COMPARATIVE ANALYSIS OF SARS-CoV-2 DETECTION METHODS BY USE OF DIFFERENT CLINICAL SAMPLES

OLOO MARCELINE ADHIAMBO

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DECLARATION

This research thesis is my original work and has never been presented for the award of a degree at any other university.

Signature ……. Date …….

OLOO MARCELINE ADHIAMBO

ADM NO: S152/4133/2020

(CANDIDATE)

APPROVAL

We hereby certify that this research thesis is the original work of the candidate and that it has been submitted for examination with our approval as university supervisors

Signature…… Date….....

Dr. Benson Onyango

Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya

Signature ……. Date…….

Dr. Shehu Shagari Awandu

Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya

Signature…… Date……..…

Dr. Richard Magwanga

Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya

DEDICATION

I dedicate this work to my late dad; Luke Obiero Oloo, mom; Dorcas Anyango and Uncle Herman.

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ABSTRACT

Even with promising news on vaccine development and mass vaccination globally, tracking, tracing and mass testing of the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) continues to be very essential to public health policy responses towards Coronavirus 2019 (COVID-19) pandemic. Diagnostic testing remains vital in detection of the virus, understanding its emergence and occurrence, case management and suppression of its transmission. This study aimed at comparing diagnostic sensitivity and specificity between antigen rapid diagnostic test (Ag-RDT) and reverse transcriptase polymerase chain reaction (rt-PCR), determining the presence of SARS-CoV-2 in nasopharyngeal swab (NPs) and stool samples as well as determining the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) among symptomatic and asymptomatic individuals for COVID-19 disease in Siaya and Kisumu counties. This study recruited 92 participants each providing three clinical samples including NPs, stool and blood. The NP swab samples were tested using both Ag-RDT and rt-PCR, stool samples were run through rt-PCR and the blood samples were tested by enzyme-linked immunosorbent assay at a level 3 safety laboratory at Kenya Medical Research Institute, Kisian. The overall pooled sensitivity and specificity of Ag-RDT were 76.3% (95% CI, 59.8-88.6%) and 96.3% (95% CI, 87.3-99.5%) respectively with a negative and positive predictive value of 85% (95% CI, 73.8%-93.0%) and 93% (95% CI, 78.6%-99.2%) respectively. A Cohen's kappa value of 0.75 at (95% CI, 0.74-0.77) was obtained, showing a substantial agreement between rt-PCR and Ag-RDT. The area under the receiver operating characteristic (ROC) curve was 0.87 at (95% CI, 0.80-0.94) indicating excellent performance of the Ag-RDT. The average cycle threshold (Ct) value of targeted genes; nucleocapsid (N) and open reading frames1ab (ORF 1ab) gene were 30.00 and 32.90 for NP samples and 32.74 and 33.86 for stool samples. The lowest Ct values of (19.28 and 23.11) for N gene and 23.35 and 22.48) for ORF1ab were obtained in NP swab and stool samples respectively. The mean IgM and IgG antibody response to SARS-CoV-2 was 1.11 (95% CI, 0.78-1.44) and 0.88 (95% CI, 0.65-1.11), 4.30 (95% CI, 3.30-5.31) and 4.16 (95% CI, 3.32-5.00) in asymptomatic and symptomatic individuals respectively. In conclusion, the sensitivity of Ag-RDT 76.3% was lower compared to that of rt-PCR assay, however its high specificity of 96.3%, positive and negative predictive values of 93% and 85% respectively and its fast test output implies that individuals infected with SARS-CoV-2 can be traced faster. This corroborates that Ag-RDT is important for screening COVID-19 in areas that lack suitable laboratories with rt-PCR diagnostics. The high cycle threshold in stool samples indicates low viral load which may cause transmission of the virus. IgM and IgG antibody response to SARS-CoV-2 revealed that asymptomatic individuals had higher mean antibody ratios and were considered to be the most exposed. This study is of a great significance as it addresses a pressing public health issue, and recommends the most suitable diagnostic test (rt-PCR) and clinical samples (nasopharyngeal swab) for the detection of SARS-CoV-2.

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ABBREVIATIONS AND ACRONYMS

ACE	Angiotestin Converting Enz yme
Ag	Antigen
AUC	Area Under Curve
BSL-3	Biosafety Level three
CDC	Center for Disease Contol and Prevention
CGHR	Center for Global Health Research
CLIA	Chemiluminescent Immunoassays
COVID-19	Coronavirus Disease 2019
E	Envelope region of SARS-CoV-2 Virus
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural organization
FN	False negative
FP	False positive
GDP	Gross Domestic Products.
HBIC	Home Based Isolation Care.
IFAD	International Fund for Agricultural Development
Ig	Immunoglobulin
IgG/IGM	Immunoglobulin G/ Immunoglobulin M
ILO	International Labour Organization
KEMRI	Kenya Medical Research Institute

MESR-CoV	Middle East Respiratory Syndrome Coronavirus
MOH	Ministry of Health
N	Nucleocapsid
NAAT	Nucleic Acid Amplification Test
NP	Nasopharyngeal
NPIs	Non-Pharnaceutical Intervention
ORF 1ab	Open Reading Frame
PCR	Polymerase Chain Reaction
PSO	Post Symptoms Onset
RDB	Receptor Binding Domain
RdRp	RNA dependent RNA polymerase
RDT	Rapid Diagnostic Test
RNA	Ribonucleic Acid
ROC	Receiver Operator Characteristics
Rt-PCR	reverse transcriptase-Polymerase Chain Reaction
S	Spike protein
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SDGs	Standard Development Goals.
TN	True negative
TP	True positive
WHO	World Health Organization

DEFINATION OF OPERATIONAL TERMS

Ag-RDT - A test that rapidly detects viral antigens

Antigen - A foreign substance/protein that induces an immune response to the body

Asymptomatic - The state at which an individual has a pathogen but is not exhibiting clinical symptoms

Coronavirus Disease 2019 (COVID-19) - A disease caused by severe acute respiratory syndrome corona virus 2.

Cycle threshold - The number of cycles required to amplify viral RNA to a detectable level

Detection -To identify the presence of the virus

False negative - A binary classification error in which a test result incorrectly indicates the absence of a condition

False positive - An error in a binary classification where a test result incorrectly indicates the presence of a condition

Immunoglobulins -Also called antibodies- these are proteins produced by the immune system to help fight foreign materials within the body

Infection- Invasion of the body by harmful microorganism

Negative Predictive Value- The probability that the individuals detected to be negative do not have the disease

Positive Predictive Value- The probability that the individuals detected to be positive truly have the disease

Prevalence- Proportion of the population found to be affected by a medical condition

Reverse transcriptase-Polymerase Chain Reaction (Rt-PCR)- A laboratory technique that combines a reverse transcription of RNA to DNA and amplifies specific DNA targets using a polymerase Chain Reaction. Measures the amount of specific RNA

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) - The virus which causes COVID-19 disease

Sensitivity - Ability of a diagnostic test to determine a true positive

Specificity - Ability of a diagnostic test to determine a true negative

Symptomatic - The state at which an individual shows characteristic of a particular disease

Transmission- The passing of a pathogen from an infected host individual to another individual

True Negative - An outcome where a negative outcome is correctly predicted

True positive - An outcome where a positive outcome is correctly predicted

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) is the causative agent for the highly infectious coronavirus disease 2019 (COVID-19) (Helmy *et al*., 2020; Shereene *et al*., 2020). The novel severe acute respiratory syndrome was first detected in December 2019 in Wuhan city of China (Liu *et al*., 2020). The disease resulted in more than 1800 deaths and over 70,000 infections within the first 50 days (Shereen *et al.,* 2020). As at 21st February 2020, the virus had been detected in various parts of China together with 28 other countries (Spiteri *et al*., 2020). It was at this point that the disease was declared a global pandemic by the World Health Organization. The COVID-19 disease spread globally, with devastating effects, owing to increased number of confirmed cases and deaths (Sanyaolu *et al.*, 2021). By 6th September, the global confirmed cases of COVID-19 infection were 610 million, with 6.51 million deaths (WHO, 2022a).

Africa's first case of COVID-19 disease was detected and reported in Egypt on February 15th, 2020 (Aluga, 2020). Three months later, SARS-CoV-2 virus had spread and had been detected throughout the continent with Lesotho being the last African country to report its first case (Ministry of Health Lesotho, 2020). By 1st August 2022, most African countries had community transmissions which led to increased number of infections (8,794,089 confirmed cases with 173,301 deaths). African countries instituted mechanisms for mitigation of the virus spread, although the testing capacity was low due to under-developed healthcare systems, which may have led to under-reporting of cases (WHO, 2021). Kenya's first case of COVID-19 was detected and reported in March 13th 2020 in Nairobi (Adamuoch *et al*., 2020). The reported cases in Kenya had reached 338,237 with 5,674 deaths by end of August, 2022 (Ministry of Health Kenya, 2022). The Kenyan government instituted stiff containment measures which included curfew, closure of schools and higher institutions of learning and mass testing in hotspot areas (Adamuoch *et al.*, 2020). However, the country continued to experience up to six waves of COVID-19 infection, with different variants of the virus, which included Alpha, Beta, Gamma, Delta and Omicron which was the dominant variant by September, 2022. The outbreak in June

2021 constituted the fourth wave of the virus and was characterized by the Delta variant which was first detected in western Kenya, affecting counties of Kisumu, Siaya, Homabay, Migori and Kakamega. Although containment and mass testing was instituted in the region, little work has been done to evaluate the efficiency of COVID-19 testing regimes adopted by the counties' health departments.

Efficient testing is a fundamental tool towards containment of transmission of SARS-CoV-2. Disease surveillance schemes require a robust evaluation of sampling techniques, and the associated testing procedures (WHO, 2021). Sampling through nasopharyngeal swabs has been used in most countries to detect COVID-19 infection (Péré *et al*., 2020). However, most patients in Kenya found it to be invasive and uncomfortable, and this affected the mass testing rolled out by the government (The Star Newspapers, 9th May 2020). New forms of human samples have since been used to detect for the presence of SARS-Cov-2. For instance, live SARS-CoV-2 was isolated from the feces of patients infected with COVID-19 disease (Amirian, 2020). Similarly, there was a positive detection of SARS-CoV-2 virus from stool specimen of COVID-19 infected patients whose results were negative from multiple SARS CoV-2 RNA profiles from oropharyngeal and nasopharyngeal swabs samples (Brogna *et al*., 2021). Thus, a comparison of test outcomes of fecal with oropharyngeal, nasopharyngeal and blood samples is a practical approach to validate COVID-19 infection, especially in cases where there are variations between clinical symptoms of COVID-19 (Brogna *et al.*, 2021). This can be adopted alongside regular clinical diagnostics including the Ag-RDT and the nucleic acid-based amplification (NAAT) tests.

Ag-RDT was recommended by WHO as an instant tool for detection of COVID-19 (WHO, 2020). It quickly detects individuals with high SARS-CoV-2 RNA load and can therefore be used in the diagnosis of COVID-19 disease (Wagenh *et al.,* 2021). Usage of Ag-RDT helps to detect and isolate possible super spreaders prior to detection using reverse transcriptase polymerase chain reaction (rt-PCR), especially for persons seeking hospitalization (Wagenh *et al.*, 2021). The test is performed on samples taken by swabbing procedures, which can be processed on-site, often providing results in less than 30 minutes (WHO, 2020). The short-turn-around-time of the test is important for real-time patient

management, and allows for screening during pre-operative management for invasive procedures, especially when the NAATs are unavailable (Tang *et al.*, 2020). However, the challenges with its ability to detect true positives together with true negatives have been reported in other parts of the world (Chaimayo *et al.,* 2020), especially if low amounts of the virus is present in the swab due to poor sampling techniques. In the Kenyan counties of Kisumu and Siaya, the health facilities adopted the Ag-RDT from nasopharyngeal swab samples for COVID-19 testing. However, a comparative evaluation of outcomes of the Ag-RDT tests with other testing regimes is yet to be determined.

The NAAT-based rt-PCR assay remains the most reliable method for the laboratory diagnosis of SARS-CoV-2 infection (Chu *et al.,* 2020) since it has minimal false positive outcomes (Kanji *et al.*, 2021). The procedure involves nucleic acid extraction followed by rt-PCR which multiplies viral copies (Blow *et al.,* 2004). However, elaborate laboratory procedures make the test relatively expensive and laborious, requiring at least four hours of operation by skilled technicians, which limits its use on a massive scale (Chaimayo *et al.,* 2020). This may hamper efforts to minimize the spread of this virus and may result to underestimation of prevalence rates (Kanji *et al.,* 2021).

Serological test is an epidemiological tool used to evaluate disease implication in order to correlate antibody responses with clinical outcomes (de Assis *et al*., 2020). The test complements rt-PCR testing of COVID-19 in the later stages of illness, and aids in evaluation of a patients' adapted immunity status (Lou *et al*., 2020). Thus, individuals that were previously infected with corona virus disease can be detected, even if they were never tested before (Sidiq *et al.,* 2020), which may create a clearer understanding of asymptomatic infections. The tests can therefore reveal community transmission of SARS-CoV-2 by identifying individuals who have been exposed and already mounted natural immune response against corona virus (Cohen *et al.,* 2020). Currently, there is insufficient information on the usefulness of SARS-CoV-2 serological testing in western Kenya settings.

This study compared rt-PCR and Ag-RDT using nasopharyngeal swab samples. Discrepancies in sensitivity, specificity, negative predictive values and positive predictive values of these tests have been highlighted in this study, and this may contribute to

significant surveillance data on COVID-19 prevalence in Siaya and Kisumu counties. This study compared COVID-19 test outcomes from clinical samples such as stool and nasopharyngeal swabs, and further examined the presence of IgM and IgG antibodies against SARS-CoV-2 in asymptomatic and symptomatic individuals using blood samples. Mean antibody ratios for both the IgM and IgG were highlighted in symptomatic and asymptomatic patients.

1.2 Statement of the Problem

The occurrence and swift spread of SARS-CoV-2 endangered global health and eroded economic gains towards attainment of the United Nations Sustainable Development Goals (SDGs). The Kenyan government instituted stiff containment measures which included curfew, closure of schools and higher institutions of learning and mass testing in hotspot areas (Wambua *et al.,* 2020). However, the country continued to experience waves of COVID-19 infection resulting to high rates of morbidity and mortality. Although several diagnostic strategies are available, the Ag-RDT test has been adopted in most county and sub-county hospitals in western Kenya. However, a comparative evaluation of the sensitivity and specificity of Ag-RDT outcomes with rt-PCR using different clinical samples in western Kenya settings is not established.

Disease surveillance schemes requires a robust evaluation of sampling techniques. Sampling through nasopharyngeal swabs was found to be invasive and uncomfortable by the Kenyan populace, and this affected the mass testing rolled out by the Kenyan government. To achieve proper mitigation and suppression of the spread of COVID-19, there is need to explore more cost effective sampling procedures, capable of mass detection of the virus in populations, and with minimal social stigma. Such approaches include, the use of alternative clinical samples namely stool samples for SARS-CoV-2 detection and serological testing, which remain largely unexplored in Kenya. Western Kenyan counties of Kisumu and Siaya became the epicenters of COVID-19 outbreak in June 2021, and the more lethal delta variant of SARS-CoV-2 was first detected in Kibos, Kisumu County. These counties are characterized with high populations and by extension infections rate are expected to be higher. These counties has two government hospitals where majority of the populations seeks medical interventions, these include Kisumu and Siaya referral hospitals.

Up to date, few surveillance studies have been conducted in Kisumu and Siaya counties, to evaluate the true spread of corona virus through the population.

1.3 Justification

Even with promising mass vaccination globally, tracking, tracing and fast testing of SARS-CoV-2 remains essential to public health policy responses towards COVID-19 pandemic. Diagnostic testing remains vital in detection of the virus, understanding its emergence and occurrence, case management and suppression of its transmission. Reverse transcriptase polymerase chain reaction remains to be the most reliable method for the detection of SARS-CoC-2 virus. However, alternative diagnostic schemes such as the Ag-RDT have been introduced. A comparative evaluation of the sensitivity and specificity of different detection method for screening SARS-CoV-2 is critical for its mitigation and control. Further, utilization of varying clinical samples such as stool, blood serum and nasopharyngeal swabs may provide accurate disease surveillance data and aid in identification of tests which best suit the sample. This may lead to the development of alternative but complimentary testing and sampling strategies. Adoption of these techniques will help contain COVID-19 transmission and act as a tool for epidemiological projections to support the utilization of Ag-RDT and stool samples for the detection of SARS-CoV-2.

1.4 Objective

1.4.1 Broad objective

To compare sensitivity and specificity of Ag-RDT and rt-PCR for the detection of SARS-CoV-2 using nasopharyngeal, stool samples and serological analysis of blood samples of patients from Siaya and Kisumu counties.

1.4.2 Specific objectives

1. To compare the sensitivity and specificity between Ag-RDT and rt-PCR RNA profiles from nasopharyngeal swabs for SARS-CoV-2 detection.
2. To compare the rt-PCR RNA profiles from nasopharyngeal swab and stool samples.
3. To determine the presence Ig-G and Ig-M antibodies in the blood of asymptomatic and symptomatic COVID-19 patients.

1.5 Null Hypothesis

1. There is no significant difference in the sensitivity and specificity of Ag-RDT and rt-PCR RNA profiles from nasopharyngeal.
2. There is no significant difference in the RNA profiles from nasopharyngeal swab and stool samples.
3. There is no significant difference in the presence of Ig-G and Ig-M antibodies from asymptomatic and symptomatic COVID-19 patients.

1.6 Study Significance

The study presents significant aspects of addressing a pressing public health issue by seeking to establish the most suitable diagnostic test for SARS-CoV-2. The study also determines the most suitable sample for detection of the virus, which is critical towards mitigation and control of COVID-19 disease. This study contributes to the existing literature by comparing various diagnostic tests for SARS-CoV-2 which improves the detection and lowers false negative rates. It also sheds light on the potential benefits of screening individuals regardless of their patient status. Finally, the thesis also advances the field of health sciences which might help in public health responses when the virus re-emerges.

1.7 Conceptual Framework

The independent variables for this study entailed the detection techniques alongside samples that were being used such as rt-PCR with nasopharyngeal and stool samples, Ag-RDT with nasopharyngeal swab and ELISA with blood samples (Figure 1). Modifying variables were age, gender, patient status and vaccination status. Dependent variables included the tests outcomes; positive/negative, Ct values, the IgG and IgM ratios.

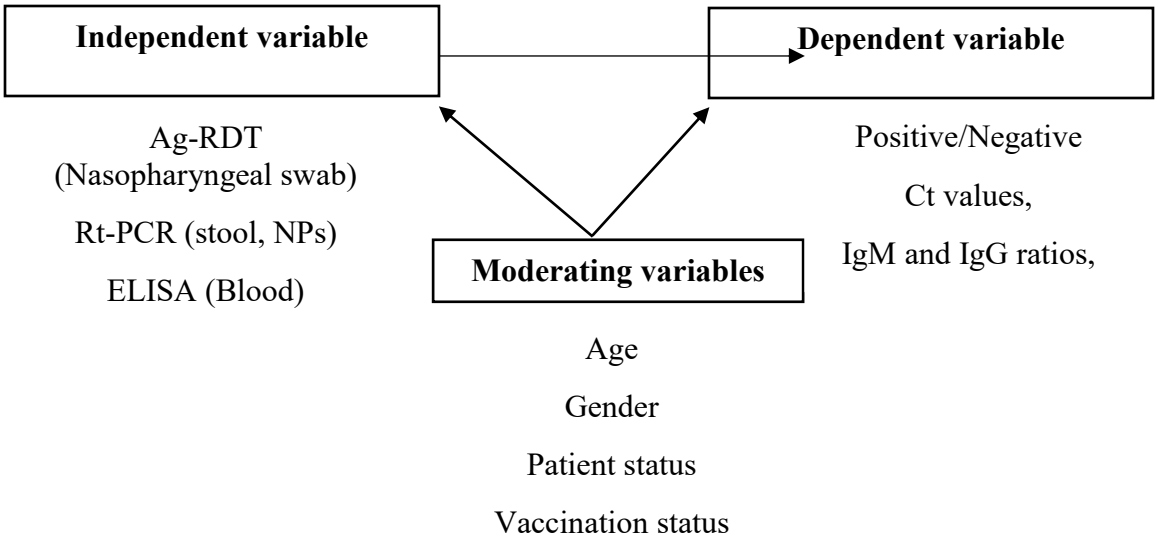

Figure 1. A conceptual frame work to determine the effectiveness and reliability of SARS-CoV-2 detection techniques using different clinical samples.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and the genomic structure of corona virus

In the year 1968, Almeida and David Tyrrel coined the name ‘‘coronaviruses’’. The name was derived from the observations made under the electron microscope that revealed a crown-like morphology of the virus (Weiss & Navas-Martin, 2005). According to (Owczarek *et al.*, 2018) the 229E and OC43 of the human coronavirus strains were first detected in the late 1960s, which led to the attestation that coronaviruses were infectious to humans. In the winter of 2002 in Southern China, an outbreak of SARS-CoV occurred which resulted in a 10% fatality rate of infected persons (Cheng *et al.,* 2007; Peiris *et al.,* 2004). In the year 2012, a new highly pathogenic Middle East Respiratory Syndrome Corona Virus (MERS-CoV) was detected in humans, demonstrating that the coronaviruses were transmissible to humans with devastating consequences to public health (Ali *et al.,* 2015). The novel corona virus 2019 disease was first detected in China in December 2019 (Liu *et al.,* 2020), where it rapidly infected more than 50 people (Shereen *et al.*, 2020). SARS-CoV-2 was classified in the family *Coronaviridae* and order *Nidovirales* (Brian and Baric, 2005). According to Yosra *et al.,* (2020), the genomic characterization of SARS-CoV-2 shares a nucleotide similarities of 82%, 96.3% and 89% with SARS-CoV, bat CoV RaTG13 and SARS-like CoVZXC21 respectively which confirms its zoonotic origin.

Coronavirus belongs in the family *Coronaviridae*, sub family *Coronaviriniae* and the subfamily contain four genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus* (Brian and Baric, 2005). The genome of Coronaviruses is about (27-32 kb) and is a single-stranded positive sense-RNA which is larger than any other RNA viruses. The genome is enclosed within the nucleocapsid protein (N) which forms the capsid outside. The genome is further packed by an envelope which consist of three structural proteins: the membrane protein (M) , the spike protein (S) and the envelope protein (E) (Brian and Baric, 2005). The genome has the ORF gene that encodes for 16 nonstructural proteins. Most of the studies prefers a combination of a structural N gene and a non-structural ORF 1ab gene due to their highly conserved nature meaning that they are less likely to mutate and their unique sequence specific for SARS-CoV-2. Situations may arise where only one out of two targets are detected (Tran *et al*., 2021). Detection of only

one gene does not necessarily indicate the presence of a new variant and may be the result of low viral load. For instance, with the high throughput assay at UC Davis Health, an ORF1ab positive, but E-gene negative result is still considered positive for SARS-CoV-2 RNA (Tran *et al*., 2021).

2.2 Prevalence and transmission of SARS-CoV-2

The transmission of SARS-CoV-2 is mostly from one person to another through small droplets via two main pathways; droplet infection via the respiratory secretions, or the stool to oral route (Jayaweera *et al.,* 2020) (de Assis *et al.*, 2020). People may get infected with this disease in case they breathe in the droplets. Alternatively, people could become infected by handling objects and surfaces such as tables, doorknobs and even handrails that might be containing SARS-CoV-2 then touching their eyes, nose or mouth (Hafeez *et al.,* 2020). SARS-CoV-2 can typically survive for an appropriate environment for several days after exiting the human body (Shang *et al.,* 2020). The most common iatrogenic corona virus may over-live on inanimate surface for a period of one month (Kramer *et al*., 2006). Transmissions risks are said to be low with regards to touching contaminated papers. For the nasopharyngeal, oropharyngeal swab (respiratory) and stool specimens, the virus at room temperature can maintain its infectivity stage for a long period of time (Ren *et al.*, 2020). Materials such as cotton are said to be safer and can protect one from viral infection because they are absorbent materials unlike non- absorptive-materials. For containment of the spread of corona virus, wearing of face masks, washing hands, keeping social distances constitute proper preventive strategies for the corona virus 2019 disease (Ren *et al.*, 2020).

Since its outbreak in 2019, Corona virus disease has spread globally, with a devastating effects, owing to the increased number of confirmed cases and deaths (Sanyaolu *et al.*, 2021). By 8th August 2021 the global confirmed cases of COVID-19 infection were 203.3 million, with 4.3 million deaths (WHO, 2021a). the first case of COVID-19 infection in Africa was reported in Egypt on 15th February, 2020 (Aluga, 2020). By June 2020, the virus had been detected throughout the continent leaving Lesotho as the last African country to report its first case (MOH (L), 2020). By 26th May 2020, most African countries had experienced community transmission which led to increased number of infections (4,635,103 confirmed cases with 124,434 deaths). African countries have instituted

mechanisms for mitigation of the virus spread, although the testing capacity is still low due to under-developed healthcare systems, which may have led to under-reporting of cases (Abdelmoneim *et al.,* 2020). The first case of COVID-19 infection in Kenya was reported in March 13th 2020 in Nairobi (Adamuoch *et al.,* 2020), but the reported cases had reached 211,028 with 4,117 deaths by end of August, 2021 (MOH Ministry of Health Kenya, 2021).

2.3 Approaches for Mitigation of COVID-19 Transmission

The globally adopted approach to fight COVID-19 epidemic is through non-pharmaceutical interventions (NPIs), aiming at cutting down the rate at which people may come into contact with each other or rather the virus itself. These NPIs helps in reducing the transmission of the virus (WHO, 2020c). This can be achieved through mitigation to reduce the spread of the epidemic (closure of school and work places, social distancing and restricted intra and international movement) and through disease suppression strategies (such as self-isolation and quarantine) which aim to reverse the epidemic growth (WHO, 2020c). For fast and accurate laboratory testing, the diagnosis of viral pneumonias caused by SARS-CoV-2 involves collecting the correct specimen from the patient at the right time (Ben *et al.*, 2020).

One of the most difficult challenges is testing, tracking the initial contacts, the spread and the changing trends of coronavirus 2019 disease at the population level together with the need to map its magnitude and distribution in near real time (Daughton, 2020). The most significant tool therefore is the ability to quickly identify the individuals infected with the virus other than the Non-pharmaceutical interventions and therapeutic treatments (Daughton, 2020). The application of diagnostic tests at an individual case level has been the main approach towards eradication of epidemics. However, this approach that uses rt-PCR assays, faces overwhelming challenges in providing fast test results of large populations (Azman *et al.,* 2020).

2.3.1 Real time Reverse transcriptase polymerase chain reaction (rt-PCR)

The NAAT based real time reverse transcriptase polymerase chain reaction is the most widely used diagnostic method for SARS-CoV-2 infection (Daniel K.W *et al*., 2020). The rt-PCR can be used on different clinical samples: nasopharyngeal swab, saliva, sputum, oropharyngeal and even stool samples(Torretta *et al.,* 2020). The negative results of the rt-

PCR do not rule out the infection. The test is associated with minimal false positive cases and therefore known to be highly reliable (Kanji *et al.*, 2021) and involves nucleic acid extraction followed by rt-PCR which multiplies the viral copies (Blow *et al*., 2004; Chaimayo *et al.,* 2020). From a public health point of view rt-PCR is characterized by a number of challenges such as high cost ,it requires highly trained personnel and the sub-optimal turnaround time (Bruzzone *et al*., 2021). This may hamper the efforts to control the spread of this virus and may lead to the underestimation of prevalence rates (Kanji *et al.*, 2021). To discourse these rt-qPCR shortcomings, an increasingly common Ag-RDT were developed and are cost effective with a short turnaround (WHO, 2020b; ECDC, 2020; (Bruzzone *et al*., 2021). Several diagnostic methods target structural and non-structural regions including a combination of nucleocapsid (N), spike (S) and/or the envelope (E) and non-structural ORF 1ab region of the SARS-CoV-2 gene along with the two controls; positive and negative controls (Ching *et al*., 2020). This strategy ensures that the diagnostic gene targets combine structural and non-structural protein which are highly conserved across coronavirus and highly specific for SARS-CoV-2 (Ching *et al.*, 2020).

2.3.2 Antigen-Detecting Rapid Diagnostic test

The antigen rapid diagnostic test (Ag-RDT) is a qualitative membrane-based immunoassay that detects the nucleocapsid (N) protein of SARS-CoV-2 in nasopharyngeal, sputum and Saliva (Linares *et al.,* 2020); (Onsongo *et al.*, 2021a). It was recommended as an instant tool for detection of COVID-19 (WHO, 2020b) and are increasingly being employed by most of the low- and middle-income countries for high demand for SARS-CoV-2 testing (Onsongo *et al.*, 2021a). The antigens detected are protein markers on the outside of the virus (Linares *et al.,* 2020). The tests are performed on samples taken by swabbing procedures, which can be processed on-site, often providing results in less than 30 minutes (WHO, 2020). The short-turn-around-time of the test plays an important role in real-time patient management, and also allows for screening during pre-operative management for invasive procedures, especially when the NAATs are unavailable (Tang *et al.*, 2020). However, challenges associated with specificity and sensitivity of Ag-RDT test have been reported in other parts of the world (Chaimayo *et al.,* 2020) especially if low amounts of the virus are present in the swab due to poor sampling techniques.

The Ag-RDT allows rapid and qualitative detection of SARS-CoV-2, additionally, it is a ready to use test which detects antigen in a nasopharyngeal secretions (Scohy *et al.,* 2020). The kit is marked with two lines, a control line region (C) and a test line region (T) used in interpretation of results based on expected outcomes. According to the Kenyan Ministry of Health, (2020) reviews of the Ag-RDT have revealed considerable good performance in terms of sensitivity and specificity using different samples including Nasopharyngeal swab samples (Olalekan *et al*., 2020). The World Health Organization on Interim evaluation of the Ag-RDT highlighted that its accuracy as clinical diagnostic tool for SARS-CoV-2 detection has not been well established (WHO, 2021c). Indeed, appropriate application of the test varies depending on the goal and stage of disease (MOH, 2020). Therefore, a comparative evaluation of sensitivity and specificity outcomes of Ag-RDT with molecular based tests such as the rt-PCR is necessary.

2.3.3 Serological Analysis

Serological test is a blood test, that accurately determine the type and levels of antibodies for SARS-CoV-2 and correlates them with clinical outcomes (de Assis *et al.,* 2020). Serological test commonly uses blood serum or plasma to a number of circulating antibodies generated by B lymphocytes. (Juliet *et al.*, 2020). Apparently, majority of health institutions laboratories are progressively developing platforms for serological testing, putting into consideration technologies such as enzyme-like immunosorbent assay which is widely explored and classical immunoassays (Galipeau *et al.*, 2020). For subclinical infection, serology plays a very crucial role in determining the true prevalence of COVID-19 disease at a population level and particularly for subclinical infections. These tests identifies (Immunoglobulin G (IgG), Immunoglobulin A (IgA) and Immunoglobulin M (IgM), which are responsible for humoral-mediated response to a viral infection. IgM immunoglobulins are the largest and are first expressed during naive B cell development (Galipeau *et al.*, 2020), which can as well be used to distinguish the duration of infection in a patient (Okba *et al.*, 2020). IgM antibodies accounts for 10% of the total antibodies and are always detectable 1-4 weeks of Post-Symptoms Onset (PSO)(Harry & Lisa, 2010). In contrast, IgG are the smallest (exists in monomeric form), most abundant circulating antibody and makes up of about 80% of total antibody count (Galipeau *et al.*, 2020). IgG typically appears later in infection stage when the mature B-Cells receives signals to switch

from production of IgM to IgG (Harry & Lisa, 2010). The IgA antibodies are detectable 2-3 weeks closely after the IgM, (Galipeau *et al*., 2020). Currently, published papers uphold the fact that SARS-CoV-2 triggers a classical viral model, in which the IgM antibodies (primary antibodies) are produced 1-4 weeks of post-symptom onset (PSO), IgA follows closely which peaks at 2-3 weeks before diminishing. (Galipeau *et al*., 2020).

Finally, the IgG antibodies are produced later and remains undetectable for several months of post-symptoms onset. The tests can reveal the extent of community transmission by identifying individuals who have been exposed and already mounted an immune response against SARs-CoV-2 (Cohen *et al.,* 2020) (de Assis *et al.*, 2020). Serological detection of antibodies against SARS-COV-2 is anticipated to be helpful. However there is little scientific information on antibody response to SAR-CoV-2 infection (Lou *et al*., 2020). The most important thing about these tests is that they can identify individuals who had previously been covid 19 positives even if they never underwent testing while acutely ill (Sidiq *et al.,* 2020). This creates an understanding on asymptomatic infections. Serological tests are rapid in contrast to molecular tests, they will demonstrate whether an active immune response against the virus exists and will reduce instances of false positives or false negatives (Cohen *et al.*, 2020).

2.3.4 SARS-CoV-2 antibody detection

In the second week of SARS-CoV-2 infection, the viral load reduces and may be undetectable during a natural infection by the SARS-CoV-2 (Roman *et al*., 2020). Under such circumstance, immunoglobulins becomes the primary and the most appropriate method to detect a past infection or a recently cured infections to the covid 19 disease (Borremans *et al*., 2020). Antibody tests are mapped out to detect if antibodies against various pathogens inclusive of SARS-CoV-2 are present or absent. A positive outcome of SARS-CoV-2 serological test is an indication that an individual had been exposed to the antigenic epitopes of the pathogen and the individual had been infected with the virus epitopes and therefore depending with the type of antibody detected, for example the presence of IgM antibodies indicates that there was an active infection. Furthermore, if the pathogen of interest shares antigenic epitope sequences with the proteins of other microbes or even that of vaccine antigens, a test can be reported as false positive. False negative may

arise due to late release of the antibodies (Galipeau *et al*., 2020). According to CDC, an ELISA test has been established by coating with SARS-CoV-2 recombinant spike protein (purified SARS-CoV-2 S protein (no live virus) as antigen and has been used to detect serum IgM and IgG antibodies against SARS-Cov-2 in COVID-19 patients. These serological tests are designed to reduce cross-reactivity to the antibodies generated to other common types of corona virus such as cold which causes less severe illnesses.

2.4. Sampling for SARS-CoV-2 Testing

2.4.1 Nasopharyngeal Swabs

Patients infected with COVID-19 with respiratory tract symptoms such as fever, fatigue, cough and shortness of breath, usually reflects the infection of respiratory epithelial cells and the human-to-human transmission via the airways (Guan *et al.,* 2020). The most recently used mode of testing for corona virus 2019 involves a swab of the upper respiratory specimen and lower respiratory specimens (in patients with more severe respiratory conditions(Olalekan *et al.,* 2020);(Loeffelholz & Tang, 2020). The distance between the nostril and posterior wall of the nasopharynx differs between adults and children. In children, the nasal cavity is slightly shorter 6-10 cm while in adults its slightly longer 8-10 cm (Pondaven-Letourmy *et al*., 2020). The nasopharyngeal swab is performed by inserting the 2-3 cm swab into a nostril until a resistance is felt at the turbinate, while gently rotating, the swab is gently rubbed and rolled then left in a place for several seconds to absorb secretions.(Pondaven-Letourmy *et al*., 2020). The nasopharyngeal swab sample collection method is coupled with a number of challenges including its sensitivity, unpleasant experience for the patient and the personal protective equipment (PPE) required for collectors (Péré, *et al.*, 2020). This method is invasive and uncomfortable and has led to reluctance in seeking voluntary testing which could be a big threat in containing the spread of COVID-19 in Kenya. Therefore, there is need to seek alternative testing regimes, that will be acceptable to improve detection of COVID-19.

2.4.2 Sampling of Stool Matter

Confirmed COVID-19 infected patients report gastrointestinal symptoms like vomiting and diarrhea (Guan *et al.*, 2020). Studies have reported that SARS-CoV-2 RNA could be detected from stool specimen (Holshue *et al.,* 2020; Xiao *et al.*, 2020). Indeed, live SARS-

CoV-2 was detected and isolated from stool sample of individuals infected with SARS-CoV-2. (Amirian, 2020). Concerns of stool contamination as source of communal spread has been raised, although the sample size in previously reported studies has been too small (Wong *et al.,* 2020). Notably, detection of SARS-CoV-2 in feces has raised concerns about the potential fecal–oral or fecal–respiratory transmission route (Donà *et al.,* 2020). This is because stool samples may cause infection by invading the respiratory mucosa and mouth following contamination of hand, water and food (Chen *et al.,* 2020). Thus, a confirmation of virus shedding in human stool through analysis of SARS-COV2 RNA in stool is necessary. It is therefore critical to design optimal sampling and RNA extraction procedures.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The samples for this study were obtained from Kisumu and Siaya counties. The counties are neighboring each other and are located near the shores of Lake Victoria as shown in Figure 2. These two counties are neighboring each other and are located on the shores of Lake Victoria. Kisumu and Siaya have a total population of 2,148,757 (Adminurs, 2019) accounting for approximately 4 percent of the total population in Kenya. The population consist of both urban and rural dwellers. These counties have two main county hospitals, one in each county, Kisumu County Referral Hospital located at a latitude and longitude of 0.0990° S, 34.7553° E respectively. Siaya County Referral Hospital is located at a latitude and longitude of 0.0639° N, 34.2870° respectively. The samples were analyzed at KEMRI CGHR Kisian.

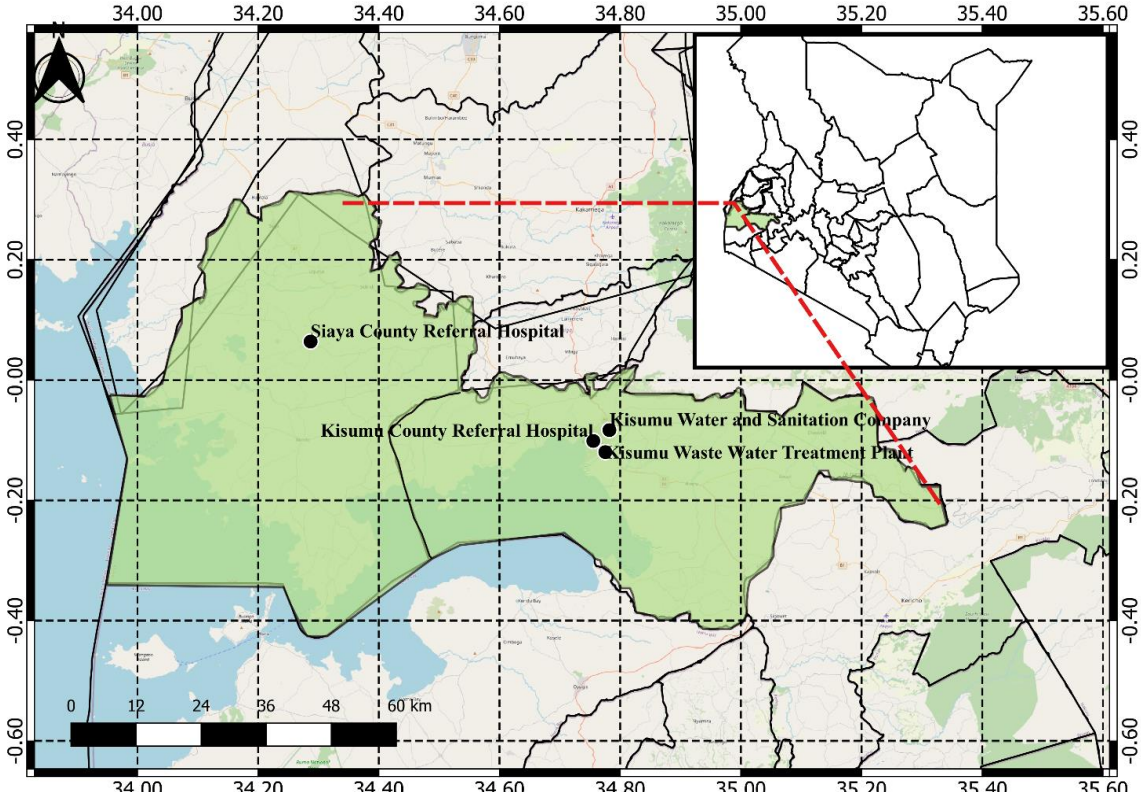

Figure 2: A map of Siaya and Kisumu counties showing the exact Sampling Sites.

Map drawn using QGIS Version 3.16 Hannover

3.2 Sampling Design

The purposive sampling technique was adopted to recruit a total of 92 consented (Appendix II) study participants out of 100 intended from Siaya and Kisumu County Referral Hospitals, 50 participants from each hospital giving a total of 100 study participants. A cross-sectional study design was employed to obtain samples from Siaya and Kisumu County referral hospital. The study cohorts consisted of individuals who voluntarily presented themselves for testing, including those at risk of contracting COVID-19 disease such as public health workers and people in isolation centers (patients suspected to be infected and their close contacts). Demographics details of the recruited participants such as name, age, gender, patient status, vaccination status, comorbidities and dwelling places were recorded.

3.3 Calculation of sample size

Sample size calculation was achieved through an online platform http://www.raosoft.com/samplesize.html, at 95% confidence interval with a margin of error of 10.19 and with 50% response distribution resulting to a total of 92 samples.

3.4 Inclusion and Exclusion criteria

Inclusion criteria involved all walk-in patients who voluntarily presented themselves to the hospital regardless of age and only individuals coming from the two counties were eligible to participate in the study.

Exclusion criteria-Individual who were unable to provide consent, unable to provide all the three clinical samples from different anatomical and those who were coming from different counties apart from Kisumu and Siaya were excluded from study.

3.5 Sample Collection and Storage

Sample collection, handling and storage were done according to protocols described by CDC, (2020, and 2021). Human blood, feces and nasopharyngeal samples were taken by well-trained health practitioners with the required PPEs following the guidelines of the Ministry of Health, Kenya (MOH, 2020). Nasopharyngeal specimens were collected using an aluminum or plastic shafts swabs with synthetic tip. This was done by gently inserting the swab 2-3 cm in the nasal cavity and holding it parallel to the palate, gently rubbed and

rolled then taken out. The swabs were then placed in sterile tubed supplied with 2-3 ml of Gibco™ viral transport media in cooler boxes. Stool samples were collected by requesting the recruited individuals to provide stool samples with sterile plastic containers. The plastic containers had spatulas which were used to transfer the stool into well labelled collection bottles with Gibco™ virus transport media.

The swab, serum and stool specimens were then transported to KEMRI CGHR BSL 3 laboratory for analysis. Nucleic acid extraction was done after sample processing. Aliquots of samples were made then stored at -80 degrees for samples that took more than 7 days before analysis, samples that took less than 7 days were refrigerated at 2-8 ^{o}c. Extracted RNA was stored at -80^{o}c for further reference. The laboratory work was carried out in Biosafety level 3(BSL 3) Laboratories at KEMRI/CGHR Kisumu Kenya.

3.6 Rapid Antigen Diagnostic Test

The Panbio™ COVID-19 Ag Rapid Test (Abbott Point of Care Test kits, Germany) were obtained from the county referral hospitals. Swab specimens and detection buffer were applied to the test cartridges. A dropper pipette (supplied) was used to aspirate the extracted specimen into the lateral-flow inlet. Results were read after 15 minutes. The presence of only the control line (C) indicated negative results whereas, the presence of test line (T) and control line (C) indicated positive result. Absence of a control line rendered test results as indeterminate.

3.7 Serological Analysis

Blood samples were collected according to the guidelines provided by the Centre for Disease Control and Prevention (CDC, 2021). Venous blood samples were collected using a 10 ml syringe and a needle. Sample procedures were reviewed as per the recommendations to ensure that not any person was placed in their care at risk for infection. To avoid the risk of transmission of other blood borne infectious diseases, syringe and needle devices were provided for each individual and the blood samples were collected by trained technicians.

3.7.1 Antibody Testing

The IgM and IgG antibodies against SARS-CoV-2 S proteins in serum specimens were detected using the qualitative indirect SCoV-2 Detect™ IgG ELISA kit and SCoV-2

Detect™ IgM ELISA kit (InBios International, Seattle, USA). The serum samples and controls were diluted by adding 4 µL of each serum and controls up to 369 uL of sample dilution buffer for SARS-CoV-2. About 50 µL each of the diluted serum samples, positive, negative and cut -off controls in duplicates were added into the SCoV-2 Antigen coated microtiter ELISA plates. The plates were then covered using microfilm and was incubated at 37°C for an hour. About 300 uL of 1X Wash Buffer was used to subsequently wash the plates 6 times. About 50 uL of the conjugate was added to the wells. Then, the plates were covered with parafilm and incubated again for at 37 °C for 30 in an incubator. The plates were again washed 6 times using 300 uL of 1X Wash Buffer and 75 uL of liquid tetramethylbenzidine was added into the wells. The uncovered plates were then incubated at room temperature in the dark for 20 minutes. About 50 uL of stop solution was then added to the wells and the plates were left to stand uncovered at room temperature for 1 minute. The plates were read on a BIOTEK ELX800 absorbance microplate reader at 450 nm ($OD_{450)}$ absorbance. The raw (Optical Densities) ODs were recorded and ratios computed in relation to the average ODs of the cut off controls. Samples with IgG or IgM ratio greater than or equal to 1.1 were considered positive and IgG or IgM ratio less than or equal to 0.9 were considered negative. Neither positive nor negative samples were classified as indeterminate results

3.8 Pretreatment of Samples

Swab samples were vortexed thoroughly into the transport medium, 200 µl was used for extraction. For fecal sample pretreatment, about 180-220 mg of fecal samples were put into 1.5 ml tube, stool lysis buffer of 1 ml was added and was thoroughly vortexed for 1 minute. The mixture was incubated at room temperature for 10 minutes then centrifuged for 3 minutes at 13000 rpm and 200 µl of the supernatant was used for RNA extraction.

3.9 RNA Extraction from Nasopharyngeal Swabs

Extraction of RNA from nasopharyngeal swab samples were performed with QIAGEN viral RNA kit using spin column from Thermo-scientific® Kingfisher. The protocol for isolating viral nucleic acids from 200 µl of nasopharyngeal swab samples were as follows: AVL/AVE/Carier RNA (Lysis buffer with carrier RNA) of 560 µl was added into one set of the tubes, the samples were then vortexed and 200 µl of the samples added to the tubes,

the mixture was then vortexed for 15 seconds the incubated for 10 minutes at room temperature to ensure efficient and complete lysis. Absolute ethanol measuring 285 µl was added then the mixture was centrifuged briefly at 8000 rpm for 1 minute. Of about 630 µl of each sample previously centrifuged was transferred into the column supplied in the kit. The column was centrifuged for 1 min at 8000 rpm at room temperature. The column was then placed in a clean collection tube while the filtrate was discarded. The remaining samples were transferred into the column, centrifuged for 1 min at 8000 rpm at room temperature. Again, the column was placed in a clean collection tube and filtrate discarded. About 500uL of Wash buffer 1 was added to each column, centrifuged for 1 min at 8000 rpm at room temperature. The spin column was then placed in a new clean collection tube then 500 µl of wash buffer 2 was added to the column. The column was again centrifuged for 3 min at the highest speed. The column was place in a clean collection tube, centrifuged for 1 min at 14000 rpm (dry spin). The column was placed in a clean collection tube. Of about 60 µl of equilibrated elution buffer was lastly added then incubated for 10 min. The column was then centrifuged for 1 min at 8000rpm at room temperature. The eluted RNA was placed on ice awaiting PCR reactions.

3.10 RNA Extraction from Stool Samples

Extraction of RNA from stool samples were performed with QIAGEN RNA viral kit using spin column from Thermo-Scientific® Kingfisher. The protocol for isolating viral nucleic acids from 200 µl of stool and nasopharyngeal swab were done as follows; Stool and nasopharyngeal swab of 200 µl were added to a nuclease-free 1.5 ml micro-centrifuge tube together with a working solution, freshly prepared (carrier RNA/l-supplemented binding buffer) then 50 µl proteinase K solution was added and mixed immediately. The solution was then incubated for 10 min at +72°C after which 100 µl binding buffer was added then mixed well. To transfer the sample to a high pure filter tube, one high pure filter tube was inserted into a collection tube. The entire sample was pipetted into the upper reservoir of the filter tube. The entire high pure filter tube assembly was then inserted into a standard table-top centrifuge. Centrifugation was then done for 1min for 8000 rpm. After centrifugation, the filter tube was retained while the filtrate was discarded. The filter tube with a new collection tube was combined and 500 µI inhibitor removal buffer then was added to the filter tube then centrifuged for 1 min at 8,000 rpm. After centrifugation the

filter tube was removed from the collection tube, the flow through and the collection tube were again discarded. The filter tube was placed in a new collection tube, the collection tube containing the filtrate was discarded. Approximately 500 µl of Wash Buffer 1was added to the upper reservoir of the filter tube. Centrifugation was then done for 1 min at 8,000 rpm and flow through were discarded. The filter tube was then removed from the collection tube after the first wash and centrifugation. The flow through and the collection tube were discarded and the filter tube was again combined with another new collection tube, of about 500 µl of wash buffer 2 was added to the upper reservoir of the filter tube then centrifuged 1 min at 8,000 rpm and the flow through were discarded. The filter tube-collection tube assembly were then left in the centrifuge and were spined for 10s at maximum speed 14,000 rpm to remove any residual wash buffer. The extra centrifugation time ensured removal of residual Wash Buffer. The collection tube was then discarded and the filter tube inserted into a nuclease free, sterile 1.5 ml micro-centrifuge tube. For the viral nucleic acids' elution, 60 µl elution buffer will be added to the upper reservoir of the filter tube then the tube assembly was centrifuged for 3 min at 14,000 × g. The micro-centrifuge tube therefore contained the eluted, purified viral nucleic acids. The eluted Viral RNA was used directly in RT-qPCR (5 µl viral RNA), for later analysis, and was stored at –80°C or elution volume of 100µL awaiting rt-PCR analysis.

3.11 rt-PCR Reactions

The presence of SARS-CoV-2 from the extracted nucleic acid was established using 7500 fast real time PCR system (Applied Biosystems) in accordance with manufacturer instructions. The RT-qPCR reactions procedures were adapted from Triplex detection kit for 2019 novel corona virus PCR DAAN GENE CO. LTD kit instructions (Fisher Scientific). The master mix preparation procedures were as presented on **Table 1**.

3.11.1. The rt-PCR Reaction Mix Preparation

The detection kit for COVID-19 RNA (PCR fluorescence probing) technology was used. The kit is supplied with two reaction solutions reaction solution A and B.

Table 1: rt-PCR Master Mix Reaction Volumes

	Number of samples	1	100
Reaction mix	NC (ORF1ab/N) PCR reaction solution A- Specific primer probes, $MgCl_2$, $(NH_4)_2SO_4$, KCL, HCL	17 µL	1700 µL
	NC (ORF1ab/N) PCR reaction solution B- Hot start Taq DNA polymerase, CMMLV enzyme, DNTPs and Rnasin	3 µL	300 µL
Total reaction mix volumes		20 µL	2000 µL

3.11.2. Performing the rt-PCR

About 10 µL of the reaction mix was pipetted into each well of a MicroAmp^R Fast Optical 96-well reaction plate with barcode 0.2 mL. The sealed plate containing the purified sample RNA and negative control from the RNA extraction procedure were vortexed gently and centrifuged to collect liquid at the bottom of the plate. The plate containing the purified sample RNA and negative control from the RNA extraction procedure were then unsealed and 5 microliters of purified RNA from each sample transferred to the plate with master mix. All the downstream PCR steps (master mix preparation, template addition, and positive control addition) were done in dedicated workspaces. The plates were then fully sealed using MicroAmp Optical 96-Well strip. The plate was vortexed at the highest setting speed for 10–30 seconds with medium pressure, this ensured proper mixing, removal of bubbles and to allow the liquid to settle at the bottom of the reaction

Table 2: RT-qPCR reaction plate volumes

Component	Volume per reaction		
	RNA Sample reaction	Positive Control	Negative Control
Reaction Mix	20 µL	20 µL	20 µL
Purified sample RNA (from RNA extraction)	5.0 µL	—	—
Positive Control (diluted Taq Path™ COVID-19 Control, from step 3)	—`	5.0µL	—
Purified Negative Control (from RNA extraction)	—	—	5.0 µL
Total volume	25 µL	25 µL	25 µL

3.11.3. Analysis of Real Time PCR Results

Real time 7500 PCR software version 2.3 was used during amplification, targets and samples were defined i.e., the gene targets were ORF 1ab gene which had VIC as the reporter dye and N gene having the FAM as the reporter dye, the internal control A had CY5 as the reporter dye, the quencher was selected as none and ROX dye was used as the passive reference dye. The cycling conditions were 50º for 15 minutes, 95º for 15 minutes, 94º for 15 seconds and 55º for 45 seconds. Upon completion of the rt-PCR run; Linear amplification plot views were adjusted at the exponential phase for final results analysis. For assay verification, positive and negative controls were ensured that all had passed the designated threshold. Analysis for each target was then done individually, while recording the cycle threshold for both the N gene and ORF 1ab gene. The cycle threshold values ranged between 15-40, where 15-25 were considered low CTs having high viral loads (strong positives), 25-32 were considered to have a medium CT and 33-40 being considered to have high CTs translating to low viral loads (weak positives). Confirmations for true positives was done by analyzing the samples individually. The amplification of the positive control showed that the reagents used in the assay were valid. The amplification of samples that turned out to be undetermined were recorded to be negative cases.

3.12 Data Analysis

The data obtained from the participants were analyzed using descriptive statistics. The sensitivity, specificity, negative predictive value and positive predictive values, Kappa coefficient analysis and Area Under the Receiver Operating Characteristic (ROC) curve was determined. The sensitivity of a diagnostic test was defined by the proportion of correctly identified COVID-19 positive patients by rt-PCR while specificity was defined by the proportion of participants who were classified as negative by rt-PCR. Cohens Kappa statistics was used to qualitatively measure the inter rater. A ROC curve and area under the curve was then constructed illustrating the overall diagnostic performance of the diagnostic test by plotting the true positive rate (sensitivity) against the false positive rate (1-specificity) at various threshold settings was done using STATA software. To estimate sensitivity and specificity of the Ag-RDT, rt-PCR was considered as the reference diagnostic test. Two tailed t- test, (STATA software Version 17) was used to compare the Cycle thresholds for the N gene and ORF 1ab gene between the nasopharyngeal and stool samples and also to compare the presence of Ig-M and Ig-G between asymptomatic and symptomatic patients. Statistical significance were performed at 95% confidence intervals.

3.13 Ethical Approvals

Ethical approval for this study was granted by Jaramogi Oginga Odinga University of Science and Technology (ERC/21/5/21-4). The research license was granted by Kenya National Commission for Science and Technology (NACOSTI/P/22/17543). Administrative approval were provided by the county governments of Kisumu and Siaya. All the participants were provided with well written informed consent forms before they were enrolled.

CHAPTER FOUR

4.0 RESULTS

4.1 Characteristics of Samples Analyzed

This study recruited a total of 92 patients who walked into KCRH and SCRH for COVID-19 testing. The median age of the 92 patients as obtained from the demographic data was 28 ranging from 9 to 73 years. Among the patients there were 36 males and 56 females. From each individual, three different clinical samples (nasopharyngeal swab, stool and blood) were taken for the test, giving out a total 276 samples. The nasopharyngeal swabs were analyzed using both the Ag-RDT and rt-PCR, stool samples analyzed using rt-PCR, blood serum samples were analyzed through ELISA test. Table 3 shows a summary performance of the three detection methods using three different clinical samples

Table 3: Ag-RDT, rt-PCR and ELISA tests with different clinical samples

Test type/ samples	Ag-RDT	rt-PCR		ELISA (blood serum)	
	Nasopharyngeal swab	Nasopharyngeal swab	Stool	IgM	IgG
Positive	38(41.30)	31(33.70)	28(30.43)	28(30.43)	71(77.17)
Negative	54 (58.70)	61(66.30)	64(69.57)	64(69.57)	21(22.83)
Total	92	92	92	92	92

NOTE: Antigen Rapid Diagnostic test (Ag-RDT), Enzyme Linked Immunosorbent Assay (ELISA) Reverse Transcriptase-Polymerase Chain Reaction (rt-PCR)

Of the 92 NP samples, 58.7% (n = 54) were found negative and 41.38% (n=38) positive by Ag-RDT. With rt-PCR 66.3% (n=61) tested negative and 33.7% (n=31) tested positive while 69.57% (n=64) tested negative and 30.43% (n=28) tested positive with NP swabs and stool samples respectively. Blood serum analysis showed a positivity rate 30.43% (n=28) and 77.17% (n=71) for the IgM and IgG respectively and a negativity rate of 69.57% (n=64) and 33.83% (n=21) for the IgM and IgG respectively.

Table 4: Ag-RDT and rt-PCR test results for COVID-19 patients.

RT-qPCR			
Ag-RDT	**Positive**	**Negative**	**Total Ag-RDT results**
Positive	32.0(29) **TP**	9.8 (9) **FP**	41.3(38)
Negative	2.2 (2) **FN**	56.2(52) **TN**	58.7(54)
Total PCR results	33.7(31)	66.3(61)	(100)92

Of the 31 rt-PCR positive patients, rapid antigen test classified 29 subjects correctly indicating that the individuals had the disease. Both the Antigen and the rt-PCR reported a total of 52 cases to be negatives and were considered true negatives. Contrasting results between Ag-RDT and rt-PCR were observed. The proportion of false negative results was (n=2) 2.2% while that of false positive was (n=9) 9.8% as illustrated in table 4.

Table 5: Sensitivity specificity, NPV and PPV for Ag-RDT and rt-PCR diagnostic techniques

Test type	**N**	**TP**	**FP**	**TN**	**FN**	**PPV**	**NPV**	**Sensitivity(95% CI)**	**Specificity(95 %CI)**
Ag-RDT	92	32%	10 %	56%	2%	93%	85%	76.3% (59.8-88.6%)	96.3% (87.3-99.5%)
PCR	92	32%	2%	56%	10%	76%	96%	93.5% (78.6-99.2%)	85.2% (73.8-93.0%)

NOTE: n-Total population; TP -True positives; FP- False positives; TN-True negatives; FN -False negative; NPV-Negative predictive values; PPV- Positive Predictive Values.

Table 5. The overall pooled sensitivity and specificity were 76.3% (95%CI; 59.8% -88.6%) and 96.3% (95% CI; 87.3% - 99.5%) respectively. The proportion that tested positive and negative with the rt-PCR were 33.7% (n=31) and 66.3% (n=61) respectively. Of the 31 rt-PCR, 29 tested positive with the Ag-RDT, resulting to a sensitivity of 76.3% (95% CI; 59.8% - 88.6%). Out of the 61 rt-PCR negatives, Ag-RDT classified a total of 54 as negatives, resulting to a specificity of 96.3% (95% CI; 87.3% - 99.5%). The positive predictive value of the Ag-RDT in this population was at 93% (95% CI; 78.6% - 99.2%)

while the negative predictive value was 85.2% (95 CI; 73.8% - 93.0%). Rt-PCR had higher sensitivity compared to Ag-RDT, while Ag-RDT had a higher specificity compared to rt-PCR

Table 6: Kappa coefficient analysis for Ag-RDT and rt-PCR

Kappa	**SE of kappa**	**95% CI**	**Z**	***p* value**
0.7465	0.1029	0.707–0.868	7.26	<0.001

NOTE: The interpretations of *K*-value were as follows: <0.20 as poor; 0.21-0.40 as fair; 0.41-0.60 as moderate; 0.61-0.80 aw substantial and >0.81 was considered almost perfect.

The inter-rater agreement between rt-PCR and Ag-RDT diagnostic test was determined using Kappa coefficient analysis. The exhibited K was 0.75 thereby indicating a substantial agreement with (p<0.001). **Table 6.**

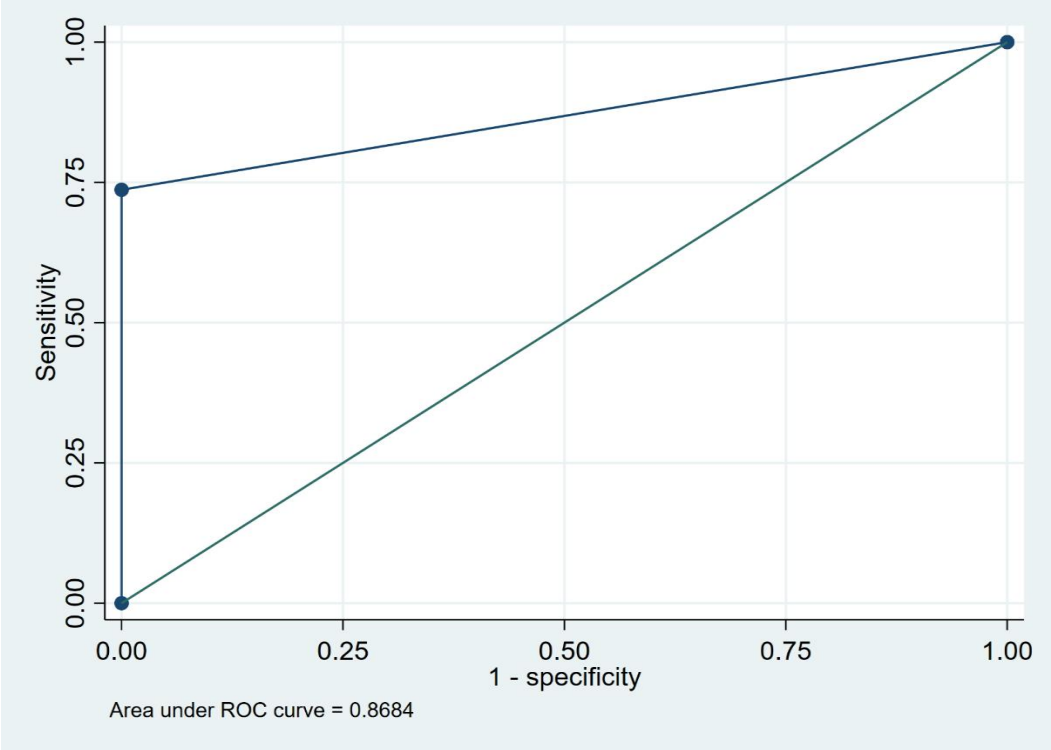

Figure 3: Receiver operator characteristics (ROC) curve of Antigen rapid test compared with rt-PCR as the reference standard. Area under the curve 0.8684.

The receiver operator characteristic (ROC) curve of Ag-RDT and the corresponding area under the curve is shown in Figure 3. The obtained area under the ROC curve was 0.8684 (95% CI; 0.79% - 0.94%). The curve featured true positives rate (sensitivity) on the Y axis and false positive rate (1-Specificity) on the X axis for different cut-off points of a parameter. AUC summarizes the overall diagnostic accuracy of a test and it ranges in value from 0 to 1. A value of 0.5 and below indicates that the ROC curve have fallen on the diagonal and therefore suggests that the diagnostic test has no discriminatory ability. In general, while a value higher than 0.5 suggests that the diagnostic test have reasonable discriminating ability to diagnose patients with or without the disease. AUC value of 0.6-0.7 is considered moderate, 0.7 to 0.8 is considered acceptable, 0.8 to 0.9 is considered excellent, and more than 0.9 is considered outstanding. Obtained AUC was 0.8684 which suggested an 87% chance that the Ag-RDT was able to correctly distinguish between a true positive from a true negative hence having an excellent performance.

4.2 SARS-CoV-2 RNA profiles from Nasopharyngeal Swab and Stool Samples

Among the two clinical samples, nasopharyngeal swabs showed a higher detection rate with a total of 31 (33.7%) positives; (male=12, female=19), while stool had 28 (30.4%) positives (male 9, female 19). Among recruited participants, 31 (33.7%) tested positive in nasopharyngeal swabs. Of these cases, 22(70.9%) also tested positive for SARS-Cov-2 in stool. In total, 6(7%) tested positive only in stool and 55 (59.8%) showed a negative result in both the samples.

Table 7: Comparison between the positive cases identified by rt-PCR using stool and NP

Total population (n=92)	**NP Swab**	**Stool/fecal**
Male (n=36)	12(38.7%)	9(32.1%)
Female (n=56)	19(61.3%)	19(67.9%)
Total	31	28

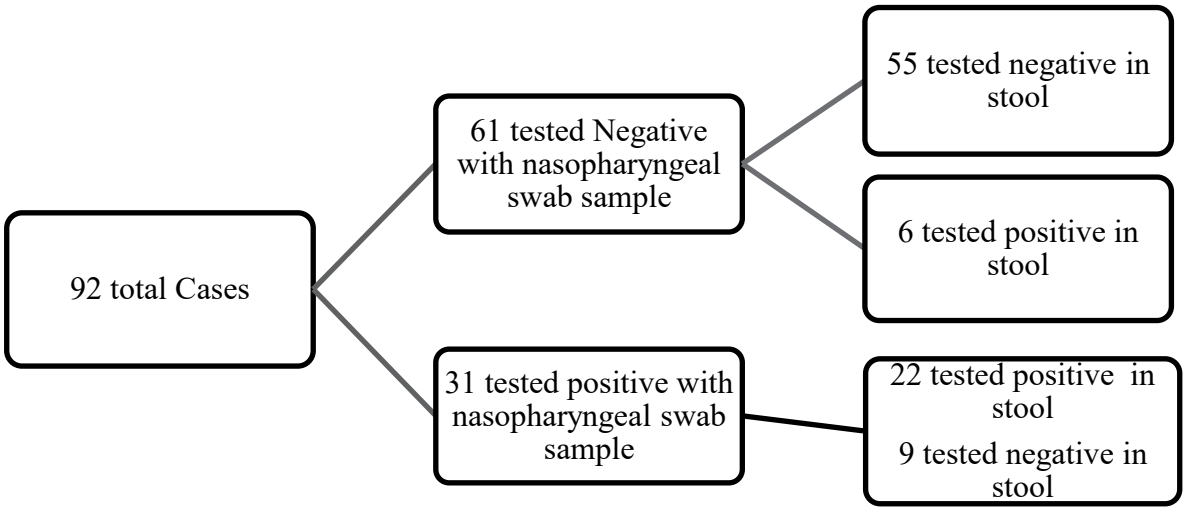

Figure 4. Rt-PCR test results from Stool and NPs among 92 participants

4.3 Comparison of SARS-Cov-2 Cycle threshold (Ct) values between stool and Nasopharyngeal Swab samples

The ORF1ab gene and N gene cycle threshold were compared between nasopharyngeal swab and stool samples Figure 5. The Ct value for N gene ranged from 19 to 36 for nasopharyngeal swab and from 23 to 39 for stool samples. For the ORF 1ab gene, the Ct values ranged from 22 to 40 and 23 to 39 for Nasopharyngeal and stool samples respectively. There was a significant difference between the Ct values obtained for nasopharyngeal swab and stool samples for the N gene in. *P= (0.0038)* at 95% confidence interval. These results therefore confirms higher viral load in nasopharyngeal swab samples compared to stool samples.

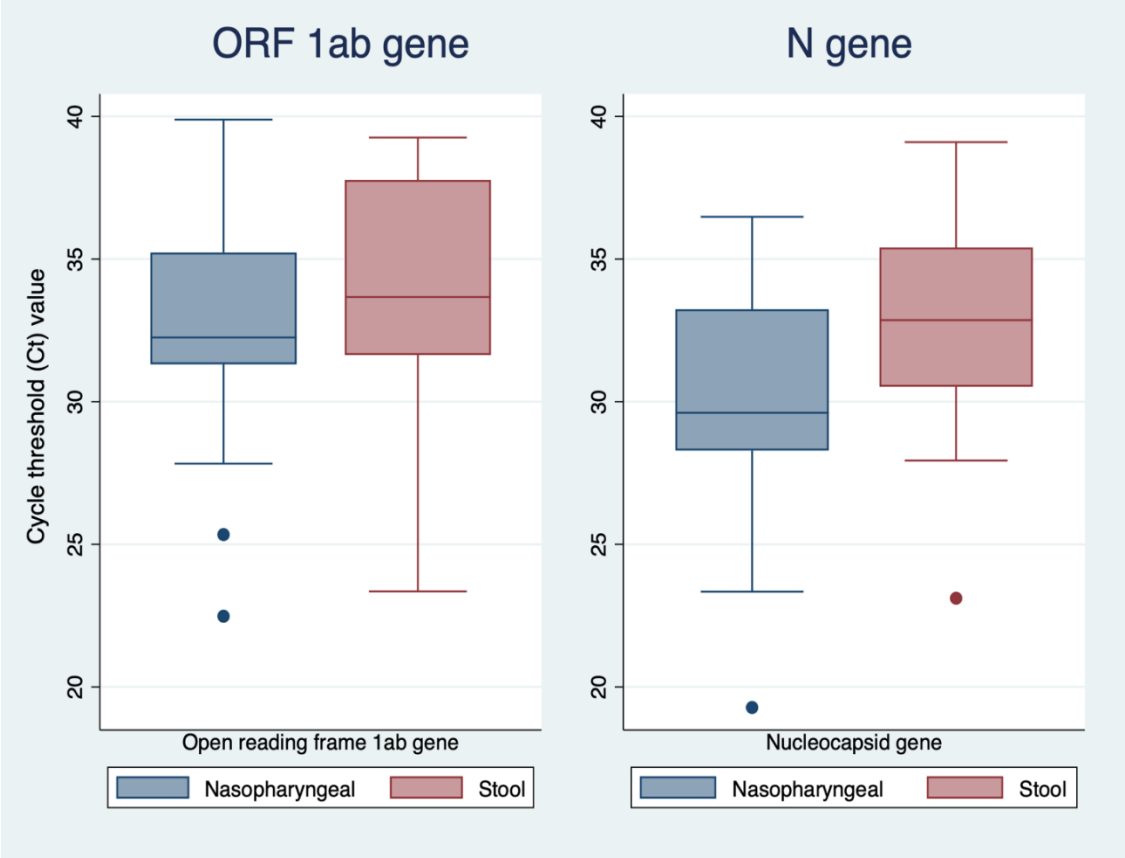

Figure 5: Comparison between cycle thresholds in stool and Nasopharyngeal swab samples. (a) Comparison between cycle threshold in stool and nasopharyngeal swab for ORF1ab gene target; (b) comparison between cycle threshold in stool and nasopharyngeal for the N gene targets.

Further analysis comparing the average cycle threshold of the N and ORF 1ab gene between nasopharyngeal swab and stool samples was performed. The average Ct values for the N gene in Nasopharyngeal swab was shown to be lower (30.00) with the Ct values falling between 19 and 32, for the stool sample, the average cycle threshold for the N gene obtained was (32.74) with the Ct values falling between 23 and 39. The nucleocapsid gene had a significantly lower cycle threshold value from the nasopharyngeal swab compared to stool sample (p=0.0038). The average Ct value for ORF 1ab gene was 32.9 and 33.86 for nasopharyngeal swab and stool samples respectively and with the Ct values ranging between 22 and 40 for nasopharyngeal swab and 23 and 39 for stool samples. There were no observed significant difference on the Ct values of the ORF 1ab gene between nasopharyngeal and stool samples (p=0.3223).

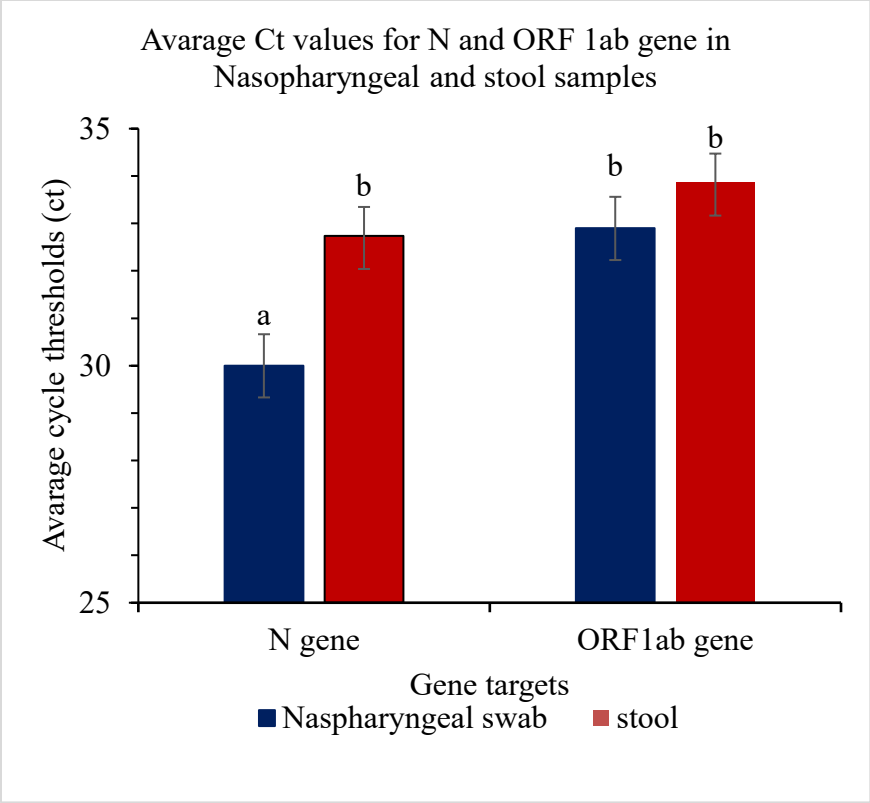

Figure 6: Mean cycle thresholds between Nasopharyngeal and stool samples for N-gene and ORF 1ab gene targets.

4.4 Ig-G and Ig-M antibodies profiles from symptomatic and asymptomatic COVID-19 patients

Blood serum samples were obtained from the same 92 recruited individuals and were used to test for the immune responses to SARS-CoV-2 among symptomatic and asymptomatic participants antibody targets were IgM and IgG. The presence and absence of antibodies were compared between patient status (symptomatic and asymptomatic patients), age group and gender and vaccination status in terms of those that had received full dose, partial dose and those who had not been vaccinated). Majority of the patients were symptomatic with 61.95% (57/92) with which 16 (28.07%) and 43(75.44%) tested positive for IgM and IgG respectively. Asymptomatic patients were 38.04% (35/92), 12(34.29%) and 28(80.00%) tested positive for IgM and IgG respectively. Table 8. Amidst the asymptomatic participants, there were 5(14.29%) positive cases with the rt-PCR and 30 (85.71%) negatives. Symptomatic individual constituted of 26(45.61%) rt-PCR positives and 31(54.39%) negatives. Fully vaccinated individuals were 31(33.69%). Of the 31

participants, 15 tested positive with rt-PCR and 16 negatives, 15.22% (95% CI 8.04 – 22.8) were partially vaccinated with 5 testing positive with the rt-PCR and 9 testing negative while 51.08% with 11 rt-PCR positive and 36 of the participants who did not receive any vaccine tested negative. In terms of vaccination status and rt-PCR positivity rate, especially those that were fully vaccinated, there were no significance difference between the positives and negatives (p=0.072).

A two-sample t-test was used to determine the mean IgG and IgM antibody profiles between the asymptomatic and symptomatic patients. Figure 7. The mean between IgM and IgG profiles differed significantly (p=0.002) in asymptomatic and symptomatic individuals, with IgM profiles averaging to 1.11 (95%CI, 0.78 - 1.44) and 0.88 (95%CI, 0.65 - 1.11) for asymptomatic and symptomatic patients respectively. The IgG mean on the other hand had a mean of 4.30 (95% CI =3.30 - 5.31) and 4.16 (95% CI, 3.32 - 5.01) for asymptomatic and symptomatic patients respectively. Out of the 31 fully vaccinated, 14 partially vaccinated and 47 not vaccinated 19(61.29%), 7(50.00%), 38(80.85%) and 4(12.90%), 4(28.57%), 13(27.66%) tested negative for IgM and IgG respectively.

Table 8: Test outcomes for IgM, IgG antibody profiles by patient and vaccination status.

	n = 92	IgM test results		IgG test results	
Patient status /vaccination status		**Positive**	**Negative**	**Positive**	**Negative**
Asymptomatic	35	12(34.29)	23(65.71)	28(80.00)	7(20.00)
Symptomatic	57	16(28.07)	41(71.93)	43(75.44)	14(26.56)
Full dose	31	12(38.71)	19(61.29)	27(87.10)	4(12.90)
Partial dose	14	7(50.00)	7(50.00)	10(71.43)	4(28.57)
Not vaccinated	47	9(19.15)	38(80.85)	34(72.34)	13(27.66)
Mean antibody profiles by patient status					
		Mean	**SE.**	**Std. dev.**	**95% conf. intvl**
IgM Asymptomatic	35	1.1096	0.1644	0.9726	0.7755 - 1.4437
Symptomatic	57	0.8821	0.1157	0.8735	0.6503 - 1.1138
IgG Asymptomatic	35	4.3041	0.4949	2.9278	3.2984 - 5.3099
Symptomatic	57	4.1627	0.4211	3.1788	3.3192 - 5.0062

4.4.1 IgM and IgG Antibody Response by Age group and Gender

The participants were grouped into separate groups. Antibody responses were compared based on age and gender. Participants aged 16 - 35 years considering both genders detected the highest levels of IgG antibodies, alternatively, those that aged less than 15 years and above 60 years had recorded lowest amounts of IgM antibodies.

None of those aged above 46 years in both the genders tested negative for the IgG. Figure 5. Conversely IgM response was seen to be very low across all the genders and age group except for those aged between 16-45 years which had slightly high IgM response, an indication that the infection rates might be similar across all age groups. Figure 5.

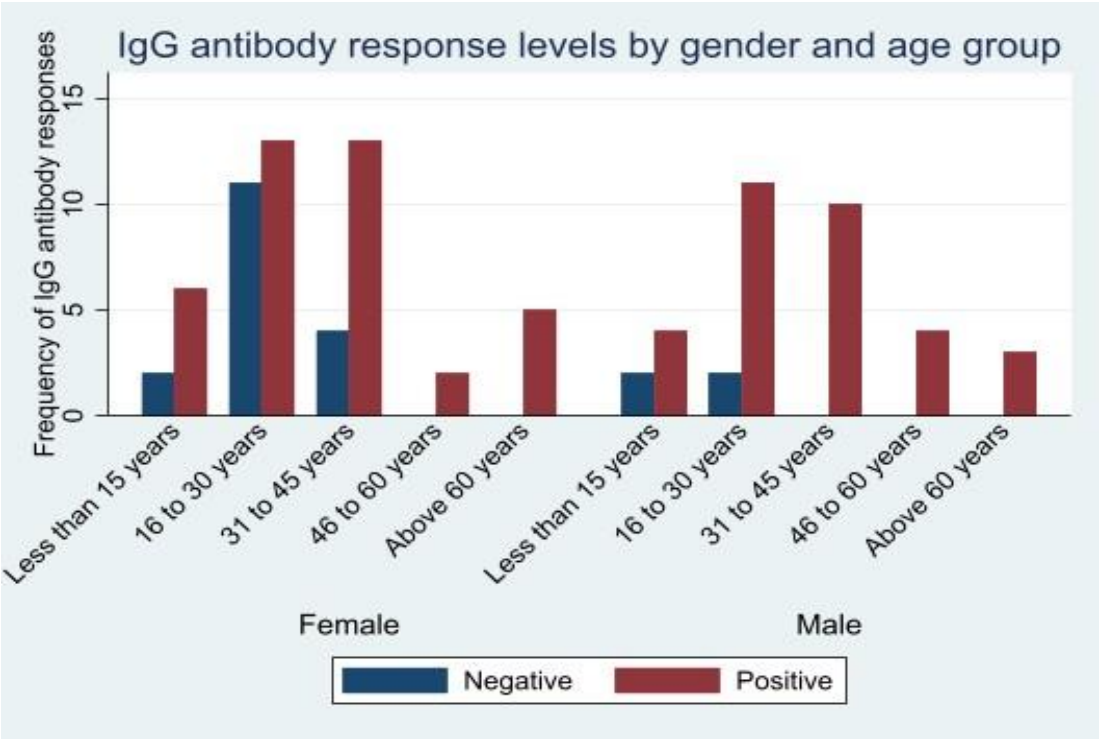

Figure 7: IgG antibody response by age group and gender

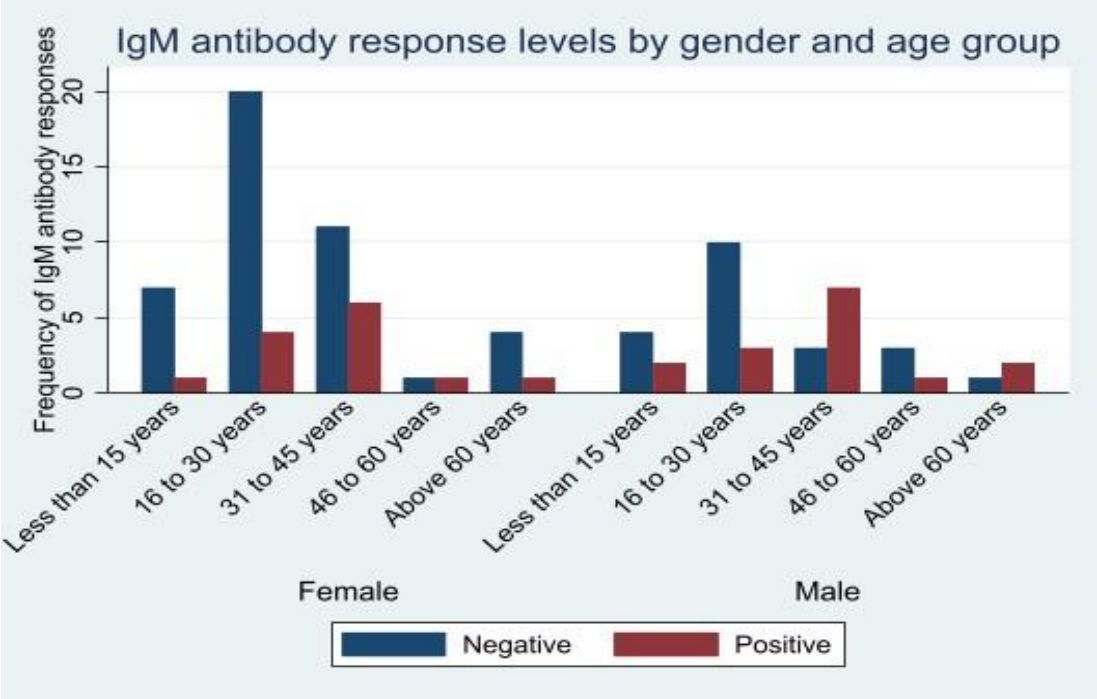

Figure 8: IgM antibody response levels by age group and gender

Mean IgM and IgG antibody response levels between asymptomatic and symptomatic COVID-19 patients.

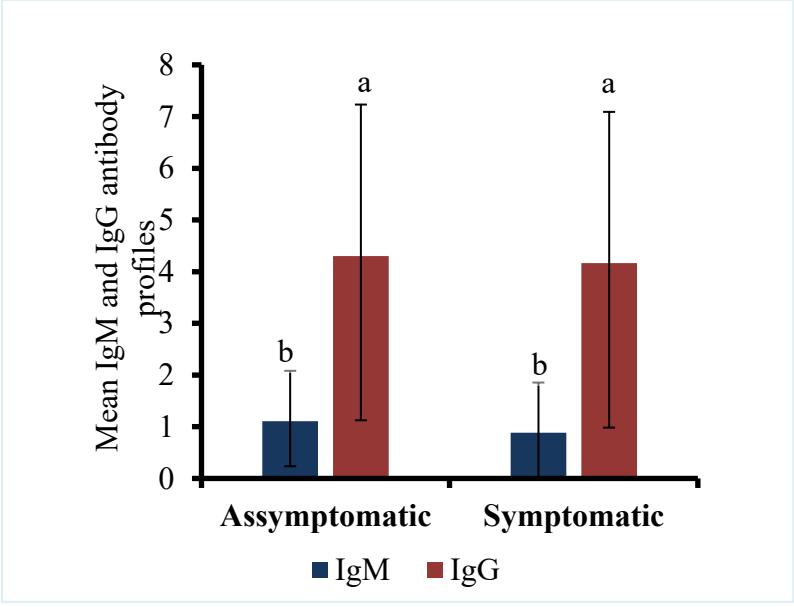

Figure 9: Mean antibody levels by patient status

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Diagnostic Sensitivity and specificity of Ag-RDT and rt-PCR

Poor choice of SARS-CoV-2 diagnostic technique alters early detection, prophylactic responses and first mitigation approaches to the COVID-19 pandemic (Cascella *et al*., 2022). Proper choice of a detection method on the other hand allows first detection, early contact tracing, isolation and removal of infectious COVID-19 patients which minimizes transmission opportunities (Wiersinga *et al.*, 2020). The use of Nasopharyngeal swab has been recommended by World Health Organization as the reference sample for the detection of SARS-CoV-2 (Péré, Péré, *et al*., 2020). Up to date, the most explored and reliable diagnostic test for SARS-CoV-2 virus is by use of rt-PCR along with nasopharyngeal swab sample (Mandal *et al*., 2022). However, the delay in turnaround time of the rt-PCR during the outbreak and the stigmatization that was brought by nasopharyngeal swab sampling technique affected mass screening in the population and disease surveillance (Mandal *et al*., 2022). To mitigate this challenge, Ag-RDT which is cheap with short turnaround time and do not require well-trained personnel neither expensive machines, were introduced to help detect COVID-19 (Ghasemi *et al.*, 2022). This study employed the use of nasopharyngeal swab sample to compare the diagnostic sensitivity and specificity of the Ag-RDT and rt-PCR considering rt-PCR as the standard diagnostic regime.

A lower sensitivity of 76.3% and a higher specificity of 96.3% was achieved with the Ag-RDT. However, the sensitivity of rt-PCR was higher with 93.5% with a lower specificity of 85.2% with a Ct cut-off value ≤40. Ag-RDT recorded a high positive and negative predictive value. The low sensitivity and high specificity of Ag-RDT observed in this study may be as a result of low viral load in the sample at the time of sample collection and detection of the virus. This was confirmed in a study carried out by Onsongo *et al*., (2021b) which evaluated Ag-RDT at Ct cut off of ≤40. Alternatively, the outcomes of this study suggests that rt-PCR still remains the gold standard test in the detection of SARS-Cov-2 (Mandal *et al.*, 2022). The Center for Disease Control and Prevention (CDC, 2021) has recommended the use of Ag-RDT, putting in considerations that a sensitivity of <80% (73.6%) for the Ag-RDT should not replace the gold standard test in diagnosis and

surveillance of SARS-CoV-2 infections. In a field evaluation study carried out by Albert *et al.,* (2021) and Torres *et al.*, (2021), similar to this study, Ag-RDT kit displayed 76% sensitivity and a 100% specificity. Onsongo *et al.*, (2021b) in their field evaluation study, recorded a sensitivity of 71.5% (95%CI; 62.3% - 78.7%) and specificity of 97.5% (95%CI; 96.2% - 98.5%) for Ag-RDT when Ct cut-off of ≤40 was used. Indeed, Torres *et al*., (2021) recorded a 48.1% sensitivity and 100% specificity for Ag-RDT kit and a negative predictive value (NPV) of 93.1%. These observations are in line with this study, except for the very low sensitivity of the Ag-RDT.

The high specificity (96.3%) of Ag-RDT can be of significant value despite its low sensitivity, they can play an important role in guiding patients at point of care and in public health decision making which may reduce the transmission rates.(Albert *et al.*, 2021). The 93% positive predictive value of the antigen rapid diagnostic test implies that both asymptomatic and symptomatic patients that had positive antigens were infected and do not require real time rt-PCR for confirmation (Albert *et al.*, 2021). Similarly, the negative predictive value of Ag-RDT (85%), indicates that persons with negative Ag-RDT do not have COVID-19 disease. Receiver Operating Curve of Ag-RDT and its corresponding area under the curve suggested an 87% chance that the Ag-RDT was able to correctly distinguish the true positives from the false negatives rating the Ag-RDT to have an excellence diagnostic capability.

There was a discrepancy with 2 false-negative and 9 false-positives results between the Ag-RDT and rt-PCR. The discrepancy might have been due to inconsistent factors that determines the performance of Ag-RDT including site of sample collection, the type of antigen kit used, sample handling during collection and antigen extraction process, the Ct values and the viral load (Chaimayo *et al.*, 2020; Mak *et al.*, 2020). Contrastingly, in a cross-sectional study that was carried out on COVID-19 infected individuals at Kathmandu in Nepal, Mandal *et al.*, (2022) obtained a discrepancy of 43 false negatives and 2 false positives out of 213 COVID-19 patients. Diagnostics agreement data between ag-RDT and rt-PCR showed a Cohen's kappa value of 0.75 displaying substantial agreement. This shows that there was consistency during data collection, and the data collected are proper representations of the variables measured. The findings obtained by Lee *et al.*, (2021)

recorded a Cohen kappa value of 0.75 which are similar to the findings of this study. Contrastingly, another study observed a Cohens kappa value of 0.59 which translated to a moderate agreement (Mandal *et al*., 2022). These differences may be because of the presence of bias between the observers and data distribution which highly affected the kappa values (Sim and Wright, 2005).

5.2 rt-PCR RNA Profiles from Nasopharyngeal Swab and Stool samples

The findings of this study showed that 30.43% and 33.7% of the individuals were having the virus in stool and nasopharyngeal swab samples respectively, 21.4% of the proportion that tested positive with stool tested negative with nasopharyngeal swab. This approach was efficient, especially when a proportion of patients who receive negative rt-PCR test results from the nasopharyngeal swabs still tested positive for COVID-19 when stool-derived viral RNA was analyzed. Also, 29% of the patients that tested positive in nasopharyngeal swab tested negative in stool derived RNA. This suggests the possibility of different virus incubation periods for respiratory and enteric infections, a differing rate of viral replication in each organ/system together with different rates of viral shedding (Daou *et al*., 2022). These finding are in line with prior data obtained by Chen *et al.*, (2020) that suggested a total of 28 (66.67%) patients out of 42 having detectable SARS-CoV-2 RNA in stool samples. Similarly, other studies had reported the presence of SARS-CoV-2 RNA in anal/oral swabs from 16 COVID -19 patients in Hubei province, swabs from throat and sputum from 17 patients (Wang *et al*., 2020). In their study they also found out that 18 (64%) of the patients turned negative with nasopharyngeal swabs but were positive when stool samples were used.

The mean cycle threshold for the target genes; N and ORF1 ab were lower in nasopharyngeal swab compared to stool. The inverse relationship that exists between the viral load and the cycle threshold values and the viral load declaratively shows that specimens with low cycle threshold value have high viral load and that those with high cycle threshold values have low viral load. Therefore, it can be inferred that the nasopharyngeal swab samples had maximum viral load compared to that of stool samples with a significance difference of (p=0.0038) for the N gene. The amplification of the ORF 1ab gene showed no differences in the two samples (p=0.3223). This therefore indicates

high presence of viral titers in the nasopharyngeal swab compared to that of stool sample (Zheng *et al*., 2020). That makes collection through nasopharyngeal swab more reasonable (Sharma *et al.*, 2021). Although many concepts based on hygiene currently bank on the supposition that the likeliness of SARS-CoV-2 to establish an infection depends on the Ct values and that the infectivity rate decreases with an increase in the Ct values (Platten *et al.*, 2021), this assumption may be true to some extent though the conclusions made that patient having low viral load/high Ct value may not transmit the disease affects successful case managements. This is because there is no reliably determined infectious dose for the minimum viral loads and various researches are still underway to determine the number of virions of SARS-CoV-2 that are likely to cause an infection. Although many studies have recommended the use of Nasopharyngeal swab (Sharma *et al.*, 2021), other studies have pointed out the disadvantages of the nasopharyngeal swab. For instance, collections through nasopharyngeal swab can be invasive and uncomfortable to individuals taking the test (Kinloch *et al.*, 2020). Again, it requires highly trained healthcare workers, which can manipulate potential infectious samples.

SARS-CoV-2 RNA in stool when correlated with the obtained Ct values indicated low viral loads (average Ct value 33) both for the N gene and ORF1ab gene. To date, few studies have addressed the possible viral shedding from stool samples directly (Foladori *et al.,* 2020). Presently, it is presumed that SARS-CoV-2 might be contagiously transmitted through the fecal-oral route (Cerrada-Romero *et al.*, 2022), which shows a clear indication that the virus can still be transmitted from one person to another despite insignificant SARS-CoV-2 RNA in stool samples. The findings of this study also pointed out that only 10 % of the individuals that tested positive for the virus in stool had non-respiratory symptom diarrhea. Contrary to the findings of this study, this symptom was highly seen on patients that detected positive for the virus in stool samples (Daou *et al*., 2022). The same observation was previously reported in several small case series and large case studies reviewed by (D'Amico *et al*., 2020). Inferences can therefore be made that the presence of the virus can be detected in persons having or not having gastrointestinal symptoms (Szymczak *et al*., 2020). Although studies indicate that the stool sample is easier to obtain since the patients can collect the samples by themselves and comfortably following instructions from healthcare professionals (Cerrada-Romero *et al.*, 2022). Notably, the

accessibility of the stool samples may be challenging (Guo *et al.*, 2021). Collection of stool samples is also characterized by a number of challenges, for instance in this study, majority of the individuals had their specific time when they could produce stool, others with underlying conditions were not capable of producing stool. Children below the age of 5 years could not produce stool within the facility during sample collection.

5.3 Ig-G and Ig-M antibody profiles from symptomatic and asymptomatic COVID-19 patients.

With regards to IgM and IgG antibodies against SARS-CoV-2, 28(30.43%) and 71(71.17%) of the cases were positive for IgM and IgG respectively. The significant differences seen between IgM and IgG antibodies may be attributed to the fact that the IgM antibodies are primarily produced to help fight for the current infections and lasts for a shorter period of time within the body. Therefore, it is possible that many of the cases observed in this study were not at an acute phase of infection. Alternatively, it can be inferred that after the infection had already passed and the individuals had recovered, the IgG antibodies remained in the blood. In most patients, these antibodies develops within 7 to 10 days after the onset of symptoms of COVID-19 (Shirin *et al.*, 2020). The presence of IgG antibodies within the blood is an indication that one might have had the virus in the resent past and was capable of developing these antibodies for protection from future infections. Similar study by Y. Tang *et al*., (2022) showed a lower and higher percentages in IgM and IgG respectively. Contrastingly, studies carried out in Aracaju and Itabaiana obtained higher percentage in IgM sero-prevalence compared to that of IgG (Borges *et al.*, 2020). Several factors that affects antibody levels as reported by Mitani *et al.,* (2021) includes age, comorbidities and smoking habits.

Regarding antibody responses in asymptomatic and symptomatic individuals, the mean IgM did not differ significantly between the asymptomatic and symptomatic individuals (p=0.2486) similarly to mean IgG (p=0.8315). The high percentages and higher mean antibody levels of IgM and IgG antibodies were shown within asymptomatic individuals. This suggests earlier infections and ongoing community transmission within the two counties during the study period. These findings correlates with those of Borges *et al.*,(2020) which reported a high SARS-CoV-2 antibody prevalence among asymptomatic

individuals in Sergipe. Interestingly, according to Oran & Topol (2020), asymptomatic individuals are said to account for 40%-50% of SARS-CoV-2 infections. Therefore, they are capable of transmitting the disease to others over a very long period of time. Conclusions can therefore be made that substantial missing of asymptomatic infections based on the rt-PCR alone cannot be found out in time, this also experimentally confirm the view of the prediction model with the number of undiscovered infections (Jun *et al.*, 2021). Thus, epidemiological monitoring of symptomatic individuals can only control a portion of all infections.

There was a variation by age and gender on the proportion that tested positive for IgM and IgG antibodies. In agreement, highest prevalence for IgM and IgG antibodies for both, men and women were seen within age group of 16-45 years. Those below 15 years and above 60 years of age revealed a low prevalence for IgM and IgG antibodies. The decline of antibodies in elderly individuals is faster and may be due to the aging effect of the immune system, factors including deficiency in T- lymphocytes and reduced support for memory plasma located in the born marrow may also affect the antibody quantity within the elderly individuals (Howard *et al.*, 2006). The low quantity of the antibodies found on the younger and elderly individuals may suggest that despite age group, the infections rates might be similar across age groups and social mixing of older adults had not been efficiently controlled (Bi *et al.*, 2020). These findings are similar to those of Borges *et al*., (2020) in Brazil noting that age group (35-50 years of age) showed higher percentages of IgM and IgG antibodies (*Coronavirus Brasil*, 2020).

Among the vaccinated individuals, only 12.09% had no detectable antibodies. The detection of antibodies targets different parts of the virus, for instance, the spike proteins of the virus which are produced to fight the viral infection and in response to the vaccine as well, whereas other serological tests targets the nucleocapsid protein of the virus (Bausch *et al.*, 2021). The S protein is mostly used as the target in COVID-19 vaccine (Dai & Gao, 2020). According to Gavi, The Vaccine Alliance (2021), the small proportion that had no detectable antibodies might have either received the vaccine within the past two week and the body had not produced antibodies against SARS-CoV-2 and might have not had the infection yet. Additionally, it is still unclear in the duration that the antibodies exists

in an individual after COVID-19 infection and the number of antibodies needed to neutralize SARS-CoV-2 following another attack (Chvatal-Medina *et al.*, 2021). Alternatively, apart from humoral immune response that produces antibodies, the body also uses cell mediated immunity that partially induces response against the infection (Guihot *et al.*, 2020). Interestingly, evidence indicates that specific T-cell responses to SARS-CoV-2 is essential for clearing the virus and may and may prevent infection without seroconversion and yield sensible memory (Moss, 2022). Thus, conclusions cannot be made that those who had no detectable antibodies had no previous infection or rather have no longer had the infection. They might have had an infection and on the other hand, the better proportion that had superior immune response maybe due to hybrid immunity (Pilz *et al*., 2022). After vaccination, there is high introduction of responses in individuals that were previously infected and may significantly have high levels of antibodies (Karachaliou *et al.*, 2022). Although a number of individuals not vaccinated tested positive for antibodies, it may be inferred that majority of the populations had contracted the virus and their immune system had produced antibodies against the infection (CDC, 2020).

5.4 Limitation of the Study

This study had some limitations, though did not affect the quality of the study. One, the number of recruited individuals was small and was confined to patients who attended Siaya and Kisumu referral hospitals, there are other several hospitals within the two counties and therefore it is impossible to generalize the findings of this study to the population of Kisumu and Siaya counties. Two, elimination of false positive antibody titers was not achieved.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. With the high specificity, positive and negative predictive values in individuals together with rapid test results that implies faster tracing of infected individuals, these results can attribute to and provide policymakers with a comprehensible evidence that rapid antigen tests may play an interesting role in testing and contact tracing strategies that will in turn help in the control of COVID-19 pandemic, especially in those areas that lack suitable laboratories with rt-PCR machines to perform SARS-CoV-2 detection. Additionally, Ag-RDT can be used in areas where results take more than 24-48 hours and also in areas with large populations.
2. The presence of viral RNA from stool specimen suggests viral replication in the gastrointestinal system and viral shedding in stool as observed in this study. The implication that SARS-CoV-2 RNA could be detected in stool regardless the viral load could still be a concern for the SARS-CoV-2 transmissibility.
3. The positivity rate of the IgM and IgG response against SARS-CoV-2 were higher in asymptomatic patients compared with symptomatic covid-19 patients, which might have been due to several factors including age, vaccination status, and the kind of developed immunity. The study therefore concludes that the asymptomatic patients were highly exposed.

6.2 Recommendations

6.2.1 Recommendations from the study.

1. The study recommends the use of Ag-RDT at point of care and screening of vulnerable population before confirmation using rt-PCR.
2. The study also recommends that nasopharyngeal swab samples be used during the diagnosis of SARS-CoV-2 due to their high viral load and cab be supplemented with stool samples to reduce the rates of false negatives.
3. The study recommends screening of both asymptomatic and symptomatic individuals, this will help in the determination of true spread of the virus.

6.2.2 Recommendation for further studies

1. Further evidence is required on the reliability and use of newly introduced types of antigen rapid test kits as well as its comparison with standard test should be conducted to reach a definitive conclusion about their use.
2. Future studies should assess stool samples to complement other clinical samples for other infectious viral diseases.
3. Future studies on epidemiological monitoring of pandemics should target both symptomatic and asymptomatic individuals as a means of mitigating disease spread.

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APPENDICES

Appendix I: JOOUST Ethical approval

JARAMOGI OGINGA ODINGA

UNIVERSITY OF SCIENCE AND TECHNOLOGY

DIVISION OF RESEARCH, INNOVATION AND OUTREACH JOOUST-ETHICS REVIEW OFFICE

Tel. 057-2501804 P.O. BOX 210-406

Email: erc@jooust.ac.ke BONDO

Website: www.jooust.ac.ke

OUR REF: JOOUST/DVC-RlO/ERC/E3 **19 May 2021**

Prof Benson B. Estambale

DVC-RIO

JOOUST

Dear Prof Estambale,

RE: APPROVAL TO CONDUCT RESEARCH TITLE ‘‘TOWARDS COVID-19 CONTAINMENT: SEROLOGICAL, FAECAL AND WASTEWATER

EPIDEMIOLOGICAL SURVEILLANCE OF SARS-C0V-2 IN SUPPORT OF HOME-BASED ISOLATION AND CARE IN KENYA"

This is to inform you that JOOUST ERC has reviewed and approved your above research proposal Your application approval number is ERC/21/5/21-4. The approval period is from 9May 2021 to 18th May 2022. This approval is subject to compliance with the following requirements:

1. Only approved documents including (informed consent, study materials, MTA) will be used
2. All changes including (amendments, deviations and violations) are submitted for review by JOOUST IERC.
3. Death and life promising problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to NACOSTI IERC within 72 hours of notification.
4. Any changes, anticipated or otherwise that may increase the risk of affected safety or welfare of the study participants and others or affect the integrity of the research must be reported to NACOSTI IERC within 72 hours.
5. Clearance of export of biological specimens must be obtained from relevant institutions.
6. Submissions for request of renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
7. Submissions of an executive summary report within 90 days upon completion of the study to the JOOUST IERC

Prior to commencing your study, you will be expected to obtain a research permit from National Commission for Science Technology and Innovation (NACOSTI). Https: oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

Prof. Francis Anga'wa
Chairman, JOOUST IERC

Appendix II: Ethical Approvals

NACOSTI Permit

NATIONAL COMMISSION FOR

SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 797616 Date of Issue: 19/May/2022 RESEARCH LICENSE

This is to Certify that Jaramogi Oginga Odinga University of Science and Technology, has been licensed to conduct research in Kisumu, Siaya on the topic: Towards COVID-19 containment: Serological, Faecal and Waste Water Epidemiological Surveillance of SARS-CoV-2 and Home-Based Care in Kenya for the period ending: 19/May/2023

License No: NACOSTI/P/22/17545

Applicant Identification Number Director General 797616

NATIONAL COMMISSION FOR

SCIENCE, TECHNOLOGY &

INNOVATION

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National Commission for Science, Technology and Innovation off Waiyaki Way, Upper Kabete, P. O. Box 30623, 00100 Nairobi, KENYA. Land line: 020 4007000, 020 2241349, 020 3310571, 020 8001077. Mobile: 0713 788 787 / 0735 404 245, E-mail: dg@nacosti.registry@nacosti.go.ke Website: www.nacosti.go.ke

Appendix III: Consent form

COVID-19 Consent Form Version 1 23 Feb 2021

Title of the Research Study: Towards COVID-19 Containment: Serological, Faecal and Wastewater Epidemiological Surveillance of SARS-COV-2 in Support of Home-Based Care in Kenya.

Investigator(s) Prof Benson Estambale, Jaramogi Oginga Odinga University of Science and Technology PI.

Study location: Kisumu and Siaya Counties

You are being asked to take part in a study. The box below tells you important things you should think about before deciding/allowing your child to join the study. We will provide more detailed information below the box. Please ask questions about any of the information before you decide to participate. You may also wish to talk to others (for example, your family, friends, or colleagues) about this study, before agreeing to join.

Key Information for You to Consider

- **Voluntary Consent.** You are being asked to volunteer for a research study. You can choose whether you would like to participate or not. If you do agree you can change your mind at any time and withdraw from the research. This will not affect you in any way.
- **Purpose**. We are conducting this study to find alternative ways of detecting COVID19 in the community. We want to understand the transmission dynamics in local populations and assess the effectiveness of Home-Based Isolation and Care in containing COVID-19. The data generated by this study will also be used to develop a real-time platform for COVID-19 surveillance.
- **Duration**. Your participation in this study will require you to provide us with single venous blood sample from your arm, a stool sample, and a nasopharyngeal sample at only time point.
- **Procedures and Activities**. We will request for 1 venous blood sample. The total blood volume that is taken is 4 mL (1.5 teaspoons). You will also provide a stool

sample and give a nasopharyngeal sample for COVID-19 testing.

- **Risks or disadvantages**. Most studies have possible harms that could happen to you if you join. In this study, we expect that You might feel a small amount of discomfort during blood sampling and may have a small amount of bruising or bleeding on arm where the blood sample was taken. This is considered not to be harmful. We will use sterile equipment to collect the blood sample and the small wound that may arise from the procedure will be treated adequately. The volume of blood is too small to influence your health/the child's health and the blood will quickly be replaced by your/your child's body.
- **Benefits.** There are no direct benefits in this study. However, your participation will inform the development of COVID 19 surveillance strategies and we will provide you with your COVID-19 test results. Your participation will help in understanding the spread of COVID-19 in the country and ways of strengthening its containment.

Purpose of the Research: This study is being undertaken by Ministry of Health, Kenya Medical Research Institute (KEMRI), Jaramogi Oginga Odinga University of Science and Technology (JOOUST), Masinde Muliro University of Science and Technology (MMUST), and Kenya Industrial Research and Development Institute (KIRDI). The coronavirus disease 2019 (COVID-19) caused by coronavirus (SARS-CoV-2) is affecting many livelihoods with the elderly and people with underlying health conditions like diabetes and hypertension most at risk. At the moment we know very little about the disease presentation in our population. We want to examine how the body fights the disease though the immune response. We will recruit people who are infected with COVID-19 but do not experience COVID-19 symptoms and those with symptoms in Kisumu and Siaya, counties. To do this we will take venous blood sample (about 1.5 teaspoons of blood), stool and nasopharyngeal samples from you at a single time point. You may experience some discomfort when the blood is taken and a small bruise may occur, however we will use sterile equipment to minimize infections. We will use the blood to compare the IgG and IgM antibody responses in individuals, and test for COVID-19 in your stool and nasopharyngeal samples. These tests will be performed at the Kenya Medical Research

Institute laboratories in Kisumu. The findings of the study will be shared with the community.

Study procedures This study will involve 250 individuals with COVID-19 symptoms and another 250 with no COVID-19 symptoms who will be invited to participate in this study. If you or your child chooses to participate, you are/your child is asked to participate for a single day. On this day, a venous blood sample, a stool sample and nasopharyngeal samples are taken. The blood sample is used to examine human antibody responses against COVID-19 and determine if the individual has been infected before and raised a response against the virus. In total the venous blood volume that is taken is 4 mL (1.5 teaspoons). If COVID19 symptoms occur, you/your child will receive medical support according to the Ministry of Health guidelines. A qualified doctor will ask questions on health, determine temperature, and perform clinical examination. If the clinician thinks that you/your child requires additional health care, this will be provided. You will not be charged for this treatment.

Voluntary participation. Your decision not to participate or to withdraw from participation will not affect the care you/your child will receive at the clinic in any way. Even if you do agree to become a study participant, you can withdraw from the study at any time. If you chose not to participate, you and your child will have access to the same level of clinical care.

Discomforts and Risks You/your child might feel a small amount of discomfort during blood sampling and may have a small amount of bruising or bleeding on or arm where the blood sample was taken. This is considered not to be harmful. We will use sterile equipment to collect the blood sample and the small wound that may arise from the procedure will be treated adequately. The volume of blood is too small to influence your health/the child's health and the blood will quickly be replaced by your/your child's body. Should your child experience any of these adverse events you may contact Prof. Benson Estambale on +254 722700185.

Benefits. There are no personal benefits to taking part, but your blood samples will contribute towards a better understanding of COVID-19 presentation in the population and

inform the development of COVID 19 surveillance strategies. We will provide you with your COVID-19 test results.

Confidentiality statement. The records concerning your /your child's participation are to be used only for the purpose of this research project. Your / your child's name will not be used on labels on laboratory specimens or in any report resulting from this study. If data are shared as part of publications, your name and other personal information will be removed. At the beginning of the study, we will give you/your child a study identification number and this number will be used on the forms and on the laboratory specimens. Any information obtained in connection with this study will be kept strictly confidential and under lock and key. Only senior members of the study team will have access to information linking your/your child's name with his/her study number.

Questions and freedom to withdraw from the study If you or your child have/has any question concerning this study, do not hesitate to contact Principal Investigator Benson Estambale. Results from the study will be communicated to your community. In case you want to contact an independent person, not related to the study, about the research study itself, you or your child's rights as a research subject or any research-related injury, you can contact the Chairperson of Jaramogi Oginga Odinga University of Science and Technology (JOOUST) Ethics Committee on Telephone number 057-2501804 or on postal address of P.O. Box 210-40601, Bondo.

Jaramogi Oginga Odinga University of Science and Technology: official Consent Form STUDY TITLE: Towards COVID-19 Containment: Serological, Faecal and Wastewater Epidemiological Surveillance of SARS-COV-2 in Support of Home-Based Care in Kenya. I have had the study explained to me. I have understood all that has been read/explained and had my questions answered satisfactorily.

I agree to participate in the study by providing a venous blood sample

□ Yes □ No

I agree participate in the study by providing a stool sample

□ Yes □ No

I to agree to participate in the study by providing a nasopharyngeal sample

□ Yes □ No

I understand that I can change my mind at any stage, and it will not affect me in any way.

Signature:_____ Date _____

Participant name_____ Time _____ (Please print name)

Where participant cannot read, ensure a witness* observes consent process and signs below:

I *attest that the information concerning this research was accurately explained to and apparently understood by the participant and that informed consent was freely given by the participant.

Witness' signature: _____ Date_____

Witness' name: _____ Time_____ (Please print name) *A witness is a person who is independent from the study or a member of staff who was not involved in gaining the consent. Thumbprint of the participant as named above if they cannot write: I have followed the study SOP to obtain consent from the participant. He apparently understood the nature and the purpose of the study and consents to the participation in the study. He has been given opportunity to ask questions which have been answered satisfactorily. Designee/investigator's signature_____

Date_____ Designee/investigator' Name: _____ Time_____ (Please print name) THE PARTICIPANT SHOULD NOW BE GIVEN A SIGNED COPY TO KEEP

Appendix VI: Case investigation form

MINISTRY OF HEALTH

Division of Disease Surveillance and Response

Case investigation form for 2019 Novel Coronavirus (2019-nCoV)

Date of reporting to national health authority: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_] Reporting Health Facility-Sub Country----------Country: ……

Detected at point of entry □ No□ Yes□ Unknown If yes, date[_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]

Section 1: Patient information

Unique Case Identifier (used in country): ……. Date of Birth: [_D_] [_D_]/[_M_] [_M_]/[_Y_][_Y_][_Y_][_Y_]or estimated age: [ ] in years if < 1 year old, [ ][ ] in months or if < 1 month, [ ][ ] in days . Sex at birth: □ Male □ Female Place where the case was diagnosed: Health Facility: ……

Admin Level 1 (County): ---------- Admin Level 2 (Sub County): ……...

Patient usual place of residency: Country: ……

Admin Level 1 (province): ……Admin Level 2 (district): ……..

Section 2: Clinical information

Patient clinical course Date of onset of symptoms: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]

□ Asymptomatic □ Unknown Admission to hospital: □ No □ Yes □ Unknown First date of admission to hospital: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_] Name of hospital: ……

Date of isolation: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]
Was the patient ventilated: □ No□ Yes□ Unknown
Health status (circle) at time of reporting: Stable / Severely ill / dead / unknown
Date of death, if applicable: [_D_] [_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]

Patient symptoms (check all reported symptoms):
□History of fever / chills □ Shortness of breath □ General weakness □ Diarrhea
□ Runny nose □ Cough □ Sore throat □ Nausea/vomiting □ Headache□
Irritability/Confusion □ Pain (check all that apply) □ Joint □Chest □Abdominal □
Muscular Other, specify
…….

Patient signs:

Temperature: [ ][ ][ ] □°C / □ F
□Pharyngeal exudate
□Abnormal lung X-Ray findings
□ Coma □Conjunctival injection
□Seizure
□ Abnormal lung auscultation
Other, specify: ……

Underlying conditions and comorbidity (check all that apply):

□Pregnancy (trimester: ----------) □ Post-partum (< 6 weeks)
□Cardiovascular disease, including hypertension □ Immunodeficiency, including HIV
□Diabetes □ Renal disease □Liver disease □ Chronic lung disease
□Chronic neurological or neuromuscular disease □ Malignancy
Other, specify: ……

Section 3: Exposure and travel information in the 14 days prior to symptom onset (prior to reporting if asymptomatic)

Occupation: (tick any that apply)

Student □ Health care worker □ Working with animals □ Health laboratory worker □Other, specify: ……

Has the patient travelled in the 14 days prior to symptom onset? □ No□ Yes □ Unknown

If yes, please specify the places the patient travelled:

Country	City
1……	……..
	2……
……..	
	3……
…….	

Has the patient **visited any health care facility (ies**) in the 14 days prior to symptom onset? □ No □ Yes□ Unknown

Has the patient **had close contact1** with a person with acute respiratory infection in the 14 days prior to symptom onset? □ No □ Yes □ Unknown

If yes, contact setting (check all that apply): □ Health care setting □ Family setting □ Work place □ Unknown □ Other, specify…….

Has the patient had contact with a probable or confirmed case in the 14 days prior to symptom onset? □ No□ Yes □ Unknown If yes, please list unique case identifiers of all probable or confirmed cases:

Case 1 identifier…… Case 2 identifier…… Case 3 identifier…… If yes, contact setting (check all that apply): □ Health care setting □ Family setting □ Work place □ Unknown □ Other, specify: …….. If yes, location/city/country for exposure:

Have you visited **any live animal markets** in the 14 days prior to symptom onset? □ No □ Yes □ Unknown

If yes, location/city/country for exposure:

Have you been vaccinated? □ No□ Yes□ Unknown If yes, how many doses have you received: _____

Section 4: Laboratory Information

Specimen collection (*To be completed by the health facility*) Was specimen collected? 1=Yes2=No If no, why _____ Date(s) of specimen collection: [_D_] [_D_]/[_M_] [_M_]/[_Y_] [_Y_] [_Y_] [_Y_]

Specimen type: □NP Swab □ OP Swab □Serum □Sputum □ Tracheal Aspirate Other(specify): _____

Date specimen send to the lab: [_D_] [_D_]/[_M_] [_M_]/[_Y_] [_Y_] [_Y_] [_Y_]

(To be completed by the confirming lab) Name of confirming lab: _____

Please specify which assay was used:…… Sequencing done? □ Yes □ No □ Unknown Preliminary lab results……..……

Date of laboratory confirmation: [_D_] [_D_]/[_M_] [_M_]/[_Y_] [_Y_] [_Y_] [_Y_]

[1] Close contact' is defined as: 1. Health care associated exposure, including providing direct care for nCoV patients, working with health care workers infected with novel coronavirus, visiting patients or staying in the same close environment of a nCoV patient. 2. Working together in close proximity or sharing the same classroom environment with a with nCoV patient. 3. Traveling together with nCoV patient in any kind of conveyance. 4. Living in the same household as a nCoV patient.