalid
	CT-value	
3e	Repeat Cobas 8800® test result:	○ SARS-CoV-2 RNA is Detected ○ Presumptive Positive ○ SARS-CoV-2 RNA is Not Detected ○ Not all Target Results were valid. ○ All Target Results were invalid.
3f	If result is invalid Invalid Code Description	[_ _]
3g	Remark	
Test results: Xpert® **Xpress**		
4a	Xpert® Xpress test done on nasopharyngeal sample?	○ Yes, date: _ _ / _ _ / _ _ _ _ *dd mm yyyy* ○ No, reason: ○ sample lost ○ leaking container ○other,specify: _____
	N2	○ Positive ○ Negative ○ Invalid
	CT-value	
	E	○ Positive ○ Negative ○ Invalid
	CT-value	
	SPC	○ Positive ○ Negative ○ Invalid
	CT-value	

4b	Xpert® Xpress test result	○ SARS-CoV-2 Positive ○ SARS-CoV-2 Presumptive Pos ○ SARS-CoV-2 Negative ○ Invalid ○ Error ○ No Result
4c	Analyte Result E N2 SPC	 [_ _] [_ _] [_ _]
4d	If Error Error description Specify Error Code Error detail	 [_ _] [_ _]
4e	If no test result was obtained, or presumptive Positive was the test repeated?	○ Yes, date: _ _ / _ _ / _ _ _ _ *dd mm yyyy* ○ No, reason: ○ sample lost ○ not enough sample left ○ other, specify: _____
	N2 result	○ Positive ○ Negative ○ Invalid
	CT-value	
	E result	○ Positive ○ Negative ○ Invalid
	CT-value	
	SPC result	○ Positive ○ Negative ○ Invalid
	CT-value	

4f	Repeat Xpert® Xpress test result	○ SARS-CoV-2 Positive ○ SARS-CoV-2 Presumptive Pos ○ SARS-CoV-2 Negative ○ Invalid ○ Error ○ No Result
4g	Analyte Result E N2 SPC	 [_____] [_____] [_____]
4h	If Error Error description Specify Error Code Error detail	 [_____] [_____]
4i	Remark	

Annex X: Laboratory data collection form for pool sample

UPIC: *paste barcode sticker here*

General information			
1.	Data completed by:		
2.	Pool ID		
3.	Positive Sample ID		
4.	Group of Positive sample ID		○ Very Low(VL) ○ Low(L) ○ Medium(M) ○ High(High)
5.	Result of Positive samples		
	Cobas 8800®	Target 1	○ Positive ○ Negative ○ Invalid
		Target 2	○ Positive ○ Negative ○ Invalid
		Final result	○ SARS-CoV-2 RNA is Detected ○ Presumptive Positive ○ SARS-CoV-2 RNA is Not Detected ○ Not all Target Results were valid. ○ All Target Results were invalid.
	Xpert® Xpress	N2	○ Positive ○ Negative ○ Invalid
		E	○ Positive ○ Negative ○ Invalid
		SPC	○ Positive ○ Negative ○ Invalid
		Final result	○ SARS-CoV-2 Positive ○ SARS-CoV-2 Presumptive Pos ○ SARS-CoV-2 Negative ○ Invalid ○ Error ○ No Result
6.	CT-value of Positive sample		

	Cobas 8800®	Target 1	
		Target 2	
	Xpert® Xpress	N2	
		E	
		SPC	
7.	Negative Samples ID		
Test results: Cobas 8800®			
7a	Cobas 8800® test done on Pool sample?		○ Yes, date: _ _ / _ _ / _ _ _ _ *dd mm yyyy* ○ No, reason: ○ sample lost ○ leaking container ○ Insufficient sample to pool ○other,specify: _____
	Target 1 result		○ Positive ○ Negative ○ Invalid
	CT-value		
	Target 2 result		○ Positive ○ Negative ○ Invalid
	CT-value		
7b	Cobas 8800® test result:		○ SARS-CoV-2 RNA is Detected ○ Presumptive Positive ○ SARS-CoV-2 RNA is Not Detected ○ Not all Target Results were valid. ○ All Target Results were invalid.
7c	If result is invalid Invalid Code Description		[_____]

7d	If no test result was obtained, was the test repeated?	○ Yes, date: _ _ / _ _ / _ _ _ _ *dd mm yyyy* ○ No, reason: ○ sample lost ○ not enough sample left ○ other, specify: _____
	Target 1	○ Positive ○ Negative ○ Invalid
	CT-value	
	Target 2	○ Positive ○ Negative ○ Invalid
	CT-value	
7e	Repeat Cobas® 8800® test result:	○ SARS-CoV-2 RNA is Detected ○ Presumptive Positive ○ SARS-CoV-2 RNA is Not Detected ○ Not all Target Results were valid. ○ All Target Results were invalid.
3f	If result is invalid Invalid Code Description	[_____]
7g	Remark	
Test results: Xpert® **Xpress**		
8a	Xpert® Xpress test done on Pool sample?	○ Yes, date: _ _ / _ _ / _ _ _ _ *dd mm yyyy* ○ No, reason: ○ sample lost ○ leaking container ○ Insufficient sample to pool ○other, specify: _____
	N2	○ Positive ○ Negative ○ Invalid
	CT-value	

	E	○ Positive ○ Negative ○ Invalid
	CT-value	
	SPC	○ Positive ○ Negative ○ Invalid
	CT-value	
8b	Xpert® Xpress test result	○ SARS-CoV-2 Positive ○ SARS-CoV-2 Presumptive Pos ○ SARS-CoV-2 Negative ○ Invalid ○ Error ○ No Result
8c	Analyte Result E N2 SPC	 [_____] [_____] [_____]
8d	If Error Error description Specify Error Code Error detail	 [_____] [_____]
8e	If no test result was obtained, was the test repeated?	○ Yes, date: _ _ / _ _ / _ _ _ _ *dd mm yyyy* ○ No, reason: ○ sample lost ○ not enough sample left ○ other, specify: _____
	N2 result	○ Positive ○ Negative ○ Invalid
	CT-value	

	E result	○ Positive ○ Negative ○ Invalid
	CT-value	
	SPC result	○ Positive ○ Negative ○ Invalid
	CT-value	
8f	Repeat Xpert® Xpress test result	○ SARS-CoV-2 Positive ○ SARS-CoV-2 Presumptive Pos ○ SARS-CoV-2 Negative ○ Invalid ○ Error ○ No Result
8g	Analyte Result E N2 SPC	 [_____] [_____] [_____]
8h	If Error Error description Specify Error Code Error detail	 [_____] [_____]
8i	Remark	

Declaration

I, the undersigned candidate, affirm that this M.Sc. thesis is entirely my own work and has not been previously submitted for a degree at this or any other university. I further acknowledge that all sources of materials utilized for this thesis have been appropriately acknowledged.

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Signature: _____

Date of submission: _____

This Thesis has been submitted with my approval as advisor.

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Date: _____

The comparison of Xpert® Xpress with Cobas® SARS-CoV-2 assay on individual and pooled nasopharyngeal swabs and whole genome sequencing of SARS-CoV-2 samples collected in selected hospitals of Addis Ababa, Ethiopia.