EVALUATION OF LABORATORY TESTS FOR COVID-19 IN SOUTH AFRICA

Presented by

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DOCTOR OF PHILOSOPHY

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December 2023

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SUPERVISOR'S DECLARATION

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text. The research described in this dissertation was conducted at the Centre for the AIDS Programme of Research in South Africa (CAPRISA), School of Laboratory Medicine and Medical Science, College of Health Sciences, University of KwaZulu-Natal, under my supervision.

Signed: [redacted] Date: 01 December 2023

Professor Ayesha B.M. Kharsany (Supervisor)

Co-supervisor's Declaration

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text. The research described in this dissertation was conducted at the Centre for the AIDS Programme of Research in South Africa (CAPRISA), School of Laboratory Medicine and Medical, College of Health Sciences, University of KwaZulu-Natal, under my supervision.

Signed: [redacted] Date: 01 December 2023

Dr Aida Sivro (Co-supervisor)

DECLARATION 2 – PUBLICATIONS

The publications presented here originated from two studies, one funded by the European & Developing Countries Clinical Trials partnership (EDCTP): RIA2020EF-2928 - Mobilisation of research funds for COVID-19 as a Public Health Emergency and the South African Department of Science and Innovation (DSI) Top-up funding to strengthen EDCTP COVID-19 regional networks, which I was a co-investigator with Professor Ayesha BM Kharsany as Principal Investigator and Dr Aida Sivro as co-investigator. The studies were undertaken at the Centre for the AIDS Programme of Research in South Africa (CAPRISA) in collaboration with the Department of Health, Province of KwaZulu-Natal, and the King Dinuzulu Hospital complex and KwaMashu community health centre, both located in Durban, South Africa between July 2020, and December 2021. Subsequent studies for the field evaluation of the rapid antigen kits were funded by the Foundation for Innovative New Diagnostics (FIND): CV21-0261-Evaluation of the performance of antigen rapid diagnostics for SARS-CoV-2 detection, for which I was Principal Investigator and Dr Aida Sivro co-investigator. The studies were undertaken in Hillcrest and central Durban, KwaZulu-Natal. The study protocols for CAPRISA 225, CAPRISA 228, and CAPRISA 230 studies are available online (www.caprisa.org/Pages/CAPRISAStudies). This thesis constitutes my original work and complies with the stipulations set out for the degree of Doctor of Philosophy by research manuscripts.

The following publications contributed to this thesis:

Manuscripts Published

1. **Samsunder N**, Devnarain N, Sivro A, Kharsany ABM. The performance of diagnostic tests for severe respiratory syndrome coronavirus 2 (SARS-Cov-2) in the South African population: A scoping review. *Trop. Med. Infect. Dis*. 2023, 8, 514.

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2. **Samsunder N**, Lustig G, Ngubane S, Maseko TG, Rambaran S, Ngcapu S, Magini SN, Lewis L, Cawood C, Kharsany ABM, Karim QA, Karim SA, Naidoo K, Sivro A. Field evaluations of four SARS-CoV-2 rapid antigen tests during SARS-CoV-2 Delta variant wave in South Africa. *Diagn Progn Res*. 2023 Jul 25;7(1):14.

Contribution: As Principal Investigator, together with Dr Aida Sivro and other co-authors listed, I conceptualized the study, performed the experiments, conducted the data analysis, and prepared the first draft. All authors critically reviewed and approved the final version of the manuscript.

3. **Samsunder N**, de Vos M, Ngcapu S, Giandhari J, Lewis L, Kharsany ABM, Cawood C, de Oliveira T, Karim QA, Karim SA, Naidoo K, Escadafal C, Sivro A. Clinical Evaluation of Severe Acute Respiratory Syndrome Coronavirus 2 Rapid Antigen Tests During the Omicron Wave in South Africa. *J Infect Dis*. 2022 Oct 17;226(8):1412-1417.

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4. **Samsunder N**, Lustig G, de Vos M, Ngcapu S, Giandhari J, Tshiabuila D, San EJ, Lewis L, Kharsany AB, Cawood C, de Oliveira T, Abdool Karim Q, Abdool Karim S, Escadafal C, Naidoo K, Sivro A. Performance of rapid antigen tests in identifying Omicron BA.4 and BA.5 infections in South Africa. *J Clin Virol*. 2023 Aug; 165:105498.

   Contribution: As Principal Investigator, together with Dr Aida Sivro and other co-authors listed, I conceptualized the study, performed the experiments, conducted the data analysis, and prepared the first draft. All authors critically reviewed and approved the final version of the manuscript.

Manuscripts under Peer review

5. **Natasha Samsunder**, Thando Glory Maseko, Santhuri Rambaran, Slindile Ngubane, Sinaye Ngcapu, Stanley Nzuzo Magini, Razia Hassan-Moosa, Lara Lewis, Ayesha BM Kharsany, Quarraisha Abdool Karim, Salim Abdool Karim, Kogieleum Naidoo, Aida Sivro. Evaluations of two SARS-CoV-2 rapid antigen tests during first and second wave of COVID-19 infections in South Africa. *This manuscript is under peer review at the Journal of Public Health in Africa, ref number: JCVPLUS-D-22-00177.*

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6. **Natasha Samsunder**, Aida Sivro, Razia Hassan-Moosa, Lara Lewis, Zahra Kara, Cheryl Baxter, Quarraisha Abdool Karim, Salim Abdool Karim, Ayesha BM Kharsany, Kogieleum Naidoo, Sinaye Ngcapu. Usability of saliva as a reliable and non-invasive sample for SARS-CoV-2 detection in Durban, South Africa. *This manuscript is under peer review at the journal BMC Diagnostic and Prognostic Research, ref number DAPR-D-23-00040.*

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Manuscripts in preparation

7. **Natasha Samsunder**, Jienchi Dorward, Thando Glory Maseko, Zahra Kara, Sinaye Ngcapu, Razia Hassan-Moosa, Lara Lewis, Quarraisha Abdool Karim, Salim Abdool Karim, Ayesha BM Kharsany, Kogieleum Naidoo, Nigel Garrett, Aida Sivro. Evaluation of the Xpert® Xpress SARS-CoV-2 assay for SARS-CoV-2 diagnosis in South Africa. [Manuscript in preparation].

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Manuscripts related to (but not directly from) the doctoral research (Appendix A).

9. Khan K, Karim F, Cele S, Reedoy K, San JE, Lustig G, Tegally H, Rosenberg Y, Bernstein M, Jule Z, Ganga Y, Ngcobo N, Mazibuko M, Mthabela N, Mhlane Z, Mbatha N, Miya Y, Giandhari J, Ramphal Y, Naidoo T, Sivro A, **Samsunder N**, Kharsany ABM, Amoako D, Bhiman JN, Manickchund N, Abdool Karim Q, Magula N, Abdool Karim SS, Gray G, Hanekom W, von Gottberg A, COMMIT-KZN Team, Milo R, Gosnell BI, Lessells RJ, Moore PL, de Oliveira T,

Moosa MS, Sigal A. Omicron infection enhances Delta. antibody immunity in vaccinated persons. *Nature.* 2022 Jul;607(7918):356-359.

Contribution: I contributed as a co-author to this project. I critically reviewed the draft manuscript and approved the final version.

10. Khan K, Karim F, Ganga Y, Bernstein M, Jule Z, Reedoy K, Cele S, Lustig G, Amoako D, Wolter N, **Samsunder N**, Sivro A, San JE, Giandhari J, Tegally H, Pillay S, Naidoo Y, Mazibuko M, Miya Y, Ngcobo N, Manickchund N, Magula N, Karim QA, von Gottberg A, Abdool Karim SS, Hanekom W, Gosnell BI; COMMIT-KZN Team. Lessells RJ, de Oliveira T, Moosa MS, Sigal A. Author Correction: Omicron BA.4/BA.5 escape neutralizing immunity elicited by BA.1 infection. *Nat Commun.* 2022 Oct 13;13(1):6057.
    Contribution: I contributed as a co-author to this project. I critically reviewed the draft manuscript and approved the final version.

11. Sandile Cele, Mallory Bernstein, Farina Karim, Khadija Khan, Yashica Ganga, Zesuliwe Jule, Kajal Reedoy, Gila Lustig, **Natasha Samsunder**, Matilda Mazibuko, Shi-Hsia Hwa, Nithendra Manickchund, Anne von Gottberg, Nombulelo Magula, Richard J. Lessells, Tulio de Oliveira, Salim S. Abdool Karim, Willem Hanekom, COMMITKZN Team, Bernadett Gosnell, Mahomed-Yunus S. Moosa, Alex Sigal. Beta infection combined with Pfizer BNT162b2 vaccination leads to broadened neutralizing immunity against Omicron.
    Published on MedRxiv preprint doi: https://doi.org/10.1101/2022.04.15.22273711, this version posted April 15, 2022.

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12. Razia Hassan-Moosa, Kegaugetswe Motsomi, Radhamoney Narisimmulu, Aida Sivro, Kevindra Naidu, Ayesha BM Kharsany, **Natasha Samsunder**, Rubeshan Perumal, Kogieleum Naidoo. HIV-Drug Resistant TB and COVID-19 Co-infection: case reviews from Kwa-Zulu Natal, South Africa. *This manuscript has been accepted for publication at Infection and Drug Resistance Journal.* Ref number ID:433695
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13. Aida Sivro*,Thandeka Moyo-Gwete*, Simone I Richardson*, Nonhlanhla Mkhize, Razia Hassan-

Moosa, **Natasha Samsunder**, Derseree Archary, Lara Lewis, Ayesha BM Kharsany, Quarraisha Abdool Karim, Salim Abdool Karim, Penny L Moore, Richard J Lessells, Kogieleum Naidoo. Early evolution of Beta-like mutations in SARS-CoV-2 infection in immunocompromised individuals: case reviews from KwaZulu Natal, South Africa. [Manuscript in preparation]

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14. Aida Sivro, Razia Hassan-Moosa, Lara Lewis, **Natasha Samsunder**, Sinaye Ngcapu, Avik Haridutt, Kevindra Naidu, Patricia Jabulile Malunga, Cheryl Baxter, Lyle R. McKinnon, Quarraisha Abdool Karim, Salim Abdool Karim, Ayesha BM Kharsany, Kogieleum Naidoo. Persistent SARS-CoV-2 viral shedding in people living with HIV. [Manuscript in preparation]

    Contribution: I contributed as a co-author to this project. I critically reviewed the draft manuscript.

Natasha Samsunder, MT, BTech, MMedSc
Durban, 01 December 2023

DEDICATION

This thesis is dedicated to my dearest friend, sister, my Day one,

Lutchmee Eswarlal.

(12 July 1974 - 12 February 2021)

You existed beyond personal gains, always in pursuit of the well-being of others.

You are dearly missed, but always in our thoughts and I know that you walk besides us.

Miss you lots.

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Table of Contents

LIST OF ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
Ab	Antibody
Ag	Antigen
ART	Antiretroviral Therapy
BREC	Biomedical Research Ethics Committee
BSL-3	Biosafety Level 3
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CAPRISA 225	COVID-19 transmission and natural history in KwaZulu-Natal, South Africa: Epidemiological Investigation to Guide Prevention and Clinical Care: **BREC/00001195/2020 – Date of approval 31 March 2020**
CAPRISA 228	A prospective cohort study to establish the association between COVID 19 and influenza on TB incidence, prevalence, and severity: **BREC/00001195/2020 – Date of approval 31 March 2020**
CAPRISA 230	Natural History And Laboratory Tests for COVID-19 in South Africa HALT_COVID 19: **BREC/00003106/2021 -Date of approval 6 October 2021**
CLIA	Chemiluminescence immunoassay
COVID-19	Coronavirus disease 2019 (Clinical disease caused by SARS-CoV-2)
CRISPR	Clustered regularly interspaced short palindromic repeats
DBS	Dried blood spot samples
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
HIV	Human Immunodeficiency Virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQR	Interquartile range
KZN	KwaZulu-Natal
LAMP	Loop-mediated isothermal amplification
LFA	Lateral Flow assay
MFI	Median fluorescent intensity
MERS	Middle East Respiratory Syndrome
NAAT	Nucleic acid amplification tests
NPV	Negative predictive value
PPV	Positive predictive value
PCR	Polymerase chain reaction
POC	Point-of-care-tests
RBD	Receptor binding domain
RdRp	RNA-dependent RNA polymerase
RDT	Rapid diagnostic tests
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction

SAPHRA	South African Health Products Regulatory Authority
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TB	Tuberculosis
TMA	Transcription-mediated amplification
UKZN	University of KwaZulu-Natal
WHO	World Health Organization

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Abstract

The emergence and unprecedented spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) across the globe resulted in the urgent need for rapid diagnosis, followed by clinical management, to facilitate prevention of onward transmission control and development of Coronavirus disease 2019 (COVID-19). Accurately diagnosing individuals with infection was paramount to limit transmission of the virus and reduce morbidity and mortality. During the course of the pandemic, laboratory testing of individuals was critical to guide care provision and is key to controlling the spread of SARS-CoV-2. Thus, there was an urgent need for the reliable and rapid diagnosis of infection to implement strategies to contain the spread of infection.

The overall aim of this study was to evaluate a combination of diagnostic tests including point-of-care (POC) tests and sample types to assess and validate the performance of tests, determine the turnaround time to generate a result and aid in the rapid diagnosis of asymptomatic and symptomatic infections, across different stages of clinical disease among individuals with SARS-CoV-2 infection.

To better understand the landscape of the pandemic in South Africa, a scoping review was undertaken to determine the evolution, performance, and reliability of diagnostic tests for the identification of SARS-CoV-2 infection in South Africa. Of the 17 peer-reviewed articles published between March 2020 and August 2022, the scoping review highlighted that no single SARS-CoV-2 gene/s and or antigen- or antibody-based assay was sufficiently sensitive and specific simultaneously and the performance of the tests was highly dependent on the timing of sample collection over the course of SARS-CoV-2 infection, thus the earlier the collection of clinical samples of nasopharyngeal and/or oropharyngeal and tracheal swabs, the greater the sensitivity. The antibody-based tests on serum and plasma samples showed better sensitivity from later stages of infection.

To improve the turnaround for testing for SARS-CoV-2, the sensitivity and specificity of the rapid antigen tests were evaluated against the gold standard of reverse transcription polymerase chain reaction (RT-PCR). The evaluation of SD Biosensor, STANDARD Q COVID-19 Ag Test (n=604), and the Abbott Panbio™ COVID-19 Ag Rapid Test (n=692) during the first (Ancestral variant with D614G mutation) and second (Beta variant) waves in South Africa showed an overall sensitivity of 53.5% for SD Biosensor Ag test and 52.8% for the Panbio™ Ag test. The specificity was 100% for the SD Biosensor Ag test and 99.4% for the Panbio™ Ag test. For samples with cycle threshold (Ct) values <20, the test sensitivity increased to 88.5% for the SD Biosensor Ag test and 83.7% for the Panbio™ Ag test. Sensitivity was highest at 68.8% for the SD Biosensor Ag test and 64.0% for the Panbio™ Ag test when samples were collected within the first week post symptom onset.

Whilst the use of rapid diagnostic tests (RDT) for the detection of SARS-CoV-2 Ag increased dramatically over time, there was significant variation in the performance of these tests across settings with fewer reports on the performance of these tests in low- and middle-income countries (LMIC). The field evaluation of the nasal Panbio™ COVID-19 Ag Rapid Test Device (Abbott) was evaluated in parallel with the nasopharyngeal (NP) Espline SARS-CoV-2 Ag test (Fujirebio, nasopharyngeal) and was followed by the evaluation of nasal RightSign COVID-19 Antigen Rapid test Cassette (Hangzhou Biotest Biotech) in parallel with the NP STANDARD Q COVID-19 Ag test (SD Biosensor) with the Abbott RealTi*m*e SARS-CoV-2 assay as the reference test. Evaluation of Panbio™ and Espline Ag tests were performed on 494 samples while the evaluation of Standard Q and RightTest Ag tests was performed on 539 samples. The overall sensitivity for all four tests ranged between 60-72% with specificity values of >98% for both tests. Sensitivity increased to >80% in all tests for samples with Ct values <20. All four tests performed best for samples from patients presenting within the first week of symptom onset.

Despite the emergence of new SARS-CoV-2 variants, the Panbio™ and SD Biosensor rapid antigen tests demonstrated sensitivity values ranging from 78% to 81% and specificity values of >99%. The sensitivity of rapid antigen tests increased above 90% in samples with Ct values <20, and all tests had higher sensitivities when performed within first week of symptom onset. The sensitivity of the Panbio™ antigen test for Omicron 21L/BA.2 was 100.00%, (95% CI 90.82-100.00), for Omicron 21K/BA.1 sensitivity was 77.61% (95% CI: 69.84-83.84) and for Omicron 21M infections sensitivity was 63.64% (95% CI 35.38-84.83).

As subvariants of SARS-CoV-2 Omicron variants evolved, the SD Biosensor Ag test and AllTest rapid Ag tests were evaluated for diagnosing patients with Omicron BA.4 and BA.5 infections. The objective was to evaluate the performance of two widely used SARS-CoV-2 rapid Ag tests during the SARS CoV-2 BA.4/BA.5 circulating variants in South Africa between May to June 2022. A prospective field evaluation of the SARS-CoV-2 Ag Rapid test from Hangzhou AllTest Biotech (nasal swab) and the Standard Q COVID-19 Rapid Ag test from SD Biosensor (NP swab) to the Abbott RealTi*m*e SARS-CoV-2 assay (NP swab) was undertaken on samples collected from 540 study participants. The overall sensitivity of the AllTest SARS-CoV-2 Ag test and Standard Q COVID-19 Ag test was 73.4% (95% CI 65.9-79.7) and 74.0% (95 % CI 66.6-80.3) rand the specificity was 97.4% (95% CI 95.3-98.6) and 99.2% (95 % CI 97.7-99.7) respectively. Sensitivity was >90% for samples with Ct values <20. The sensitivity of both rapid tests was >90% in samples with Omicron sub-lineage BA.4 and BA.5. Accuracy of tested rapid Ag tests that target the nucleocapsid SARS-CoV-2 protein, were not adversely affected by BA.4 and BA.5 Omicron sub-variants.

The rapid diagnostic Xpert® Xpress SARS-CoV-2 assay was evaluated for diagnostic accuracy. Using NP samples from health care workers suspected for COVID-19 at healthcare facilities in Durban, South

Africa. With Abbott RealTi*m*e SARS-CoV-2 as the reference assay, the sensitivity and specificity of the Xpert® Xpress SARS-CoV-2 assay was assessed in 39 consecutive NP specimens. The Ct values for the Abbott RealTi*m*e SARS-CoV-2 assay (*RdRp* and *N* genes) were significantly lower compared to those of the *E* and *N2* gene values obtained using the Xpert® Xpress assay ($p<0.0001$), suggesting that the Xpert® Xpress assay results should be interpreted with caution.

Whilst the NP swab samples were recommended as the sample of choice for the detection of SARS-CoV-2 infection, the challenges with adequate sample collection procedures including procedures potentially increasing the risk of onward transmission, shipping and laboratory processing necessitated the need for alternative samples and collection procedures that were less invasive, acceptable to patients, and simultaneously producing reliable results. The study therefore investigated the use of saliva samples at different volumes compared to NP swab samples and found no significant difference in cycle number (CN) values on the Abbott m2000 RealTi*m*e System, between saliva and NP swab specimens, irrespective of saliva volume.

The use of serology has numerous beneficial properties, and the studies were expanded to evaluate one point of care immunoassay Orient Gene Biotech COVID-19 IgG/IgM Rapid test and the MILLIPLEX® MAP Kit SARS-CoV-2 Antigen Panel 1 IgG, a multiplex bead assay that targets various SARS-CoV-2 antigens against the routinely used diagnostic platform, the Abbott SARS-CoV-2 IgG-II (Quant) and Abbott Architect IgG (Qual). These tests were selected based on availability in South Africa at the time of testing. The study found the POC Orient Gene Rapid test had comparable sensitivity, specificity and detectability to the Abbott SARS-CoV-2 IgG-II test, while the MILLIPLEX® MAP Kit SARS-CoV-2 Antigen Panel 1 IgG demonstrated higher detectability that the Abbott SARS-CoV-2 IgG-II test. This study's outcome adds valuable insight into the performance of different serological tests.

In conclusion, the series of studies showed that despite the considerable amount of testing for SARS-CoV-2 in South Africa during the pandemic, the sensitivities of the tests were limited. Even with the advances in the POC Ag tests to rapidly diagnose individuals with SARS-CoV-2 infection, the performance of these tests remained limited. Thus, the improvements in test performance are recommended to enable the reliable and accurate use of tests to diagnose and manage SARS-CoV-2 infection and importantly to limit transmission.

CHAPTER 1

INTRODUCTION

Chapter 1: Introduction

1.1 BACKGROUND AND RATIONALE OF THIS STUDY

1.1.1. Thesis background

In late December 2019, the emergence of severe acute respiratory syndrome coronavirus 2 (**SARS-CoV-2**) in the Wuhan district of China marked the inception of a consequential health crisis. Within two months, the virus exhibited unparalleled dissemination, culminating in the designation of the ensuing disease, namely Coronavirus Disease 2019 (**COVID-19)**, as a pandemic. Most of the affected countries reported exponential growth in the number of new cases. The high rates of transmission, morbidity, and mortality resulted in a public health emergency of international concern in a short space of time(WHOa, 2020).

In South Africa, the first case of COVID-19 was reported in early March 2020 (WHOa, 2020). Despite the implementation of mitigation strategies aimed at curtailing viral transmissions, new cases increasingly emerged. The trajectory of the COVID-19 epidemic in South Africa followed resurgences of infections resulting from highly transmissible viral strains emerging due to genetic mutations; hence the need for reliable and rapid diagnosis of infection to implement strategies to contain the spread of infection.

Despite the availability of antigen- and antibody-based tests, delays in the turnaround time of test results contributed to the ongoing transmission of SARS-CoV-2. Furthermore, there had been a surge in the development of newer diagnostic tests, with little or no reliable data on the test performance among individuals with COVID-19 especially in settings that also bear a disproportionate burden of Human Immunodeficiency Virus (HIV) and Tuberculosis (TB). Thus, the objective of this study was to comprehensively evaluate and validate the performance of the rapidly evolving diagnostic antigen- and antibody-based tests for SARS-CoV-2 infections among individuals in settings with a high background burden of HIV and TB.

The overall aim of the study was to evaluate a combination of diagnostic tests including point-of-care (POC) tests and sample types to assess and validate the performance of tests, determine the turnaround time to generate as result and aid in the rapid diagnosis of asymptomatic and symptomatic infections, across different stages of clinical among individuals with SARS-CoV-2 infection.

1.1.2. Problem statement

The importance of early and large-scale testing for SARS-CoV-2 infection has facilitated the management and containment of the virus in countries like Singapore and South Korea, unlike the rest of the world where the spread of infection had been rampant. South Africa bears a disproportionate burden of HIV and TB; hence it was imperative that the diagnostic capabilities of POC tests were enhanced to address and improve prevention and treatment efforts for SARS-CoV-2 infection. Thus, there was an urgent need for newer, rapid, reliable, efficient POC tests to be fully validated and implemented in contrast to the original time-consuming RT-PCR testing. As infections continued to rise, POC tests with high levels of sensitivity and specificity would aid in the rapid diagnosis of infections and facilitate the implementation of measures to minimize the spread of SARS-CoV-2 infections. Furthermore, improving the efficiency of diagnostic capabilities, including testing of any significant emerging laboratory markers, were undertaken for a deeper understanding of infectiousness, transmission dynamics and the natural history of SARS-CoV-2 infection and COVID-19 disease progression. The ability to evaluate newer diagnostics tests that were developed and emerged in the market against existing laboratory tests and the anonymised evaluation and performance of laboratory tests on routinely collected samples from public sector settings enhanced diagnostic capabilities. Since the first recognition of the virus, the virus has mutated, resulting in multiple SARS-CoV-2 variants across the world. It was critical that existing and new diagnostic tests be evaluated against emerging variants. Therefore, the overall aim of the study was to evaluate a combination of diagnostic tests including point-of-care (POC) tests and sample types to assess and validate the performance of tests, determine the turnaround time to generate as result and aid in the rapid diagnosis of asymptomatic and symptomatic infections, across different stages of clinical among individuals with SARS-CoV-2 infection.

Therefore, this study aimed to validate existing and emerging new diagnostic tests, determine sensitivity, specificity, positive and negative predictive values in the context of SARS-CoV-2 infection alone and in combination with HIV and/or TB.

1.1.3. Research questions

Comprehensive laboratory validation of antigen and antibody based diagnostic tests for SARS-CoV-2 infection will supplement the gold standard RT-PCR and provide a set of tests for the rapid detection of COVID-19.

The **objectives** of the study were:

a) To evaluate the performance of newer laboratory POC and antigen/antibody-based tests for SARS-CoV-2 infection.

b) To evaluate the potential of saliva as an alternate sample type for the SARS-CoV-2 RT-PCR assay.

c) To evaluate SARS-CoV-2 antibody tests in individuals with COVID-19 that had co-morbid HIV and TB.

1.1.4 Thesis overview

This thesis consists of eight chapters.

Chapter 1 forms the introduction to the thesis and provides the background, rationale, and the research questions.

Chapter 2 presents the literature review on SARS-CoV-2 infections, COVID-19 global pandemic, the pandemic in South Africa, the evolution of pandemics that South Africa is experienced. This chapter also provides a comprehensive review on the origin, evolution, virus structure, transmission, symptoms and clinical manifestation, pathogenesis, host responses, diagnosis, and the diagnostic testing landscape in South Africa.

Chapter 3 presents the methods that were used for the studies with details on the study design, location, inclusion criteria, procedures for sample collection and processing and the laboratory testing procedures. Data collection, management and analyses are also described in this chapter.

Chapter 4 presents the scoping review of the literature on the diagnostic testing for SARS-CoV-2 in South Africa. The following manuscript focusses on this review.

4.1 "*The performance of diagnostic tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the South African population: a scoping review".* This manuscript has been published in the journal Tropical Medicine and Infectious Disease, 2023, 8, 514.

Chapter 5 addresses the objective - To evaluate the performance of newer laboratory POC and antigen/antibody-based tests for SARS-CoV-2 infection. The following manuscripts focus on this objective.

5.1 "*Evaluations of two SARS-CoV-2 rapid antigen tests during first and second wave of COVID-19 infections in South Africa*". This manuscript is under review by the Journal of Public Health in Africa. Reference Number: JCVPLUS-D-22-00177.

5.2 "*Field evaluations of four SARS-CoV-2 rapid antigen tests during SARS-CoV-2 Delta variant wave in South Africa*". This manuscript has been published in the Diagnostic and Prognostic Research, 2023; 7:14

5.3 "*Clinical evaluation of SARS-CoV-2 rapid antigen tests during the Omicron wave in South Africa*". This manuscript has been published in the Journal of Infectious Diseases, 2022, 226: 1412-1417.

5.4 *"Performance of rapid antigen tests in identifying Omicron BA.4 and BA.5 infections in South Africa".* This manuscript has been published in Journal of Clinical Virology, 2023, 165: 105498.

5.5 "*Evaluation of the Xpert® Xpress SARS-CoV-2 assay for SARS-CoV-2 diagnosis in South Africa*". This manuscript is in preparation and has been included in this chapter.

Chapter 6 addresses the objective - To evaluate the potential of saliva as an alternate sample type for the SARS-CoV-2 RT-PCR assay. The following manuscripts focus on this objective.

6.1 "*Usability of saliva as a reliable and non-invasive sample for SARS-CoV-2 detection in Durban, South Africa".* This manuscript is under review by the BMC Diagnostic and Prognostic Research Reference Number: DAPR-D-23-00040

Chapter 7 addresses the objective- To evaluate SARS-CoV-2 antibody tests in individuals with COVID-19 that has co-morbid HIV and TB. The following manuscript focusses on this objective.

7.1 *"Evaluation of COVID-19 antibody tests in KwaZulu-Natal, South Africa".* This manuscript is in preparation and has been included in this chapter.

Chapter 8 provides the overall summary, discussion, and conclusions of the studies undertaken and highlights key strengths, limitations and provides recommendations for future research. ` .

CHAPTER 2

LITERATURE REVIEW

Chapter 2: Literature review

2.1 THE COVID-19 GLOBAL PANDEMIC

In November 2019, the first cases of a new disease, later named Coronavirus Disease 2019 (**COVID-19)** by the World Health Organisation (WHO), were reported by health care workers from Wuhan, China. In December 2019, researchers from Wuhan reported a cluster of pneumonia cases caused by a novel severe acute respiratory syndrome coronavirus 2 **(SARS-CoV-2)** (Huang et al., 2020). Within a matter of weeks, the rapid transmission of this virus resulted in an exponential increase in the number of cases and corresponding deaths globally. While the true case-fatality rate from SARS-CoV-2 infection was unknown due to the uncertainty of the true number of people who had contracted the virus, the WHO estimates the case fatality rate to have been between 2 to 3% (WHOa, 2020). This case fatality rate has been considerably higher than the typical seasonal flu which was around 0.1%; however, it was much lower than previous coronavirus outbreaks, such as severe acute respiratory syndrome (SARS), which was about 10%, and Middle East respiratory syndrome (MERS), which was about 30% (WHOa, 2020).

Whilst everyone exposed to SARS-CoV-2 appeared to be equally at risk of acquiring infection, the severity of the resulting clinical disease differed markedly by age, with the case fatality rate of <1% for people <60 years and sequentially increasing to 14.8% among those 80 years or older. The high rates of SARS-CoV-2 transmissions, morbidity, and mortality resulted in a "public health emergency of international concern" in a short space of time. **Figure 2.1** shows that as of 4 September 2022, there were more than 603 million confirmed cases of COVID-19, 4,372,582 active cases and ~6.5 million deaths globally. Exactly a year later, despite fewer people testing and reporting, the cumulative number of cases across the world was 770 million, with 6.9 million deaths recorded (WHOb, 2020).

On the African continent, the first case of SARS-CoV-2 infection was reported in Egypt in February 2020, followed by the rapid increase in the spread of infections across all countries in Africa, including South Africa. As of 9 September 2023, Africa has experienced five waves of infection **(Figure 2.2)** and has recorded over 8.9 million cases with 174,211 deaths and 8,270,280 recoveries (WHOb, 2020).

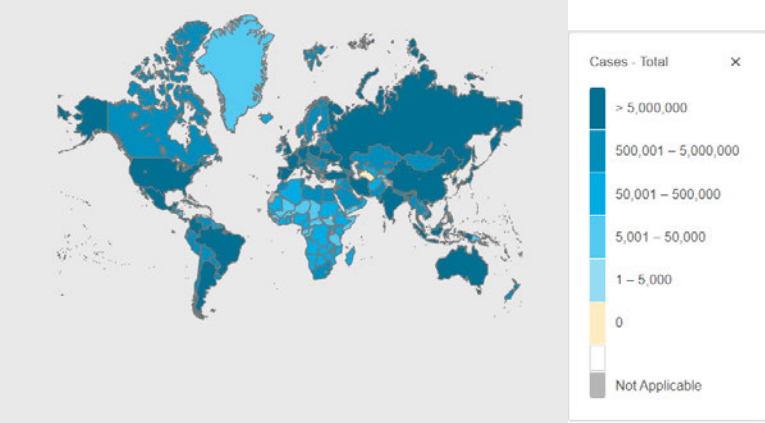

Figure 2.1: Cumulative confirmed cases of COVID-19 globally. (WHO COVID-19 Dashboard). Geneva: World Health Organization, 2020. Available online: https://covid19.who.int/ (last cited: 4 September 2022).

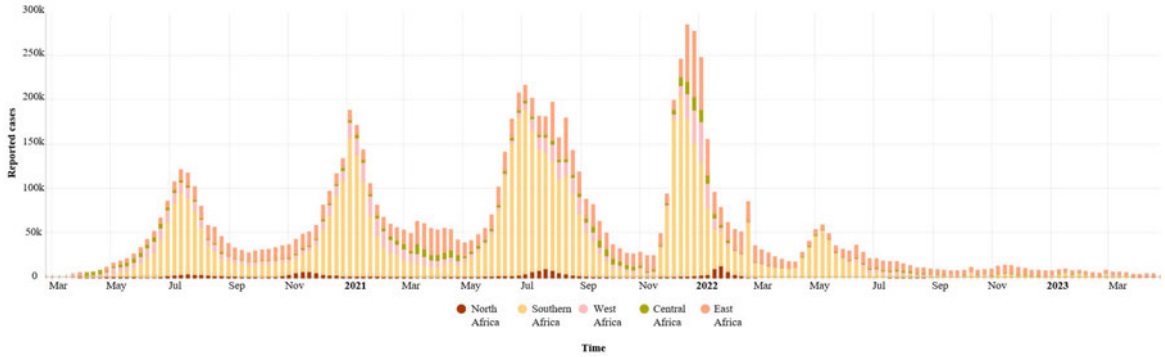

Figure 2.2: Epidemiology curve of laboratory-confirmed COVID-19 cases in the WHO African sub regions as at 9 September 2023 (adapted from the WHO weekly bulletin on Outbreaks)(WHO, 2022).

2.2 COVID-19 PANDEMIC IN SOUTH AFRICA

South Africa documented the first confirmed case of SARS-CoV-2 infection on 5 March 2020. Non-pharmaceutical measures to reduce viral transmission proved futile resulting in an exponential increase in cases with numbers peaking in late July 2020, with 12,584 cases being reported within a 24-hour period. This wave was dominated by D614G variant of SARS-CoV-2. Whilst infections subsided around September 2020, a second wave driven by the Delta variant of SARS-CoV-2 which saw an increase in the number of cases in early January 2021 with 19,042 cases reported daily, followed by yet a third wave dominated by the Delta variant of SARS-CoV-2 in July 2021 with 19,956 cases reported

daily. The subsequent fourth wave of infection was driven by the Omicron subvariants, BA.1 and BA.2, with 23,437 cases being reported within a 24-hour period. The most recent fifth wave was driven by the Omicron BA.4 and BA.5 subvariants, peaking at 7,685 cases reported within a 24-hour period, as illustrated in **Figure 2.3** (WHOb, 2020).

Since the start of the pandemic, South Africa has recorded just over four million cases and approximately 102,531 deaths. Although the recovery rate in South Africa had been relatively high at around 96%, ongoing local community transmission continued to substantially burden communities, with limited access to regular healthcare and severely straining healthcare services. Since SARS-CoV-2 replication occurs mainly in the upper and lower respiratory tract, transmission occurred mainly through respiratory droplets and aerosols (WHOc, 2020), and infection varied considerably from being asymptomatic, oligosymptomatic to moderate to severe disease (Salzberger et al., 2021). However, existing co-morbidities, such as hypertension, chronic cardiac, obesity or pulmonary disease or immunosuppression increase the risk of susceptibility to, including severe disease, or death (Salje et al., 2020, Wang et al., 2021). ***Thus, urgent efforts to diagnose infection rapidly and reliably were needed to interrupt ongoing transmission, and to enhance the understanding of infectiousness, transmission dynamics and the natural history of COVID-19***

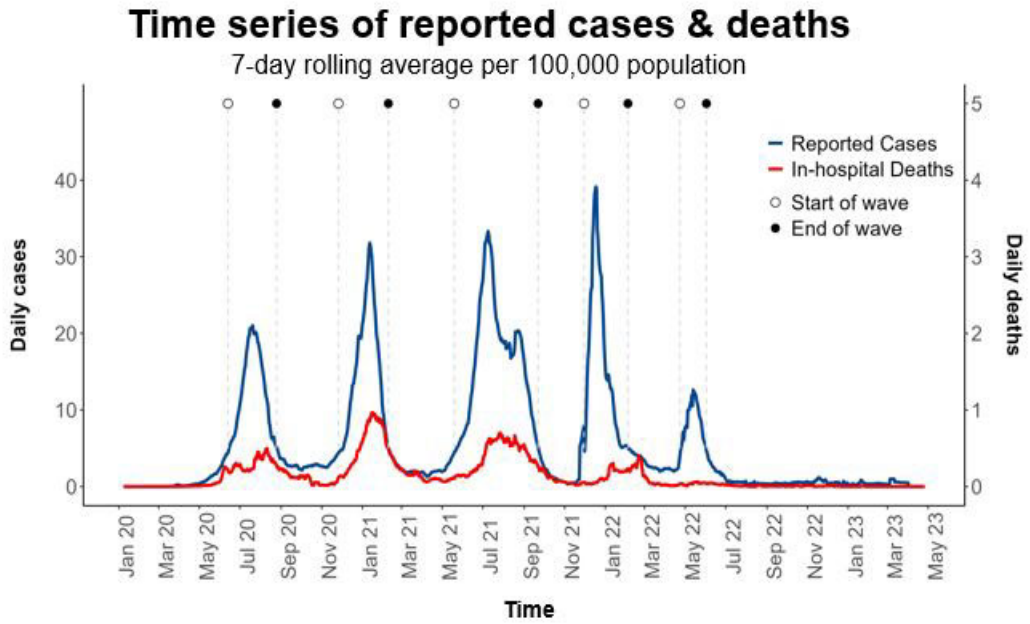

Figure 2.3: 7-day rolling average of SARS-CoV-2 cases and COVID-19 related in-hospital deaths in South Africa, 05 March 2020 to 08 May 2023. *Source World Health Organization (WHO) & Our World in Data/ Data to 29 April 2023. Source of hospital admissions data: Lucille Blumberg, Richard Welch and Waasila Jassat - DATCOV, NICD*

2.3 THREE PANDEMICS IN SOUTH AFRICA: TB, HIV, AND COVID-19

Globally tuberculosis (TB), HIV, and COVID-19 are all pandemics with ongoing, sustained community transmissions. In 2018, TB was the leading cause of mortality with 1.2 million deaths, whilst 770,000 people died due to AIDS-related illnesses, and in the first quarter of 2020, COVID-19 resulted in over 125,000 deaths. As SARS-CoV-2 spreads throughout Africa, and more importantly in sub-Saharan Africa, where the burden of TB and HIV is the heaviest, several unknowns about the clinical and epidemiological interactions between TB, HIV and COVID-19 remain. Despite antiretroviral therapy (ART) and the associated immune-reconstitution, HIV positive individuals are at a significantly higher risk of viral and bacterial infections compared to HIV negative individuals. Whilst the impact of COVID-19 infection on latent TB reactivation remains unknown, there is increasing recognition that individuals previously treated for TB have residual, long-term lung damage (Meghji et al., 2020) and are therefore likely to be at higher risk for severe COVID-19 disease. Thus, it is expected that individuals with COVID-19 and co-infected with HIV and/or TB may have poorer outcomes and the overlap of the three diseases may lead to a significant increase in morbidity and mortality across. Thus, in settings with generalised high burden of HIV and TB, the natural history of the SARS-CoV-2 infection is likely to differ in countries with lower burden of HIV and TB. Furthermore, many countries with generalized HIV and TB epidemics already have an overburdened health care system and the impact of COVID-19 is likely to put additional severe strain on health services.

2.4. SARS-COV-2 VIRUS CLASSIFICATION, ORIGIN, AND STRUCTURE

2.4.1 History of Coronaviruses

The word “coronavirus” is derived from the Latin word “corona” meaning “crown”, referring to the distinctive spikes projecting on the outside of the virus. The SARS-CoV-2 virus is a member of the *Coronaviridae* family and *Nidovirales* order (Harapan et al., 2020). Coronaviruses are simply haboured or cause asymptomatic disease or infect a wide variety of mammalian and avian species (Li, 2016). SARS-CoV-2 is a novel Beta corona virus (Corman et al., 2021) causing a human outbreak since January 2020. The first was the SARS-CoV in 2003 (Rosling and Rosling, 2003) that emerged in China with approximately 8,000 cases and 800 deaths, originating from a Himalayan palm civet, followed by MERS-CoV that was prevalent in Saudi Arabia in 2012, which had approximately 2,500 cases and 800 deaths, originating from the dromedary camel (Cascella et al., 2022, Alanagreh et al., 2020). The burden of mortality of SARS-CoV-2 was greater than that of SARS-CoV and MERS-CoV (Zhang and Holmes, 2020).

Coronaviruses have become the major pathogen of emerging respiratory diseases, though it is unclear how these viruses could cross species barriers and cause in-human illnesses ranging from the common cold to severe disease, such as SARS-CoV and MERS (Cascella et al., 2022). The zoonotic origin of

SARS-CoV-2 is currently unclear but phylogenetic analysis of the novel coronavirus genomes indicated that the virus shares about 96% nucleotide sequence identity to a bat coronavirus, suggesting that the natural animal host could possibly be a horseshoe bat (Mittal et al., 2020, Jiang and Wang, 2022) with the intermediate host being the pangolin (Lu et al., 2020, Lam et al., 2020), but the specific route of transmission from the natural reservoir to humans remains unclear (Zhou et al., 2020, World Health Organization, 2021).

2.4.2 Emergence

The emergence of SARS-CoV-2 was first observed when the world was alerted in late December 2019 to cases of unexplained cases of pneumonia that clustered around the Huanan wet market in Wuhan, China (World Health Organization, 2021, Huang et al., 2020). There was controversy as to whether the market was indeed the epicenter of COVID-19 pandemic. Investigation by the WHO and other researchers have shown evidence that initial cases were geographically centered on the wet market and that no prior samples tested from the area tested positive for SARS-CoV-2 prior to December 2019 (World Health Organization, 2021, Worobey et al., 2022, Jiang and Wang, 2022). The genomic diversity of SARS-CoV-2 before February 2020 showed that there were likely two distinct viral lineages with phylodynamic rooting methods coupled with epidemic simulations. This revealed that these lineages were the result of at least two separate cross-species transmission events to humans with the first, lineage B, most likely transmitted around 18 November 2019 (23 October to 8 December 2019) and a second, lineage A, likely transmitted across species within weeks of this event (Pekar et al., 2022, Xiong et al., 2020). From 18-29 December 2019, five patients were hospitalized with acute respiratory distress syndrome (Ren et al., 2020). The SARS-CoV-2 virus was first isolated in the bronchoalveolar lavage fluid of three COVID-19 patients from Wuhan Jinyintan Hospital on 30 December 2019 (Zhu et al., 2020). By the end of 2019, based on clinical manifestation, pathology and chest radiographs, COVID-19 emerged in several local hospitals in Wuhan, Hubei Province, China (Jin et al., 2020). The virus spread rapidly to other parts of China and globally through air travelers, with subsequent cases being reported in the following months in Italy, the United Kingdom, Australia, and Spain.

The WHO commissioned report established that SARS-CoV-2 emerged from the Huanan wet market. In a retrospective study of stored samples and sewage samples collected prior to December 2019, there was a batch of sewage samples collected in Barcelona Spain on 12 March 2019 that tested positive for SARS-CoV-2, but this finding was isolated with retrospective and prospective samples also tested with no evidence of the findings being replicated leading to speculation that this result may be a false positive (World Health Organization, 2021, Chavarria-Miró et al., 2020).

On sequencing and evolutionary tree analysis, the then named 2019 novel coronavirus was established as a member of the Beta coronavirus family (Zhu et al., 2020). The WHO announced that the official

name of the 2019 novel coronavirus is Coronavirus Disease 2019, shortened to COVID-19 and named the virus "SARS-CoV-2" due to its close linkages to SARS-CoV (Zhu et al., 2020).

2.4.3 Virus structure

Coronaviridae are large, enveloped, single-stranded ribonucleic acid (RNA) viruses with SARS-CoV-2, being larger than other RNA viruses, at approximately 27-32 kilobases (kb) encoding 9,860 amino acids, with a diameter of 60-140 nm, and distinctive spikes (9-12 nm) that are responsible for the crown-like appearance of virions under electron microscopy (Cui and Zhang, 2020). The genome structure of the virus is organized with a 5'-cap structure and 3'-poly-A tail. Genome replication produces two large open reading frames (ORFs) that work as templates to produce sub genomic mRNAs. The first OFR (OFR1a/b) encompasses approximately 66% of the whole genome and encoding 16 non-structural proteins (nsp-16), while the remaining 33% comprises four structural proteins namely membrane protein (M), spike protein (S), and envelope protein (E) and the nucleocapsid protein (N) as illustrated in **Figure 2.4** (Chen et al., 2020b, La Marca et al., 2020).

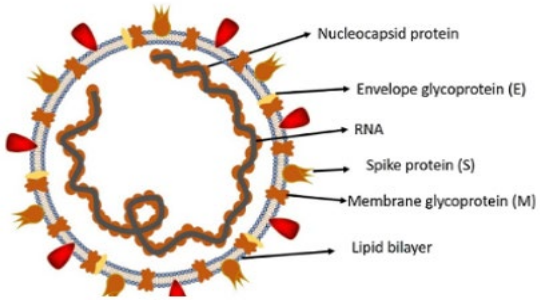

Figure 2.4: Structure of SARS-CoV-2 virus adapted from (Shereen et al., 2020)

The four structural proteins are essential for assembly of the virion, viral RNA synthesis and immune evasion (Shereen et al., 2020, Snijder et al., 2020, La Marca et al., 2020). The S protein, which are spikes on the virus surface, is made up of S1 and S2 domains, facilitates virus entry into the host cells and is critical for SARS-CoV-2 replication. The S protein contains a receptor-binding domain (RBD) that recognizes the angiotensin-converting enzyme 2 (ACE2) receptor specifically. This RBD is a critical component for the binding of antiviral compounds and antibodies. The M protein shapes the virions, promotes membrane curvature, and covers the nucleocapsid. The E protein is involved in virus assembly and release, and viral pathogenesis, while the N protein is responsible for viral replication and genome packaging (La Marca et al., 2020, Mittal et al., 2020).

2.5 TRANSMISSION

According to the WHO, SARS-CoV-2 is transmitted by exposure to infectious respiratory fluids, including droplets that are expelled during close unprotected contact, exposure during talking, coughing, sneezing, airborne, faecal-oral and animal-to-human transmission. Another mode of transmission is fomites with evidence that SARS-CoV-2 could remain dormant on surfaces and metals for prolonged periods (WHOc, 2020). Symptomatic and asymptomatic patients could both transmit the virus since viral load in the upper respiratory tract appears to peak at the time of symptom onset while viral shedding would have commenced 2 or 3 days prior to the onset of symptoms. Pharyngeal shedding seems to be the highest during the week of infection when symptoms are still less severe (WHOd, 2020, Salzberger et al., 2021). There have been reports that asymptomatic transmission is as high as 48-62% (Wiersinga et al., 2020, WHOd, 2020) resulting in the recommendation to maintain social distancing, wearing of face mask and sanitizing of hands and surfaces to minimize transmission of the virus.

2.6 CELL ENTRY

Early in infection, the process of SARS-CoV-2 entering the host cells is initiated by the attachment of the viral structural S glycoprotein to the ACE2 receptor found in the host nasal and bronchial epithelial cells and pneumocytes. The type 2 transmembrane serine protease (TMPRSS2), present on the surface of host cell, promotes viral uptake by cleaving the S protein at the S2 position. This process is followed by fusion of the viral membrane and host cell and allows the coronavirus entry into the host cell. The presence of both ACE2 and TMPRSS2 are essential for viral entry (Hoffmann et al., 2020, Wan et al., 2020). This mechanism is the reason why the spike glycoprotein is a favourable target for vaccines, antibodies and therapeutics (Okoh et al., 2022).

Cell surface vimentin and binding of heparan sulphate enhances binding to ACE2. To sustain its high infectivity, SARS-CoV-2 relies on a second strategy, which is host protease activation. Cell surface protease TMPRSS2 and lysosomal cathepsin activate SARS-CoV-2 pseudovirus entry (Hoffmann et al., 2020). Once the virus has entered the host cell, it releases its genomic mRNA material into the cytoplasmic compartment and translation of ORF-1a and ORF-1b begins (Astuti, 2020). This is followed by viral RNA expression and replication to produce full-length copies that are incorporated into newly produced viral particles. Translation of viral structure proteins followed by translocation into endoplasmic reticulum (ER) membranes and transit through the ER to Golgi inter-mediate compartment, where interaction with N-encapsidated newly produced genomic RNA results in budding into the lumen of secretory vesicular compartments. Fully assembled virions are secreted from the infected cell by exocytosis and are accessible to infect other healthy cells and are released into the surrounding environment via respiratory droplets and onward transmission of the virus (V'kovski et al., 2021, Astuti, 2020, Shah et al., 2020). This process is illustrated in **Figure 2.5**.

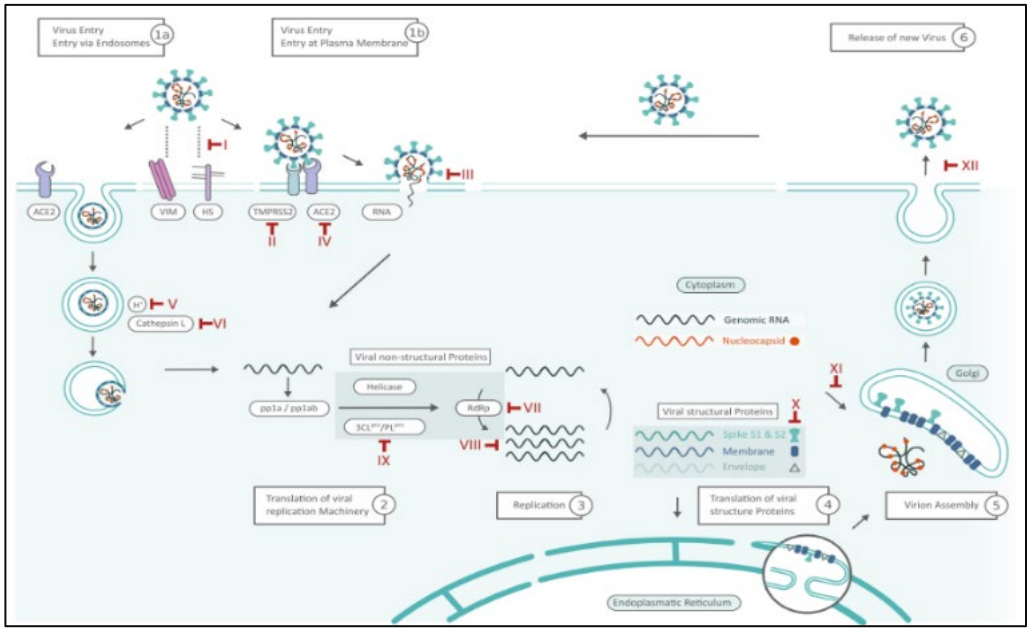

Figure 2.5: SARS-CoV-2 cell entry and replication cycle (adapted from https://www.antibodies-online.com/resources/18/5410/sars-cov-2-life-cycle-stages-and-inhibition-targets/)

2.7 SYMPTOMS AND CLINICAL MANIFESTATION

SARS-CoV-2 enters the nasal and oral cavities and infects the host cells. The initial asymptomatic phase lasts for 1-2 days, while the virus multiplies in the upper respiratory tract with minimal hinderance from the innate immune cells. Between day 2 and 14 the host starts presenting with symptoms of COVID-19, which include fever, dry cough, shortness of breath, joint pain, and fatigue. It is during this early phase that transmission of the virus is likely to occur (Feng et al., 2021). The virus continues to move towards the lower respiratory tract via airways to activate a strong innate immune response. The patients start to present with strong pro-inflammatory responses that manifest as difficulty in breathing or shortness of breath, and associated chest pain. The condition could progress to viral sepsis, accompanied by other complications that include pulmonary oedema, upregulation of cytokines, acute respiratory distress syndrome leading to multi-organ failure and death (WHOb, 2020, Wiersinga et al., 2020, Shah et al., 2020). Laboratory markers of inflammation include lymphopenia, elevated inflammatory markers such as erythrocyte sedimentation rate and C-reactive protein, ferritin, interleukin (IL)-6, IL-1, abnormal coagulation parameters, elevated D-dimer, and low fibrinogen. Radiology observations include bilateral, lower lobe predominate infiltrate on chest radiographic imaging. Older patients and patients with existing co-morbidities, such as hypertension, chronic cardiac conditions, pulmonary disease or immunosuppression, exhibit increased risk of susceptibility to severe disease or death (Salje et al., 2020, Wang et al., 2021, Hakim et al., 2021).

2.8 HOST IMMUNE RESPONSE

The innate immune response is activated within hours of viral exposure as the body releases antiviral molecules at the site of infection. This defense commences with the release of non-specific physiochemical factors such the mucosal barrier, secreted by mucosal epithelial cells, that trap and eliminate the virus (Russell et al., 2020, Smith et al., 2021). Once this defense barrier is breached the host's innate immune sensors called pattern recognition receptors recognize pathogen-associated molecular patterns and initiate the release of innate immune proteins within hours of exposure (Mistry et al., 2021). Toll-like receptors 3, 7, and 8 are the first to recognize the virus, which leads to enhanced interferon production (Shah et al., 2020).

The humoral response is triggered upon the onset of SARS-CoV-2 infection where B cells elicit an early response against the N protein, while antibodies against S protein can be detected after 4-8 days from the onset of initial symptoms. SARS-CoV-2-specific Immunoglobulin A (IgA), Immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies are detectable at various times as the infection progresses as illustrated in **Figure 2.6.** IgM is the early antibody response that is detected between 0-3 days after exposure and peaks within 7 days. IgM continues as the acute phase of the disease continues. SARS-CoV-2-specific IgA and IgG antibodies develop several days after IgM and do not decrease to undetectable levels and are assumed to continue lifelong as protective antibodies (Azkur et al., 2020).

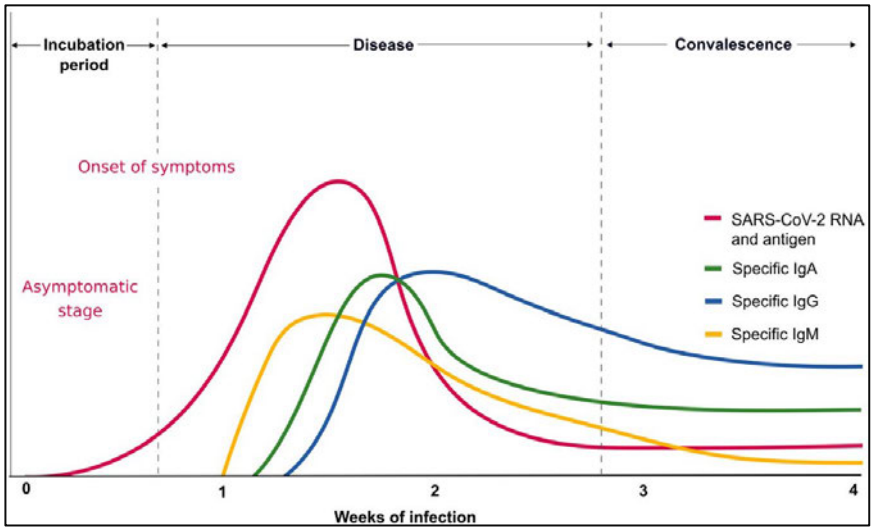

Figure 2.6: Specific antibody response to SARS-CoV-2 (adapted from (Azkur et al., 2020)).

In addition to neutralizing antibodies, there are numerous non-neutralizing antibodies in the host immune system that may respond to the infection. During viral infection, T cells also recognize the viral antigens and elicit a robust immune response against S, M and N protein.

The adaptive immune response occurs days after infection after the system is triggered by components of the innate immune system. This leads to the activation of pathogen-specific cellular and humoral immune responses. Once viral clearance has been achieved, immune memory cells remain, thereby conferring the presence of immunity. When reinfection with SARS-CoV-2 occurs, the memory response is triggered and recognition of the virus surface epitopes or virus as a whole activates (Mistry et al., 2021).

2.9 ANTIBODY RESPONSES TO SARS-COV-2 INFECTION

Infection with SARS-CoV-2 virus initiates a cell-mediated and humoral immune response with the production of antibodies against the specific viral antigens, such as the N protein and S protein. Antibodies can be detected in the serum and plasma samples within 7-10 days post viral exposure. The profile of specific antibodies to the SARS-CoV-2 virus has been assessed in multiple studies (Zhao et al., 2020). Early studies assessed the profile of antibodies in 34 patients (Xiao et al., 2020) and showed that IgM and IgG in 32 individuals (94%) were present after 2 weeks from symptom onset, whilst another study among 82 confirmed and 58 probable SARS-CoV-2-positive cases (RT-PCR negative but with typical manifestations) showed that the median time for IgM and IgA detection was 5 days (Interquartile range (IQR) 3-6 days) and IgG detection was 14 days [IQR 10-18 days] with a positive rate of 85.4%, 92.7% and 93.1% respectively (Guo et al., 2020). Detection of specific IgM and IgG was based on using recombinant viral N protein in an enzyme linked immunosorbent assay (ELISA). This study also found that combining the RT-PCR with the IgM/IgG ELISA increased the positive detection rate significantly to 98.6% as compared to 51.9% for the RT-PCR alone. These data suggest that if RT-PCR is combined with measurement of IgM/IgG, the diagnosis of COVID-19 including subclinical cases could be markedly improved and the false-negative RT-PCR results based on insufficient amounts of the viral genome or poor technique used to obtain throat or NP swabs could be partially overcome.

Combining RNA and antibody detections has been shown to significantly improve the sensitivity of the pathogenetic diagnosis for COVID-19 (as early as one week since onset) and a higher titre of Ab was independently associated with a worse clinical classification (Zhao et al., 2020). Data from several studies show that within 10-14 days after the initial onset of symptoms, RT-PCR is important for confirmation of viral infection and that the combination of serological assay could enhance diagnostic efficacy. After that, the diagnosis for viral infection depends largely on serology results. Thus, a combination of antigen/antibody-based tests provides a reliable, efficient, and optimal diagnosis of COVID-19 for clinical management and enable intervening timeously to reduce transmission.

2.10 DIAGNOSIS

Rapid diagnosis based on clinical assessment, symptoms and laboratory assays is critical for identifying those infected and control viral transmission. The diagnosis of COVID-19 constitutes review of radiology, laboratory, clinical and epidemiological findings. There are several types of tests that can be used for the detection of SARS-CoV-2 and the choice of test is dependent on the stage of the infection and whether virus antigen is being detected or antibodies to SARS-CoV-2. **Figure 2.7** illustrates at which stage viral RNA and SARS-CoV-2 specific antibodies can be detected. Recent publications allude to the half-life of SARS-CoV-2 antibodies and the duration of antibody response may be as long as 180 days and is dependent on whether the individual experienced SARS-CoV-2 infection and/or vaccine induced antibody production (Bobrovitz et al., 2023). Importantly, the test selected also depends on access to specialized equipment, trained personnel, cost and access to reagents, kits and consumables.

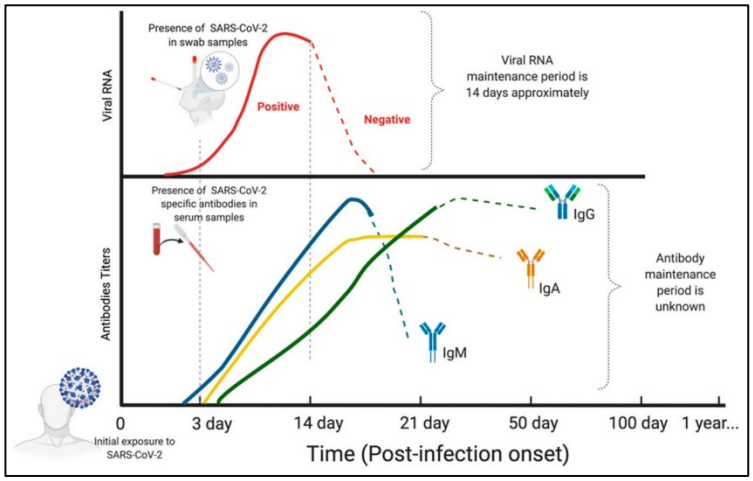

Figure 2.7: Detection of SARS-CoV-2 by RNA Testing and the production of the specific antibodies (IgA, IgG and IgM plotted according to the time since initial exposure to the virus. The dashed lines denote the variable dynamics of adequate levels of SARS-CoV-2 specific antibodies (Guevara-Hoyer et al., 2021).

2.10.1 RT-PCR for SARS-CoV-2 gene/s

Nucleic Acid Amplification Tests (NAATs) can detect current infection with SARS-CoV-2 and include RT-PCR, which is the gold standard and most used diagnostic test for the detection of SARS-CoV-2. In early January 2020, the whole sequence of SARS-CoV-2, SSrna-29870 bp, was uploaded by the Shanghai Public Health Clinical Center and School of Public Health, Fudan University, Shanghai, China on Virology.com (Cascella et al., 2022). This was subsequently confirmed by several laboratories and uploaded with GenBank Accession number MN908947. This became the basis for the development of RT-PCR based methods for SARS-CoV-2 detection (Wu et al., 2020, Lu et al., 2020, Wang et al.,

2020a). Upon the release of the sequence and gene targets of SARS-CoV-2, there was a rapid development of primers and probes for SARS-CoV-2 detection by biotechnology companies and molecular/virology laboratories. Since RT-PCR is a commonly used assay in most laboratories, pivoting to RT-PCR testing for SARS-CoV-2 when the pandemic emerged was simple. The assay requires specialized equipment, trained personnel, can be a lengthy process, but is highly sensitive and specific, and can detect one or more viral RNA genes that the virus presents. Recent updates on the technology have resulted in the RT-PCR test being more accessible, simpler to conduct, POC, and a shorter turnaround time. Most NAATs result in qualitative data with a cycle threshold (Ct) value. Molecular targets include the genes that encode for the nucleocapsid (N), envelope (E), spike (S) and RNA-dependent RNA polymerase (RdRp) proteins. The assay can be performed on a variety of sample types that include preferred NP, oropharyngeal and nasal swabs, and less common sputum, tracheal aspirates, bronchoalveolar lavage and saliva. There have also been reports of SARS-CoV-2 being detected in faecal samples, urine, breast milk and semen (CDC, 2022). Once collected, the samples can be placed in viral transport medium if testing is delayed, or dry swabs can be transported to the laboratory for testing. The samples can be stored at 2-8 ^{o}C for up to 72 hours and can be stored at -70 ^{o}C or below if testing is delayed. Sample results are very dependent on accurate and acceptable sample collection, handling and storage as false negative results could be expected (CDC, WHOe, 2020, Wang et al., 2020b).

2.10.2 Antigen testing for SARS-CoV-2 infection

It is imperative that individuals with SARS-CoV-2 infections are diagnosed rapidly and reliably such that appropriate measures are taken for clinical management of individuals and to control and halt the spread of infection. The detection of SARS-CoV-2 viral RNA is performed via RT-PCR using NP and throat swab samples. Whilst the performance of the RT-PCR test for SARS-CoV-2 antigen is complex, time consuming, requiring specialised equipment, resources, and trained laboratory personnel, it is highly sensitive and specific with a turn-around-time of 24 hours.

Rapid diagnostic tests are assays designed for use at the POC as a screening tool and can be adapted for use in low-resource settings. The advantage of RDTs is that they are of low-cost, simple to operate and read, sensitive, specific, stable at high temperatures, and works in a short period of time. Therefore, the RT-PCR-based POC is less complex, less expensive, quicker turnaround time and could be performed outside of the laboratory. Whilst the RDT are unable to amplify the virus like the RT-PCR, the test detects the presence of viral proteins on the surface of the virus (Guglielmi, 2020, CDC, 2022) and identifies individuals at the time point when they are highly infectious and most likely to be shedding virus. Thus, RDTs have been prioritised as they are sensitive and specific with quick turn-around time, minimal costs and can advantageously be performed outside of the laboratory setting (Guglielmi, 2020).

A negative result on these tests does not rule out an infection with SARS-CoV-2 virus and further testing in conjunction with symptom assessment and in country algorithms is advised (CDC, 2022). Transcription-mediated amplification (TMA) is another technique used instead of RT-PCR. Loop-mediated isothermal amplification (LAMP) is a NAAT assay that utilizes isothermal reaction that does not require the thermocycling process of RT-PCR (Baldanti et al., 2022).

2.10.3 Antibody testing for SARS-CoV-2 infection

The COVID-19 pandemic resulted in an urgent need for rapid and reliable diagnosis to: i) identify the infected; ii) contain the spread of the virus; iii) aid in identifying individuals who may be exposed to SARS-CoV-2 infection and may be asymptomatic or experiencing mild disease; iv) track the recovery of COVID-19 patients; v) characterise antibody responses in infected individuals, vi) diagnose patients who seek medical attention more than seven days after the onset of symptoms when RT-PCR may miss diagnosis, vii) diagnose COVID-19 where RT-PCR specimens could not be obtained; and viii) establish the exposure of the local population to the virus by population based seroprevalence and incidence studies.

Antibodies to SARS-CoV-2 infection develop around 7-14 days following infection (Zhao et al., 2020, Wolfel et al., 2020), and assist in identifying individuals who would have been exposed to SARS-CoV-2 infection and may be asymptomatic or experiencing mild disease. Development of measurable antibody response after infection is termed seroconversion (WHOe, 2020). However, antibody testing does not identify individuals at the peak of their infection and who are most likely to be shedding and transmitting virus. Thus, antibody testing is useful for assessing exposure to SARS-CoV-2 infection at a population level. During SARS-CoV-2 infection, the viral load increases and decreases at various stages of the infection and IgM is the first antibody to emerge, followed by IgG. It is, therefore, critical to utilise diagnostic tests that provide the required information in determining the status of individuals, such that the appropriate responses are taken to mitigate further spread of SARS-CoV-2 virus, to provide clinical support to affected individuals and for a deeper understanding of infectiousness, transmission dynamics and the natural history of the disease. Importantly, the relationship between viral concentration and/or immunological responses with COVID-19 disease progression and severity needs to be explored and better understood as this is likely to vary among individuals with COVID-19 and coinfected with HIV and/or TB. **Figure 2.8** shows the trajectory of the currently available SARS-CoV-2 laboratory testing panel. Furthermore, as newer diagnostic tests are commercialised, it is critical to evaluate the performance of these tests.

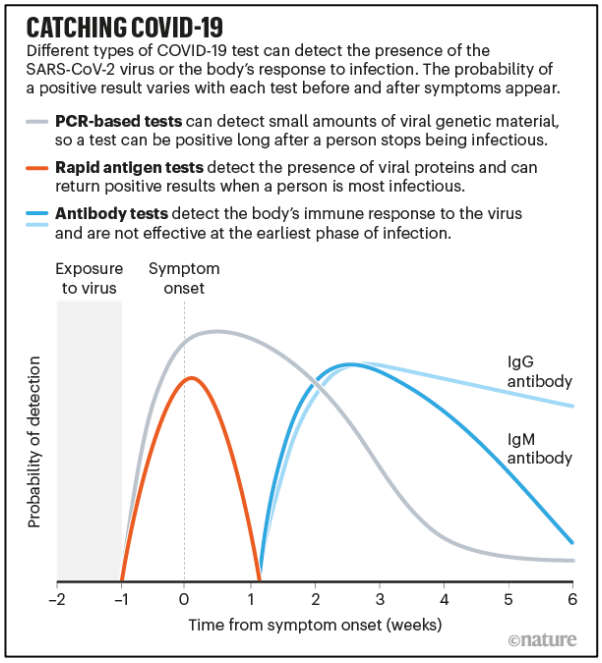

Figure 2.8: Trajectory of SARS-CoV-2 laboratory antigen and antibody testing panels (Guglielmi, 2020).

Non-quantitative antibody assays include the **lateral flow assay** (LFA), which is designed to be a rapid test that detects the presence of SARS-CoV-2 specific antibodies. However, the test does not quantify the antibodies present and are not recommended for diagnosis during the acute stages of infection nor for the clinical management. The advantage of the assay is for epidemiological surveillance (WHOe, 2020). Measurement of SARS-CoV-2 viral structure protein specific antibodies (Total immunoglobulins (Ig), IgG, IgM, and/or IgA) in various combinations can be measured using ELISA and **Chemiluminescence immunoassays** (CLIA). Antibody assays have been validated with blood as the preferred specimen type. **Table 2.1.** presents a summary of diagnostic testing methods for COVID-19.

2.10.4 Other methods for diagnosing COVID-19

1. Imaging technology – imaging techniques such as chest X-rays, pulmonary computed tomography scans, and lung ultrasound. However, these are expensive, not easily accessible and could lead to misdiagnosis.
2. Virus isolation – culture-based methods have been used in research and public health laboratories, but is time consuming, highly complex, has low sensitivity, requires specialized facilities and requires Biosafety level 3(BSL-3) facilities (da Silva et al., 2020).
3. Electron microscopy – the virus has a distinct morphology and can be observed using electron microscopy but requires skilled personnel, access to electron microscopes, is time consuming, is

not for high through-put, and SARS-CoV-2 viral particles could easily be missed (da Silva et al., 2020).

Table 2.1. Summary of diagnostics testing methodology for COVID-19 (da Silva et al., 2020, Baldanti et al., 2022, Zhao et al., 2020, WHOe, 2020).

Type of Test	**Measures**	**Optimal time for testing (days)**	**Sample type**	**Platform/ technologies**	**Detection Target**	**Turnaround time for results (range)**
NAAT for SARS-CoV-2 gene/s	Direct detection of SARS-CoV-2 RNA	At least 2 days after infection	Nasopharyngeal swab, oropharyngeal swab, nasal swab, sputum, saliva, bronchoalveolar lavage, others	High-throughput RT-PCR Point-of-care RT-PCR High through-put TMA POC LAMP High through-put LAMP CRISPR/LAMP lateral flow	SARS-CoV-2 RNA	1.5-8 hours 20 min 3 hours 26-60 min 45 min 15 min
Antigen detection	Detection of SARS-CoV-2 viral antigens	At least 2 days after infection	Nasopharyngeal, oropharyngeal, nasal swab, saliva	High throughput centralised. Point-of-care lateral flow	SARS-CoV-2 viral antigens	From 18 min 15-30 min
Antibody detection	Detection of immune response i.e., post exposure to SARS-CoV-2	After 7-14 days of infection	Serum, plasma, whole blood	High throughput centralised (ELISA), CLIA POC (lateral flow)	Presence and level of antiviral antibodies (qualitative or quantitative) IgG/IgM	From 18 min to 24 hours 15 min

Abbreviations: NAAT: Nucleic acid amplification tests; RNA: Ribonucleic acid; RT-PCR: Reverse transcription polymerase chain reaction; TMA: Transcription-mediated amplification; CRISPR: Clustered regularly interspaced short palindromic repeats; LAMP: Loop-mediated isothermal amplification; ELISA: Enzyme linked immunosorbent assay; CLIA: Chemiluminescence immunoassay; POC: Point of care; IgG: Immunoglobulin G; IgM: Immunoglobulin M, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

2.10.5 Testing in South Africa

South Africa's readiness to respond to the SARS-CoV-2 pandemic was tested due to reliance on the already established extensive molecular capacity in RT-PCR platforms that had been established and expanded for large HIV programme testing. This enabled managing high volumes with rapid turnaround time (Hans et al., 2021), which was in line with the WHO declaration that the SARS-CoV-2 virus was a variant of concern and testing on a mass scale was recommended to identify individuals infected for quarantine and isolation, so that testing of contacts could be achieved (WHOa, 2020).

2.11. STUDY JUSTIFICATION

The significance of early and extensive testing for detecting SARS-CoV-2 infections has played a pivotal role in effectively managing and containing the virus in countries such as Singapore and South Korea, in contrast to many other regions where the virus has spread widely. South Africa carries a disproportionate global burden of HIV and tuberculosis (TB), which underscores the urgent need to enhance point of care diagnostic capabilities to address and enhance prevention and treatment efforts for SARS-CoV-2 infection.

To address these imperatives, there was an urgent need to develop, validate, and implement newer, rapid, reliable, and efficient POC tests as an alternative to the time-consuming conventional RT-PCR methods. As infection numbers continued to rise, POC tests with high sensitivity and specificity became essential for the swift diagnosis to identify individuals with SARS-CoV-2 to facilitate measures to limit the spread of SARS-CoV-2. There was a concerted effort to enhance the efficiency and effectiveness of diagnostic capabilities, including the understanding of how infections, transmission and disease progression occurred. The ability to assess and evaluate new diagnostic tests emerging in the market against existing laboratory tests would contribute to the improvement of diagnostic capabilities. Since its emergence in December 2019, the virus has undergone mutations, resulting in multiple SARS-CoV-2 variants and sub-variants. In this context, it became critical to evaluate both existing and new diagnostic tests against these emerging variants and sub-variants. Therefore, this study aimed at validating existing and emerging diagnostic tests, and to determine their diagnostic parameters in the context of SARS-CoV-2 infection alone and in combination with HIV and/or TB.

2.12. GENERAL STUDY APPROACH

Testing of stored samples obtained from participants in longitudinal follow-up cohorts for SARS-CoV-2 infection in combination with HIV and/or TB were tested using a combination of diagnostic tests to assess and validate the performance of tests and determine the turnaround time to generate a result for screening for a rapid diagnosis of asymptomatic and symptomatic infection, across different stages of clinical disease progression and clinical outcomes.

2.13. HYPOTHESES

Individuals for SARS-CoV-2 infection differ in clinical, virological, immunological, and serological marker responses. Thus, comprehensive laboratory validation testing studies will supplement RT-PCR and provide a set of tests for the rapid diagnosis of SARS-CoV-2 infection. Second, the evaluations of longitudinally collected clinical samples from SARS-CoV-2 infected individuals improves our understanding of SARS-CoV-2 antigen and antibody responses, transmission dynamics, natural history and disease progression.

CHAPTER 3

METHODS

CHAPTER 3: METHODS

3.1. STUDY APPROVALS AND FUNDING

The thesis utilised data from the CAPRISA 225, CAPRISA 228 (BREC/00001195/2020) and the CAPRISA 230 studies (HALT_COVID-19) (BREC/00003106/2021). All studies were approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (Appendix B). The study was approved by the Department of Health (DoH) (Appendix C). The CAPRISA 225 study was funded by the South African Medical Research Council, the CAPRISA 228 and CAPRISA 230 studies were funded by the EDCTP (RIA2020EF) – Mobilisation of research funds for COVID-19 as a Public Health Emergency and the South African Department of Science and Innovation (DSI) Top-up funding to strengthen EDCTP COVID-19 regional networks and the Foundation for Innovative New Diagnostics (FIND) (CV21-0022 and CV21-0261) grants.

3.2. STUDY DESIGN

The study was designed as a prospective, observational cohort study that was conducted between July 2020 and July 2022. In addition to data collected during the study, stored blood and swab specimens were collected, stored, and tested retrospectively. In addition, samples collected at a drive through testing facility in Durban, South Africa was used for the rapid antigen test evaluations.

3.3. STUDY LOCATION

Participants were recruited from government facilities adjacent to CAPRISA's research facilities in Durban, South Africa, between July 2020 and January 2021. The drive through facility was located at an independent site in Durban, South Africa and participants were recruited between July 2021 and June 2022.

3.4. STUDY POPULATION

Patients accessing routine health care services were offered SARS-CoV-2 testing irrespective of the presence of symptoms. Patients with positive test results were offered enrolment into the study. At the drive through facility, adults > 18, meeting the inclusion criteria and seeking testing for SARS-CoV-2 diagnosis were included.

3.5. INCLUSION AND EXCLUSION CRITERIA

Stable, ambulant adults ≥ 18 years of age, inclusive of men and women with a confirmed SARS-CoV-2 PCR test result, who were willing to participate in the study and able to provide written informed consent (Appendix D) were eligible for study participation.

3.6. STUDY PROCEDURES

Participants were screened and enrolled within three days of COVID-19/SARS-CoV-2 test result and were then followed up at day 7, 14, 28 and 3, 6, 9, 12 and 18 months post screening visits. At the enrolment visit, trained staff administered questionnaires, obtained medical history inclusive of HIV and TB co-infection diagnosis and treatment, other pre-existing chronic illnesses, and concomitant medication history. Participants were assessed for COVID-19 symptoms, disease severity including routine pulse oximetry, occurrence of intercurrent adverse events, and receipt of COVID-19 specific therapy were assessed at enrolment and at each follow-up visit by trained clinicians. Participants were referred for clinical care as required. Disease severity was defined using the National Institute of Health clinical and functional criteria (Sup. Table 1). Adherence to national COVID-19 isolation regulations was maintained with enrolment visits and relevant follow-up visits conducted at participants' homes or isolation facility. All post-isolation study follow-up visits were conducted at the CAPRISA Springfield Clinical Research Site.

At the drive through testing facility, all adults, > 18 years old, seeking testing provided two or three swabs, depending on the rapid antigen kit or method being assessed. If a nasal swab was required, that swab was collected first followed by the NP swab/s. On site testing using the comparator rapid antigen test was conducted and the results read by two independent trained readers. A photograph of the results obtained was taken and archived for review in instances where inconsistencies in results were observed. A NP swab was always sent to the testing laboratory for RT-PCR testing using the Abbott RealTi*m*e SARS-CoV-2 assay. All confirmed COVID-19 cases were reported to the South African National Department of Health using the National Medical Conditions (NMC) surveillance system.

3.7. SAMPLE COLLECTION AND PROCESSING.

Clinical staff collected NP swabs for SARS-CoV-2 RT-PCR testing at screening, enrolment, day 7, 14 and 28. Blood samples were collected at enrolment for routine haematology, chemistry, and inflammatory markers. Samples from all available timepoints were used in this study. The blood samples were centrifuged at 1,200 revolutions per minute (rpm) for 10 minutes, aliquoted and stored at -85^{o}C until use. Control samples for specificity testing was derived from samples stored at the laboratory prior to December 2019. At the drive through testing facility, trained professionals collected NP swabs. The NP swabs were resuspended in 2 mL viral transport media (VTM) on receipt in the laboratory. The Abbott RealTi*m*e SARS-CoV-2 (Des Plaines, IL) assay was performed on the m2000 system, which consists of a m2000sp sample preparation unit and m2000rt amplification and detection unit. Viral RNA was isolated and amplified (targeting sequences in the SARS-CoV-2 *RdRp* and *N* genes

of the SARS-CoV-2 genome). Samples with a Ct <40 was indicative of the presence of SARS-CoV-2 RNA.

3.8. LABORATORY TESTING PROCEDURES

All samples from the CAPRISA 225, 228 and 230 studies were tested using existing and newer tests as and when they became available to guide the applicability, rapidity, reliability, and efficiency on their performance for large scale use in the field.

3.8.1. SARS-CoV-2 RT-PCR

Swabs were inactivated at 60°C for 30 minutes, after which the viral RNA was isolated using the Abbott mSample Preparation System (Abbott GmbH & Co, Germany) for extraction. We used the TaqPath COVID-19 CE-IVD RT-PCR Kit (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions for amplification. The assays target genomic regions (ORF-1 antibodies (ORF1ab), S protein and N protein) of the SARS-CoV-2 genome. RT-PCR was performed on a QuantStudio 7 RT-PCR instrument (Life Technologies, Carlsbad, CA). The MS2 Phage Control provided in the kit was used to verify the efficacy of the sample preparation and the absence of inhibitors in the RT-PCR reaction. The target not detected samples were assigned a Ct value of 40 for the purposes of further analysis.

As technologies became available in South Africa, we expanded our testing schedule to include:
Abbott RealTi*m*e SARS-CoV-2 assay (Des Plaines, IL) using M2000 system *sp/rt* (Abbotta, 2020). The Abbott RealTi*m*e SARS-CoV-2 assay is a RT-PCR test detecting dual target *RdRp* and *N* genes with a limit of detection of 100 copies/mL. Abbott RealTi*m*e SARS-CoV-2 internal control is added to the lysis buffer. The lysis buffer is added to each sample and amplified during the process. The resultant standard curves post amplification illustrates the sample curve in relation to the standard internal curve. Annealing fluorescent probes targeted to the amplified viral sequence indicates a positive test. The assay was performed using swabs to which VTM was added and assayed according to the manufacturer's instructions.

The Cepheid Xpress SARS-CoV-2 assay (Cepheid, Sunnyvale, CA, USA) is a rapid real time RT-PCR assay targeting the *E* gene and the N2 region of the *N* gene, with a detection limit of 250 copies/mL and an approximate running time of 45 minutes (Cepheid, 2021a). The assay is a single use cartridge-based nucleic acid amplification assay, with each cartridge containing a built-in internal control. The basic method followed for each of the RT-PCR assays is illustrated in the **Figure 3.1.** below.

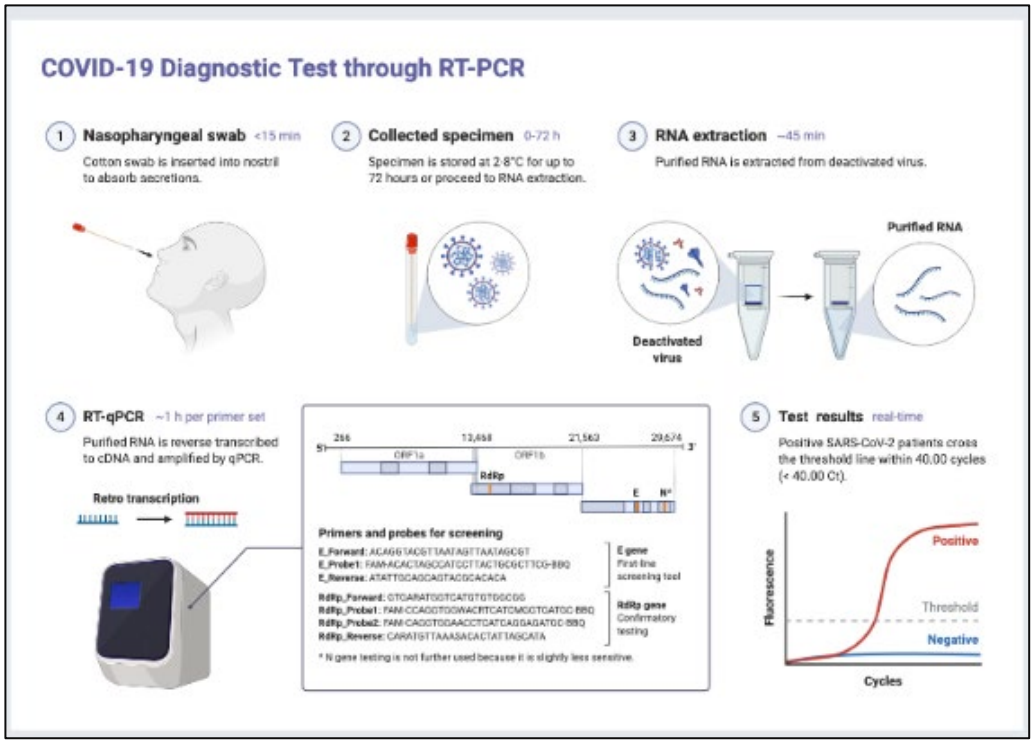

Figure 3.1: RT-PCR assay. Image created by Bio Render (https://biorender.com/)

3.8.2. HIV antibody, viral load and CD4 cell count measurement.

HIV rapid testing was performed at enrolment using the Advanced Quality One Step Anti-HIV Test (Colloidal Gold, Amada Diagnostics, China) and First Response HIV1-2 Card Test (Rapid Immunochromatographic Premier, India). Plasma samples collected at enrolment were tested to confirm HIV status using the Abbott Diagnostics (Abbott Park, IL) RealTi*m*e HIV type 1 (HIV-1) assay on the m2000sp/m2000rt automated extraction platform (Abbott Molecular Inc., Des Plaines, IL) with a dynamic range of 20-10 million copies per mL. CD4 cell counts were measured at enrolment using Becton Dickinson (BD) FACS Calibur flow cytometry (BD Biosciences, San Jose, CA, USA).

3.8.3. Antigen testing by Point of Care (POC) testing

Nasal and/or NP swabs were used for COVID-19 antigen testing by POC and compared to the gold standard RT-PCR. **Figure 3.2** describes the fundamental design and methodology of the SARS-CoV-2 POC rapid antigen tests. All tests were performed according to manufacturer's instructions. The POC that were included in the evaluation are shown in **Table 3.1.**

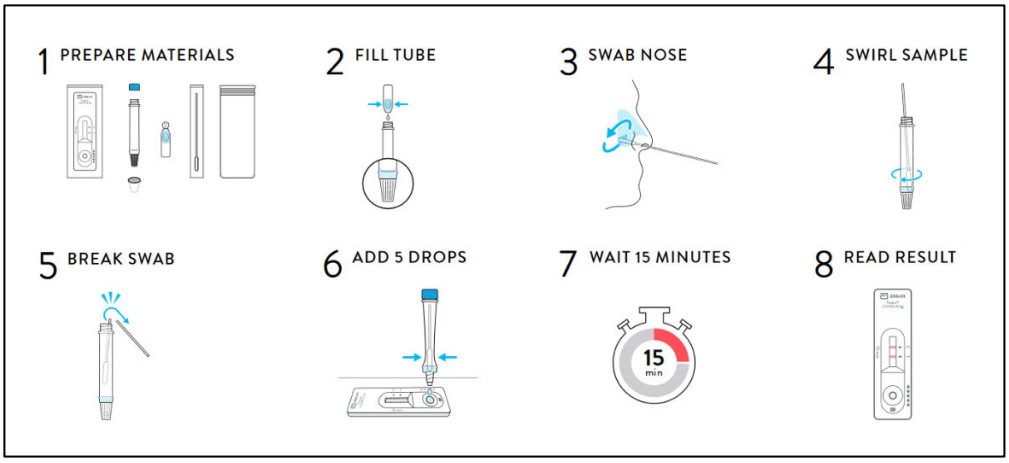

Figure 3.2: Procedure of a rapid antigen test https://www.globalpointofcare.abbott/en/product-details/panbio-covid-19-antigen-self-test-au.html

Table 3.3. Point-of-Care antigen detection tests for SARS-CoV-2 antigen.

TEST NAME	SUPPLIER	METHOD
Panbio™ COVID_19 Ag Rapid Test Device (Abbott, SAPHRA approved)	Abbott Rapid Diagnostics Jena GmbH	Panbio™ COVID-19 Ag Rapid Test Device contains a membrane strip, which is pre-coated with immobilized anti-SARS-CoV-2 antibody on the test line and mouse monoclonal anti-chicken IgY on the control line. Two types of conjugates (human IgG specific to SARS-CoV-2 Ag gold conjugate (binds to the nucleocapsid protein) and chicken IgY gold conjugate) move upward on the membrane chromatographically and react with anti-SARS-CoV-2 antibody and pre-coated mouse monoclonal anti-chicken IgY, respectively. For a positive result, human IgG specific to SARS-CoV-2 Ag gold conjugate and anti-SARS-CoV-2 antibody will form a test line in the result window.
STANDARD Q COVID-19 Ag test (SD Biosensor, SAPHRA approved)	SD Biosensor, Inc	STANDARD Q COVID-19 Ag Test has two pre-coated lines, "C" Control line, "T" Test line on the surface of the nitrocellulose membrane. Both the control line and test line in the result window are not visible before applying any specimens. Mouse monoclonal anti-SARS-CoV-2 antibody is coated on the test line region and mouse monoclonal anti-Chicken IgY antibody is coated on the control line region. Mouse monoclonal anti-SARS-CoV-2 antibody conjugated with colour particles are used as detectors for SARS-CoV-2 antigen device. During the test, SARS-CoV-2 antigen in the specimen interact with monoclonal anti-SARS-CoV-2 antibody conjugated with colour particles making antigen-antibody colour particle complex. This complex migrates on the membrane via capillary action until the test line, where it will be captured by the mouse monoclonal anti-SARS-CoV-2 antibody. A coloured test line would be visible in the result window if SARS-CoV-2 antigens are present in the specimen

ESPLINE SARS-CoV-2 (Fujirebio, not SAPHRA approved)	Fujirebio Inc., Tokyo, Japan	This product is a kit for detecting SARS-CoV-2 antigen in nasopharyngeal swabs using immunochromatography with enzyme immunoassay as the measurement principle. Liquids containing a test sample are dropped into the cassette, and within about 30 minutes, the presence or absence of the indicator line on the cassette is checked to determine positive or negative test result.
Biotest COVID-19 Antigen (RightSign, not SAPHRA approved)	Biotest Biotech Co. Ltd Hangzhou	The COVID-19 Antigen Rapid Test Cassette (Nasal Swab) is a rapid chromatographic immunoassay for the qualitative detection of COVID-19 antigen in Nasal Swab. The identification is based on the monoclonal antibodies specific for the Nucleocapsid (N) protein of SARS-CoV-2.
AllTest SARS-CoV-2 Antigen Rapid Test (Nasal swab)	Hangzhou AllTest Biotech Co, Ltd.	The SARS-CoV-2 Antigen Rapid Test (nasal swab) is a rapid chromatographic immunoassay for the qualitative detection of SARS-CoV-2 nucleocapsid protein antigen present in the nasal swab specimen.

3.8.4. SARS-CoV-2 antibody testing

Exposure to SARS-CoV-2 infection was measured on stored EDTA plasma specimens or from DBS samples for antibodies to assess the dynamics of antibody responses over time. Participants testing positive from the CAPRISA 225, 228 and 230 studies whose samples were collected at different time points were tested. Control plasma samples were selected from stored EDTA plasma specimens collected prior to December 2019 and from individuals testing SARS-CoV-2 positive to assess the dynamics of antibody responses. SARS-CoV-2 antibody testing for this study included the tests listed in **Table 3.2.**

Table 3. 4. SARS-CoV-2 antibody tests used in this study.

TEST NAME	SUPPLIER	METHOD
Orient Gene LFA	Zhejiang Orient Gene Co Ltd	The test uses anti-human IgM antibody (test line IgM), anti-human IgG (test line IgG) and rabbit IgG (control line C) immobilized on a nitrocellulose strip. The burgundy-coloured conjugate pad contains colloidal gold conjugated to recombinant COVID-19 antigens (SARS-CoV-2 Spike S1 antigen) conjugated with colloid gold (COVID-19 conjugates).
Abbott Architect SARS-CoV-2 IgG assay (Qual)	Abbott Architect	The Abbott assay detects IgG antibodies reactive against the nucleocapsid protein using a two-step sandwich immunoassay employing microparticle-bound antigen and acridinium-labelled human anti-IgG.
Abbott SARS-CoV-2 IgG-II (quant)	Abbott Architect	The Abbott assay detects IgG antibodies reactive against the nucleocapsid protein S using a two-step sandwich immunoassay employing microparticle-bound antigen and acridinium-labelled human anti-IgG-S

MILLIPLEX® MAP Kit	MILLIPLEX®	The panel is designed to measure antibodies by median fluorescent intensity (MFI). The four antigens are recombinant poly-his-tagged.

Orient Gene *COVID-19 IgG/IgM kit:* Plasma samples were tested on the Zhejiang Orient Gene Biotech COVID-19 IgG/IgM Rapid Test Cassette (OG) (Huzhou China), a LFA. Orient Gene COVID-19 IgG/IgM assay is a rapid, qualitative, and differential detection of IgG and IgM for SARS-CoV-2. Serum, plasma, or whole blood may be used for this assay. 5µL of plasma was deposited into the specimen well (S) on the test cassette. Two drops of sample buffer were immediately added to the buffer well (B). The reaction was allowed to proceed and read after 10 minutes. The cassette displays a blue control line that turns red when the test is performed correctly. IgM and IgG are represented by 2 separate bands that turn red when present. The principles of rapid antibody test lateral flow assays as illustrated in **Figure 3.3.**

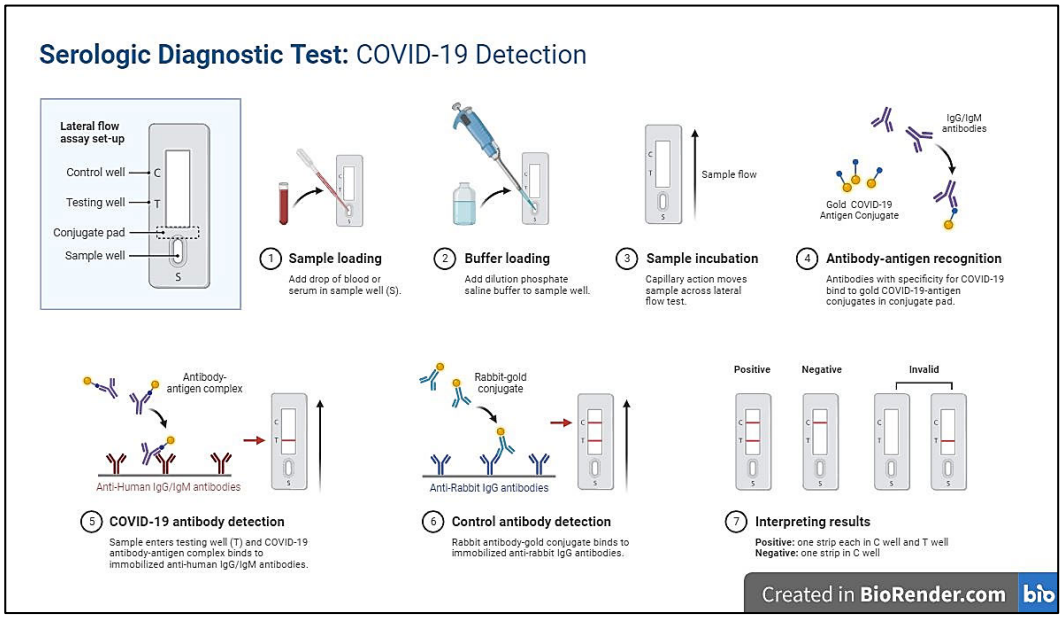

Figure 3.3: Rapid antibody test procedure**.** Created in Bio Render on 14 August 2022.

MILLIPLEX® MAP Kit: IgG, IgA and IgM antibody levels against 89 SARS-CoV-2 spike protein subunits S1 and S2, RBD and N were measured in duplicate plasma samples from the 103 patients (320 samples) using novel MILLIPLEX® 90 SARS-CoV-2 Antigen Panel 1 IgG, SARS91 CoV-2 Antigen Panel 1 IgA and SARS-CoV-2 Antigen Panel 1 IgM (Millipore, MA, USA, 92 Catalogue Numbers: HC19SERG1-85K, HC19SERA1-85K, and HC19SERM1-85K respectively; For 93 Research Use

Only. Not For Use in Diagnostic Procedures). The kits were used in accordance with manufacturer's instructions. This panel is designed to measure 94 antibodies by MFI.

Plasma samples were thawed completely on removal from storage, vortexed and centrifuged at 1200 rpm for 10 minutes. Samples were diluted 1:100 in assay buffer. All reagents were allowed to warm to room temperature and prepared as per manufacturers guidelines. 96-well plates were pre-wetted with 200µL wash buffer, covered with plate sealer, and incubated for 10 minutes at room temperature with plate shaking, then decanted with the removal of residual amount from the wells by inverting the plate and gently tapping onto absorbent paper towel several times. 25µL of assay buffer was added to each sample well followed by 25µL of each diluted sample and 25µL assay buffer was added to background wells. The mixing bottles were vortexed and 25µL of the mixed beads added to each well.

The plate was sealed and incubated for 2 hours at room temperature with constant shaking. Thereafter, the well contents were removed, and the plate washed 3 times with 200µL wash buffer using the plate washer. Thereafter, 50µL of phycoerythrin-anti-human immunoglobulin (IgG, IgA, or IgM per kit in use) detection antibody was added to each well, plate sealed and incubated 90 minutes at room temperature with constant shaking. Plates were washed three more times with magnetic plate washer. 150µL sheath fluid was added to each well, the plate was then sealed and shaken at room temperature for 5 minutes. The plates were then read on a Bio-Rad Laboratories (Hercules CA) Bio-Plex ® 200 suspension array system, post calibration passing.

Abbott Architect: Plasma samples were run on the Abbott Architect instrument using the Abbott SARS-CoV-2 IgG assay (Abbott Diagnostics, Abbott Park Illinois, United States), qualitative testing IgG directed against the nucleocapsid (N) protein (Abbott, 2020) and Abbott Architect SARS-CoV-2 IgG-II (CoV-2 IgG II, Abbott Laboratories, Sligo, Ireland), quantitative, testing for IgG directed against the spike (S) protein (Abbott, 2021). Both assays are chemiluminescent microparticle immunoassay (CMIA) for detection of IgG in human serum or plasma against the SARS-CoV-2 nucleoprotein. The assay requires a minimum of 100 µL of serum or plasma. Depending on the kit used, qualitative and quantitative results and index values reported by the instrument were used in analyses.

3.9. DATA COLLECTION AND ANALYSIS

The CAPRISA 225, 228 and 230 collect detailed participant information, all laboratory evaluations were anonymised except for use of information on gender, age, SARS-CoV-2 infection, HIV and TB infection status to address the study objectives. Each participant's laboratory and questionnaire data were merged using a unique linking number. The analyses included assessment of sample type, handling procedures, equipment, and reagents needed and cost, trained personnel needed, assay processing duration, target population, practicality in the field, optimization, standardization and

determine the performance characteristics of the assay. The performance of the tests was assessed in comparison with RT-PCR. The following test-performance metrics was assessed: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, the limit of detection, coefficient of variation (intra- and inter-assay), reproducibility, repeatability, and reliability. Detailed analyses are included in each manuscript.

3.10. ETHICAL CONSIDERATIONS

Study related information and procedures were provided to all volunteers. All procedures were undertaken following written informed consent including consent for future testing on stored samples. Samples collected through public sector settings and tested through public and private laboratories also provided written consent for testing (Appendix D). Participant identity and information remained confidential and laboratory staff did not have access to any of study participants personal information. Participants received no direct benefits, though study staff facilitated access to follow-up care if required. Confidentiality was maintained for all study records.

CHAPTER 4

SCOPING REVIEW

CHAPTER 4: SCOPING REVIEW

A scoping review was undertaken to determine the evolution, performance, and reliability of diagnostic tests for identification of SARS-CoV-2 infection in South Africa. We identified published studies in the English language undertaken from March 2020 to August 2022 that evaluated the performance of antigen- and antibody-based diagnostic tests for SARS-CoV-2 in South Africa. We identified 17 relevant peer-reviewed articles; six reported on antigen detection whilst 11 reported on antibody detection. Of the antigen-based tests, sensitivity ranged from 40% to 100%, whilst for the antibody-based tests, sensitivity ranged from 13% to 100%. All tests evaluated were highly dependent on the stage of infection and timing of sample collection. This scoping review demonstrated that no single antigen- or antibody-based assay was sufficiently sensitive and specific simultaneously. The sensitivity of the tests was highly dependent on the timing of sample collection over the course of SARS-CoV-2 infection. In the case of antigen detection, the earlier the collection of samples, the greater the sensitivity, while antibody detection tests showed better sensitivity using samples from later stages of infection. The South African Government rolled out and intensified HIV prevention strategies to reach eventual epidemic control. The manuscript from this scoping review titled "*The performance of diagnostic tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in South African population: a scoping review"*, was published by the journal Tropical Medicine and Infectious Disease.

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Review

The Performance of Diagnostic Tests for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in the South African Population: A Scoping Review

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Abstract: To determine the performance and reliability of diagnostic tests for the identification of SARS-CoV-2 infection in South Africa, we conducted a scoping review to identify published studies undertaken in the English language from March 2020 to August 2022 that evaluated the performance of antigen- and antibody-based diagnostic tests for SARS-CoV-2 in South Africa. We identified 17 relevant peer-reviewed articles; six reported on SARS-CoV-2 gene and/or antigen detection whilst 11 reported on antibody detection. Of the SARS-CoV-2 gene and/or antigen-based tests, sensitivity ranged from 40% to 100%, whilst for the antibody-based tests, sensitivity ranged from 13% to 100%. All tests evaluated were highly dependent on the stage of infection and the timing of sample collection. This scoping review demonstrated that no single SARS-CoV-2 gene and/or antigen- or antibody-based assay was sufficiently sensitive and specific simultaneously. The sensitivity of the tests was highly dependent on the timing of sample collection with respect to SARS-CoV-2 infection. In the case of SARS-CoV-2 gene and/or antigen detection, the earlier the collection of samples, the greater the sensitivity, while antibody detection tests showed better sensitivity using samples from later stages of infection.

Keywords: SARS-CoV-2; SARS-CoV-2 gene/s; diagnostic testing; RT-PCR; antigen; antibody; scoping review

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1. Introduction

The unprecedented spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in the urgent need for rapid and reliable diagnostic tests. Accurately diagnosing individuals with infection was paramount to limit the transmission of the virus and to reduce morbidity and mortality. Whilst individuals exposed to SARS-CoV-2 appear to be equally at risk of acquiring infection, the severity of the resulting clinical disease differs markedly by age with a case fatality rate of <1% for people <60 years and sequentially increasing to 14.8% among those 80 years or older [1].

Based on the first available SARS-CoV-2 viral sequences, the World Health Organization (WHO) issued guidance on polymerase chain reaction (PCR)-based assays to be performed from upper respiratory tract specimens as the "gold standard" for the detection of SARS-CoV-2 infection [2]. Africa, and in particular, South Africa, relied on the existing PCR-based platforms that had been established for human immunodeficiency virus (HIV) and tuberculosis (TB), enabling the rapid introduction and scale-up of testing for SARS-CoV-2 infections [3]. Notwithstanding South Africa's diagnostic capabilities to undertake

testing, commercially available diagnostic tests and consumables including competitive first-world pricing and prioritisation to specific institutions were a major challenge in accessing and scaling up testing services to address the rapidly growing needs of the country to determine the extent of current and past infection.

The evolution of the rRT-PCR is based on primers and probes (nCoV_IP2 and nCoV_IP4) that were designed to target the genes that encode for the nucleocapsid (N), envelope (E), spike (S), and RdRp proteins [4]. Rapid diagnostic tests (RDT) were designed and to be point-of-care (POC) tests for the detection of SARS-CoV-2 gene/s and/or antigens that are simpler to perform and have a shorter turnaround time. There is minimal evolution of the N gene and therefore most POC tests target the nucleocapsid. Once the virus has entered the host cell, it releases its genomic mRNA material in the cytoplasmic compartment and the translation of ORF-1a and ORF-1b begins [5]. This is followed by viral RNA expression and the replication of genomic RNA to produce full-length copies that are incorporated into newly produced viral particles [6]. Individuals with SARS-CoV-2 infection elicit an innate immune response within hours of viral exposure, followed by the development of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies at around 7 to 14 days [2,7]. Thus, the detection of SARS-CoV-2 gene/s and/or antigen and antibody responses helps in understanding the infectiousness, transmission dynamics, and natural history of the disease.

Viral shedding has been found to occur in oropharyngeal and nasal or sputum, tracheal aspirates, bronchoalveolar lavage and saliva [8], faeces, urine [9], and semen samples [10]. These findings highlight the need for alternative sampling approaches to improve diagnostic performance and to understand the magnitude and/or duration of viral shedding that could correlate with disease severity and viral dynamics to influence infection and transmission outcomes. The rapidly evolving SARS-CoV-2 pandemic with the emergence of new SARS-CoV-2 variants and subvariants has led to complex diagnostic testing challenges, especially in settings with limited access to diagnostic tests.

This scoping review evaluated the laboratory performance of SARS-CoV-2 diagnostic tests in South Africa to identify knowledge gaps and enhance the accuracy of these tests.

2. Materials and Methods

This scoping review followed the PRISMA-ScR (Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews) checklist [11] and followed the framework of Levac et al. [12].

2.1. Eligibility Criteria

Included in this review were articles reporting original studies undertaken in South Africa between March 2020 to August 2022, peer-reviewed and published in the English language. Articles examining other diseases or behaviours related to mitigating SARS-CoV-2 transmission, with incomplete data, or which were either opinion pieces, reviews, or guidelines and not undertaken in South Africa were excluded.

2.2. Information Sources

Two independent reviewers (NS and ND) designed a search strategy and systematically searched bibliographic databases—the PubMed, Web of Science, and Scopus electronic databases—for published articles. Manual searches were conducted by reviewing the references of published articles. The final search results were exported into Endnote^{TM}20 (Thomson Reuters, New York, NY, USA) software, a reference management tool for citations.

2.3. Search Strategy

The search strategy terms used individually and/or in combination included "severe acute respiratory syndrome coronavirus 2", "SARS-CoV-2", "testing for SARS-CoV-2 in South Africa", "SARS-CoV-2 antibody", "testing for COVID-19", real-time reverse transcriptase–polymerase chain reaction for SARS-CoV-2, rRT-PCR for SARS-CoV-2, "SARS-CoV-2 PCR", "SARS-CoV-2 GeneXpert".

2.4. Data Charting and Extraction

Each reviewer (NS and ND) screened the article titles and abstracts independently, excluded duplicate articles, merged the results of the review, and resolved discrepancies. The final set of full articles was reassessed for the pre-set inclusion criteria. The reviewers prepared the data charts, standardised the data abstraction process, and independently charted the data for discussion and the final selection. Disagreements were resolved through discussion for the final selection of articles.

2.5. Quality Assessment

Eligible articles were evaluated using the quality appraisal tool [13] and scored to assess clarity, the sampling and data collection strategy, the sample representative of the target population, measurements, the risk of non-response, and statistical analysis to address the research question. NS assessed the quality of the studies as being of low quality (score $\leq$ 50%), average quality (51% to 75%), and high quality (76% to 100%). No articles were excluded based on quality.

3. Results

3.1. Study Selection

Figure 1 shows the identification, screening, eligibility, and inclusion of the studies for the scoping review [14–30]. The quality assessment of the 17 articles resulted in four articles scoring 71% and the rest scoring 86% and above. Table 1 provides an overview of the testing kits or methodology evaluated. Seven of the 17 studies included populations from Gauteng [17,19,21,22,24,29,30], four from the Western Cape [15,16,26,28], one from Limpopo [14], one from Eastern Cape [18], and one from the Free State [23]. Of the remaining three studies, two studies included samples from more than one province [20,27] and one study undertook testing on stored samples [25]. All studies were cross-sectional in design and tested samples retrospectively or prospectively. The majority of the studies were undertaken with well-characterised samples from patients experiencing COVID-19 at different stages of the disease, thus allowing for sensitivity assessment of the tests over specific time periods. Several studies describing antibody kit evaluations included archived pre-COVID-19 samples to assess specificity. Samples were collected starting from the origin of the D614G strain, followed by the Beta, Delta, and Omicron variants. Table 2 provides the performance characteristics of the SARS-CoV-2 diagnostic tests, which are either commercially available or are in-house assays, the manufacturer, country of manufacture, analytes measured, sample type, sample volume required per test, time taken to perform the test to obtain the result, and the complexity of the testing procedure. Not all studies included the volume of sample required, though they indicated the sample type used. Table 3 shows the analytical assessment and testing parameters of SARS-CoV-2 gene(s) and antigen- and antibody-based diagnostic tests.

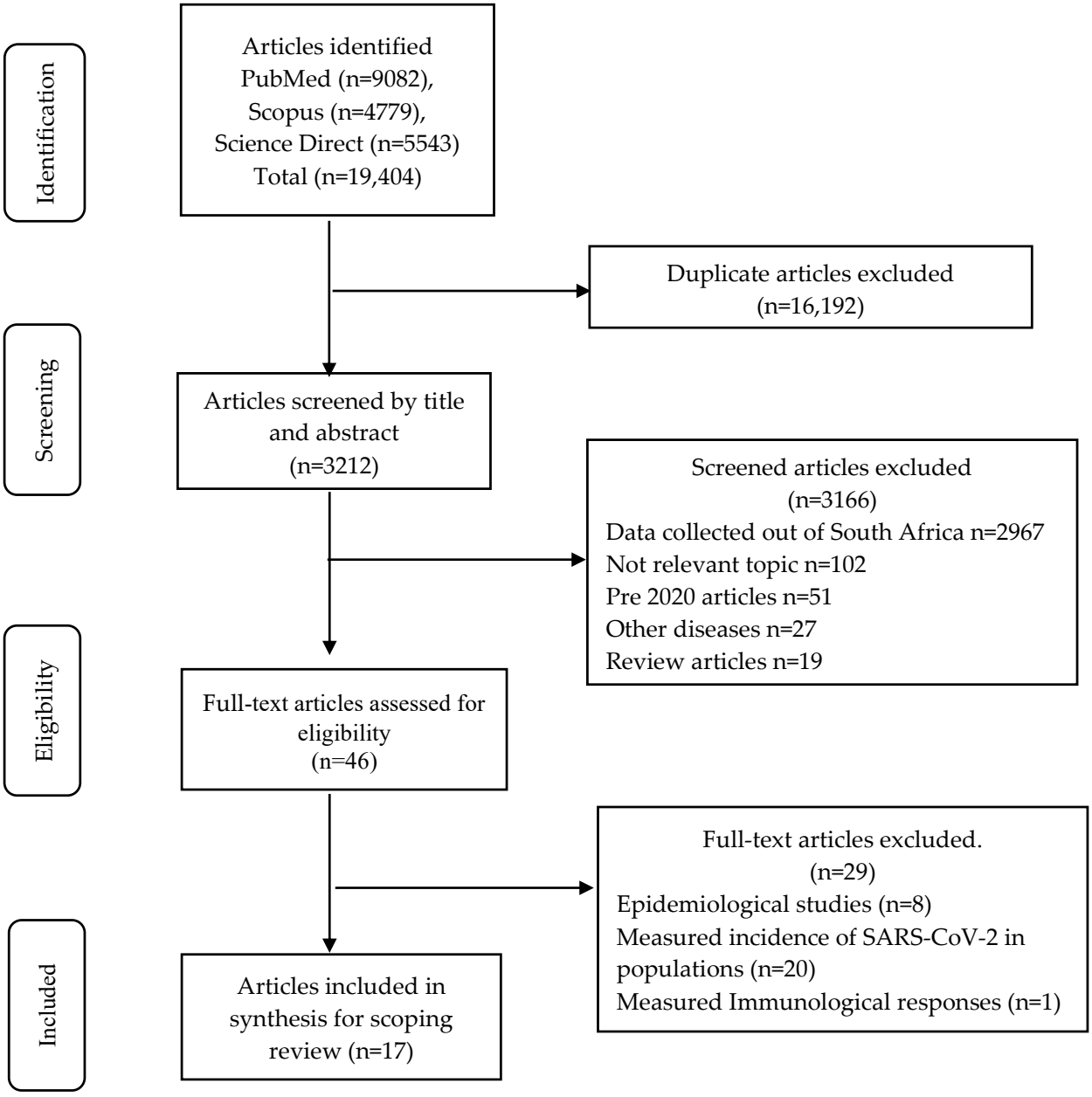

Figure 1. Search strategy and selection of articles for data extraction, analysis, and reporting for a scoping review according to PRISMA-ScR guidelines.

Table 1. Overview of articles on diagnostic testing for SARS-CoV-2 in South Africa.

Study (Author, Ref)	Province	Study Design	Date Range	Clinical Disease Stage	SARS-CoV-2 Viral Variants
Genotyping					
Umunnakwe et al., 2022 [14]	Limpopo	Cross-sectional random sampling of samples from biorepository	April–Oct 2021	Not reported	Beta, Delta
RT-PCR					
Marais et al., 2020 [15]	Cape Town, Western Cape	Cross-sectional retrospective testing of preselected samples	Not reported	Not reported	Not reported
Marais et al., 2022 [16]	Cape town, Western Cape	Prospective cross-sectional	20 August–19 November 2021 19 November 2021– 7 February 2022	Ambulatory outpatients	Delta, Omicron
Omar et al., 2021 [17]	Johannesburg, Gauteng	Retrospective descriptive cross-sectional	20 May–8 August 2020	Symptomatic Asymptomatic	D614G
Rapid antigen testing					
Akingba et al., 2021 [18]	Nelson Mandela Bay, Eastern Cape	Prospective cross-sectional evaluation	17–20 November 2020	Symptomatic	Beta
Majam et al., 2022 [19]	Johannesburg, Gauteng	Prospective evaluation	June–September 2021	Random sampling	Delta
Serological testing					
Makatsa et al., 2021 [20]	Gauteng and Western Cape	Prospective evaluation	10 April–26 May 2020	Symptomatic Asymptomatic	D614G
Gededzha et al., 2021 [21]	Braamfontein, Gauteng	Retrospective cross-sectional evaluation of volunteer samples	Not reported	87% symptomatic, 13% asymptomatic	Not reported
Jugwanth et al., 2022 [22]	Braamfontein, Gauteng	Cross-sectional sample of volunteers	Not reported	87% symptomatic, 13% asymptomatic	Not reported
Matefo et al., 2022 [23]	Bloemfontein, Free State	Retrospective cross-sectional of patient samples	March–October 2020		D614G
Grove et al., 2021 [24]	Johannesburg, Gauteng	Prospective analytical evaluation	May–August 2020	Not reported	D614G
David et al., 2021 [25]	Not reported	Retrospective cross-sectional testing on previously confirmed samples	Not reported	Not reported	Not reported

Table 1. *Cont.*

Study (Author, Ref)	Province	Study Design	Date Range	Clinical Disease Stage	SARS-CoV-2 Viral Variants
Serological testing					
Shaw et al., 2021 [26]	Cape Town, Western Cape	Cross-sectional volunteers	17 August–4 September 2020	Volunteers	D614G
Wolter et al., 2022 [27]	Mitchell's Plain Western Cape, Pietermaritzburg KwaZulu-Natal, Klerksdorp Northwest	Prospective cross-sectional household seroprevalence survey in 3 communities	March and April 2021	Not reported	Beta
Maritz et al., 2021 [28]	Stellenbosch, Western Cape	Retrospective cross-sectional of volunteer samples	Not reported	Asymptomatic	Not reported
Kwatra et al., 2022 [29]	Soweto, Gauteng	Retrospective sampling of participants testing positive	April–December 2020	Hospitalised Symptomatic	D614G, Beta
Irwin et al., 2021 [30]	Johannesburg, Gauteng	Cross-sectional of randomly selected in- and out-patients	Not reported	Symptomatic Asymptomatic	Not reported

Table 2. Performance characteristics of the diagnostic tests for SARS-CoV-2.

Study (Author, Ref)	Product Name	Manufacturer /Country	Analyte Measured	Sample	Sample Volume	Read Time	Complexity
Genotyping							
Umunnakwe et al., 2022 [14]	Allplex™ SARS-CoV-2 Variants II multiplex real-time PCR genotyping assay	Seegene/South Korea	Specific primers and probes for Beta and Delta variants	Nasopharyngeal swab	Not applicable	~2 h	Slightly complex
RT-PCR							
Marais et al., 2020 [15]	Rapid sample preparation (RSP) Abbott RealTime SARS-CoV-2 Assay or Allplex™ 2019-nCoV assay	Abbott Laboratories and Seegene	E, N, RdRp genes	Nasopharyngeal and oropharyngeal swabs	Not applicable	Not reported	Slightly complex

Table 2. *Cont.*

Study (Author, Ref)	Product Name	Manufacturer /Country	Analyte Measured	Sample	Sample Volume	Read Time	Complexity
RT-PCR							
Marais et al., 2022 [16]	RT-PCR on saliva and mid-turbinate sample vs. respiratory swab	Abbott Laboratories/USA and Seegene/South Korea	E, N, RdRp genes	Saliva, mid-turbinate swab	Not applicable	8–12 h	Medium complex
	FlowFlex SARS-CoV-2 N protein lateral flow assay	ACON Laboratories Inc./USA	Nucleocapsid protein	Saliva, mid-turbinate swab	Not applicable	15 min	Technically simple
Omar et al., 2021 [17]	Thermocycler (Genechecker; and a 2400 SARS-CoV-2 Smartchecker PCR kit	Genesystem/South Korea	N, RdRp gene	Nasopharyngeal, oropharyngeal, nasal swabs, tracheal aspirates	Not applicable	45 min	Slightly complex
Rapid antigen testing							
Akingba et al., 2021 [18]	PanBio COVID-19 antigen test	Abbott Rapid Diagnostics/USA	Nucleocapsid protein	Nasopharyngeal swab	Not applicable	15 min	Technically simple
Majam et al., 2022 [19]	PanBio COVID-19 antigen test Device	Abbott Rapid Diagnostics/USA	Nucleocapsid protein	Nasopharyngeal swab	Not applicable	15 min	Technically simple
Serological testing							
Makatsa et al., 2021 [20]	Indirect in-house ELISA using recombinant plant-derived viral proteins	Cape BioPharms/South Africa	IgG	Serum	Not reported	Not reported	Slightly complex
Gededzha et al., 2021 [21]	EUROIMMUN Anti-SARS-CoV-2 IgA and IgG	EUROIMMUN Medizinische Labor diagnostika AG/Germany	IgG, IgA	Serum, plasma	Not reported	Not reported	Slightly complex
Jugwanth et al., 2022 [22]	Abbott SARS-CoV-2 IgG Architect	Abbott Diagnostics/USA	IgG	Serum, plasma	Not reported	Not reported	Slightly complex

Table 2. *Cont.*

Study (Author, Ref)	Product Name	Manufacturer /Country	Analyte Measured	Sample	Sample Volume	Read Time	Complexity
Serological testing							
Jugwanth et al., 2022 [22]	Abbott SARS-CoV-2 Alinity	Abbott Diagnostics/USA	IgG	Serum, plasma	Not reported	Not reported	Slightly complex
Matefo et al., 2022 [23]	Laboratory-developed ELISA and IFA	Not applicable	IgG	Serum	Not reported	Not reported	Complex
Grove et al., 2021 [24]	Roche Elecsys™ chemiluminescent immunoassay	Roche Diagnostics/Switzerland	IgM, IgG	Serum, plasma	Not reported	Not reported	Slightly complex
David et al., 2021 [25]	Zheihang OrientGene COVID-19 IgG/IgM	Orient Gene Biotech/China	IgM, IgG	Venous blood	5 µL	10 min	Technically simple
	Genrui Novel Coronavirus (2019-nCoV) IgG/IgM	Genrui Biotech Inc/China	IgM, IgG	Venous blood	10 µL	10 min	Technically simple
	Boson Biotech 2019-nCoV IgG/IgM	Xiamen Boson Biotech/China	IgM, IgG	Venous blood	2 µL	10 min	Technically simple
	Biosynex COVID-19 BSS	Biosynex Swiss SA/Switzerland	IgM, IgG	Venous blood	10 µL	10 min	Technically simple
Shaw et al., 2021 [26]	Abbott SARS-CoV-2-2 IgG assay	Abbott Laboratories/South Africa	IgM, IgG	Whole blood	Not applicable	30 min	Slightly complex
Wolter et al., 2022 [27]	Wantai SARS-CoV-2 Ab ELISA	Wantai Biological Pharmacy Enterprise/China	IgM, IgG, IgA	Serum, plasma	Not reported	Not reported	Slightly complex
Maritz et al., 2021 [28]	Semi-quantitative detection for IgG, IgM, IgA and Nab in serum	Not applicable	IgM, IgA	Serum	Not reported	Not reported	Complex
Kwatra et al., 2022 [29]	DBS serology vs. plasma serology	Not applicable	RdRp gene S-protein	Dried blood spot, plasma	Not reported	Not reported	Slightly complex

Table 2. *Cont.*

Study (Author, Ref)	Product Name	Manufacturer /Country	Analyte Measured	Sample	Sample Volume	Read Time	Complexity
Serological testing							
Irwin et al., 2021 [30]	2019-nCoV-IgG/IgM Rapid Test	Dynamiker Biotechnology Company Ltd./China	IgM, IgG	Fingertip whole blood	10–20 μL	15–20 min	Technically simple
	2019-nCoV IgG/IgM Rapid Test Cassette	AllTest Biotech Company Ltd./China	IgM, IgG	Fingertip whole blood	10–20 μL	15–20 min	Technically simple
	2019-nCoV Ab Test (Colloidal Gold)	Innovita Biotechnology Company Ltd./China	IgM, IgG	Fingertip whole blood	10–20 μL	15–20 min	Technically simple
	Medical Diagnostech COVID-19 IgG/IgM Rapid Test	Altis Biologics (Pty) Ltd./South Africa	IgM, IgG	Fingertip whole blood	10–20 μL	15–20 min	Technically simple
	Cellex qSARS-CoV-2 IgG/IgM Cassette Rapid Test	Cellex/China	IgM, IgG	Fingertip whole blood	10–20 μL	15–20 min	Technically simple

RT-PCR—reverse transcriptase polymerase chain reaction; RT-qPCR—quantitative reverse transcriptase polymerase chain reaction; ELISA—enzyme-linked immunosorbent assay; IFA—immunofluorescent assay. IgG—immunoglobulin G; IgM—immunoglobulin M; IgA—immunoglobulin A. RBD—receptor-binding domain; N—nucleocapsid; E—envelope; S—spike; RdRp—RNA-dependent RNA polymerase proteins.

Table 3. Analytical assessment of antigen- and antibody-based diagnostic tests for SARS-CoV-2.

Study (Author, Ref)	Assay	Reference Comparator Assay	Sample Size	Results Dependent on Time from Symptom Onset/Age	Analyte Target	Sensitivity (95% CI)	Specificity (95% CI)
Genotyping							
Umunnakwe et al., 2022 [14]	Allplex™ SARS-CoV-2 Variants II multiplex real-time PCR genotyping assay, Seegene (Seoul, South Korea)	Illumina MiSeq (Illumina Inc., San Diego, CA, USA), PacBio Sequel IIe (Pacific Biosciences Inc., Menlo Park, CA, USA) or Genexus Ion Torrent (Thermo Scientific, Waltham, MA, USA) platforms.	187	Not reported	K417N (Beta), K417T (Gamma). L452R (Delta), W152C (Epsilon)	Not reported	No cross reactivity
RT-PCR							
Marais et al., 2020 [15]	Rapid sample preparation for RT-PCR	Standard nucleic acid purification protocol for RT-PCR	195	Not reported	E, N and RdRp genes	41.7%–100% dependent on dilution factor PPA: 97.37% (92.55–99.28) NPA: 97.30% (90.67–99.52)	Not reported
Marais et al., 2022 [16]	RT-PCR saliva and mid-turbinate swab	Allplex ™ 2019-nCoV SARS-CoV-2 PCR Abbott RealTime SARS-CoV-2 or Abbott Alinity m SARS-CoV-2 (Abbott Laboratories, Chicago, IL, USA)	453 [304 (Delta), 149 Omi-cron]	Yes	E, N and RdRp genes	Delta PPA on saliva: 73% (53–84) Omicron PPA on saliva: 96% PPA on mid-turbinate: 93%	Not reported
	FlowFlex SARS-CoV-2 N protein lateral flow assay	Allplex ™ 2019-nCoV SARS-CoV-2 PCR Abbott RealTime SARS-CoV-2 or Abbott Alinity m SARS-CoV-2 (Abbott Laboratories, Chicago, IL, USA)	372 including 30 Delta, 29 Omi-cron	Yes	N gene	Delta variant: 93% Omicron variant: 68%	Not reported
Omar et al., 2021 [17]	Thermocycler (Genechecker; and 2400 SARS-CoV-2 Smartchecker PCR kit	Standard RT-PCR	315	Not applicable	N and RdRp genes	95% PPA: 82.4% NPA: 99.2%	97%

Table 3. *Cont.*

Study (Author, Ref)	Assay	Reference Comparator Assay	Sample Size	Results Dependent on Time from Symptom Onset/Age	Analyte Target	Sensitivity (95% CI)	Specificity (95% CI)
Rapid antigen testing							
Akingba et al., 2021 [18]	Abbott PanBio COVID-19 antigen RTD	Allplex ™ 2019-nCoV SARS-CoV-2 PCR	677	Ct-dependent	N gene	69.17% (61.44–75.80)	99.02% (98.78–99.26)
Majam et al., 2022 [19]	Abbott PanBio COVID-19 antigen RTD	QuantStudio 5 Real-Time PCR System, Firmware version 1.3.3) using the TaqPath SARS-CoV-2 (Thermo Fisher Scientific, Waltham, MA, USA)	569	Ct-dependent	N gene	40% (30.3–50.3) PPA: 85.1% (71.7–93.8) NPA: 88.5% (85.5–91.1)	98.5% (96.9–99.4)
Serological testing							
Makatsa et al., 2021 [20]	Indirect in-house ELISA using recombinant plant-derived viral proteins	Euroimmun IgG S1	77	Not reported	Spike protein (S1 and RBD regions)	Reactivity same for both assays PPA: 89.4% (82.18–94.39) NPA: 88.4% (80.53–93.83)	Not reported
Gededzha et al., 2021 [21]	EUROIMMUN Anti-SARS-CoV-2 IgA and IgG	RT-qPCR	355	Not reported	Spike protein	IgG: 64.1% (59.1–69.0) IgA: 74.3% (69.6–78.6)	IgG: 95.2% (90.8–98.4) IgA: 84.2% (77–89.2)
Jugwanth et al., 2022 [22]	Abbott SARS-CoV-2 IgG Architect	RT-qPCR	526	Not reported	Nucleocapsid N protein	69.5% (64.7–74.1)	95% (89.9–98)
	Abbott SARS-CoV-2 Alinity	RT-qPCR	425	Not reported	Nucleocapsid N protein	64.8% (59.4–69.9)	90.3% (82.9–95.2)
	Abbott SARS-CoV-2 IgG Architect	In-house ELISA	197	Not reported	Spike protein	94.7% (88.8–98)	88.1% (79.2–94.1)
	Abbott SARS-CoV-2 Alinity	In-house ELISA	191	Not reported	Spike protein	92.5% (85.8–96.7)	91.7% (83.6–96.6)
Matefo et al., 2022 [23]	Laboratory-developed ELISA and IFA assay	Elecsys™ Anti-SARS-CoV-2 ELISA and COVID-19 IgG/IgM Orient Gene	48	Not reported	Spike protein	ELISA: 100% IFA: 98.8% PPA for ELISA: 92.1% NPA for IFA: 91.0%	ELISA: 96% IFA: 100%
Grove et al., 2021 [24]	Roche Elecsys™ chemiluminescent immunoassay	RT-PCR	434	Sensitivity increased >14 days	Nucleocapsid N protein	65.2% (59.57–70.46)	100% (97.07–100)

Table 3. *Cont.*

Study (Author, Ref)	Assay	Reference Comparator Assay	Sample Size	Results Dependent on Time from Symptom Onset/Age	Analyte Target	Sensitivity (95% CI)	Specificity (95% CI)
Serological testing							
David et al., 2021 [25]	Zheihang OrientGene COVID-19 IgG/IgM	IgG versus PCR and IgG versus formal serology	150	Not reported	Spike protein	IgG versus PCR: 90.7% (81.7–96.2) IgG versus Formal Serology: 100% (94.5–100)	IgG versus PCR: 100% (95.2–100) IgG versus Formal Serology: 96.5% (90.0–99.3)
	Genrui Novel Coronavirus (2019-nCoV) IgG/IgM	IgG versus PCR and IgG versus formal serology	150	Not reported	Not reported	IgG versus PCR: 89.3% (80.1–95.3) IgG vs. Formal Serology: 98.5% (91.7–100)	IgG versus PCR: 97.3% (90.7–99.7) IgG versus Formal Serology: 94.1% (86.8–98.1)
	Boson Biotech 2019-nCoV IgG/IgM	IgG versus PCR and IgG versus formal serology	150	Not reported	Not reported	IgG versus PCR: 85.3% (75.3–92.4) IgG versus formal serology: 98.5% (91.7–100)	IgG versus PCR: 97.3% (90.7–99.7) IgG versus formal serology: 97.6% (91.8–99.7)
	Biosynex COVID-19 BSS	IgG versus PCR and IgG versus formal serology	150	Not reported	Not reported	IgG versus PCR: 84.3% (73.6–91.9) IgG versus formal serology: 98.2% (90.4–100)	IgG versus PCR:100% (91.4–100) IgG versus formal serology: 92.7% (82.4–98.0)
		In-house ELISA	111	Not reported	Spike protein	IgG: 80% (71.5–86.9) IgA: 87.8% (80.4–93.2)	IgG: 86.9% (77.8–93.3) IgA: 73.8% (63.1–82.8)
Shaw et al., 2021 [26]	Abbott SARS-CoV-2 IgG assay	Not reported	137	Not reported	Nucleocapsid N protein	Not reported	98.54% (94.82 -99.82)
Wolter et al., 2022 [27]	Wantai SARS-CoV-2 Ab ELISA	Elecsys™ Anti-SARS-CoV-2 ELISA	7479	Not reported	Spike protein (RBD region)	91.0%	97.2%

Table 3. *Cont.*

Study (Author, Ref)	Assay	Reference Comparator Assay	Sample Size	Results Dependent on Time from Symptom Onset/Age	Analyte Target	Sensitivity (95% CI)	Specificity (95% CI)
Serological testing							
Maritz et al., 2021 [28]	Semi-quantitative detection for IgG, IgM, IgA and Nab in serum	Not applicable	Not reported	Not reported	Spike protein (S1 region)	Range 83.2%–99.7%	Range 90.5%–99.1%
Kwatra et al., 2022 [29]	Dried blood spot sample serology vs. plasma serology	Not applicable	16	Not applicable	Spike protein (RBD region)	Correlation: RBD: 93.5% (81.4–97.8) S-protein: 96.5% (89.5–98.8)	Not reported
Irwin et al., 2021 [30]	2019-nCoV-IgG/IgM Rapid Test	Not reported	102	Age-dependent	Nucleocapsid N protein	IgM 67% IgG 69%	Not reported
	2019-nCoV IgG/IgM Rapid Test Cassette (whole blood, serum, or plasma),	Not reported	102	Age-dependent	Nucleocapsid N protein	IgM 15% IgG 65%	Not reported
	2019-nCoV Ab Test (Colloidal Gold)	Not reported	102	Age-dependent	Nucleocapsid N protein	IgM 13% IgG 36%	Not reported
	Medical Diagnostech COVID-19 IgG/IgM Rapid Test	Not reported	102	Age-dependent	Nucleocapsid N protein	IgM 26% IgG 66%	Not reported
	Cellex qSARS-CoV-2 IgG/IgM Cassette Rapid Test	Not reported	102	Age-dependent	Nucleocapsid N protein	IgM 64% IgG 67%	Not reported

95% CI—95% confidence interval; Ct—cycle threshold; PPA—positive per cent agreement; NPA—negative per cent agreement. RT-PCR—reverse transcriptase polymerase chain reaction; RT-qPCR—quantitative Reverse transcriptase polymerase chain reaction; ELISA—enzyme-linked immunosorbent assay; IFA—immunofluorescent assay. IgG—immunoglobulin G; IgM—immunoglobulin M; IgA—immunoglobulin A. RBD—receptor-binding domain; N—nucleocapsid; E—envelope; S—spike; RdRp—RNA-dependent RNA polymerase proteins.

3.2. SARS-CoV-2 Gene and Antigen-Based Diagnostic Tests

Studies that evaluated diagnostic tests for the detection of SARS-CoV-2 genes and antigens included RT-PCR variant genotyping [14], followed by the Allplex^{TM} SARS-CoV-2 Variants II multiplex real-time PCR genotyping assay by Seegene (Seoul, South Korea). The testing was based on circulating Beta and Delta variants prior to the emergence of the Omicron variant and utilised specific primers and probes for each variant. The results were available in two hours as opposed to the time-consuming next-generation sequencing. This assay delineated the Beta and Delta variants and had the ability to determine the rapid rate at which the Delta displaced the Beta variant in the study setting of Limpopo, and thus the capability of the assay to rapidly monitor circulating variants [14]. The reproducibility of the assay was identical across operators with near identical cycle threshold (Ct) values, whilst the overall average Pearson correlation for linearity between the SARS-CoV-2 median Ct and variant typing Ct values for the samples analysed was 0.976 (standard deviation (SD) $\pm$0.019) with 96.4% concordance for repeatability. However, testing was restricted to known circulating variants.

To improve the turnaround time and to be less reliant on reagents, equipment, and staff, Marais et al. [15] from the Western Cape applied a revised workflow using rapid sample preparation (RSP) with a key modification that included sample centrifugation and heating prior to RT-PCR for either the Abbott RealTime SARS-CoV-2 assay or the Allplex^{TM} 2019-nCoV assay platforms. This modification showed a 97.37% (95% confidence interval (CI):92.55–99.28) positive per cent agreement (PPA) and a 97.30% (95% CI:90.67–99.52) negative per cent agreement (NPA) compared to nucleic acid purification-based testing. In confirmed Delta variant infections, the PPA of RT-PCR on saliva was 73% (95% CI:53.0–84.0).

In Omicron variant infections, saliva performed as well as or better than mid-turbinate samples up to day 5, with an overall PPA of saliva swabs of 96% and mid-turbinate samples of 93%, demonstrating the altered kinetics in viral shedding [16].

As the demand for diagnostic testing overwhelmed the capacity to deliver, Omar et al. assessed the utility of a mobile laboratory staffed with non-laboratory healthcare personnel to undertake PCR testing [17]. Using the 2400 SARS-CoV-2 Smartchecker PCR kit (Genesystem, Daejeon, South Korea) targeting the *N* and *RdRp* genes and processed using the thermocycler (Genechecker; Genesystem, Daejeon, South Korea) showed a median turnaround time of 152 min (interquartile range 123–184) with sensitivity and specificity of 95% and 97% and positive and negative predictive values of 82.4% and 99.2%, respectively, when compared to a clinical diagnosis of COVID-19.

With increasing demands on testing for SARS-CoV-2 infection, two studies [18,19], evaluated the field performance of the Abbott Panbio Antigen Rapid Test Device (Ag-RDT) (Abbott, San Diego, Carlsbad, CA, USA) against the available SARS-CoV-2 RT-PCR, which detects the Beta and Delta variants [18,19]. In the Eastern Cape province, the test had a sensitivity of 69.17% (95% CI:61.4–75.8) and specificity of 99.02% (95% CI:98.8–99.3) among symptomatic individuals [18], whilst among members of the public at three taxi ranks in Johannesburg, the test had a sensitivity of 40.0% (95% CI:30.3–50.3) and specificity of 98.5% (95% CI:96.9–99.4) with a positive predictive value of 85.1% (95% CI:71.7–93.8) and a negative predictive value of 88.5% (95% CI:85.5–91.1) [19]. The sensitivity of the test was dependent on the amount of viral RNA in clinical samples, as reflected by the PCR Ct value [19].

3.3. SARS-CoV-2 Antibody-Based Diagnostic Tests

Serological assays for the detection of IgG, IgM, or Immunoglobulin A (IgA) against SARS-CoV-2 infection provide important information for surveillance, antibody persistence, infection rate, and vaccine coverage. Serological assays, including enzyme-linked immunosorbent assays (ELISA) and rapid lateral flow assays, are available commercially; however, the high cost limits their accessibility in resource-limited countries. Although several assays have been developed, field evaluations have been limited. Testing was

performed on serum samples, plasma, fingerstick, and dried blood spot (DBS) samples. The analytical assessments of antibody-based diagnostic tests for SARS-CoV-2 are shown in Table 3. Of the twelve studies, five (38.5%) compared serological outcomes to RT-PCR, whilst in seven (54%), a comparison was made to either in-house or commercially available serological tests.

Makatsa et al. (2021) [20] developed an in-house indirect ELISA using plant-derived recombinant viral proteins by means of the S1 and receptor-binding domain (RBD) portions of the spike protein from SARS-CoV-2, expressed in *Nicotiana benthamiana* [20]. This test measured antibody responses among SARS-CoV-2 PCR-positive patients. Samples taken at a median of 6 weeks from diagnosis from patients with mild and moderate COVID-19 disease showed that the in-house ELISA, when compared to the S1 IgG ELISA kit (EUROIMMUN), detected immunoglobulins; S1-specific IgG was detected in 66.2% and RBD-specific IgG in 62.3% of samples and were concordant with the EUROIMMUN assay.

To optimise the diagnostic algorithm for SARS-CoV-2 infection, Gededzha et al. (2021) [21] evaluated the diagnostic performance of the EUROIMMUN Anti-SARS-CoV-2 ELISA for the semi-quantitative detection of IgA and IgG antibodies in serum and plasma samples targeting the recombinant spike (S1) domain of the SARS-CoV-2 spike protein as the antigen. The sensitivity of EUROIMMUN was higher for IgA (74.3%, 95% CI:69.6–78.6) than for IgG (64.1%, 95% CI:59.1–69.0), though specificity was lower for IgA (84.2%, 95% CI:77–89.2) than IgG (95.2%, 95% CI:90.8–98.4) and both sensitivity and specificity improved in symptomatic individuals [21].

The performance of the Abbott SARS-CoV2 Architect and Abbott SARS-CoV2 Alinity IgG when compared to RT-qPCR showed the sensitivity of the assays to be 69.5% (95% CI:64.7–74.1) and 64.8% (95% CI:59.4–69.9), respectively, whilst the specificity of the assays was 95% (95% CI:89.9–98) and 90.3% (95% CI:82.9–95.2), respectively. When the assays were compared to the in-house ELISA, the sensitivity for the Architect and Alinity assays was 94.7% (95% CI:88.8–98) and 92.5% (95% CI:85.8–96.7), respectively, whilst specificity was 88.1% (95% CI:79.2–94.1) and 91.7% (95% CI:83.6–96.6), respectively. The sensitivity for both assays was highest at 31–40 days post-presentation and lowest at time points of less than 7 days. These findings highlight the futility of testing for antibody responses during the acute and early stages; that is, within less than 14 days of infection [22].

Matefo et al. (2022) investigated two in-house ELISAs and an in-house immunofluorescent assay (IFA), developed using the SARS-CoV-2 S1 protein, for use in South African populations [23]. The tests were compared with Roche Elecsys™ Anti-SARS-CoV-2 (Roche Diagnostics GmbH, Mannheim, Germany) and a commercial lateral flow assay, COVID-19 IgG/IgM Rapid Test cassette (Zhejiang Orient Gene Biotech Co., Ltd., Zhejiang, China). Based on IgG antibodies, specificity was 96% and 100% for ELISA and IFA, respectively, and sensitivity was shown to be 100% and 98.8% for ELISA and IFA, respectively, for samples collected one week after the onset of illness. Positive predictive values were 92.1% for ELISA and 91.0% for IFA. The in-house ELISA and IFA were positive for IgG antibodies, regardless of circulating variants, therefore demonstrating the potential of these tests for high throughput screening in resource-constrained environments [23].

The performance of the Roche Elecsys™ chemiluminescent immunoassay (Rotkreuz, Switzerland) to detect antibodies to SARS-CoV-2 N as antigen was evaluated by Grove et al. [24]. Among patients from Johannesburg, serum samples from SARS-CoV-2 RT-PCR positive and negative individuals showed a sensitivity of 65.2% (95% CI:59.57–70.46) and specificity of 100% (95% CI:97.07–100). The sensitivity of the test improved to 72% among those with >14 days and to 88.6% in those 31–50 days post diagnosis. Nevertheless, using the in-house ELISA assay utilising the plant-based S1 and RBD as antigens [20], the overall PPA was 89.4% (95% CI:82.18–94.39) and NPA was 88.4% (95% CI: 80.53–93.83). However, among individuals at earlier time points post-infection and among asymptomatic individuals, the sensitivity was lower with the Roche Elecsys™ chemiluminescent immunoassay and the in-house ELISA [24].

David et al. (2021) evaluated 30 lateral flow immunoassays using serum or plasma samples from patients with confirmed SARS-CoV-2 infection [25]. Of these, 26 assays did not meet the predefined operational acceptance criteria for kits to be approved for use in South Africa. Whilst the performance of the lateral flow tests was similar to the sensitivities and specificities reported in other studies, only four (13%) assays (Zheihang Orient Gene COVID-19 IgG/IgM, Genrui Novel Coronavirus (2019-nCoV) IgG/IgM, Biosynex COVID-19 BSS IgG/IgM, Boson Biotech 2019-nCoV IgG/IgM) were recommended for South Africa Health Products Regulatory Authority (SAHPRA) approval [25].

Among volunteers in Cape Town, 23.7% tested positive for IgG antibodies with the Abbott SARS-CoV-2 IgG assay. Of those who tested positive, 47.9% reported no symptoms of COVID-19 in the past 6 months. Seropositivity was significantly associated with living in informal housing, residing in a subdistrict with low income per household, and having a low-earning occupation. The specificity of the assay was 98.54% (95% CI:94.82–99.82) [26].

In the household survey undertaken in three communities across three provinces in South Africa, the burden of SARS-CoV-2 infections was measured using two ELISA kits: Wantai SARS-CoV-2 Ab ELISA (Beijing Wantai Biological Pharmacy Enterprise), measuring total antibodies (IgM, IgG and IgA) against the RBD in the spike protein, and Roche Elecsys^{TM} Anti-SARS-CoV-2 ELISA (Roche Diagnostics), measuring total antibodies to the N protein. There was 94.5% PPA with a Cohen κ statistic of 0.89. The Wantai assay, compared with the Roche Elecsys^{TM} assay, had a sensitivity of 91.0% and a specificity of 97.2% [27]

To monitor antibody responses to SARS-CoV-2 following a vaccine rollout, Maritz et al. (2021) assessed a ligand binding-based serological assay for the semiquantitative detection of IgG, IgM, IgA, and neutralising antibodies (nAb) in serum [28]. The assay demonstrated high levels of diagnostic specificity and sensitivity (85–99% for all analytes). Serum IgG, IgM, IgA, and nAb correlated positively (R2 = 0.937, R2 = 0.839, R2 = 0.939 and R2 = 0.501, $p < 0.001$, respectively) with those measured in DBS samples. In vitro SARS-CoV-2 pseudotype neutralisation correlated positively with the solid phase nAb signals in convalescent donors (R2 = 0.458, $p < 0.05$), highlighting the potential use of the assay in efficacy studies, infection monitoring, and post-marketing surveillance following vaccine rollout [28].

To enable large-scale testing for SARS-CoV-2 antibodies, DBS samples were evaluated against plasma samples with a correlation of r = 0.935 and 0.965 for RBD and full-length S-protein of SARS-CoV-2 [29]. A Bland–Altman assessment showed agreement between IgG mean fluorescence intensity (MFI) values with 6.25% of observations for both RBD IgG and spike IgG, falling outside the 95% limit of agreement. Therefore, DBS samples are a useful medium for population screening and field studies in resource-constrained settings, as they are non-invasive and ideal for storage, transportation, and processing [29].

As the need for testing increases, especially for surveillance or during outbreak situations, rapid antibody testing assays are useful in such situations. Irwin et al. (2021) evaluated the sensitivity of five rapid antibody assays and explored factors influencing their sensitivity in detecting SARS-CoV-2-specific IgG and IgM antibodies [30]. In addition, finger-prick blood samples from participants within 2–6 weeks of PCR-confirmed COVID-19 diagnosis were included in the evaluation. Overall sensitivity for IgG and IgM antibodies was below 70% and ranged from 13% to 67% for IgG and markedly lower for IgM. Whilst rapid tests in resource-constrained settings are a promising tool in COVID-19 diagnosis, the sensitivity was reduced for those under 40 compared with those over 40 years of age. These findings show significant variability when used in real-world settings, limiting their application [30].

4. Discussion

The scoping review yielded 17 studies that assessed the performance of diagnostic tests in South Africa for the detection of SARS-CoV-2 infection. These studies were important, especially during the unprecedented spread of SARS-CoV-2 in diverse populations and in a

high HIV- and TB-burden setting. Therefore, this scoping review provided an opportunity for an analytical assessment of rapidly emerging diagnostic tests, especially for a newly identified virus, complicated by the rapid evolution of novel variants and sub-variants.

Whilst the international community made considerable progress to produce and distribute diagnostic tests, access to high-quality testing platforms was extremely limited in low- and middle-income countries. Additionally, the need for in-country regulatory approvals by SAHPRA prior to the utilisation of diagnostic tests contributed to substantial delays in the availability of such tests, diminishing the urgency of testing and perpetuating the risk of onward community transmission.

Several important insights and themes emerged from this scoping review. All studies identified were deemed to be of high quality and were from diverse studies. These studies demonstrated that the timing of the collection of clinical samples with respect to symptom onset had a major impact on assay sensitivity. The sensitivity of the SARS-CoV-2 gene/s and antigen detection tests improved when samples were collected during the earlier stages of infection when the SARS-CoV-2 viral load was the highest. As expected, sensitivity declined when samples were collected during the advancing stages of infection. However, there was no consensus on the precise timing of sample collection, and therefore, SARS-CoV-2 negative rapid test results in suspected cases require further testing to confirm the results [14–16,29]. Importantly, the quality of the nasopharyngeal, oropharyngeal, and nasal swabs or tracheal aspirates collected is dependent on the skills and expertise of the medical personnel, directly impacting test performance. Furthermore, sampling for POC self-tests by untrained, non-medical staff would be an influencing factor for the sensitivity of the tests resulting in misleading results.

For the SARS-CoV-2 gene/s and antigen testing evaluations, the Allplex^{TM} SARS-CoV-2 Variants II multiplex real-time PCR genotyping assay addressed the genotyping challenges, was simpler to perform, easier to interpret, and was less expensive than conventional genotyping. Improving the sample preparation had the added advantage of improving the turnaround time for test results with the capability of handling samples during peak testing periods. Furthermore, the assay provided evidence of recombination or mixed populations of variants identified through low *S* gene Ct values that failed to be assigned a variant, thus harbouring novel mutations and highlighting the need for confirmation with next-generation sequencing.

The testing kits applied in the field nevertheless had a shortened turnaround time with improved sensitivities and specificities [17]. A limitation of these tests was the design of target molecules based on primers and probes to identify existing known variants, thus making them likely to miss new variants as and when they emerge. It is important that assays are designed to improve variant detection capacity and identify multiple variants, including novel variants, or include targets that are likely to be common to variants and subvariants of SARS-CoV-2.

Our scoping review has some limitations. Whilst studies were of high quality, the sample sizes in several studies were relatively small. Furthermore, the utility of different sample types such as saliva demonstrated lower levels of sensitivity, reducing their use in settings that may require high throughput even though saliva sample collection might be easier. This review highlights the limited number of test evaluations that have been conducted in South Africa, with the majority of studies taking place in Gauteng and the Western Cape. With the diverse population, different environmental conditions, and other infectious diseases prevailing in various parts of South Africa, it is important that diagnostic tests be evaluated prior to implementation in local settings and specifically across all provinces. The variability in the studies included in the scoping review makes it difficult for direct comparisons, therefore the selection of laboratory tests should be based on the laboratory evaluation of the test kits as part of the initial evaluation to include panels of samples collected over time from more than one province. This should be followed by field evaluation of the tests, as real-life use may highlight certain challenges and nuances that may not be observed in controlled laboratory testing by trained laboratory

staff. In addition, the evaluation of test kits should be expanded to two or more provinces, considering the epidemiology of the analytes being tested, especially in settings with low and high prevalence to provide robust performance data prior to roll-out of testing. Another limitation was the lack of peer-reviewed publications appearing as preprints that were not included in this review.

5. Conclusions

Our review indicates that timely diagnosis of SARS-CoV-2 is critical to reduce transmission, morbidity, and mortality. Although diagnostic tests for SARS-CoV-2 varied considerably in sensitivity, the duration of infection, the timing of sample collection, and SARS-CoV-2 Variants of Concern all impacted the sensitivity of diagnostic tests. These findings therefore highlight the importance of improvements to existing diagnostic tests or the application of broad-based epitopes in the next generation of diagnostic tests to enhance sensitivity and specificity.

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Results

CHAPTER 5

EVALUATION OF THE PERFORMANCE OF POC ANTIGEN AND NUCLEIC ACID AMPLIFICATION BASED DIAGNOSTIC TESTS

CHAPTER 5: EVALUATION OF THE PERFORMANCE OF POC ANTIGEN AND NUCLEIC ACID AMPLIFICATION BASED DIAGNOSTIC TESTS

Chapter 5 addresses the objective -To evaluate the performance of newer laboratory POC and antigen/antibody-based tests among COVID-19 individuals. The initial introduction of rapid antigen tests, their use was restricted to laboratory testing only which led to the evaluation of two SARS-CoV-2 rapid tests during the first and second wave of infections in South Africa. The evaluations and findings are described in the manuscript entitled *"Evaluations of two SARS-CoV-2 rapid antigen tests during first and second wave of COVID-19 infections in South Africa"* which is under peer review at Journal of Public Health in Africa. Following approval of these tests for field use, four rapid antigen tests were evaluated in field testing centres in Durban, South Africa from July to December 2021 during the Delta wave of infections. The evaluation and findings are described in the manuscript entitled *"Field evaluations of four SARS-CoV-2 rapid antigen tests during SARS-CoV-2 Delta variant wave in South Africa"* was published in BMC Diagnostic and Prognostic Research.

With the emergence of new variants of concern, there was a concern as to whether the rapid antigen tests would be proficient in detecting emerging variants. To address this concern, two rapid antigen tests were evaluated during the Omicron BA.1 and BA.2 wave of infections and subsequently evaluated a second two tests during the Omicron BA.4 and BA.5 wave of infections. The findings from these evaluations are presented in two manuscripts entitled *"Clinical evaluation of SARS-CoV-2 rapid antigen tests during the Omicron wave in South Africa"* which has been published in the Journal of Infectious Diseases. The second manuscript entitled "*Performance of rapid antigen tests in identifying Omicron BA.4 and BA.5 infections in South Africa"* was published in Journal of Clinical Virology.

During the first year of the COVID-19 pandemic in South Africa, the acquisition of kits and reagents for SARS-CoV-2 RT-PCR presented major challenges resulting in laboratories testing for SARS-CoV-2 on a single platform. Testing was facilitated and evaluated included the Xpert® Xpress SARS-CoV-2, as an alternate platform. The Xpert® Xpress SARS-CoV-2 was an attractive option as most laboratories housed a form of GeneXpert instrument that had primarily been used for *Mycobacterium tuberculosis* testing or in use for the diagnosis of sexually transmitted diseases and HIV viral load measurement as part of the point of care testing platform. The evaluation of the Xpert® Xpress SARS-CoV-2 is described in the manuscript entitled "*Evaluation of the Xpert® Xpress SARS-CoV-2 assay for SARS-CoV-2 diagnosis in South Africa".* This manuscript is in preparation and is included in the thesis.

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5.1. EVALUATIONS OF TWO SARS-COV-2 RAPID ANTIGEN TESTS DURING FIRST AND SECOND WAVE OF COVID-19 INFECTIONS IN SOUTH AFRICA

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Designed the study: AS, NS;
Performed the experiments: NS, SN2, SR, TGM, SN1, SNM, AS;

Analysed the data: AS, LL, TGM, SN1;
Wrote the first draft of the paper: NS, AS;
Collected specimens and clinical data: RHM, KN;
Supervised clinical and/or experimental aspects of the study: AS, RHM, NS, SN2, KN, ABMK, QAK, SAK;
All authors contributed to the editing and finalisation of the manuscript.

Competing interest
The authors declare no conflict of interests.

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Abstract
Efficient testing strategies are key for diagnosis, clinical management, and outbreak control of COVID-19. We evaluated two rapid antigen tests, the STANDARD Q COVID-19 Ag Test (n=604) and the Panbio™ COVID-19 Ag Test (n=692) during the first (Ancestral variant with D614G mutation) and second (Beta variant) waves in South Africa. Two nasopharyngeal swabs were collected and shipped to central laboratory for testing. Abbott RealTi*m*e SARS-CoV-2 assay was used as a reference. We observed low sensitivity for both tests: 53.45% for the SD Biosensor and 52.79% for the Panbio™ test. The specificity was 100% for SD Biosensor Ag test and 99.39% for Panbio™ Ag test. In the samples with cycle threshold values <20, the test sensitivity rose to 88.46% for SD Biosensor Ag and 83.65% for Panbio™ Ag test. Sensitivity was highest within the first week post symptom onset: 68.75% for SD Biosensor Ag test and 63.96% for Panbio™ Ag test.

1. Introduction

Collection and rapid testing of appropriate specimens is a priority for the diagnosis, clinical management, and outbreak control of SARS-CoV-2/COVID-19. Routine diagnosis of COVID-19 cases is based on the detection of viral RNA by nucleic acid amplification tests (NAAT) such as real-time reverse-transcription polymerase chain reaction (RT-PCR) (WHOe, 2020). The turnaround time for standard RT-PCR based tests is around 24-48 hours and the assays require significant manpower and resources. There is a continuing need for sensitive and specific high-throughput tests that can be used in resource-limited settings and in field clinics.

The early months of the epidemic in South Africa demonstrated the importance of local diagnostic capacity and specifically the importance of rapid diagnostics in identifying cases or prior exposure to infection (Baxter et al., 2021). In the context of global competition for PCR diagnostic kits and reagents, available kits were prioritised for severely ill / hospitalized patients and saving lives took precedence over the public health imperatives in understanding sources of infection and breaking chains of transmission. Notwithstanding rationing of diagnostic kits, the overwhelmed laboratory capabilities resulted in further delays in obtaining test results highlighting the importance of expanding diagnostic testing platforms beyond PCR that are more rapid and can be undertaken at health facilities. Use of antigen -detecting rapid diagnostic tests (RDTs) for COVID-19/ SARS-CoV-2 has expanded rapidly and RDTs are currently available in clinic, community and home-based settings (Drain et al., 2020). There continues to be a need to better understand their diagnostic performance in various settings and clinical indications of the results.

Here we report on the off-site evaluation of two commonly used rapid antigen diagnostics tests for SARS-CoV-2/ COVID-19 during the first and second waves of infection in South Africa.

2. Materials and Methods

2.1. Ethical Considerations

The study was approved by the KwaZulu-Natal Biomedical Research Ethics Committee (BREC approval No: BREC/00001195/2020).

2.2. Clinical specimens

Rapid antigen test evaluations were preformed from 25th of August 2020 until 02 March 2021 in Durban, South Africa. Patients were recruited from Centre for the AIDS Programme of Research in

South Africa (CAPRISA) clinics located adjacent to the government facilities including the King Dinizulu Hospital complex and KwaMashu community health centre, both located in Durban, South Africa. Patients presenting at the clinics were offered SARS-CoV-2 testing irrespective of the presence of symptoms. Following informed consent, study participants provided the samples, demographic, and clinical data. All confirmed COVID-19 cases were reported to the South African National Department of Health using the National Medical Conditions (NMC) surveillance system.

2.3. Sample collection and processing.

Two nasopharyngeal swabs were collected from different nostrils by trained medical staff and were shipped (without additives) at room temperature to the central laboratory. Upon arrival at the laboratory swabs were stored at 4^0C and processed within 6 hours of sample collection. The first nasopharyngeal swab was resuspended in 2mL of viral transport media (VTM) and used for the Abbott RealTi*m*e SARS-CoV-2 assay (target sequences in the SARS-CoV-2 *RdRp* and *N* genes of the SARS-CoV-2 genome). The second swab was used for the evaluation of the rapid antigen tests. The following kits were evaluated: NP Standard Q COVID-19 Ag tests (SD Biosensor) referred to as Standard Q Ag test and the NP Panbio™ COVID-19 Ag Rapid Test Device (Abbott) referred to as Panbio™ Ag test. Upon arrival at the laboratory all swabs were processed as per manufacturer's instructions.

2.4. Statistical analysis

Test performance characteristics were calculated in reference to Abbott RealTi*m*e SARS-CoV-2 assay results. The 95% confidence intervals were calculated to assess the level of uncertainty induced by sample size, using the Wilson's score method. A t-test was used to assess differences in Ct values between true positive and false negative results. GraphPad Prism version 8.3.1 (GraphPad software, La Jolla, CA) and SPSS version 27 were used to perform the statistical analysis.

3. Results

3.1. Study sample characteristics

The evaluation of Standard Q Ag test was performed on 604 samples, while the evaluation of the Panbio™ Ag Test was performed on 692 samples. Study participant/sample characteristics are summarised in **Table 1**. The study period corresponds to end of first wave (dominated by ancestral variant with D614G mutation) and the second wave (dominated by Beta variant) in South Africa (Tegally et al., 2021a).

For the Standard Q Ag test overall SARS-CoV-2 positivity was 9.60% (58/604) with median cycle threshold (Ct) value of 21.40 [interquartile range (IQR): 14.26-25.71]. The median age of study participants was 39 (IQR 30-50) with 35.98% self-identifying as HIV positive. At screening 34.24% of study participants did not report any COVID-19 related symptoms (pre-or asymptomatic), with 38.98% being <7 days post-symptom onset (PSO) and 26.78% being more than 7 days PSO.

For the Panbio™ Ag Test evaluation the overall SARS-CoV-2 positivity was 28.5% (197/692) with the median Ct value of 19.76 (IQR: 13.84-25.37). The median age of study participants was 35 (IQR 27-46) with 25.37% self-identifying as HIV positive. At screening 28.32% of study participants did not report any COVID-19 related symptoms, with 43.95% being within the first week PSO, and 27.73% being more than a week PSO.

3.2. Test performance evaluation

The test performance characteristics for both assays are summarised in **Sup. Table 1** and % sensitivity across different categories is presented in **Figure 1**. The overall sensitivity and specificity for Standard Q Ag test were 53.45% (95%CI: 40.80-65.67) and 100.00% (99.30-100.00), respectively. For the Panbio™ Ag test the sensitivity was 52.79% (95%CI: 45.83-59.64) and the specificity was 99.39% (95%Ci: 98.23-99.79). Analysis for lower Ct values showed sensitivity of 66.67% (95%CI: 51.55-78.99) for Ct<25 and sensitivity of 88.46% (95% CI: 71.02-96.00) for Ct<20 for Standard Q Ag test. Similarly, increase in sensitivity in samples with lower Ct values was observed for Panbio™ Ag Test, 70.63% (95%CI: 62.70-77.48) for Ct<25, and 83.65% (95%CI: 75.37-89.54) for Ct<20.

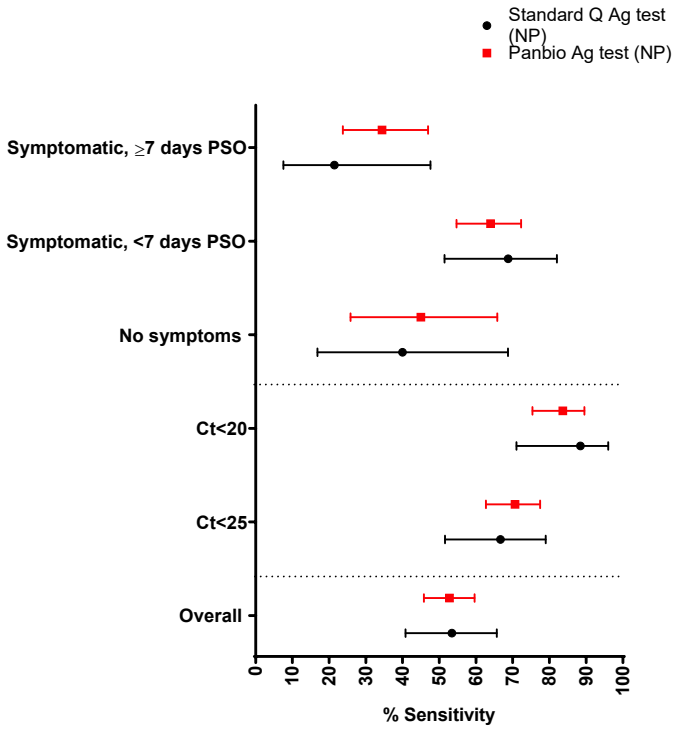
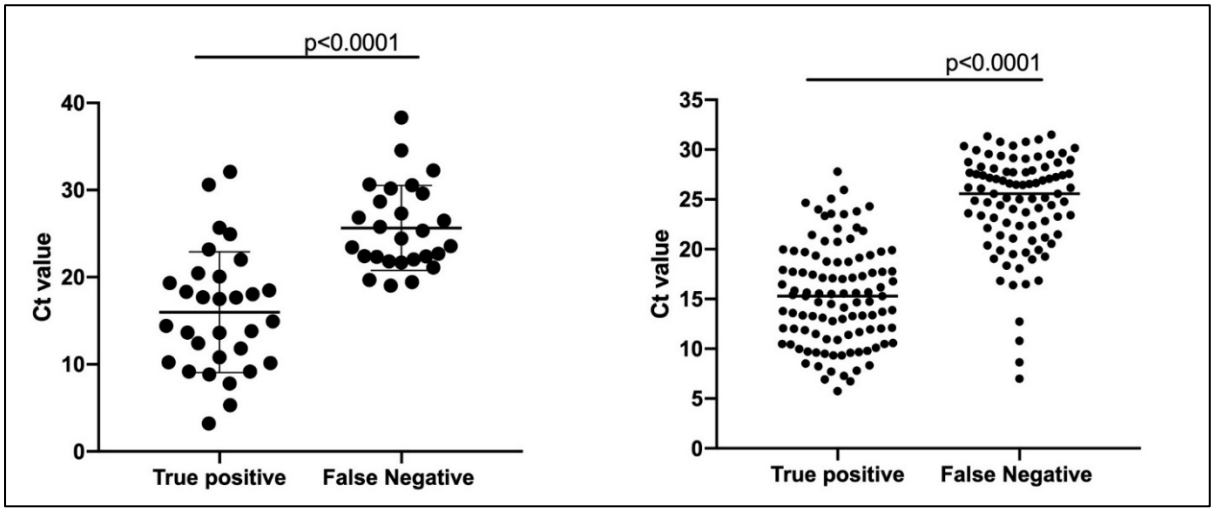


Figure 1. Sensitivity of the Standard Q Ag test and Panbio™ Ag test across different categories: SARS-CoV-2 Ct value and presence and duration of symptoms

With respect to symptom onset, both tests performed best in participants presenting within first week PSO, with Standard Q Ag test sensitivity of 68.75% (95%CI: 51.43-82.05) and Panbio™ Ag test sensitivity of 63.96% (95%CI: 54.70-72.29). Worst test performance was seen in samples from participants presenting more than a week PSO, with Standard Q Ag test sensitivity of 21.43% (95%CI: 7.57-47.59), and Panbio™ Ag test sensitivity of 34.43% (95%CI: 23.75-46.95). Compared to symptomatic participants presenting within first week PSO, the sensitivity was lower in both tests for participants not reporting any COVID-19 related symptoms: 40.00% (95% CI: 16.82-68.73) for Standard Q Ag test and 45% (95%CI: 25.82-65.79) for Panbio™ Ag test.

As expected, the Ct values were significantly higher in false negative compared to true positive samples for both assays (**Supplementary Figure 1**).



Supplementary Figure 1. Differences in SARS-CoV-2 Ct values between true positive and false negative samples for **A**) Standard Q Ag Test **B**) Panbio™ Ag Test

4. Discussion

Efficient testing strategies are key in the COVID-19 responses, enabling rapid identification and isolation of positive cases to reduce transmission and provide appropriate medical care to those affected. Here we evaluated two commonly used rapid antigen tests, the SD Biosensor, STANDARD Q COVID-19 Ag Test and the Panbio™ COVID-19 Ag Rapid Test.

Previous studies have reported varying performance values of rapid antigen tests depending on the setting, overall SARS-CoV-2 prevalence and the clinical presentation of the patients (McDonald et al., 2020, Ryan et al., 2022, Pandey et al., 2022). We observed overall low sensitivity around 50% for both tests with high specificity values (>99%). The overall low sensitivity observed in this study is likely due to delay in sample processing and the associated protein degradation. According to the manufacturers instruction direct swab specimens should be tested immediately after collection to ensure optimal performance. Delay in sample processing was shown to negatively impact performance of rapid antigen tests in previous reports (Mertens et al., 2020, Lambert-Niclot et al., 2020, Corman et al., 2021, Andreani et al., 2021). Consistent with previous studies, low Ct values (<20) resulted in significantly higher sensitivity (>80%) for both tests, satisfying the minimum performance requirement outlined by WHO (WHO, 2021). Ct values and antigen concentrations were shown to be highly correlated in NP samples (Pollock et al., 2021). While we cannot completely relay on Ct value as a marker of infectiousness (Platten et al., 2021), our data supports the notion that rapid antigen tests have high sensitivity in identifying individuals with high SARS-CoV-2 viral load (Pekosz et al., 2021). As

observed previously, both tests performed best in symptomatic individuals presenting within first week post symptom onset when the viral load is highest (Cevik et al., 2021). The sensitivity of each test was low in asymptomatic individuals highlighting the need for repeat testing.

Limitations: Our study has several limitations including deviation from the original rapid antigen kit protocols, with samples being shipped to the central laboratory for processing rather than being done as POC tests, as well as a small number of RT-PCR positive samples in STANDARD Q COVID-19 Ag Test evaluation.

Availability of data and materials

The generated datasets will be made available to any investigator on a reasonable request.

Acknowledgments

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Table 1. Study participant characteristics

	NP Standard Q Ag test evaluation	NP Panbio™ Ag Test evaluation
Sample size (N)	604	692
Age, years (median, IQR)	39 (30-50)	35 (27-46)
Gender (%, n/N Female)	55.79, 337/604	54.19, 375/692
% Positivity (n/N)	9.60, 58/604	28.47, 197/692
Presence of symptoms (%, n/N)		
No symptoms	34.24, 202/590	28.32, 192/678
Symptomatic, <7 days PSO	38.98, 230/590	43.95, 298/678
Symptomatic, => 7 days PSO	26.78, 158/590	27.73, 188/678
HIV positive (%, n/N)	35.98, 213/592	25.37 ,170/670
Ct value (median, IQR)	21.40, 14.26-25.71	19.76, 13.84-25.37
Oxygen saturation (median, IQR)	98.00, 97.00-99.00	98.0, 97.00-99.00

Missing data- Standard Q Ag: Age =4; presence of symptoms=14, HIV status: 12; Panbio™ Ag: Age = 2, presence of symptoms =14, HIV status= 22, oxygen saturation= 6).

Sup. Table 1. Estimations of test performance with respect to RT-PCR Ct (A) value and days post symptom onset (B)

	NP Standard Q Ag test			NP Panbio™ Ag Test		
A. Ct category	**Overall (Ct<39)**	**Ct<25**	**Ct<20**	**Overall (Ct<32)**	**Ct<25**	**Ct<20**
True positive	31	28	23	104	101	87
False positive	0			3		
True negative	546			492		
False negative	27	14	3	93	42	17
Sensitivity (%, 95% CI)	53.45, 40.80-65.67	66.67, 51.55-78.99	88.46, 71.02-96.00	52.79, 45.83-59.64	70.63, 62.70-77.48	83.65, 75.37-89.54
Specificity (%, 95% CI)	100.00, 99.30-100.00			99.39, 98.23-99.79		
PPV (%, 95% CI)	100.00, 88.97-100.00			97.20, 92.08-99.04		
NPV (%, 95% CI)	95.29, 93.23-96.74			84.10, 80.92-86.84		
B. Symptom category	**Asymptomatic**	**Symptomatic <7 days**	**Symptomatic >7 days**	**Asymptomatic**	**Symptomatic <7 days**	**Symptomatic >7 days**
True positive	4	22	3	9	71	21

False positive	0	0	0	1	2	0
True negative	192	198	144	171	185	127
False negative	6	10	11	11	40	40
Sensitivity (%, 95% CI)	40.00, 16.82-68.73	68.75, 51.43-82.05	21.43, 7.57-47.59	45.00, 25.82-65.79	63.96, 54.70-72.29	34.43, 23.75-46.95
Specificity (%, 95% CI)	100.00, 98.04-100.00	100.00, 98.10-100.00	100.00, 97.40-100.00	99.42, 96.78-99.90	98.93, 96.18-99.71	100.00, 97.06-100.00
PPV (%, 95% CI)	100.00, 51.01-100.00	100.00, 85.13-100.00	100.00, 48.85-100.00	90.00, 59.58-98.21	97.26, 90.55-99.25	100.00, 84.54-100.00
NPV (%, 95% CI)	96.97, 93.55-98.60	95.19, 91.38-97.37	92.90, 87.74-95.99	93.96, 89.50-96.59	82.22, 76.70-86.66	76.05, 69.04-81.89

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Diagnostic and Prognostic Research

RESEARCH

Open Access

Field evaluations of four SARS-CoV-2 rapid antigen tests during SARS-CoV-2 Delta variant wave in South Africa

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Abstract

Background Rapid antigen tests detecting SARS-CoV-2 were shown to be a useful tool in managing the COVID-19 pandemic. Here, we report on the results of a prospective diagnostic accuracy study of four SARS-CoV-2 rapid antigen tests in a South African setting.

Methods Rapid antigen test evaluations were performed through drive-through testing centres in Durban, South Africa, from July to December 2021. Two evaluation studies were performed: nasal Panbio COVID-19 Ag Rapid Test Device (Abbott) was evaluated in parallel with the nasopharyngeal Espline SARS-CoV-2 Ag test (Fujirebio), followed by the evaluation of nasal RightSign COVID-19 Antigen Rapid test Cassette (Hangzhou Biotest Biotech) in parallel with the nasopharyngeal STANDARD Q COVID-19 Ag test (SD Biosensor). The Abbott RealTime SARS-CoV-2 assay was used as a reference test.

Results Evaluation of Panbio and Espline Ag tests was performed on 494 samples (31% positivity), while the evaluation of Standard Q and RightTest Ag tests was performed on 539 samples (13.17% positivity). The overall sensitivity for all four tests ranged between 60 and 72% with excellent specificity values (> 98%). Sensitivity increased to > 80% in all tests in samples with cycle number value < 20. All four tests performed best in samples from patients presenting within the first week of symptom onset.

Conclusions All four evaluated tests detected a majority of the cases within the first week of symptom onset with high viral load.

Keywords COVID-19, SARS-CoV-2, Antigen rapid diagnostic test, Performance evaluation

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Introduction

Diagnostic testing has proven to be imperative for the management of SARS-CoV-2/COVID-19 in the context of reducing transmission and outbreak control [1]. While the gold standard RT-PCR test is highly sensitive and specific, there are several disadvantages including cost, complexity and length of the process, and need for specialised equipment and trained personal. The use and availability of rapid antigen tests (RDTs) for the diagnosis of SARS-CoV-2 infection have significantly increased over the last year of the pandemic. RDTs are affordable, fast (10–30 min), simple, and do not require specialised laboratory facilities or highly trained personal. Although their sensitivity is lower compared to a laboratory-based RT-PCR, antigen-based RDTs can detect infection early following symptom onset when the viral load is high, thereby offering quick screening and detection of SARS-CoV-2/COVID-19 among high-risk groups [2].

As of May 2022, South African Health Products Regulatory Authority (SAHPRA) has approved 53 RDT test for use in South Africa [3]. While the World Health Organization (WHO) recommends a minimum of 80% sensitivity and 97% specificity for rapid antigen diagnostics tests to be approved, there is significant variation in RDT performance depending on the study settings [4–10] As an example, the reported performance of commonly used Standard Q COVID-19 Ag test varies between 28.7 [11] and 89.2% [12] depending on the prevalence and patient group. Furthermore, there continues to be a limited number of reports on RDT field performance in low- and middle-income country (LMIC) settings. Here, we evaluate the performance of four rapid antigen tests in comparison with the Abbott RT-PCR assay during the B.1.617.2 Delta variant wave in KwaZulu-Natal, South Africa.

Methods

Study participant recruitment

Rapid antigen test evaluations were performed between July and December 2021 through drive-through testing centres in Durban, South Africa. Adult participants (age = > 18) meeting any of the following criteria were enrolled in the study: tested COVID-19 positive in the previous 7 days, the presence of COVID-19 symptoms in the previous seven days, exposed to COVID-19 5–10 days ago, healthcare worker, or doctor referral for testing. Drive-through testing centres were freely accessible with no referral necessary for testing. Data on screened out individuals that did not fit the study enrolment criteria is not available. Two separate evaluation studies were performed: first, the Espline SARS-CoV-2 Ag test [Fujirebio, nasopharyngeal (NP)] [13] was evaluated in parallel with the Panbio COVID-19 Ag Rapid Test Device (Abbott, nasal) [14]; this was followed by the evaluation of Right-Sign COVID-19 Antigen Rapid test Cassette (Hangzhou Biotest Biotech, nasal) [15] in parallel with the STANDARD Q COVID-19 Ag test (SD Biosensor, NP) [16] on a different group of participants. The Abbott RealTime SARS-CoV-2 assay (target sequences in the SARS-CoV-2 RdRp and N genes of the SARS-CoV-2 genome) [17] was used as a reference test. The Abbott RealTime SARS-CoV-2-positive results are reported with cycle number (CN) values that are equivalent to cycle threshold values more commonly used by other assays [18]. Following informed consent, participants filled in a questionnaire on basic demographic and clinical data and provided samples for the study. Study participants were provided with the results of the South African Health Products Regulatory Authority (SAHPRA) and approved rapid antigen test on site, and Abbott RT-PCR results were reported within 24 h of sample collection. At the time of the study, the nasal Panbio Ag test and NP STANDARD Q Ag test were SAHPRA approved. The study protocol was written before recruitment began. The protocol was not published. Ethics for the parent study was obtained on 24 March 2020. The evaluated tests were selected and provided by the Foundation for Innovative New Diagnostics (FIND) based on the availability and evaluation needs at the time of the study. Minimum of 50 positive cases for each test was required for evaluation. Test evaluations were continued until study ran out of available kits.

Sample collection and processing

Study participants provided three swabs: one nasal and two nasopharyngeal. Nasal swab was collected first in order to avoid cross-contamination between sites, followed by a NP swab for second rapid antigen test, and followed by the NP swab for SARS-CoV-2 RT-PCR. Swabs were collected, and rapid antigen tests were performed and interpreted by trained medical staff on site. The results of the tests were interpreted and recorded by two staff members independently. Swabs for RT-PCR were sent to the central laboratory at room temperature without additives. Swab was resuspended in 2 ml of viral transport media (VTM) and processed within 3 h of sample collection. All assays were performed as per manufacturer's protocol.

Statistical analysis

Statistical analysis was performed using SPSS version 27 and GraphPad Prism version 8.3.1 (GraphPad software, La Jolla, CA, USA). Study analysis was prespecified by FIND. Test performance characteristics [sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)] were calculated in reference to Abbott RealTime SARS-CoV-2 assay

results. Wilson's score method was used to calculate the 95% confidence intervals to assess the level of uncertainty induced by sample size. Test performance was assessed across different categories including the presence and duration of symptoms (no symptoms, symptoms = > 7 days, and symptoms < 7 days) and CN values (< 20 and < 25) as indicator of viral load and infectiousness [19]. In case of missing data, a complete-case analysis approach was used. The measure of agreement between the assays was evaluated using Cohen's kappa coefficient [20]. A *t*-test was used to assess differences in CN values between true-positive and false-negative results.

Results

Study sample characteristics

The evaluation of nasal Panbio and NP Espline Ag tests was done on 494 samples (Table 1) between 21st of July and 19th of August 2021. The median age of study participants was 34 [interquartile range (IQR) 24–47] with 57.29% of participants being female. The SARS-CoV-2 positivity was 31.00% with median CN value of 9.06 (*IQR* 5.90–16.90), with all positive samples having a *CN* < 31. Majority of the study participants (57.36%) presented for testing during the first week post-symptom onset.

The evaluation of NP Standard Q and nasal RightSign Ag tests was done on 539 samples (Table 1) collected between 15th of September and 8th of December 2021. The median age of study participants was 36 (*IQR* 24–50) with 51.76% being female. The SARS-CoV-2 positivity was 13.17% with a median CN value of 15.08 (*IQR* 11.53–23.86), with all positive samples having a CN value < 31. Majority of the study participants presented for testing during first week post-symptom onset (64.01%).

Test performance evaluation

The overall performance of nasal Panbio and NP Espline Ag tests is summarised in Sup. Table 1 and Fig. 1. The overall sensitivity and specificity of nasal Panbio Ag test were 67.97% (95% *CI* 60.22–74.85) and 98.53% (95% *CI* 96.61–99.37), respectively. The overall performance of NP Espline Ag tests was slightly higher with overall sensitivity and specificity of 72.00% (95% *CI* 64.33–78.67) and 99.71% (95% *CI* 98.35–99.95), respectively. The sensitivity of both tests increased in samples with lower CN values (increased viral load): for samples with *CN* < 20 sensitivity of nasal Panbio Ag, test was 80.16% (95% *CI* 72.35–86.18), and sensitivity of NP Espline Ag test was 82.11% (95% *CI* 74.40–87.88). Both tests performed best in samples from individuals presenting during the first week of symptom onset, with sensitivities of 74.16% (95% *CI* 64.20–82.12) for Panbio and 76.14% (95% *CI* 66.26–83.83) for Espline Ag tests, and worst in individuals presenting more than 7-day post-symptom onset, with sensitivities of 37.50% (95% *CI* 21.16–57.29) for Panbio and 47.37% (95% *CI* 27.33–68.29) for Espline Ag test.

The performance of NP Standard Q and nasal RightSign Ag test is summarised in Sup. Table 2 and Fig. 1. The overall sensitivity and specificity of NP Standard *Q*-test were 60.56% (95% *CI* 48.94–71.11) and 99.79% (95% *CI* 98.80–99.96), respectively. The overall sensitivity of nasal RightSign Ag test was 63.38% (95% *CI* 51.76–73.63) with specificity of 100.00% (95% *CI* 99.19–100.00). The sensitivity of both tests increased in samples with lower CN values, being 87.23% (95% *CI* 74.83–94.02) for both Standard Q and RightSign Ag test in samples with *CN* < 20.

Table 1 Study participant/sample characteristics

	Evaluation 1	Evaluation 2
Sample size (*N*)	494	539
Age, years (median, IQR)	34, 24–47	36, 24–50
Gender (%, n/N female)	57.29, 283/494	51.76, 279/539
% positivity (n/N)	31.00, 153/494	13.17 (71/539)
Presence of symptoms		
Asymptomatic/presymptomatic (%, n/N)	33.33, 129/387	29.50, 159/539
< 7-day PSO (%, n/N)	57.36, 222/387	64.01, 345/539
= > 7-day PSO (%, n/N)	9.30, 36/387	6.49, 35/539
HIV positive (%, n/N)#	0.42, 2/481	0.20, 1/539
CN value (median, IQR)	9.06, 5.90–16.90	15.08, 11.53–23.86
Oxygen saturation (median, IQR)	96, 95–98	96, 95–97

Missing data: evaluation 1 (HIV status, 13; oxygen saturation, 72; days of symptom onset, 107); evaluation 2 (oxygen saturation, 26). *PSO* post-symptom onset. #Self-reported

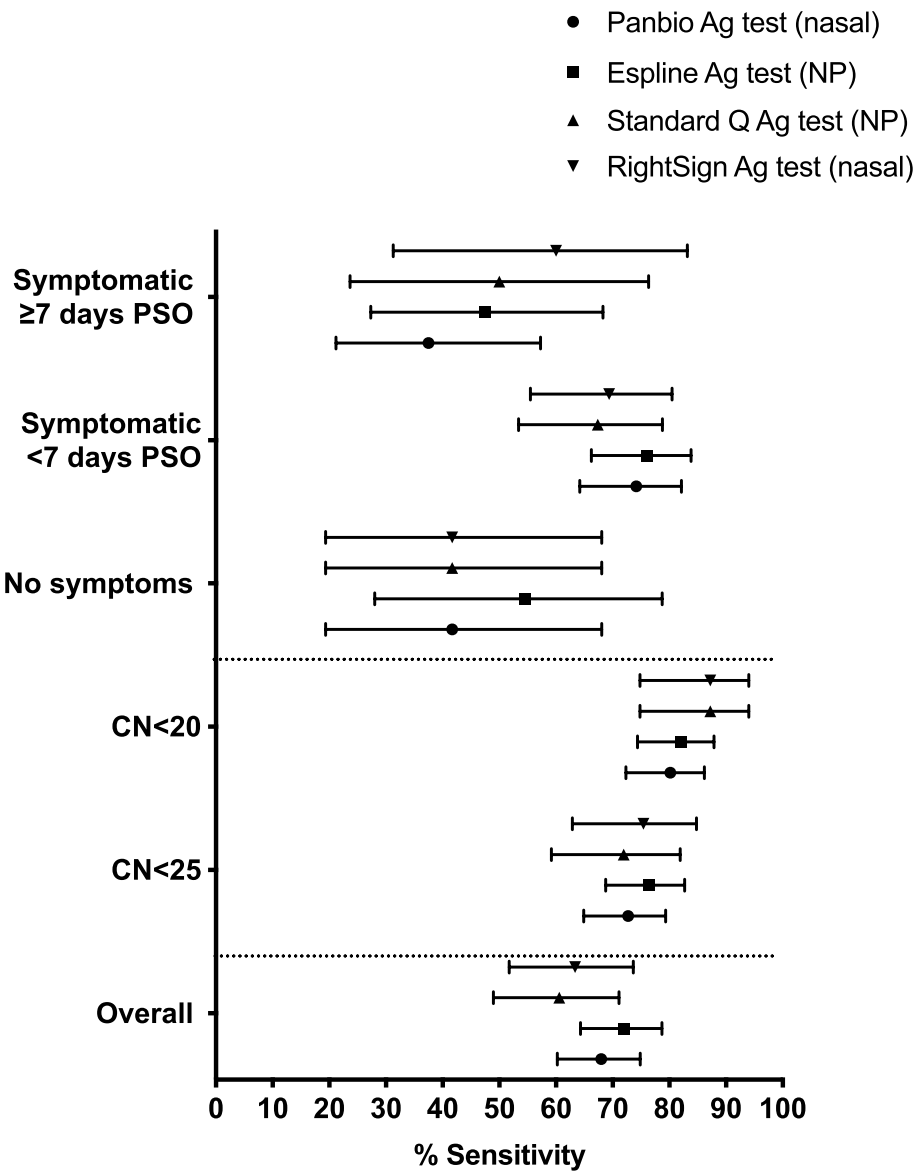

Fig. 1 Sensitivity for the Panbio COVID-19 Ag Rapid Test Device (nasal), Espline SARS-CoV-2 Ag test (NP), STANDARD Q COVID-19 Ag test (NP), and RightSign COVID-19 Antigen Rapid test Cassette (nasal) across different sample categories: SARS-CoV-2 CN value and days post-symptom onset. Error bars represent 95% confidence intervals. PSO, post-symptom onset

The kappa coefficient ranged between 0.72 and 0.78 indicating substantial agreement between the tested RDTs and the Abbott RT-PCR assay. As expected, majority of false-negative (FN) results for all 4 tests occurred in samples with higher CN values (Sup. Figure 1). There were 4 invalid test results with Espline Ag test (0.8%), with no invalid results for the remaining three tests.

Discussion

In this study, we determined the sensitivity and specificity of four RDTs for the detection of SARS-CoV-2 virus in respiratory specimens during the Delta wave of infections in Durban, South Africa.

The sensitivity of the four evaluated tests ranged from 60.55 to 87.23% with high specificity (ranging from 83.33

to 100%). The observed sensitivity of the evaluated tests is comparable with previously published studies from different settings [2, 21]. As reported previously, we observed similar results between the nasal and nasopharyngeal assays performed on equivalent samples [22, 23]. The sensitivity of each of the RDTs increased in samples with lower CN values, increasing above 80% in samples with $CN < 20$. As previously reported, all four tests performed best in samples from individuals presenting within the first week of symptom onset when the SARS-CoV-2 viral load is highest [24–27]. While the rapid antigen tests have often been criticised for low sensitivity and high rate of false-negative results, the majority of the false-negative results are observed in samples with higher C threshold/number values (low viral load) that likely have a limited potential for fulling further viral transmission [2, 24, 28, 29]. In fact, antigen-based RDTs were shown to correlate better with replication-competent SARS-CoV-2 compared to RT-PCR [30] further supporting the use of rapid antigen tests in identifying individuals who are at high potential to transmit SARS-CoV-2. Additionally, low cost and scalability represent an important advantage over standard RT-PCR tests, especially in low- and middle-income countries.

There are several limitations to our study. We did not have access to participant vaccination status and the presence and timing of previous natural infections, and we therefore could not assess the impact of previous immunity on SARS-CoV-2 viral load and rapid antigen test performance. Even though viral sequencing was not available in our study, the period overlaps with the Delta wave of infections in KwaZulu-Natal [31]. Additionally, we have no data on patients that were screened out due to not meeting the study enrollment criteria. While none of the evaluated tests satisfied the WHO requirements for the > 80% sensitivity in the overall sample group, they are still a valuable tool in identifying infected individuals within the first week of symptom onset and those with high viral loads and could play an important role in limiting transmission and controlling the COVID-19 pandemic. Rapid antigen tests remain a useful tool for rapid screening for COVID-19 in congregate settings as well as for "test to work" strategies in order to reduce/slow down spread of the virus. This study provides valuable information of the performance of rapid antigen tests in drive-through centres in South Africa.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41512-023-00151-3.

Additional file 1: Supplementary figures: Fig. 1. Differences in SARS-CoV-2 CN values between true positive and false negative samples for A) Panbio COVID-19 Ag Rapid Test Device (nasal) B) Espline SARS-CoV-2 Ag test (NP) C) STANDARD Q COVID-19 Ag test (NP) D) RightSign COVID-19 Antigen Rapid test Cassette (nasal). **Supplementary tables: Table 1.** Estimations of test performance for Panbio COVID-19 Ag Rapid Test Device (nasal) and Espline SARS-CoV-2 Ag test (NP) with respect to RT-PCR CN values (A) and presence/duration of symptoms (B). *Represents overall sensitivity and specificity (CN values for all positive samples were <31). **Table 2.** Estimations of test performance for STANDARD Q COVID-19 Ag test (NP) and RightSign COVID-19 Antigen Rapid test Cassette (Nasal Swab) with respect to RT-PCR CN values (A) and presence/duration of symptoms (B). *Represents overall sensitivity and specificity (CN values for all positive samples were <31).

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Authors' contributions

Designed the study, AS and NS; performed the experiments, AS, NS, SN2, TGM, SR, and SNM; analysed the data, AS, GL, NS, TGM, and LL; wrote the first draft of the paper, AS, NS, SN1, and GL; supervised clinical and/or experimental aspects of the study, AS, CC, KN, ABMK, NS, SAK, and QAK; and all authors contributed to the editing and finalisation of the manuscript.

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Availability of data and materials

De-identified patient-level data can be accessed by contacting the corresponding author with a detailed description of the research question.

Declarations

Ethics approval and consent to participate

All study participants provided written informed consent. The KwaZulu-Natal Biomedical Research Ethics Committee approved the study (BREC approval No: BREC/00001195/2020 and BREC/00003106/2021). Research presented here was performed in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

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BRIEF REPORT

Clinical Evaluation of Severe Acute Respiratory Syndrome Coronavirus 2 Rapid Antigen Tests During the Omicron Wave in South Africa

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We evaluated the performance of nasal and nasopharyngeal Standard Q COVID-19 [coronavirus disease 2019] Ag tests (SD Biosensor) and the Panbio COVID-19 Ag Rapid Test Device (nasal; Abbott) against the Abbott RealTi*m*e severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) assay during the Omicron (clades 21M, 21K, and 21L) wave in South Africa. Overall, all evaluated tests performed well, with high sensitivity (range, 77.78%–81.42%) and excellent specificity values (>99%). The sensitivity of rapid antigen tests increased above 90% in samples with cycle threshold <20, and all 3 tests performed best within the first week after symptom onset.

Keywords. 21K/BA1; 21L/BA2; COVID-19; SARS-CoV-2; antigen; Omicron; sensitivity; specificity.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant B1.1.529 was first reported on 24 November 2021 by the Network for Genomic Surveillance in South Africa and later designated by the World Health Organization as the Omicron variant of concern [1]. Owing to its enhanced transmissibility, Omicron has spread quickly around the world and currently represents the dominant variant globally. The Omicron SARS-CoV-2 variant has >30 mutations in the spike glycoprotein, with 15 located in the receptor-binding domain which is key for viral entry into the cells. The evolution and fast expansion of the Omicron SARS-CoV-2 variant was first noted in South Africa through the increase of S-gene target failures, using the Thermo Fischer TaqPath COVID-19 [coronavirus disease 2019] assay, resulting from the deletion of codons 69 and 70 in the spike (S) gene. The performance of reverse-transcription polymerase chain reaction (RT-PCR) tests that are not targeting the S-gene, was not affected by the Omicron variant.

Although their sensitivity is lower compared with SARS-CoV-2 RT-PCR, rapid antigen tests offer quick and affordable results at the point of care, enabling reliable detection of high viral load samples associated with the presence of infectious virus [2]. These tests have become a crucial tool to detect cases in a timely manner, even in resource-limited settings, and they therefore represent an important tool in controlling the pandemic.

Most widely used rapid antigen tests target the nucleocapsid protein and therefore should not be affected by the high degree of mutations in the S-gene. However, in addition to >30 mutations in the S-gene, Omicron has several mutations in the nucleocapsid, including P13L, Del31–33, R203K, and G204R, with R203K and G204R associated with enhanced infectivity in human lung cells [3]. Furthermore, Omicron sublineages have additional nucleocapsid mutations including S413R found in BA.2 and BA.3. In the current study, we evaluated the performance of 3 commonly used rapid antigen tests in comparison with the Abbott RT-PCR assay during the Omicron wave in KwaZulu-Natal, South Africa.

METHODS

Clinical Specimens

The evaluation was performed in the province of KwaZulu-Natal in South Africa at drive-through testing centers from December 2021 until February 2022 (spanning the fourth wave of SARS-CoV-2 infections). Residents of the selected communities were offered SARS-CoV-2 testing if they met any of the following criteria: testing positive for COVID-19 in the previous 7 days; presence of COVID-19 symptoms in the previous 7 days; exposure to COVID-19 5–10 days earlier; healthcare worker status; or physician referral for testing. Study participants provided demographics, symptom type and onset date, vaccination status, and informed consent.

Two separate evaluation studies were performed; in the first, both nasal and nasopharyngeal (NP) Standard Q COVID-19

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Ag tests from SD Biosensor were evaluated, and in the second the Panbio COVID-19 Ag Rapid Test Device (nasal) was evaluated. Three swab specimens (1 nasal and 2 NP) were collected for evaluation of the SD Biosensor kits, and 2 (1 nasal and 1 NP) for the Panbio test evaluation. In both evaluations, the nasal swab specimen was collected first to avoid cross-contamination between sites. This was followed by the NP swab specimen for the second rapid antigen test (in the first evaluation), and then the NP swab specimen for the SARS-CoV-2 RT-PCR reference test.

Rapid antigen tests were performed immediately after sample collection on site by trained medical staff. Swab specimens for RT-PCR were shipped without additives at room temperature to the central laboratory for processing within 3 hours of collection. Results from the South African Health Products Regulatory Authority– approved rapid antigen test were reported immediately to the participants, and a confirmatory RT-PCR result was provided within 24 hours after sample collection. The study was approved by the KwaZulu-Natal Biomedical Research Ethics Committee (approval no. BREC/00001195/2020).

SARS-CoV-2 RT-PCR

On arrival at the laboratory, NP swab specimens were resuspended in 2 mL of viral transport medium. The Abbott RealTi*me* SARS-CoV-2 assay (target sequences in the SARS-CoV-2 RdRp and N genes of the SARS-CoV-2 genome) was used to test for the presence of SARS-CoV-2. All samples that tested positive (irrespective of cycle threshold [Ct] values) were sequenced at KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP) [1]. Briefly, RNA was extracted on an automated Chemagic 360 instrument (Perkin Elmer). Libraries for whole-genome sequencing were prepared using the Oxford Nanopore Midnight protocol with rapid barcoding per the manufacturer's protocol and sequenced on the GridION. Sequences with >80% coverage were deposited on the GISAID sequence database. The GISAID accession numbers of sequences are provided in Supplementary Table 1.

SARS-CoV-2 Rapid Antigen Tests

The following 3 kits were evaluated: Standard Q COVID-19 Ag test (SD Biosensor; nasal), Standard Q COVID-19 Ag test (SD Biosensor; NP), and Panbio COVID-19 Ag Rapid Test Device (nasal). According to the manufacturers, the tests detect SARS-CoV-2 nucleocapsid protein with no cross-reaction with other common respiratory pathogens except SARS-coronavirus [4, 5]. All samples were collected, and assays were performed by trained medical staff and per manufacturer protocols. All 3 tests are World Health Organization emergency use listing procedure approved and are the most procured rapid antigen tests in low- and low middle-income countries.

Statistical Analysis

GraphPad Prism software (version 8.3.1; GraphPad Software) and SPSS software (version 24) were used to perform the statistical analysis. Test performance characteristics were calculated in reference to Abbott RealTi*me* SARS-CoV-2 assay results. The 95% confidence intervals (CIs) were calculated to assess the level of uncertainty induced by sample size, using the Wilson score method. The D'Agostino-Pearson omnibus normality test was used to assess data distribution; *t* test, to assess differences in Ct values between true- and false-positive results and vaccination status groups; and Kruskal-Wallis with Dunn multiple comparisons test, done to assess differences in Ct values between symptom categories and Omicron clades. Fully vaccinated participants were classified as any participants who received either 1 dose of Johnson & Johnson's Janssen COVID-19 vaccine or 2 doses of Comirnaty/Pfizer-BioNTech COVID-19 vaccine ≥2 weeks before testing.

RESULTS

Study Sample Characteristics

The evaluation of Standard Q Ag tests was performed on 297 samples (Table 1). The median age of participants was 33 years (interquartile range [IQR], 25–49 years). The overall SARS-CoV-2 positivity in the study group was 41.75%, with a median Ct value (IQR) of 13.90 (0.40–18.09). Most study participants presented for testing within the first week after symptom onset (67.00%). Fully vaccinated participants made up 59.26% of the study cohort with 32.95% (58 of 176) having received 1 dose of Johnson & Johnson's Janssen COVID-19 vaccine and 67.05% (118 of 176) having received 2 doses of Comirnaty/Pfizer-BioNTech COVID-19 vaccine. The majority (98.39%) of the SARS-CoV-2–positive samples were classified as Omicron sublineage BA.2 (Nextstrain clade 21K). Two SARS-CoV-2–positive samples lacked sequencing data and were excluded from the SD Biosensor evaluation.

The evaluation of the Panbio Ag test device was performed on 462 samples (Table 1). The median age (IQR) of study participants was 41 (26–55) years). The overall SARS-CoV-2 test positivity was 39.83%, with a median Ct value (IQR) of 14.06 (9.79–21.07). Most study participants presented within the first week after symptom onset (62.99%), with 64.29% being fully vaccinated. Of the fully vaccinated study participants, 19.87% (59 of 297) received 1 dose of Johnson & Johnson's Janssen COVID-19 vaccine, and 80.13% (238 of 297) received 2 doses of Comirnaty/Pfizer-BioNTech COVID-19 vaccine. The majority (73.37%) of the SARS-CoV-2–positive cases were identified as Omicron sublineage BA.2 (Nextstrain clade 21K). For the Panbio Ag Test, 23 positive samples with SARS-CoV-2 clades other than Omicron were excluded from the evaluation. The 2 evaluations were comparable with respect to study participant and sample characteristics, with the exception of study

Table 1. Participant and Sample Characteristics for Evaluations of Rapid Antigen Tests

Characteristic	Participants or Samples, % (No.)[a]	
	Evaluation 1: SD Biosensor Tests (n = 297)	Evaluation 2: Panbio Test (n = 462)
Age, median (IQR), y	33 (25–49)	41 (26–55)
Female sex	56.23 (167)	50.87 (235)
PCR positivity	41.75 (124)	39.83 (184)
Presence of symptoms		
Asymptomatic or presymptomatic	27.61 (82)	31.17 (144)
<7 d after symptom onset	67.00 (199)	62.99 (291)
≥7 d after symptom onset	5.39 (16)	5.84 (27)
Vaccination status		
Fully vaccinated	59.26 (176)	64.29 (297)
Unvaccinated	32.99 (98)	26.84 (124)
Partially vaccinated	7.74 (23)	8.87 (41)
HIV positive	0.34 (1)	0.43 (2)
Oxygen saturation, median (IQR)	97 (97–99)[b]	98 (96–99)[b]
Ct, median (IQR)	13.90 (10.40–18.09)	14.06 (9.79–21.07)
Omicron lineage among SARS-CoV-2 positive		
21M	1.61 (2/124)	5.98 (11/184)
21K	98.39 (122/124)	73.37 (135/184)
21L	1.61 (2/124)	20.65 (38/184)

Abbreviations: Ct, cycle threshold; HIV, human immunodeficiency virus; IQR, interquartile range; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Data represent % (no.) of participants or samples unless otherwise specified.

[b]Oxygen saturation data were missing for 24 patients in evaluation 1 and 6 in evaluation 2.

participant age, which was significantly higher in the Panbio Ag test evaluation ($P = .001$).

Test Performance Evaluation

The overall test performance for nasal and NP Standard Q Ag tests is summarized in Figure 1 and Supplementary Table 2*A*. The overall sensitivity and specificity of the nasal Standard Q COVID-19 Ag test were 79.84% (95% CI, 71.93%–85.95%) and 100.00% (97.83%–100.00%), respectively. The sensitivity of the test increased in samples with lower Ct values: for samples with a Ct <25 the sensitivity was 85.71% (95% CI, 78.05%–91.01%), and for those with a Ct <20 it was 92.93% (86.12%–96.53%).

Similar results were obtained for the NP Standard Q Ag test (Figure 1 and Supplementary Table 2*A*), with an overall sensitivity of 79.03% (95% CI, 71.05%–85.27%) and specificity of 99.42% (96.80%–99.90%). As with the nasal kit, the sensitivity increased in samples with lower Ct values, to 84.81% (95% CI, 77.03%–90.30%) in samples with a Ct <25 and 91.92% (84.86%–95.85%) in those with a Ct <20. With respect to symptom onset time, both tests performed best in individuals presenting within the first week after symptom onset, with sensitivities of 83.51% (95% CI, 74.87%–89.58%) for the nasal and 82.47% (73.71%–88.76%) for the NP kit (Figure 1 and Supplementary Table 2*B*). Exclusion of 4 samples with Omicron clade 21M (parental lineage B1.1.528) or 21L (sublineage BA.2) did not affect the sensitivity of the tests (NP, 78.69% [95% CI, 70.60%–85.02%]; nasal, 79.52% [71.50%–85.72%]) (Figure 1 and Supplementary Table 2*C*).

For the nasal Panbio Ag test device, the overall sensitivity was 81.42% (95% CI, 75.16%–86.39%), and the overall specificity was 99.64% (97.99%–99.94%) (Figure 1 and Supplementary Table 3*A*). The sensitivity increased in samples with lower Ct values, to 88.55% (95% CI, 82.82%–92.55%) in samples with a Ct <25 and to 93.20% (87.93%–96.26%) in those with a Ct <20. As with the other 2 kits, the sensitivity was highest in patients presenting within the first week after symptom onset, 86.62% (95% CI, 80.05%–91.26%) (Figure 1 and Supplementary Table 3*B*). With respect to Omicron lineage, highest sensitivity was observed for Omicron 21L/BA.2 (100.00% [95% CI, 90.82%–100.00%]), followed by Omicron 21K/BA.1 (77.61% [69.84%–83.84%]), with the lowest sensitivity observed for Omicron 21M infections (63.64% [35.38%–84.83%]) (Figure 1 and Supplementary Table 3*C*).

As expected, the majority of false-negative results for all 3 tests were observed in samples with higher Ct values (Supplementary Figure 1). We observed similar sensitivity values across the first week after symptom onset for all tests (Supplementary Table 4). Overall, samples from study participants presenting within the first week after symptom onset had significantly lower Ct values than samples from participants with no symptoms ($P < .001$) (Supplementary Figure 2*A*). There was a significant difference in Ct values between the 3 Omicron lineages' samples with 21M had significantly higher Ct values than samples with 21L/BA.2 ($P < .001$) or 21K/BA.1 ($P = .001$), and samples with 21L/BA.2 had significantly lower Ct values than samples with 21K/BA.1 ($P < .001$) (Supplementary Figure 2*B*).

For both evaluations, all false-negative values with high SARS-CoV-2 viral load (Ct <20) occurred in infections with Omicron 21K/BA.1. Further analysis of the viral sequences with >80% coverage from false-negative samples with high SARS-CoV-2 viral loads (Ct <20) did not reveal additional amino acid changes in the nucleocapsid protein (Supplementary Table 5 [6]). In addition to P13L, Del31–33, R203K, and G204R, 1 sample had a P142S substitution that was previously found in BA.1.21 at 0.3% (19 of 6581) [7]. While the sensitivity for all 3 tests was slightly higher in unvaccinated individuals (range, 84.00%–86.05%) versus fully vaccinated individuals (78.57%–80.65%) (Supplementary Table 6), we observed no significant differences in SARS-CoV-2 Ct values or day of presentation after symptom onset between participants depending on their vaccination status (Supplementary Figure 2*C* and 2*D*).

DISCUSSION

The emergence of each novel SARS-CoV-2 variant of concern prompts the need to evaluate its potential impact on the

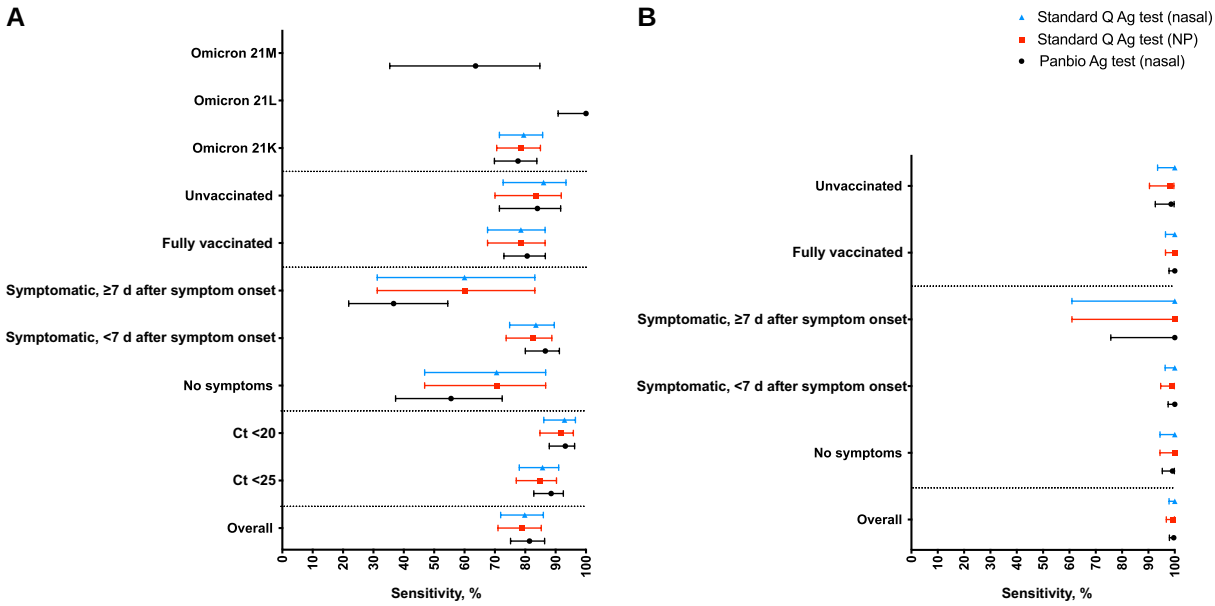

Figure 1. Sensitivity (*A*) and specificity (*B*) of the Panbio Ag test (nasal), Standard Q Ag test (nasopharyngeal [NP]), and Standard Q Ag test (nasal) across different categories. Error bars represent 95% confidence intervals.

performance of diagnostic tests currently in use. In the current study, we evaluated the performance of 3 commonly used rapid antigen kits during the Omicron wave in South Africa.

We found an high sensitivity overall (range, 79.03%–81.42%) for all 3 tests, with excellent specificity values as well. The sensitivity of rapid antigen tests increased in samples with lower Ct values (indicative of higher viral load [8]), increasing to >90% in samples with Ct values <20. As expected, all 3 tests performed best in participants presenting within the first week after symptom onset, when the SARS-CoV-2 viral load is highest [9–12]. Our results are consistent with previously published data in on the circulation of other SARS-CoV-2 variants [13–15]. As previously reported (before emergence of the Omicron variant), we observed similar performance for the nasal and NP Standard Q Ag tests performed on equivalent samples [16].

We also examined the impact of vaccination status on test performance because vaccination and preexisting immunity could potentially affect symptom presentation and timing with respect to infectiousness and viral load. We observed slightly higher sensitivity in unvaccinated individuals for all 3 tests, consistent with the previous observations showing that previous immunity is associated with a lower SARS-CoV-2 viral load on infection [17]. We did not observe significant differences in Ct values depending on the vaccination status of the study participants, but it is important to note that these results could be confounded by natural SARS-CoV-2 infections, on which we did not have data.

With respect to Omicron lineage, in the analysis of the Panbio Ag test, the highest sensitivity was observed for 21L/BA.2, followed by 21K/BA.1 and finally 21M/parental lineage B1.1.528. The observed differences in rapid Ag test performance are likely due to observed differences in Ct values between infections with the 3 lineages, with 21L/BA.2 having the lowest Ct values. Similar observations with regard to viral load differences and infectiousness between Omicron sublineages have been reported elsewhere [18, 19]. While we do not have data on BA.4 and BA.5 sublineages, based on their nucleocapsid profile and increase in infectiousness, test performance with these sublineages is likely to resemble that with BA.2 [20]. All false-negative samples with high SARS-CoV-2 viral load (defined as Ct <20) belonged to the 21K/BA.1 Omicron sublineage, with no unique additional nucleocapsid amino acid changes.

One limitation of our study is the lack of the field performance data on the evaluated tests in the same community in previous waves; however, our results are consistent with those of previously published studies performed in similar settings and in a similar manner. Overall, our data indicate that the performance of the SD Biosensor and Panbio rapid SARS-CoV-2/COVID-19 antigen test was not negatively affected by the emergence of Omicron subtypes BA.1 and BA.2, showing that rapid antigen tests remain an important tool for managing the pandemic.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not

copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Performance of rapid antigen tests in identifying Omicron BA.4 and BA.5 infections in South Africa

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ABSTRACT

Background: Concerns around accuracy and performance of rapid antigen tests continue to be raised with the emergence of new SARS-CoV-2 variants.
Objective: To evaluate the performance of two widely used SARS-CoV-2 rapid antigen tests during BA.4/BA.5 SARS-CoV-2 wave in South Africa (May – June 2022).
Study design: A prospective field evaluation compared the SARS-CoV-2 Antigen Rapid test from Hangzhou AllTest Biotech (nasal swab) and the Standard Q COVID-19 Rapid Antigen test from SD Biosensor (nasopharyngeal swab) to the Abbott RealTime SARS-CoV-2 assay (nasopharyngeal swab) on samples collected from 540 study participants.
Results: Overall 28.52% (154/540) were SARS-CoV-2 RT-PCR positive with median cycle number value of 12.30 (IQR 9.30–19.40). Out of the 99 successfully sequenced SARS-CoV-2 positive samples, 18 were classified as BA.4 and 56 were classified as BA.5. The overall sensitivities of the AllTest SARS-CoV-2 Ag test and Standard Q COVID-19 Ag test were 73.38% (95% CI 65.89–79.73) and 74.03% (95% CI 66.58–80.31) and their specificities were 97.41% (95% CI 95.30–98.59) and 99.22% (95% CI 97.74–99.74) respectively. Sensitivity was >90% when the cycle number value was <20. The sensitivity of both rapid tests was >90% in samples infected with Omicron sub-lineage BA.4 and BA.5.
Conclusion: Accuracy of tested rapid antigen tests that target the nucleocapsid SARS-CoV-2 protein, were not adversely affected by BA.4 and BA.5 Omicron sub-variants.

1. Introduction

Shortly after the identification of SARS-CoV-2 Omicron variant B1.1.529 on November 24th 2021 [1], several new sub-lineages have been identified, including BA.4 and BA.5 which were first identified in South Africa in February 2022 [2]. In addition to over 30 mutations in the S-gene, Omicron lineages are characterised by several mutations in the nucleocapsid, including P13L, Del31–33, R203K and G204R, that are characteristic to all Omicron sub-lineages and S413R found in BA.2 and BA.3. Additionally, BA.4 sub-lineage contains an additional nucleocapsid mutation P151S. Therefore, there were concerns about the performance of SARS-CoV-2 rapid antigen tests – majority of which target

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the nucleocapsid protein. While the SARS-CoV-2 testing is decreasing globally evolution of SARS-CoV-2 continues to challenge the existing prevention and diagnostic measures. In this study, we evaluated the performance of two widely used rapid antigen tests in comparison to the Abbott RealTime SARS-CoV-2 RT-PCR during the BA.4/BA.5 dominated wave of COVID-19 in KwaZulu-Natal, South Africa.

2. Methods

2.1. Study setting and specimen collection

Sample collection was performed in the province of KwaZulu-Natal in South Africa at 2 drive-through testing centers from 5 May 2022 until 26 June 2022 (spanning the 5th wave of SARS-CoV-2 infections dominated by BA.4 and BA.5 Omicron sub-variants) [2]. Residents of the selected communities falling under any of the following criteria were offered SARS-CoV-2 testing: tested SARS-CoV-2 positive in the previous seven days; presence of COVID-19 symptoms in the previous seven days; exposed to COVID-19 5–10 days ago; health care worker; or doctor referral for testing. After obtaining informed consent, study participants provided information on demographics, symptom type and onset date, and vaccination status. Three respiratory swabs were collected [1 nasal and 2 nasopharyngeal (NP)]. The collection of the nasal swab was prioritized to avoid cross contamination between sites and was followed by the collection of the 2 NP swabs. Trained medical staff performed the rapid antigen testing immediately after sample collection. The second NP swab was used for SARS-CoV-2 RT-PCR reference test and was shipped within 3 h of collections, at room temperature to the central laboratory for processing. The study was approved by the KwaZulu-Natal Biomedical Research Ethics Committee (BREC approval No: BREC/00,001,195/2020 and BREC/00,003,106/2021).

2.2. SARS-CoV-2 testing and sequencing

We evaluated two lateral-flow rapid antigen kits: SARS-CoV-2 Ag rapid test from Hangzhou AllTest Biotech (nasal, Hangzhou, China), and Standard Q COVID-19 Ag test from SD Biosensor (NP, Republic of Korea). According to the manufacturers, the tests detect SARS-CoV-2 nucleocapsid protein with no cross-reaction with other common respiratory pathogens except SARS-CoV. All samples were collected, and assays performed by trained medical staff and as per manufacturer's instructions.

Upon arrival at the laboratory, NP swabs were resuspended in 2 ml of viral transport media (VTM). The Abbott RealTime SARS-CoV-2 assay (target sequences in the RdRp and N genes of the SARS-CoV-2 genome) was the comparator (Chicago, IL. USA). Positive results are reported with cycle number (CN) values [3] . SARS-CoV-2 positive samples were sequenced at KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP) as previously described [1]. Briefly, RNA was extracted on an automated Chemagic 360 instrument (Perkin Elmer, Hamburg, Germany). Libraries for whole genome sequencing were prepared using the Oxford Nanopore Midnight protocol with Rapid Barcoding as per the manufacturer's protocol and sequenced on the Gridion. Raw reads were assembled using Genome Detective version 1.132 (https://www.genomedetective.com) against the reference NC_045512.2 (numbering equivalent to MN908947.3). Mutations were confirmed visually with . bam files using Geneious version 2020.1.2 (Biomatters). Sequences with > 80% coverage were deposited on the GISAID sequence database. The sequencing information is provided in the **Sup. Table 1.**

2.3. Statistical analysis

Fully vaccinated participants were classified as any participant that received either one dose of Ad26.CoV2. S (Johnson & Johnson) or two doses of BNT162b2(Pfizer-BioNTech) two or more weeks prior to testing. Data was analysed and graphed using GraphPad Prism version 8.3.1 (GraphPad software, La Jolla, CA), and SPSS version 24. Test performance characteristics were calculated in reference to Abbott RealTime SARS-CoV-2 assay results. The 95% confidence intervals were calculated to assess the level of uncertainty induced by sample size, using the Wilson's score method. A *t*-test was used to assess differences in CN values between true and false positive results and vaccination status groups. Kruskal-Wallis with Dunn's corrections for multiple comparisons test was done to assess differences in CN values between symptom categories.

3. Results

3.1. Study sample characteristics

The evaluation was performed on 540 samples (Table 1.). The median age of study participants was 42 [interquartile range (IQR) 30–55], with 56.66% of participants being female. Majority of study participants (74.63%) presented within the first week of symptom onset. Fully vaccinated participants made up 50.74% of the study cohort with 67.15% (184/274) having received 2 doses of BNT162b2 vaccine and 32.84% (90/274) having received one dose of the Ad26.CoV2.S vaccine. Overall SARS-CoV-2 RT-PCR positivity was 28.52% (154/540) with median CN value of 12.30 (IQR 9.30–19.40). Out of the 99 successfully sequenced SARS-CoV-2 positive samples, 18 were classified as BA.4 and 56 were classified as BA.5.

3.2. Rapid antigen test performance

Overall performance of the tests is summarised in Fig. 1 **and Sup. Table 2**. Overall sensitivity and specificity of the AllTest SARS-CoV-2 Ag test were 73.38% (95% CI 65.89–79.73) and 97.41% (95% CI 95.30–98.59) respectively. The overall sensitivity and specificity of the Standard Q COVID-19 Ag test were 74.03% (95% CI 66.58–80.31) and 99.22% (95% CI 97.74–99.74). The sensitivity of both tests increased to >80% in samples with CN values <25 and above 90% in samples with CN value <20. Both tests performed worst in patients with no symptoms with sensitivity of 33.33% (95% CI 12.06–64.58). With respect to Omicron BA.4 sub-lineage, both tests performed well with sensitivity of 100.00% (95% CI 82.41–100.00) for AllTest SARS-CoV-2 Ag test and sensitivity of 94.44% (95% CI 74.24–99.01) for Standard Q COVID-19 Ag test. Similarly, both tests performed well in samples characterised as Omicron BA.5 with sensitivity of 89.29% (95% CI 78.53–95.00) for

Table 1
Study participants/sample characteristics.

Sample size (N)	540
Age, years (median, IQR)	42, 30–55
Gender (%, n/N Female)	56.66, 306/540
% PCR Positivity (n/N)	28.52, 154/540
Presence of symptoms	
Asymptomatic/Pre-symptomatic (%, n/N)	11.85, 64/540
<7 days PSO (%, n/N)	74.63, 403/540
>=7 days PSO (%, n/N)	13.52, 73/540
Vaccination status	
Fully vaccinated (%, n/N)	50.74, 274/540
Unvaccinated (%, n/N)	34.26, 185/540
Partially vaccinated (%, n/N)	15.00, 81/540
HIV positive (%, n/N)	0.56, 3/537
Oxygen saturation (median, IQR)	98.00, 98.00–99.00
CN value (median, IQR)	12.30, 9.30–19.40
Omicron lineage	
(% of SARS-CoV-2 positive, n/N)	
B.1.1.529/BA.2, 21L	23.23, 23/99
BA.4, 22A	18.18, 18/99
BA.5, 22B	56.56, 56/99
Recombinant	2.02, 2/99

*Missing data- SARS-CoV-2 RT-PCR results:1; HIV status (self-reported): 3; SARS-CoV-2 sequencing: 55;.

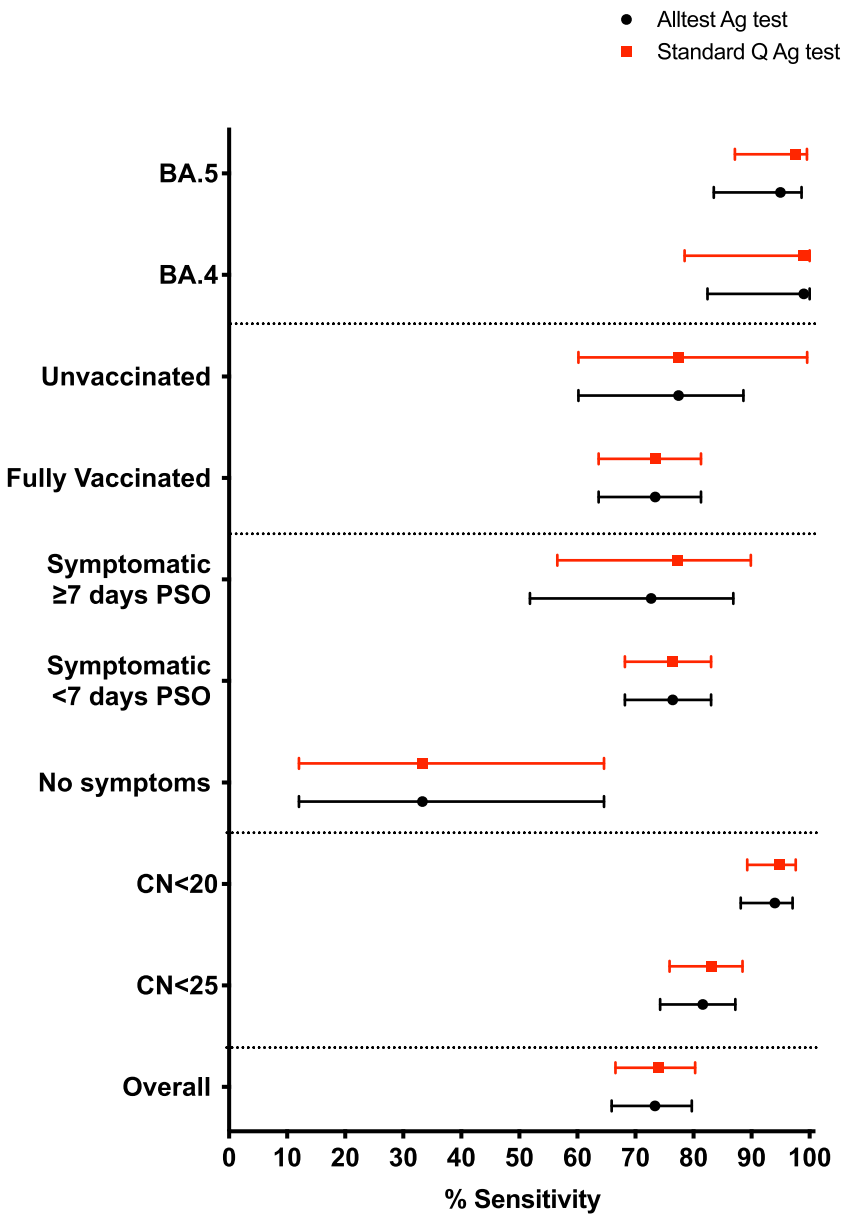

Fig. 1. Sensitivity of the AllTest SARS-CoV-2 Rapid Ag and the Standard Q COVID-19 Ag tests across different categories (CN value, presence and duration of symptoms, vaccination and SARS-CoV-2 lineage. The error bars represent 95% Confidence interval.

AllTest SARS-CoV-2 Ag test and sensitivity of 87.50% (95% CI 76.37–93.81) for Standard Q COVID-19 Ag test. This remained true in the analysis only including samples with >80% sequence coverage (**Sup. Table 2 C**). Sensitivity of both tests was slightly higher in unvaccinated (77.42% for AllTest and 77.41% for Standard Q) compared to fully vaccinated study participants (73.40% for AllTest and Standard Q), **Sup. Table 3**.

As expected, samples that resulted in false negative values for both tests had significantly higher SARS-CoV-2 CN values compared to true positive samples (**Sup. Figure 1**). We observed no difference in CN values between samples based on time post symptom onset and vaccination status (**Sup. Figure 2. A,B**).

4. Discussion

The performance of SARS-CoV-2 tests requires reassessment with the emergence of new variants carrying novel mutations that could potentially impact test performance.

Both AllTest and Standard Q Ag rapid tests performed well with an overall sensitivity >70%. Sensitivity increased to above 80% for both tests in samples with CN values < 25. As expected, both tests performed poorly in patients without symptoms [4–7]. With respect to detection of infections with the Omicron sub-lineage, sensitivity of both tests was >90% for Omicron sub-lineages BA.4 and BA.5 showing that the additional nucleocapsid mutations in these sub-variants do not impact the performance of the two evaluated rapid antigen tests. As we have previously reported [8], we observed slightly higher sensitivity in unvaccinated individuals compared to vaccinated individuals however this analysis is confounded by the lack of information on timing and presence of previous natural infections.

Our study has several limitations, including a bias towards samples with high viral load in which viral sequencing was successful. Irrespective, our data shows that both nasal AllTest and nasopharyngeal Standard Q Ag rapid test performance was not impacted by the mutations in the BA.4 and BA.5 Omicron sub-variants. The performance of rapid antigen tests for the currently dominant variant, XBB.1.5, a

recombinant of diversified BA.2 lineages, is likely to mirror the test performance previously reported for BA.2 variants [8].

CRediT authorship contribution statement

Conceptualization (AS, NS, CE), Data curation (AS, NS, SN, JG, DT, EJS, CC), Formal analysis (AS, GL, MdV, LL, JG, DT, EJS), Funding acquisition (AS, NS, CE, ABMK, KN, SAK, QAK), Methodology (AS, NS, CE, MdV), Project administration (AS, NS, CE, MdV, ABMK, KN), Resources (KN, CC, CE, TdO, SAK, QAK), Supervision (AS, NS, CC), Visualization (AS, NS, MdV), Writing - original draft (AS, NS), Writing - review & editing (AS, NS, CE, SN, JG, DT, EJS, MdV, LL, GL, CC, CE, KN, ANMK, TdO, SAK, QAK).

Data use agreement: De-identified patient-level data can be accessed by contacting the corresponding author with a detailed description of the research question.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antigen rapid diagnostic tests were provided by FIND, the global alliance for diagnostics, and FIND was involved in the study design development. CE and MdV are employees of FIND. The rest of the authors declare no conflict of interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2023.105498.

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5.5. EVALUATION OF THE XPERT® XPRESS SARS-COV-2 ASSAY FOR SARS-COV-2 DIAGNOSIS IN SOUTH AFRICA

[Manuscript in preparation]

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Abstract

Background

Rapid and accurate diagnosis of SARS-CoV-2 infection is required to manage and contain the spread of the virus. The aim of this study was to assess the diagnostic accuracy of the Xpert® Xpress SARS-CoV-2 assay.

Methods

This was a prospective diagnostic accuracy study using nasopharyngeal samples from people tested for COVID-19 in health facilities in Durban, South Africa. The sensitivity and specificity of the Xpert® Xpress SARS-CoV-2 assay were assessed in consecutive 39 consecutive nasopharyngeal specimens, in reference to the Abbott RealTi*m*e SARS-CoV-2 assay. The measure of agreement between the assays was evaluated using Cohen's Kappa coefficient. In case of positive samples, cycle threshold values were compared using paired t-tests and Pearson correlations.

Results

Out of the 39 participants, 25 (64.1%) tested positive for SARS-CoV-2 on the reference laboratory Abbott RealTi*m*e SARS-CoV-2 assay, with a median cycle number value of 20.87 (IQR 15.72-24.84). The sensitivity and specificity of the Xpert® Xpress SARS-CoV-2 assay were 100.0 (95% CI: 86.7-100.0) and 92.9 (95% CI: 68.5-98.7), respectively. The kappa coefficient was 0.94 (95% CI 0.82 -1.00) indicating almost perfect agreement. The Cycle number values for Abbott RealTi*m*e SARS-CoV-2 assay (*RdRp* and *N* genes) were significantly lower compared to the cycle threshold value for both the E and N2 gene values obtained on the Xpert® Xpress assay ($p<0.0001$). There was a strong positive correlation between Cycle number or cycle threshold values obtained for all SARS-CoV-2 targets($r>0.9$, $p<0.0001$).

Conclusion

The Xpert® Xpress assay provides a rapid, accurate and easy to use alternative to standard RT-PCR methods for the detection of SARS-CoV-2.

Key words: COVID-19; SARS-CoV-2; Xpert® Xpress; nasopharyngeal swab.

Background

Early and large-scale testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is important for countries to manage and contain the spread of the virus and successfully reduce the burden on their health care systems. Routine diagnosis of COVID-19 cases is based on the detection of viral RNA by nucleic acid amplification tests (NAAT) such as real-time reverse-transcription polymerase chain reaction (RT-PCR). The targeted SARS-CoV-2 genomic regions include ORF1b, ORF8, nucleocapsid (N), envelope (E), spike (S) protein and RNA-dependant RNA polymerase (RdRp) (WHOe, 2020), and the detection is usually performed on throat and nasopharyngeal swabs. However, lengthy processing times and the requirement for significant resources are major disadvantages of the standard RT-PCR methods.

The GeneXpert Instrument systems (Cepheid, Sunnyvale, USA) represent a faster and more efficient alternative to standard RT-PCR through automated and integrated sample preparation, nucleic acid extraction and amplification and detection of the target sequences (Cepheid). The GeneXpert individual load cartridge instruments have been used successfully for the detection of Mycobacterium tuberculosis rifampicin susceptibility (MTB/RIF) worldwide but more especially in South Africa which bears a large proportion of the world's tuberculosis (TB) burden (Ramma et al., 2015). Subsequent to this the GeneXpert has been used extensively for HIV viral load testing (Drain et al., 2020), the early detection and treatment of sexually transmitted diseases (STI) such as *Chlamydia trachomatis* (CT)/ *Neisseria Gonorrhea* (NG), *Trichomonas Vaginalis* (TV), Human papillomavirus (HPV), and Intrapartum or antepartum Group B Streptococcus (GBS) (Cepheid). The Xpert® Xpress SARS-CoV-2 tests allow for rapid detection of nucleic acid from SARS-CoV-2 specifically targeting E and N2 genes.

The Xpert® Xpress SARS-CoV-2 assay was shown to have high sensitivity and specificity in detecting SARS-CoV-2 positive samples in previous studies (Hirotsu et al., 2022, Lee and Song, 2021, Cao et al., 2022a, Hou et al., 2020, Rakotosamimanana et al., 2020, Loeffelholz et al., 2020, Cao et al., 2022b). Here we assessed its performance for diagnostic purposes in the South African setting.

Methods

Study design and setting

This was a prospective diagnostic accuracy study of the Xpert® Xpress SARS-CoV-2 assay. Participants were recruited from government health facilities including, King Dinuzulu Hospital complex and KwaMashu community health centre (CHC) located in the urban and peri-urban area of Durban, South Africa, between 26th of November 2020 and 14th of January 2021.

Participants

Patients accessing routine health care services at the study health facilities were offered SARS-CoV-2 testing irrespective of the presence of symptoms. Stable, ambulant adults ≥ 18 years of age seeking testing for SARS-CoV-2 infection who were willing to participate in the study and able to provide written informed consent were eligible for study participation.

Following informed consent study participants provided demographic and clinical data. A nasopharyngeal (NP) swab was collected by trained medical staff and sent to the central laboratory for the Xpert® Xpress and reference Abbott RealTi*m*e SARS-CoV-2 testing. Samples were transported to the central laboratory within 3 hours of collection and processed within 12 hours.

SARS-CoV-2 testing.

The Abbott RealTi*m*e SARS-CoV-2 assay using M2000 system(Abbotta, 2020) was used as a reference test. The Abbott RealTi*m*e SARS-CoV-2 assay is a RT-PCR test detecting *RdRp* and *N* genes with a limit of detection of 100 copies/mL. The Abbott RealTi*m*e SARS-CoV-2 positive results are reported with cycle number (CN) values (Hirschhorn et al., 2021). We evaluated the performance of the Xpert® Xpress SARS-CoV-2 assay, a rapid real time RT-PCR assay targeting the E gene and the N2 region of the N gene, with a detection limit of 250 copies/mL, positive results reported in cycle threshold (Ct) values and an approximate running time of 45 minutes(Cepheid, 2021a).

Upon reaching the laboratory, the NP swab was placed in 3mL of viral transport media (VTM), out of which 1mL of media was used to run the Abbott RealTi*m*e SARS-CoV-2 assay while 300µL was used to run the Xpert® Xpress SARS-CoV-2. Both assays were performed as per manufacturer's instructions.

Statistical analysis

GraphPad Prism version 8.3.1 (GraphPad software, La Jolla, CA) and SPSS version 27 were used to perform the statistical analysis. Sensitivity and specificity at detecting a positive SARS-CoV-2 result were calculated in reference to the Abbott RealTi*m*e SARS-CoV-2 assay results. The 95% confidence

intervals were calculated to assess the level of uncertainty induced by sample size, using the Wilson's score method. The measure of agreement between the assays was evaluated using Cohen's Kappa coefficient(Landis and Koch, 1977). The D'Agostino-Pearson omnibus normality test was used to assess distribution of the data. Based on the results of the normality test, a paired t test was used to assess differences in Cycle threshold (Ct) values and the Pearson correlation was used to correlate Ct values from different assays/targets in true positive samples.

Ethical approvals

The study was approved by the KwaZulu-Natal Biomedical Research Ethics Committee (BREC approval No: BREC/00001195/2020 and BREC/00003106/2021). All confirmed COVID-19 cases were reported to the South African National Department of Health using the National Medical Conditions (NMC) surveillance system.

Results

Study participants characteristics

The Xpert® Xpress and Abbott RT-PCR tests were performed on 39 patient specimens, **Table 1**. Out of the 39 patients, the median age was 35 years, and 69.2% were female with 19.7% self-reporting as HIV positive. The majority of the patients presented for testing within the first week of symptom onset (53.8%).

While no viral sequencing was performed on the samples, the evaluation period overlaps with the Beta variant wave in South Africa (Viana et al., 2022, Tegally et al., 2021b).

SARS-CoV-2 test results.

Out of 39 samples tested, 25 tested positive using the Abbott RT-PCR system with the median CN value of 20.87 (IQR: 15.72-24.84), **Table 1**. There were no invalid results from either assay. Compared to Abbott RT-PCR, the Xpert® Xpress sensitivity was 100.0 (95% CI: 86.7-100.0) and specificity was 92.9 (68.5-98.7), **Table 2**. The kappa coefficient was 0.94 (95% CI 0.82 -1.00) indicating almost perfect agreement between the assays. Out of 39 samples tested there was one false positive sample with the Xpert® Xpress with Ct values of N2: 39.2 and E: 41.4. The CN values for Abbott RT-PCR were significantly lower compared to both the E and N2 gene Ct values on the GeneXpert assay ($p<0.0001$, **Fig. 1A**). There was a strong positive correlation between Abbott CN values and Xpert® N2 and E Ct values (with N2 gene: $r= 0.9290$, $p<0.0001$; and with E: $r=0.9062$, $P<0.0001$), as well as between N2 and E gene Ct values ($r=0.9197$, $p<0.0001$)(**Fig. 1B**) in true positive samples.

Discussion

Here we evaluated the performance of the Xpert® Xpress SARS-CoV-2 assay to detect SARS-CoV-2 in South Africa. In concordance with previously published studies (Hirotsu et al., 2022, Lee and Song, 2021, Cao et al., 2022a, Hou et al., 2020, Rakotosamimanana et al., 2020, Loeffelholz et al., 2020) we found that the Xpert® Xpress was highly sensitive and specific in identifying SARS-CoV-2 positive cases.

Overall, we observed a false positive rate of 7.14% (1/14). Similar results with Xpert® Xpress were observed in previous evaluations, most often observed in specimens with no amplification of the E gene and Ct values for N2 gene greater than 40 cycles (Rakotosamimanana et al., 2020, Loeffelholz et al., 2020, Falasca et al., 2020). While both assays are marketed as qualitative, generated CN or Ct values are often used as indictors of infectiousness and for estimating epidemiologic dynamics (Hay et al., 2021). The obtained Ct values were significantly higher for the Xpert® Xpress assay compared to the CN values of Abbott RT-PCR system and this is likely due to various reasons including the different target genes, differences in the amount of sample used and the intrinsic nature of the assay itself (Navarathna et al., 2021). There was a strong positive correlation between all target CN and Ct values.

Overall, in the regions with availability of GeneXpert Instrument systems, the Xpert® Xpress SARS-CoV-2 assay provides a fast and accurate method for diagnosis of SARS-CoV-2 infection. Furthermore with the overlap in symptoms and clinical presentation with other respiratory viruses, the new combination Xpert® Xpress SARS-CoV-2/Flu/RSV cartridge which allow for testing of patient material for the presence of SARS-CoV-2, Influenza A/B and RSV A/B virus using a single cartridge (Cepheid, 2021b), allows for fast and accurate diagnosis of common respiratory pathogens improving clinical and public health decision-making (Wolters et al., 2021, Sluimer et al., 2021).

One of the limitations of this study includes the small sample size. Patient recruitment was limited due to strict isolation and quarantine regulation during early stages of the COVID-19 pandemic resulting in staff shortages. Additionally, we had limited access to Xpert® Xpress assays as these were not readily available in South Africa at the time. In this study Xpert® Xpress testing was conducted in a central laboratory, and further research is needed to confirm the diagnostic accuracy when used in primary health care clinics and other community settings.

Conclusion

The Xpert® Xpress assay provides a rapid, accurate and easy to use alternative to standard RT-PCR methods for the detection of SARS-CoV-2 in South Africa. Xpert® Xpress assay is qualitative and generated SARS-CoV-2 Ct values should be interpreted with caution.

Tables:

Table 1. Participant/sample characteristics

Sample size	39
Age, years (median, IQR)	35 (26-52)
Gender (%, n/N Female)	69.2, 27/39
% Positivity (n/N)	64.1 (25/39)
Presence of symptoms	
Asymptomatic (%, n/N)	23.08, 9/39
Symptomatic (<7 days PSO)	53.8, 21/39
Symptomatic (>= 7 days PSO	23.08, 9/39
HIV positive (%, n/N) [#]	19.4, 7/37
Ct value* (median, IQR)	20.87, 15.72-24.84
Oxygen saturation (median, IQR)	98, 96-99
Study period (dd/mm/yyyy)	26/11/2020- 14/01/2021

[#]*Missing information on 3 patients*

**Ct value obtained from the Abbott RealTime SARS-CoV-2 assay*

Table 2. Test performance of Xpert® Xpress SARS-CoV-2 assay in comparison to Abbott RT-PCR

Xpert® Xpress SARS-CoV-2 assay	**Reference**	
	Positive	Negative
Positive	25	1
Negative	0	13
Sensitivity (%, 95% CI)	100.0 (86.7-100.0)	
Specificity (%, 95 %CI)	92.9 (68.5-98.7)	
Invalid rate (%, n/N)	0, 0/0	
Cohen's k (95% CI)	0.94 (0.82 -1.00)	

Figures:

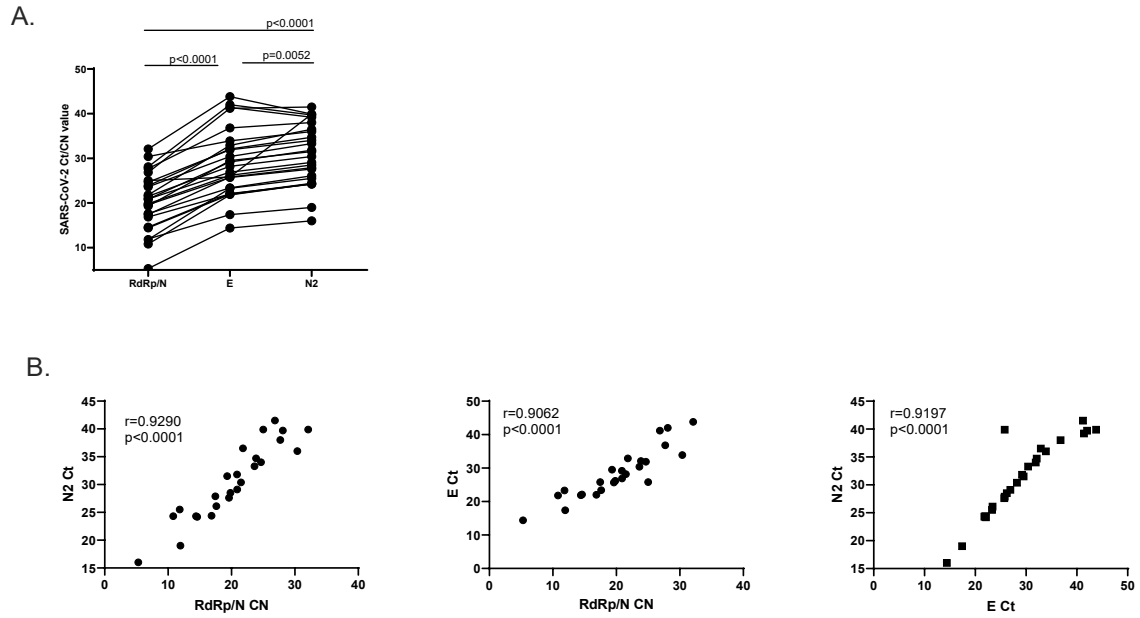

Figure 1. A) Differences in SARS-CoV-2 CN values between Abbott RT-PCR (*RdRp/N*) and SARS-CoV-2 Ct values on Xpert® (E and N2) **B)** Correlation between SARS-CoV-2 CN or Ct values for different target genes.

List of abbreviations

COVID-19: Coronavirus disease 2019

Ct: Cycle Threshold

CN: Cycle number

E: envelope

HIV: Human Immunodeficiency Virus

MTB/RIF: Mycobacterium tuberculosis/rifampin

N: nucleocapsid

PSO: post symptom onset

RT-PCR: real time polymerase chain reaction

RdRp- RNA-dependent RNA polymerase

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

TB: Tuberculosis

Declarations:

Ethics approvals and consent to participate.

All study participants provided a consent to participate, and the study was approved by the KwaZulu-Natal Biomedical Research Ethics Committee (BREC approval No: BREC/00001195/2020 and BREC/00003106/2021).

Consent for publication

Not applicable

Availability of data and materials

The generated datasets will be made available to any investigator on a reasonable request.

Competing interest

The authors declare no conflict of interests.

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Authors contributions

Designed the study: NS, AS, NG;
Performed the experiments: NS, SN, AS;
Analysed the data: AS, LL, TGM, NS;
Wrote the first draft of the paper: AS, NS, ZK;
Collected specimens and clinical data: RHM, KN:
Supervised clinical and/or experimental aspects of the study: AS, RHM, NS, JD, SN, KN, ABMK, NG;
All listed authors contributed to the editing and finalisation of the manuscript.

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CHAPTER 6

THE EVALUATION OF DIFFERENT SAMPLE TYPES FOR SARS-CoV-2 DIAGNOSTICS

CHAPTER 6: THE EVALUATION OF DIFFERENT SAMPLE TYPES FOR SARS-COV-2 DIAGNOSTICS

Chapter 6 presents the findings from the study that evaluated the use of saliva as a favourable, non-invasive, alternate sample type for the diagnosis of SARS-CoV-2 using RT-PCR. The study was conducted at the King Dinuzulu Hospital Complex and KwaMashu community health centre in Durban, KwaZulu-Natal. Outpatients seeking testing for SARS-CoV-2 infection, irrespective of symptoms were screened for SARS-CoV-2 using real-time PCR (RT-PCR). Saliva was collected at various timepoints and tested using RT-PCR and compared to nasopharyngeal swab samples. The manuscript entitled "*Usability of saliva as a reliable and non-invasive sample for SARS-CoV-2 detection in Durban, South Africa"* is under peer review by BMC Diagnostic and Prognostic.

6.1. USABILITY OF SALIVA AS A RELIABLE AND NON-INVASIVE SAMPLE FOR SARS-COV-2 DETECTION IN DURBAN, SOUTH AFRICA

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Abstract

Background: Saliva has been proposed as a potential more convenient, cost-effective, and easier sample for diagnosing SARS-CoV-2 infections, but there is limited knowledge of the impact of saliva volumes and stages of infection on its sensitivity and specificity.

Methods: In this study, we evaluated the performance of SARS-CoV-2 testing in 171 saliva samples across different volumes (50, 100, 300 and 500µL of saliva) and at different stages of disease (at screening, day 7, 14 and 28 post SARS-CoV-2 diagnosis) from 52 mostly mild symptomatic patients. Imperfect nasopharyngeal swab samples were used as a reference.

Results: Overall, 52 of the 171 samples were positive, with sensitivity of 73.2% and specificity of 81.0%. The sensitivity of saliva samples ranged from 70.6% for 50µL to 83.3% for 300µL of saliva collected. The specificity values ranged between 78.8% for 500µL and 86.4% for 100µL saliva. The overall percentage of positive results in nasopharyngeal swabs and saliva specimens remained comparable throughout the study visits. We observed no significant difference in cycle number values between saliva and nasopharyngeal swab specimens, irrespective of saliva volume tested.

Conclusions: The saliva collection offers a promising approach for population-based testing. Implementing robust saliva-based testing strategies could contribute significantly to controlling and managing the COVID-19 pandemic.

Key words: COVID-19, SARS-CoV-2, saliva, nasopharyngeal swab

Introduction

The rapid and accurate identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cases is an important strategy for controlling the spread of SARS-CoV-2 viral infection. To date, testing of nasopharyngeal (NP) swab samples for SARS-CoV-2 using the reverse transcriptase-polymerase chain reaction (RT-PCR) remains the gold standard for SARS-CoV-2 detection and diagnosis (WHOe, 2020, Wang et al., 2020c). However, the collection of NP swab samples suffers from supply chain constraints, is inclined to cause nasal discomfort, with the risk of suboptimal self-sampling, and increased risk of infection transmission via droplets or aerosol particles due to the irritation of the nasal passage (Chen et al., 2020a). Given these limitations, there is a need for alternative samples, including collection procedures that are less invasive and acceptable to patients and simultaneously produce accurate results.

Saliva samples are an attractive alternative for diagnosis due to ease of collection and patient preference and acceptability. Unlike NP swabs, saliva is most likely to increase compliance from the population for testing and decrease exposure risk to healthcare workers during the collection process (Kojima et al., 2021, Williams et al., 2020, Pasomsub et al., 2021). Studies have demonstrated that SARS-CoV-2 can be detected from the saliva of COVID-19 patients with sensitivity range from 45% to 97%, with improvement observed after RNA purification in crude sample (Riccò et al., 2020, Nagura-Ikeda et al., 2020, L'Helgouach et al., 2020, LeGoff et al., 2021). Data regarding sensitivity for SARS-CoV-2 detection in saliva and NP swab specimens are mixed, ranging from similar (Labbé et al., 2021, Butler-Laporte et al., 2021, Kojima et al., 2021), increased (Wyllie et al., 2020) or decreased (Sahajpal et al., 2021) sensitivity in saliva compared to NP swabs from COVID-19 patients.

Monitoring SARS-CoV-2 using an accurate, non-invasive, easily accessible collection method remains a public health need. Here, we assessed SARS-CoV-2 detection in paired saliva and NP swab samples collected from both asymptomatic and symptomatic COVID-19 patients attending healthcare facilities in Durban, South Africa. Additionally, we serially diluted saliva samples to determine the dilution effect on the sensitivity of SARS-CoV-2 PCR tests.

Methods

Study design, participants, and sample collection

This ancillary study nested in a prospective, observational multicentric study was conducted in the King Dinuzulu Hospital Complex and KwaMashu community health centre in Durban, KwaZulu-Natal. Outpatients seeking testing for SARS-CoV-2 infection, irrespective of symptoms were screened for SARS-CoV-2 using real-time PCR (RT-PCR). Adult patients 18 years old or over that tested positive for SARS-CoV-2 by NP swabs were invited to enrol into the main research study within three days of screening (baseline). Following informed consent, enrolled patients completed a questionnaire on basic demographics and clinical data. They were asked to return on day 7, day 14 and day 28 from positive RT-PCR test and provided NP swabs, saliva, demographic, and clinical information. NP swab was collected before saliva samples at the same visits. NP swab was placed into 2mL viral transport media (VTM)-(RPMI-1640 media, 10% foetal bovine serum plus penicillin-streptomycin). Saliva samples were collected by instructing a patient to spit into a sterile container with the obtained volume ranging between 600 to 2000µL. Four different volumes of saliva (50, 100, 300 and 500 µL) were mixed with VTM (topped up to 1100 µL) for SARS-CoV-2 testing. Saliva sensitivity was calculated using the NP RT-PCR as the imperfect reference standard. All SARS-CoV-2 positive cases were reported to the South African National Department of Health using the National Medical Conditions (NMC) surveillance system. The study formed part of studies approved by the KwaZulu-Natal Biomedical Research Ethics Committee (BREC approval Reference Numbers: BREC/00001195/2020; BREC/00003106/2021 and BREC/00003902/ 2022).

SARS-CoV-2 RT-PCR

Viral RNA was extracted from NP swabs and different volumes of saliva using the Abbott mSample Preparation System (Abbott GmbH & Co, Germany) and RT-PCR for SARS-CoV-2 RNA-dependent RNA polymerase (*RdRp*) and *N* genes were run on the Abbott m2000 RealTi*m*e System. The instrument automatically reports the results and interpretation on the Abbott m2000*rt* workstation. The Abbott RealTi*m*e SARS-CoV-2 positive results are reported with cycle number (CN) values (Hirschhorn et al., 2021).

Statistical analysis

Statistical analyses were conducted in GraphPad Prism 8.0.0 (GraphPad Software, San Diego, CA, USA) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Abbott RealTi*m*e SARS-CoV-2 assay

was used to calculate test performance characteristics (sensitivity, specificity, PPV and NPV). The 95% confidence intervals were calculated to assess the level of uncertainty induced by sample size using Wilson's score method. Paired t-test was used to compare the CN values between NP swabs and saliva samples.

Results

Study participant characteristics.

A total of 171 matching saliva and NP swab sample pairs were collected and tested for SARS-CoV-2 over 1-4 time-points from 52 SARS-CoV-2 participants screened and enrolled in the study. Screening and enrolment characteristics for the participants is detailed in Table 1, below. The majority of participants were female (57.7%, 30/52) and with an age range of 19-78 years. Overall, 63.5% had mild symptoms (33/52); 13.5% had moderate symptoms (7/52) and 23.1% were asymptomatic (12/52) Comorbidities reported were hypertension (17.3%; 9/52), diabetes (13.5%, 7/52), asthma (3.8%, 2/52), cardiac symptoms (1.9%, 1/52) and body mass index (BMI) >30 (48.1%, 25/52).

Table 1. Screening and enrolment characteristics

Participant characteristics		**Overall (N=52)** **n (%)**
Gender	Male	22(42.3)
	Female	30(57.7)
Age group	<30	7(13.5)
	30-39	13(25)
	40-49	11(21.2)
	50-59	9(17.3)
	60+	12(23.1)
Current Tuberculosis	No	50(96.2)
	Yes	2(3.8)
Previous Tuberculosis	No	45(86.5)
	Yes	7(13.5)
Hypertension	No	43(82.7)
	Yes	9(17.3)
Diabetes	No	45(86.5)
	Yes	7(13.5)

Asthma	No	50(96.2)
	Yes	2(3.8)
Cardiac	No	51(98.1)
	Yes	1(1.9)
BMI ≥ 30	No	27(51.9)
	Yes	25(48.1)
Symptoms at screening	No	5(9.6)
	Yes	47(90.4)
COVID-19 Severity assessment	Asymptomatic	12(23.1)
	Mild	33(63.5)
	Moderate	7(13.5)

Performance of saliva samples for SARS-CoV-2 testing

We assessed the performance of different saliva volumes against NP swabs in 171 matched pairs. A cycle number (CN) of <31 was regarded as a positive result. When comparing all volume of saliva tested, 71/171 (41.5%) saliva samples tested positive with a sensitivity of 73.2% (95% CI, 62.0 % - 82.2%) and specificity of 81.0% (95% CI, 72.2% -87.5%). Sensitivity was similar across different saliva volumes, ranging from 70.6% (95% CI, 46.9% - 86.7%) for 50µL of saliva to 83.3% [95% CI, 55.2% - 95.3%] for 300µL of saliva (Figure 1A). Similar specificity was observed across volumes ranging from 78.8% (95% CI, 62.3% - 89.3%) for 500µL of saliva to 86.4% (95% CI, 66.7% - 95.3%) for 100µL of saliva (Figure 1B). Full results are shown in Supplementary Table 1 and 2.

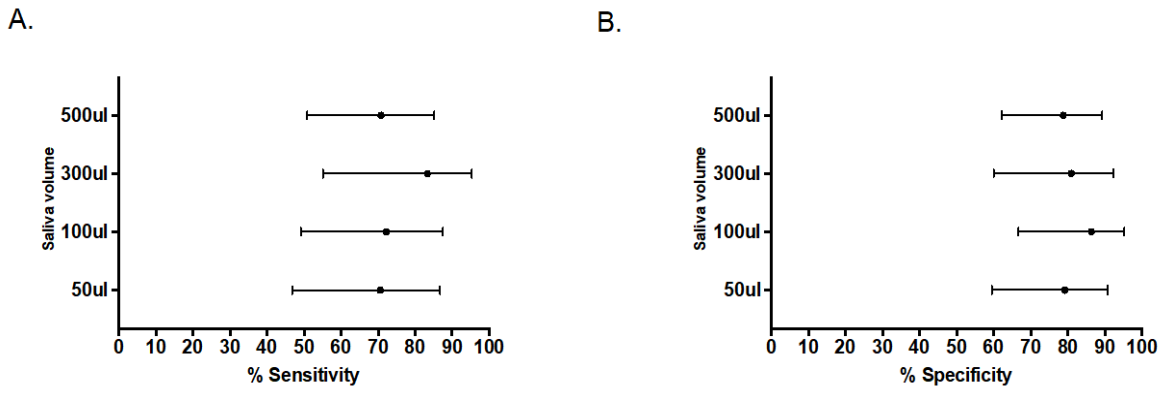

Figure 1. (A) Sensitivity and (B) Specificity values for SARS-CoV-2 results in different saliva volumes compared to imperfect NP swab reference standard.

When we compared CN values of matched SARS-CoV-2 positive saliva and NP swab samples (as an indicator of viral load), we observed no significant difference, irrespective of the saliva volume (Figure 2). While the percentage of positive NP swabs and saliva specimens was overall similar across different study visits (Figure 3), saliva showed higher sensitivity at several timepoints and volumes. A higher percentage of saliva samples tested positive for SARS-CoV-2 at day 28 post-study enrolment in 300µL and 500µL samples compared to NP swab sample; however, the sample size was small at this time point.

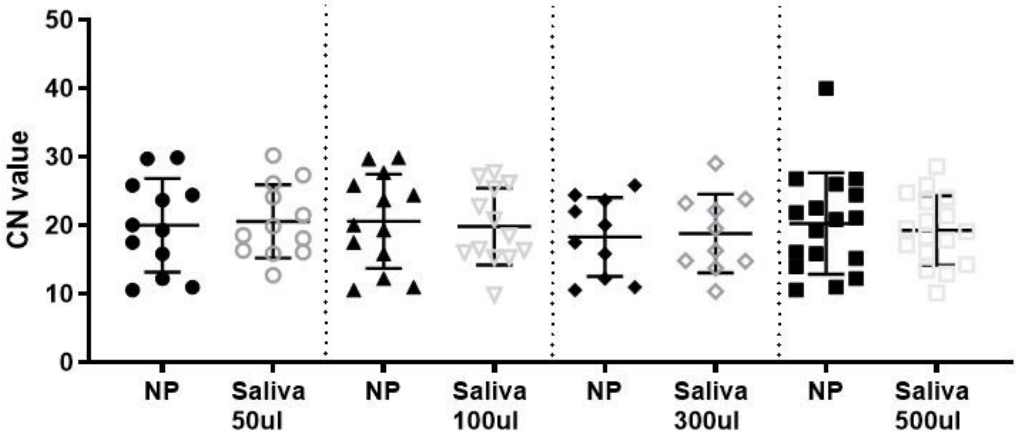

Figure 2. Differences in CN values between paired NP swab and saliva SARS-CoV-2 positive samples.

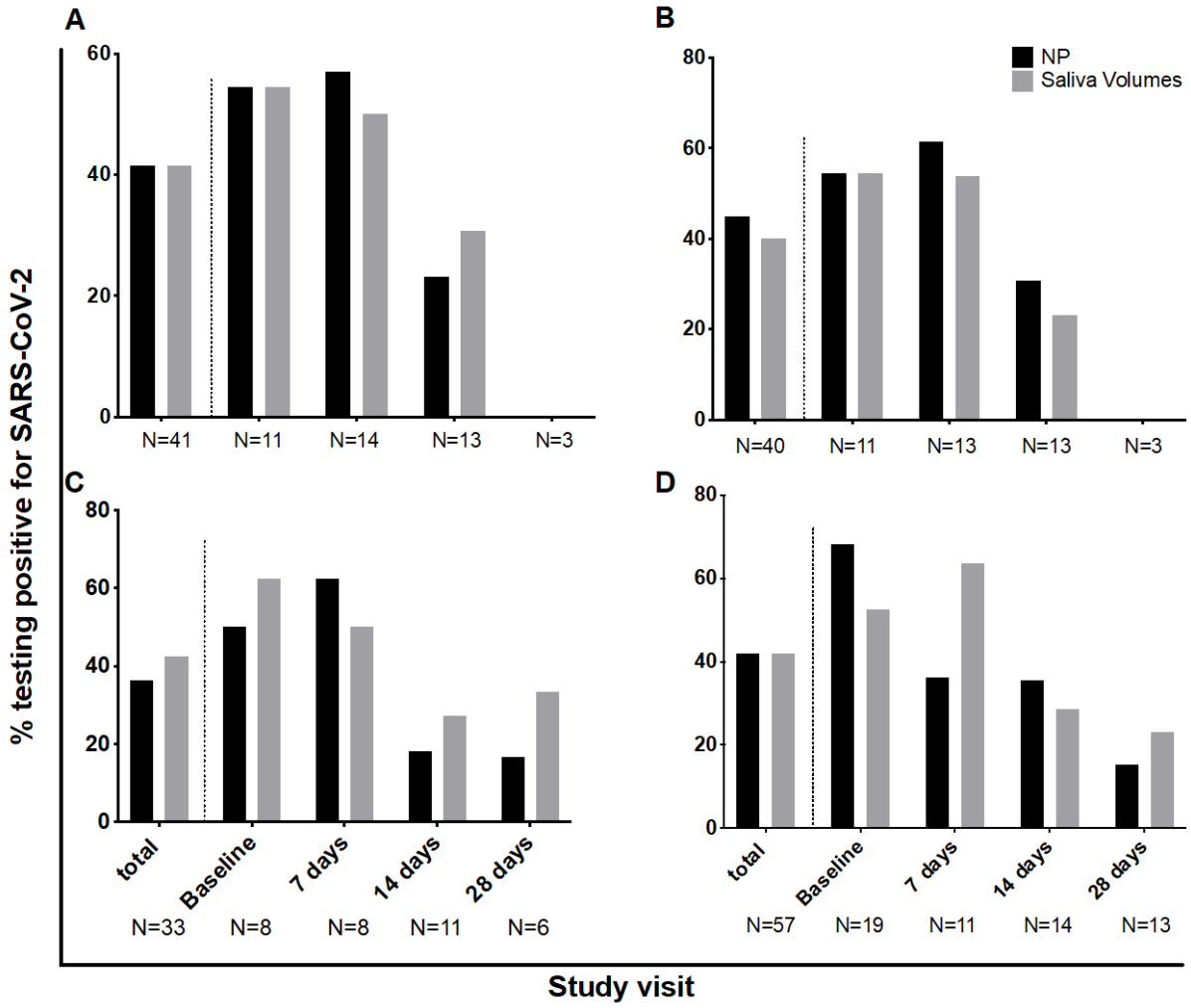

Figure 3. Precent positivity for SARS-CoV-2 in matched NP and saliva samples for total samples and across different study visits: baseline (enrolment), 7 days, 14 days, and 28 days from positive diagnosis. A) Paired NP and saliva 50µL samples. B) Paired NP and saliva 100µL samples C) Paired NP and saliva 300µL samples and D) Paired NP and saliva 500µL samples.

Discussion

The use of an optimal clinical sample is crucial for detecting and monitoring the spread of SARS-CoV-2 within the community. NP swabs have been the traditional choice, but saliva has emerged as a viable and less invasive sample for SARS-CoV-2 detection (Kojima et al., 2021, Williams et al., 2020, Pasomsub et al., 2021). In this study, we aimed to assess the utility of saliva compared to NP samples for diagnosing SARS-CoV-2 infection at different stages of the disease.

Our results demonstrate moderate to high sensitivity and specificity across different saliva volumes. The sensitivity of saliva testing increased by volume from 70.6% (50µL) to 83.3% (300µL), while the specificity decreased with increasing saliva volumes (86.4% for 100µL versus 78.8% for 500µL). Nevertheless, the overall percentage of positive results for both NP swabs and saliva specimens remained similar up to 28 days post-diagnosis. This finding aligns with those from previous studies (Lee et al., 2021, Khurshid et al., 2020, Sakanashi et al., 2021, Tahir et al., 2023). As the detection of SARS-CoV-2 in saliva was comparable to NP swabs, saliva may be a valuable alternative for identifying mild or subclinical infections. Collecting saliva is simpler, less costly, and more acceptable, especially among children and their parents, making it an attractive option for paediatric patients due to its minimally invasive nature (Azzi et al., 2020). Additionally, the safety of saliva collection by parents or adult patients eliminates the risk of exposure for healthcare workers (Sabino-Silva et al., 2020).Saliva samples would be useful for large scale surveillance programs (Marais et al., 2022). At several timepoints and volumes, the detectability of SARS-CoV-2 was slightly higher in saliva samples compared to NP swab. The implications of this on length of viral shedding and transmissibility are hard to determine due to limited sample size.

An interesting finding of this study was the comparable CN values between the nasopharyngeal sample and saliva, regardless of the volume of saliva tested. This indicates that using reduced volumes of saliva can still provide valuable information for detecting infection, monitoring its progression, evaluating intervention effectiveness, and assessing viral shedding dynamics.

Our study has some limitations that should be considered. Using a single detection system may yield different results compared to other platforms. The viscosity of saliva samples presents challenges for automated dispensing systems. Alternate methods, such as throat washing with normal saline, could potentially improve yield (Guo et al., 2020, Jamal et al., 2021). Additionally, our study's small sample size and limited geographic scope may restrict the generalization of the findings.

In conclusion, our study adds to a growing body of evidence supporting saliva as a valid and reliable alternative for diagnosing SARS-CoV-2 in patients across all stages of infection. Saliva could be considered a preferred sample in patients, particularly in challenging situations where obtaining proper nasopharyngeal swab sample is difficult, such as with children. However, a negative saliva result in symptomatic patients may warrant retesting with a different sample type to improve detection rates and reduce false negatives. Further testing, validation, and implementation of saliva-based SARS-CoV-2

diagnostics on different platforms are warranted and, if successful, will play an important role in managing COVID-19 and future pandemics.

Declarations:

Ethics approvals and consent to participate: All study participants provided a consent to participate, and the study was approved by the KwaZulu-Natal Biomedical Research Ethics Committee (BREC approval Reference Numbers: BREC/00001195/2020; BREC/00003106/2021 and BREC/00003902/ 2022). Research presented here was performed in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki

Consent for publication: Not applicable

Availability of data and materials: The de-identified patient-level data can be accessed by contacting the corresponding author with a detailed description of the research question.

Competing interest: The authors declare no conflict of interests.

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Authors contributions

Designed the study: NS, AS, SN;
Performed the experiments: NS, SN, ZK, AS;
Analysed the data: AS, LL, NS, SN;
Wrote the first draft of the paper: NS, AS, SN;
Collected specimens and clinical data: RHM, KN;
Supervised clinical and/or experimental aspects of the study: AS, CB, RHM, NS, ZK, SN, KN, ABMK, SAK, QAK.
All listed authors contributed to the editing and finalisation of the manuscript.

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Supplementary Table 1. Comparison of Real-Time RT-PCR results of paired saliva and nasopharyngeal swab samples with respective sensitivity and specificity.

n = 171; All volume								
		Nasopharyngeal swab				*Estimate*	*Lower 95% CI*	*Upper 95% CI*
		Positive	Negative	Total	*sensitivity*	73.2%	62.0%	82.2%
Saliva	Positive	52	19	71	*specificity*	81.0%	72.2%	87.5%
	Negative	19	81	100	*ppv*	73.2%	62.0%	82.2%
	Total	71	100	171	*npv*	81.0%	72.2%	87.5%

Supplementary Table 2. Comparison of Real-Time PCR results of paired saliva and nasopharyngeal swab samples at different volumes

n = 41; volume = 50µL								
		Nasopharyngeal swab				*Estimate*	*Lower 95% CI*	*Upper 95% CI*
		Positive	Negative	Total	*sensitivity*	70.6%	46.9%	86.7%
Saliva	Positive	12	5	17	*specificity*	79.2%	59.5%	90.8%
	Negative	5	19	24	*ppv*	70.6%	46.9%	86.7%
	Total	17	24	41	*npv*	79.2%	59.5%	90.8%
n = 40; volume = 100uL								
		Nasopharyngeal swab				*Estimate*	*Lower 95% CI*	*Upper 95% CI*
		Positive	Negative	Total	*sensitivity*	72.2%	49.1%	87.5%
Saliva	Positive	13	3	16	*specificity*	86.4%	66.7%	95.3%
	Negative	5	19	24	*ppv*	81.3%	57.0%	93.4%
	Total	18	22	40	*npv*	79.2%	59.5%	90.8%
n = 33; volume = 300µL								
		Nasopharyngeal swab				*Estimate*	*Lower 95% CI*	*Upper 95% CI*
		Positive	Negative	Total	*sensitivity*	83.3%	55.2%	95.3%
Sa li	Positive	10	4	14	*specificity*	81.0%	60.0%	92.3%

	Negative	2	17	19	*ppv*	71.4%	45.4%	88.3%
	Total	12	21	33	*npv*	89.5%	68.6%	97.1%
n = 57; volume = 500µL								
		Nasopharyngeal swab				*Estimate*	*Lower 95% CI*	*Upper 95% CI*
		Positive	Negative	Total	*sensitivity*	70.8%	50.8%	85.1%
Saliva	Positive	17	7	24	*specificity*	78.8%	62.3%	89.3%
	Negative	7	26	33	*ppv*	70.8%	50.8%	85.1%
	Total	24	33	57	*npv*	78.8%	62.3%	89.3%

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CHAPTER 7

EVALUATION OF COVID-19 ANTIBODY TESTS

CHAPTER 7: EVALUATION OF COVID-19 ANTIBODY TESTS

Chapter 7 addresses the third objective, evaluating SARS-CoV-2 antibody tests in COVID-19 mono and co-infected individuals. The chapter describes the laboratory evaluation of one point of care immunoassay and a multiplexed bead assay that targets various SARS-CoV-2 antigens against a routinely used diagnostic platform that provides qualitative and semi-quantitative data. Samples were collected during the first and second wave of infections in South Africa. Fresh plasma samples were used to perform the POC lateral flow test while stored plasma was used for the other tests.

The manuscript "*Evaluation of COVID-19 antibody tests in KwaZulu-Natal, South Africa*" is in preparation.

7.1. EVALUATION OF COVID-19 ANTIBODY TESTS IN KWAZULU-NATAL, SOUTH AFRICA

[Manuscript in preparation]

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Abstract

Background: The global impact of the SARS-CoV-2 pandemic has necessitated accurate and widespread diagnostic tools to assess infection and immunity. RT-PCR remains the gold standard, yet its limitations prompt the exploration of serological tests for a comprehensive understanding of the prevalence and host immune response. This study evaluated one point of care immunoassay and a multiplexed bead assay that targets various SARS-CoV-2 antigens against a routinely used diagnostic platform that provides qualitative and semi-quantitative data.

Methods: Whole blood samples were collected from 106 enrolled participants at enrolment, days 7, 14, 21 and months 3, 6 and 9. A total of 374 samples were tested on the Orient Gene Biotech COVID-19 IgG/IgM Rapid Test (Orient Gene Rapid Test) and Abbott SARS-CoV-2 IgG-II (quant) (Abbott IgG-S). The study was expanded to test 303 samples on the MILLIPLEX® MAP Kit SARS-CoV-2 Antigen Panel 1 IgG (Milliplex® IgG) specific for the Spike SI (SI), Spike S2 (S2), Receptor Binding Domain (RBD) and nucleocapsid (N) assays and the Abbott SARS-CoV-2 IgG-II (quant) (Abbott IgG-S) and Abbott Architect IgG (Qual) (Abbott IgG-N). The results of Orient Gene Rapid test and Milliplex® IgG assay were compared to the relevant Abbott assay results.

Results: An overall detectability of 79.9% was observed for the Orient Gene Rapid Test, while the detectability of the Abbott IgG-S was 79.4%. The overall sensitivity and specificity for the Orient Gene rapid test when compared to the Abbott IgG-S assay, was 93.27% (95% CI: 89.83-95.60) and 71.43% (95% CI: 60.51-80.31) respectively. For the spike protein, Milliplex® IgG-S2 demonstrated the highest detectability at 89.4%, the Milliplex® IgG-RBD 80.1%, Abbott IgG-S at 78.2% and the Milliplex® IgG-S1 58.7%. The overall detectability of SARS-CoV-2 N specific antibodies by Milliplex® IgG-N was 79.9% and 67.9% by the Abbott IgG-N.

Conclusion: The study demonstrated that the POC Orient Gene Rapid Test had comparable sensitivity, specificity, and detectability to the Abbott IgG-S while the Milliplex® IgG-S2 demonstrated higher detectability than the Abbott IgG-S test. The Milliplex® IgG-N outperformed the Abbott IgG-N platform. The study's outcome contributes valuable insight into the comparative performance of different serological tests. Overall, the study underscores the significance of rigorous serological test evaluations in enhancing our understanding of SARS-CoV-2 immunity and optimizing diagnostic approaches in the ongoing battle against the COVID-19 pandemic.

1. Introduction:

Since the first case of SARS-CoV-2 infection was announced to the world in late December 2019, we have seen the virus spread to every part of the world, with approximately 772 million infections reported and 6.9 million deaths (WHOb, 2020). Rapid, reliable diagnoses of the infected population/individuals is critical to containing the spread of the virus and to understand the extent of exposure. Real Time reverse transcription polymerase chain reaction (RT-PCR) remains the gold standard for the diagnosis of acutely infected individuals. However, that is dependent on the individual seeking testing, the sample being collected at the most acute stage of infection and the sample being collected correctly. The proportion of individuals who are symptomatic but not seeking testing; or who are asymptomatic but still transmitting the infection is evident and is estimated to be more than 30% of the population (Findeisen et al., 2021, Mizumoto et al., 2020, Sandri et al., 2020, Pollán et al., 2020). Furthermore, with patients seeking testing and care, it is estimated that there is approximately a 30% false negative RT-PCR reported (Qin et al., 2020, Li et al., 2020, Wikramaratna et al., 2020). The reason for this is multi-faceted and includes poor sample collection or delayed sample collection post the onset of symptoms. Widely available, reliable antibody detection in conjunction with adequate systems to document the tests performed and link them to the RT-PCR results would strengthen systems and would enable more accurate estimates of SARS-CoV-2 prevalence and incidence. In response to this critical need, numerous diagnostics tests were rapidly developed and commercialised. These included RT-PCR, rapid antigen tests and serological tests. Rigorous, comparative performance data are critical to inform clinical care and public heath responses.

Upon SARS-CoV-2 infection, B lymphocytes initiate an early humoral response directed towards the nucleocapsid (N) protein. Subsequently, antibodies targeting the spike (S) protein become detectable approximately 4-8 days post the onset of initial symptoms SARS-CoV-2-specific immunoglobulin M (IgM) antibodies manifest within 0-3 days following exposure, reaching peak levels around day 7. Immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies follow, emerging several days post IgM onset. Importantly, IgA and IgG exhibit sustained levels without a decline to undetectable thresholds, presumptively conferring protective immunity for a duration extending up to 6-9 months (Azkur et al., 2020). Levels of IgG can increase more than four-fold a within a week (Dong et al., 2020, Du et al., 2009, Long et al., 2020, Guevara-Hoyer et al., 2021, Zhao et al., 2020, Wölfel et al., 2020). As such the development of serological tests for SARS-CoV-2-specific antibodies allows for the detection of SARS-CoV-2 infection.

The use of serological tests offers several advantages which include i) population based seroprevalence and incidence studies to establish the exposure of the local population to the SARS-CoV-2; ii) establishing the percentage of the population that have natural immunity as opposed to vaccine induced immunity and the longevity of both; iii) characterisation of antibody responses in infected individuals; iv) aiding in identifying individuals who may have been exposed to SARS-CoV-2 infection and may be asymptomatic or experiencing mild disease; v) diagnosis of patients who seek medical attention more than seven days after the onset of symptoms when RT-PCR may miss diagnosis vi) where RT-PCR specimens cannot be obtained and: vii) tracking the recovery of COVID-19 patients. Estimation of the true burden of SARS-CoV-2 infection in the population is important as there was a large proportion that were asymptomatic or did not present for testing. In addition, understanding what percentage of the population had acquired robust immune response from natural infection as opposed to vaccine induced immunity will inform future public health policies for future outbreaks and other infectious diseases. We evaluated the sensitivities, specificities and correlations between a point of care (POC) immunoassay Orient Gene lateral flow assay (LFA) and a multiplexed bead assay (Milliplex®) used in research settings, relative to an approved diagnostic test- the Abbott Architect IgG (qual) and Abbott SARS-CoV-2 IgG-II (quant) tests in patients with confirmed SARS-CoV-2 infection.

2. Materials and Methods:

2.1. Study design, setting and population.

Participants were recruited from government facilities adjacent to CAPRISA's research facilities in Durban, South Africa, between July 2020 and January 2021 and followed up until Dec 2021. Participants accessing routine health care services were offered SARS-CoV-2 RT-PCR testing irrespective of the presence of symptoms. Participants with positive RT-PCR results were offered enrolment into the study. Stable, ambulant adults ≥ 18 years of age, with a confirmed COVID-19 RT-PCR test result, who were willing to participate in the study and able to provide written informed consent were eligible for study participation. Participants were enrolled within three days of COVID-19/SARS-CoV-2 screening and were then followed up at days 7, 14, 28 and months 3, 6, and 9 post screening visits, at which time blood samples were collected. Adherence to National COVID-19 isolation regulations was maintained with enrolment visits and relevant follow-up visits conducted at the participant's homes or isolation facility. All post-isolation study follow-up visits were conducted at the CAPRISA Springfield Clinical Research Site.

2.2. Ethical Considerations

The study was approved by the KwaZulu-Natal Biomedical Research Ethics Committee (BREC approval No: BREC/00001195/2020 and BREC/00003106/2021). All confirmed COVID-19 cases were reported to the South African National Department of Health using the National Medical Conditions (NMC) surveillance system.

2.3. Sample collection and processing.

Whole blood samples were collected from n=106 enrolled participants at enrolment, days 7, 14, 28 and months 3, 6, and 9. Samples from all available timepoints were used in this study. The blood samples were centrifuged at 1,200 rpm for 10 minutes, plasma was aliquoted. Orient Gene Biotech Rapid test was performed in the laboratory on spun down plasma samples. Remainder plasma was stored at -85ºC until use. As negative controls, plasma samples previously stored at the laboratory prior to December 2019 were used for the Milliplex® assay (Millipore, MA).

2.4. SARS-CoV-2 testing by RT-PCR

Nasopharyngeal swabs for SARS-CoV-2 PCR were collected from enrolled participants. The swabs were resuspended in 2mL viral transport media (VTM). The Abbott Real Ti*m*e SARS-CoV-2 (Des Plaines, IL) test was performed on the m2000 system with a m2000sp sample preparation unit and m2000rt amplification and detection unit. Viral RNA was isolated and amplified (targeting sequences in the SARS-CoV-2 *RdRp* and *N* genes of the SARS-CoV-2 genome). Samples with a cycle threshold (Ct) <40 was indicative of the presence of SARS-CoV-2 RNA.

2.5. Antibody tests:

Detection of SARS-CoV-2 antibodies was conducted using the following tests**: Table 1:**

Test name	Anti-SARS-CoV-2 Antibody Isotype	Type of Serological Test	Antigenic Region
Orient Gene Biotech rapid test COVID-19 IgG/IgM kit (Orient Gene Rapid Test)	IgG/IgM	Lateral Flow Assay (LFA)	N
MILLIPLEX® MAP Kit SARS-CoV-2 Antigen Panel 1 IgG (Milliplex® IgG)	IgG	Multiplexed bead assay	Spike S1 & S2, RBD, N
Abbott Architect IgG (Qual) (Abbott IgG -N)	IgG	Chemiluminescent immunoassay	N
Abbott SARS-CoV-2 IgG-II (quant) (Abbott IgG-S)	IgG	Chemiluminescent immunoassay	S

2.5.1. **Orient Gene Biotech rapid test COVID-19 IgG/IgM kit** (Orient Gene Rapid Test)***:*** Plasma samples were tested on the Orient Gene Biotech COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang, Huzhoum China), a lateral flow test (LFA), following the manufacturer's instructions.

2.5.2. **MILLIPLEX® MAP Kit SARS-CoV-2 Antigen Panel 1 IgG** (Milliplex® IgG): IgG antibody levels against SARS-CoV-2 spike protein subunits S1 and S2, RBD and N were measured in duplicate plasma samples from n=106 participants using the novel MILLIPLEX® SARS-CoV-2 Antigen Panel 1 IgG (Millipore, MA). The kits were used in accordance with manufacturer's instructions. This panel is designed to measure antibodies by median fluorescent intensity (MFI). Briefly, plasma samples were diluted 1:100 in assay buffer. Then 25µL of assay buffer was added to each sample well followed by 25µL of each diluted sample and 25µL assay buffer was added to account for background and 25µL of the vortexed and thoroughly mixed beads were added to each well. The plate was incubated for 2 hours at room temperature with constant shaking. Thereafter, 50µL of phycoerythrin-anti-human immunoglobulin was added to each well and incubated 90 minutes at RT with constant shaking. Plates were washed three more times after which 150µL sheath fluid was added to each well, the plate was then shaken at RT for 5 minutes. The plates were read on a Bio-Rad Laboratories (Hercules CA) Bio-Plex ® 200 suspension array system.

2.5.3. **Abbott Architect IgG (Qual)** (Abbott IgG-N) and **Abbott SARS-CoV-2 IgG-II (Quant)** (Abbott IgG-S): Plasma samples were run on the Abbott Architect instrument using the Abbott Architect SARS-CoV-2 IgG assay (Abbott Diagnostics, Abbott Park Illinois, United States) and Abbott Architect SARS-CoV-2 IgG-II (Abbott Laboratories, Sligo, Ireland), following manufacturer's instructions. Both tests are chemiluminescent microparticle immunoassay (CMIA) for detection of IgG in human plasma with the Abbott Architect IgG against the SARS-CoV-2 N protein and Abbott SARS-CoV-2 IgG-II for the SARS-CoV-2 S protein. The assay requires a minimum of 100µL of plasma. Depending on the kit used, qualitative and quantitative results and index values reported by the instrument were used in analysis.

2.6. Statistical analysis.

GraphPad Prism version 8.0.0 (GraphPad software, La Jolla, CA), SPSS version 27 and SAS version 9.4 were used to perform statistical analysis. Test performance characteristics [sensitivity, specificity, positive predicative value (PPV) and negative predicative value (NPV)] were calculated in reference to Abbott SARS-CoV-2 IgG-II- N or S test results, depending on the antigen. Wilson's score method was used to calculate the 95% confidence intervals to assess the level of uncertainty induced by sample size.

3. Results:

3.1. Study cohort description.

The evaluations were performed on 106 participants **(Table 2).** The majority of the participants were female (55.7%), with the median age of 45.5 [interquartile range (IQR) 33-57]. Participants presented around 5 (IQR 3-7.5) days post symptom onset for screening. Overall, 62.3% of the participants had mild symptoms, 11.3% had moderate symptoms and 26.4% were asymptomatic. The majority, 94.4%, presented oxygen saturation levels ≥ 95%. Symptoms reported at baseline were mainly cough (64.2%; 68/106), fatigue (52.8%; 56/106), shortness of breath (44.3%; 47/106), myalgia (40.6%; 43/106), sore throat (39.6%; 42/106), loss of taste (39.6%; 42/106) and loss of smell (39.6%; 42/106). The median cycle threshold of SARS-Cov-2 (RT-PCR) at screening was 20.2 (IQR 15.1-24.7). Of the subset that were HIV positive 77.8% (21/30) had HIV viral loads <40 copies/mL and 73% (22/30) were on 1st line ART regime at study enrolment.

Table 2. Baseline (screening/enrolment) study cohort characteristics

Participant characteristics		**All (N=106)**
Age at screening, median (IQR years)		45.5 (33-57 years)
Sex, N (%)	Female	59 (55.7)
Race, N (%)	Asian	24 (22.6)
	Black	71 (67)
	Caucasian	1 (0.9)
	Mixed Race	
Occupation, N (%)	Unemployed	33 (31.1)
Alcohol use, N (%)	Yes	16 (15.1)
Cigarette use, N (%)	Yes	7 (6.6)
Current TB at screening, N (%)	Yes	3 (2.8)
History of previous TB, N (%)	Yes	12 (11.3)
HIV, N (%)	Yes	30 (28.3)
Hypertension, N (%)	Yes	16 (15.1)
Diabetes, N (%)	Yes	13 (12.3)
Asthma, N (%)	Yes	5 (4.7)

Cardiac disease, N (%)	Yes	3 (2.8)
BMI, N (%)	≥30	44 (43.1)
Days since symptom onset at screening (symptomatic only), median (IQR)		5 (3-7.5)
COVID-19 screening symptoms, N (%)	Fever	56 (52.8)
	Cough	68 (64.2)
	Sore throat	42 (39.6)
	Shortness of breath	47 (44.3)
	Myalgia	43 (40.6)
	Fatigue	56 (52.8)
	Loss of taste	42 (39.6)
	Loss of smell	32 (30.2)
	No symptoms	9 (8.5)
COVID-19 severity assessment at enrolment, N (%)	Asymptomatic	28 (26.4)
	Mild	66 (62.3)
	Moderate	12 (11.3)
	Severe	0 (0)
Temperature at screening, N (%)	≥38°C	2 (1.9)
Oxygen saturation at enrolment, N (%)	≥ 95%	85(94.4)
Cycle threshold (Ct) at screening, median (IQR)		20.2 (15.1-24.7)
ART regimen at enrolment, N (%)	1st line regimen	22 (73)
	2nd line regimen	4 (13.5)
	not on ART	4 (13.5)
HIV viral load at enrolment, N (%)	<40 copies/mL	21 (77.8)

Descriptive statistics are frequencies and percentages for categorical variables and median (IQR) for continuous ones. BMI- body mass index, ART- antiretroviral treatment.

3.2. Test performance evaluation.

3.2.1. Comparisons of Orient Gene Biotech rapid test and Abbott IgG-S

The test performance characteristics for both assays are summarized in **Figure 1** and the % sensitivity across different categories is presented in **Table 3**. A total of 374 samples were tested with both the Abbott IgG-S and the Orient Gene Rapid Test. The overall detectability of SARS-CoV-2 S specific antibodies by Orient Gene Rapid test was 79.9%, and 79.4% by Abbott IgG-S. The overall sensitivity and specificity for the Orient Gene Rapid test when compared to the Abbott IgG-S test, was 93.27% (95% CI: 89.83-95.60) and 71.43% (95% CI: 60.51-80.31) respectively (**Table 3**).

True positive	277
False positive	22
True negative	55
False negative	20
Sensitivity (%, 95% CI)	93.27 (89.83-95.60)
Specificity (%, 95% CI)	71.43 (60.51-80.31)
PPV (%, 95% CI)	92.61 (89.11-95.09)
NPV (%, 95% CI)	73.33 (62.37-82.02)

Table 3. Estimations of Orient Gene Rapid test performance on plasma in comparison to Abbott IgG-S test results.

3.2.2. Comparisons of Milliplex® IgG to Abbott IgG-S and Abbott IgG -N

A total of 303 samples produced data for both Abbott IgG-S, Abbott IgG-N and the Milliplex®-IgG. tests. The control samples from the CAPRISA 002 study collected prior to SARS-CoV-2 emergence were used to set up cut off values for positive samples (**Supplementary Figure 1**).

The overall detectability of SARS-CoV-2 Spike specific antibodies was 78.2% for the Abbott IgG-S, 58.7% for the Milliplex® IgG-S1, 89.4% for the Milliplex® IgG-S2 was and 80.1% for Milliplex® IgG-RBD. The overall detectability of SARS-CoV-2-N specific antibodies was 67.9% by Abbott IgG-N and 79.9%.by Milliplex® IgG-N (Figure 2).

When compared to the Abbott IgG-S test, the overall sensitivity and specificity for of the Milliplex® IgG-S was 65.82% (95% CI: 59.57-71.57) and 65.63% (95% CI:53.40-79.08) respectively. The

sensitivity and specificity of the Milliplex® IgG-S2 test was 94.12% (95% CI: 90.37-96.46) and 26.56% (95% CI: 17.30-38.48); Milliplex® IgG-RBD was 87.82% (95% CI: 83.05-91.38) and 48.44% (95% CI:36.63-60.42). When compared to the Abbott IgG-N test the Milliplex® IgG-N had a sensitivity of 88.83% (95% CI: 83.80-92.44) and specificity of 39.18% (95% CI:30.05-49.12) (Table 4).

There was a moderate significant correlation between Abbott IgG-S antibody levels and Milliplex® IgG-S1 (r=0.4490, p<0.0001), Milliplex® IgG-S2 (r=0.3239, p<0.0001) and Milliplex® IgG-RBD (r=0.4406, p<0.0001); (**Figure 3**).

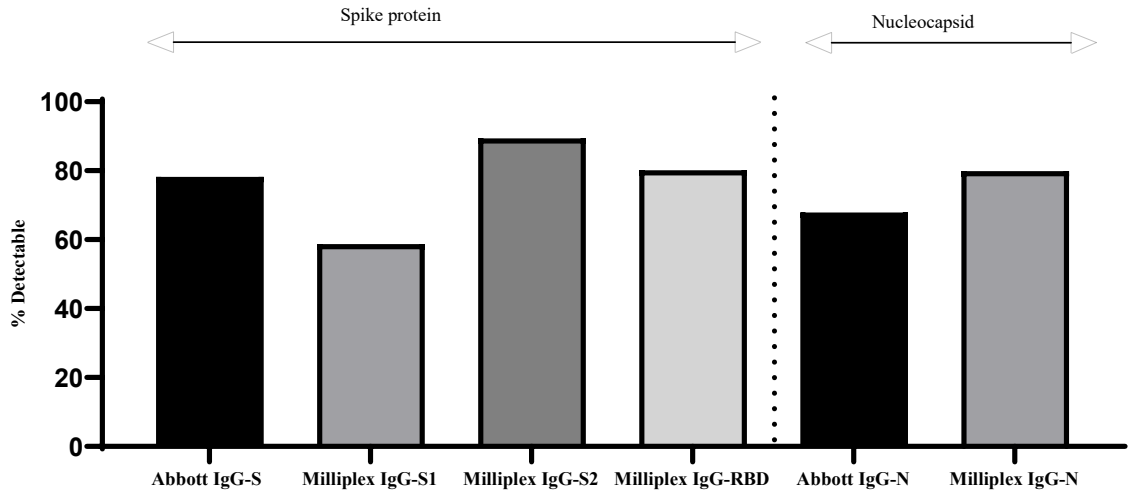

Figure 2. Precent detectability of SARS-CoV-2 S/N protein specific IgG in 303 tested specimens in Abbott and Milliplex® tests

Table 4. Estimations of test performance for MILLIPLEX® MAP Kit SARS-CoV-2 Antigen Panel 1 IgG (Milliplex® IgG) in comparisons to Abbott SARS-CoV-2 IgG-II ((Abbott IgG-S)

	MILLIPLEX® MAP Kit SARS-CoV-2 Antigen Panel 1 IgG (Milliplex® IgG)			
SARSR-CoV-2 antigen	**Milliplex® IgG-S1**	**Milliplex® IgG-S2**	**Milliplex® IgG-RBD**	**Milliplex® IgG-N**
True positive	156	224	209	183
False positive	22	47	33	59
True negative	42	17	31	38
False negative	81	14	29	23
Sensitivity (%, 95% CI)	65.82 (59.57-71.57)	94.12 (90.37-96.46)	87.82 (83.05-91.38)	88.83 (83.80-92.44)
Specificity (%, 95% CI)	65.63 (53.40-76.08)	26.56 (17.30-38.48)	48.44 (36.63-60.42)	39.18 (30.05-49.12)
PPV (%, 95% CI)	87.64 (82.00-91.69)	82.66 (77.70-86.70)	86.36 (81.47-90.12)	75.62 (69.84-80.60)
NPV (%, 95% CI)	34.15 (26.36-42.89)	54.85 (37.77-70.84)	51.67 (39.31-63.82)	62.30 (49.75-73.39

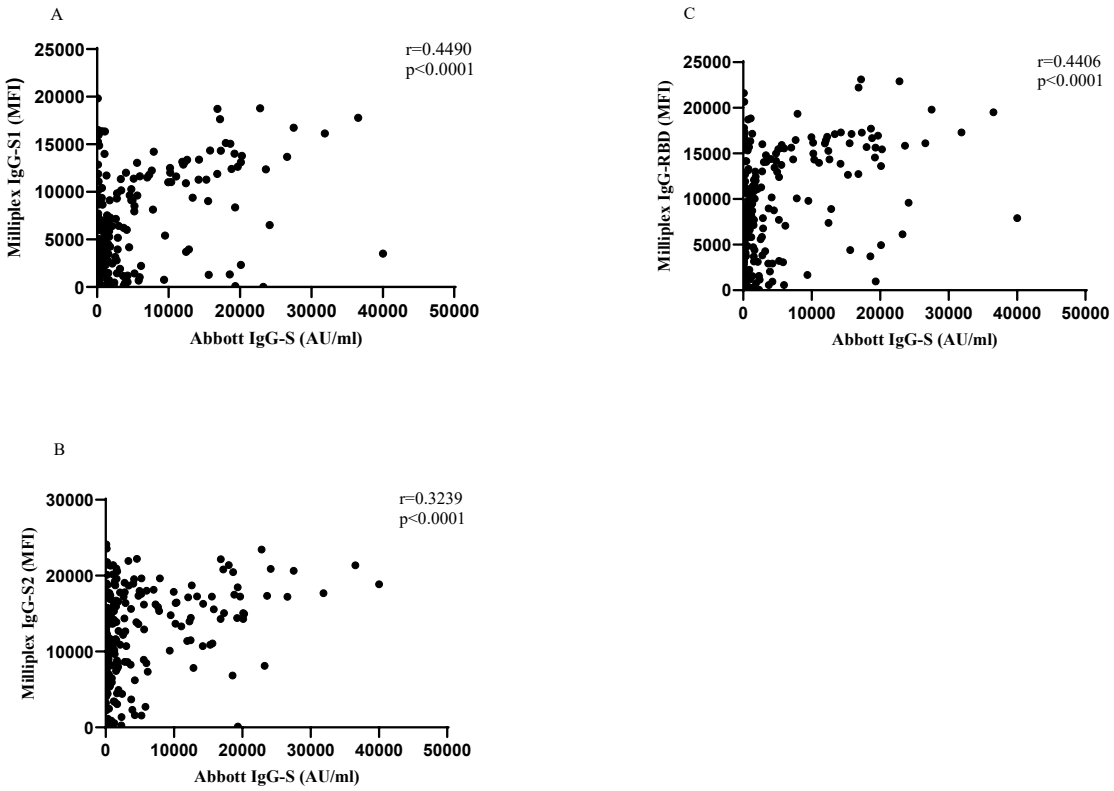

Figure 3. Correlation between Abbott IgG-S levels (AU/mL) and Milliplex® IgG S1, S2 and RBD MFI in Abbott IgG-S positive samples

4. Discussion

Accurate serological tests to detect antibodies to SARS-CoV-2 are needed to characterise the true prevalence and epidemiology of the SARS-CoV-2 infections. SARS-CoV-2 antibody testing is an established clinical diagnostic used to assess seroprevalence. Given the rate of asymptomatic infections within our communities, the roll out and availability of vaccines through large global vaccination campaigns, there is a need for the availability of sensitive and specific antibody tests that are robust and can test for both vaccines induced immunity and natural immunity from SARS-CoV-2 infection. This will result in eliminating the false negative SARS-CoV-2 diagnosis, provide accurate prevalence data, and inform future public health strategies and epidemiology studies.

This study evaluated one point of care (POC) test and one research multiplex bead test in comparison to the SAPHRA approved test in patients with diagnosed with SARS-CoV-2 infection.

Antibody responses play a crucial role in understanding the immune responses to SARS-CoV-2 for diagnosis. The temporal sequence of antibody appearance, with IgM emerging early followed by IgG, particularly against the N and S proteins, provides a valuable window into the progression of the immune response. The S protein's sub-domains, S1 and RBD, have been identified as key targets for antibody recognition.

The POC test, the Orient Gene rapid test LFA demonstrated comparable sensitivity and specificity to the reference platform standardly used for diagnostics - the Abbott IgG-S. This lateral flow immunoassay, despite its simplicity, exhibited robust performance, and highly comparable to the sensitivity of the Abbott IgG-S. The Abbott IgG-S sensitivity and specificity was reported to be between 82.4%-90.5% when samples were tested 10 days post symptom onset (Bryan et al., 2020). This finding underscores the potential utility of rapid tests in complementing diagnostic efforts, especially in resource limited setting or in large field studies where access to specialized testing platforms such as the Abbott system may be limited.

When comparing detectability and performance of the Abbott IgG-S, Milliplex® IgG-S1, IgG-S2 and IgG-RBD, we found that the Milliplex® IgG-S2 test displayed the highest detectability, surpassing the commercially available Abbott IgG-S test. Similarly for anti-N protein antibody detection, the Milliplex® IgG-N outperformed the Abbott IgG-N, highlighting higher detectability with this platform. These findings suggest that the Milliplex® IgG-N assay may provide a more sensitive means of identifying antibodies against both the Spike and nucleocapsid protein. This is in contradiction to previous published literature that reported the Abbott IgG sensitivity was ranging from 92.5% to 100% with specificity ranging from 99.6% to 100% (Bryan et al., 2020, Patel et al., 2021). This could be

attributed to these studies not being performed on the African continent or South Africa where they may be immunological factors that contribute to the test performance differently. In the samples concordant for both Abbott IgG -S and Milliplex® IgG (S1, S2 and RBD) tests, we observed a moderate but significant correlation between Abbott IgG-S S1, S2 and RBD.

The study's outcome contributes valuable insight into the comparative performance of different serological tests, specifically their accuracy and potential advantages. The limitations of the study include that it was limited to a specific population and the potential heterogeneity in antibody responses among diverse groups should be acknowledged. Further research is required to validate these findings across diverse populations and to explore the longitudinal dynamics of antibody responses over time. Furthermore, we did not account for the contribution of pre-existing HIV infection as a potential confounder for the antibody responses observed. This is due to us not be successful in enrolling adequate number of HIV infected individuals into this study. Furthermore, as all patients enrolled in the study had confirmed SARS-CoV-2 infection, the obtained specificity values are low and do not represent an accurate measure. Additionally, we did not have access to the circulating SARS-CoV-2 variant information, and this could play an important role in antibody test performance based on the affinity for the antigen being detected and the viral mutations. Finally, the study was conducted prior to SARS-CoV-2 vaccine rollout in South Africa so the antibody platforms tested samples that only had humoral responses induced through natural infection and not vaccine induced immunity. Overall, the study underscores the significance of rigorous serological test evaluations in further understanding of SARS-CoV-2 immunity and optimizing diagnostic approaches in not only the ongoing battle against the COVID-19 pandemic but against other emerging infectious diseases.

Author contributions:

Designed the study: KN, AK, AS, NS, RHM

Performed the experiments: NS; AS, TM

Analysed the data: AS, NS, LL

Wrote the first draft of the paper: AS, NS.

Collected specimens and clinical data: RHM, KN

Supervised clinical and/or experimental aspects of the study: AS, NS, DA, GL, RL, SN, KN, AK, RHM, SAK, QAK.

All authors contributed to the editing and finalisation of the manuscript.

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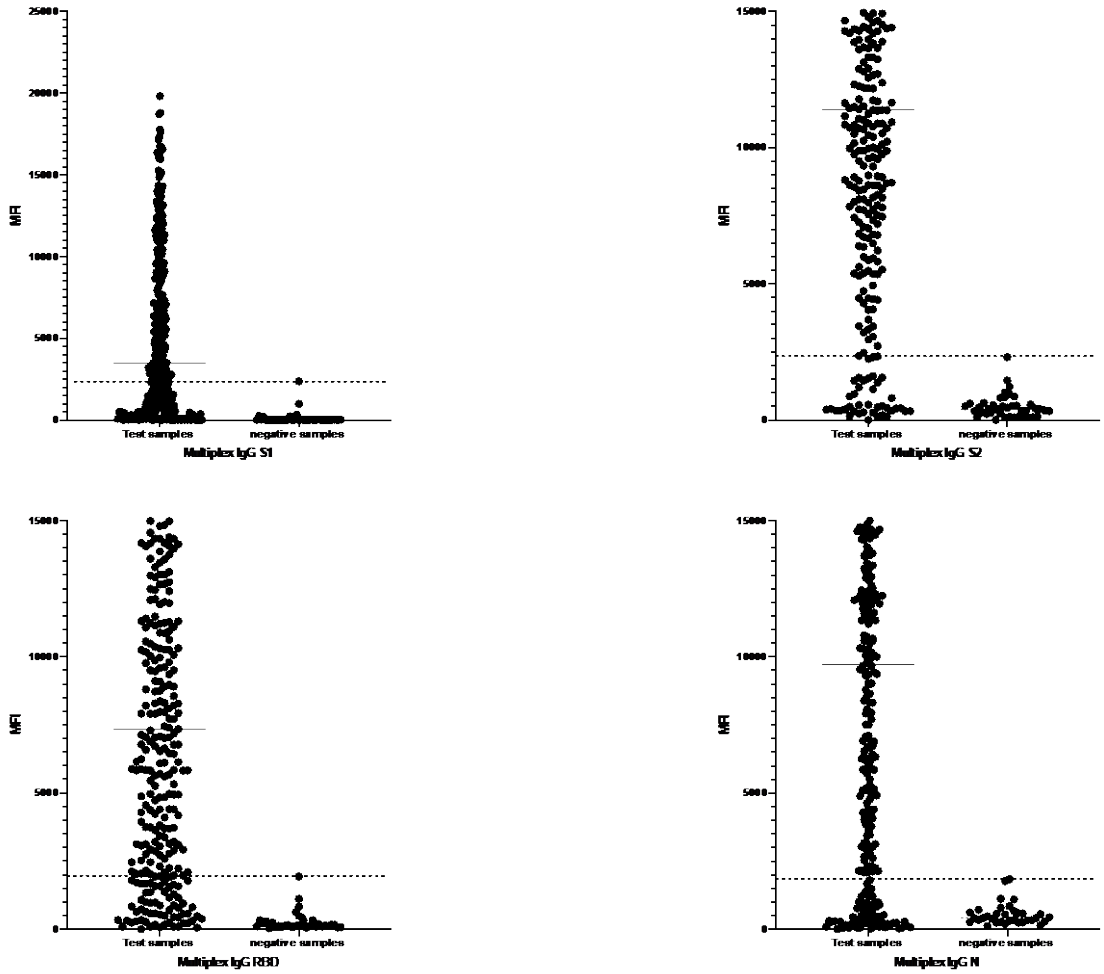

Sup. Figure 1. MFI values for test samples (CAPRISA 228 cohort) with confirmed SARS-CoV-2 infection and control samples (CAPRISA 002 cohort) collected prior to SARS-CoV-2 emergence.

CHAPTER 8

SUMMARY AND DISCUSSION OF KEY FINDINGS, CONCLUSION AND RECOMMENDATIONS

CHAPTER 8: SUMMARY AND DISCUSSION OF KEY FINDINGS, CONCLUSION AND RECOMMENDATIONS

The final Chapter summarizes and discusses the key findings from the series of analysis undertaken to achieve the objectives.

8.1. SUMMARY OF KEY FINDINGS

- **Performance of diagnostic tests:** The findings from the scoping review showed that no single diagnostic test simultaneously achieved high sensitivity for SARS-CoV-2 in South Africa. The sensitivity of the tests was highly dependent on the timing of sample collection, with antigen detection tests performing better when samples were collected earlier in the course of infection, while antibody detection tests showed better sensitivity when samples were obtained during later stages of infection. The review demonstrated the limited number of test evaluations that have been conducted in South Africa, with majority of studies taking place in Gauteng and the Western Cape.

- **Evaluation of rapid antigen tests:** The evaluation of the SD Biosensor and Abbott Panbio™ rapid antigen tests demonstrated that these tests had low sensitivity if performed in the laboratory settings with delay in sample processing. The tests performed well in the field evaluations depending on the stage of infection and viral load. The sensitivity of these tests improved for samples with lower Ct/CN values (< 20) and within the first week of symptom onset, highlighting the utility of these tests especially in outbreak settings.

- **Impact of variants:** The assessment of diagnostic tests in identifying emerging SARS-CoV-2 variants, showed that the tests maintained their performance irrespective of new variants, with sensitivity levels remaining relatively high, especially for Omicron sub-lineage BA.4 and BA.5.

- **Alternate sample collection:** Testing of saliva as an alternative sample to NP swabs for the diagnosis of SARS-CoV-2 found no significant differences in test performance, regardless of the saliva volume collected. These findings suggest that saliva is a viable alternative for SARS-CoV-2 diagnosis and could potentially address issues related to patient discomfort.

- **SARS-CoV-2 antibody tests:** The evaluation of Multiplex bead assay and POC immunoassay showed overall a similar degree of antibody detectability between tested assays. Further research is

needed to assess the impact of SARS-CoV-2 variants on antibody assay performance, especially for the assays targeting the spike antigen.

8.2. STRENGTHS AND LIMITATIONS

Overall, a key strength of these studies was the timing of the study as these coincided with the occurrence of several waves of SARS-CoV-2 infections, allowing for the rapid enrolment of participants and collection of clinical samples. However, as the number of infections increased, the simultaneous stringent "lock-down" rules to mitigate onward transmission posed a severe limitation as it hindered follow-up visits that needed to be complete as part of the study related procedures.

8.2.1. Strengths

The key strength of the studies was that they took place during the COVID-19 pandemic and provided a real-life evaluation of various diagnostic tests and samples. The performed evaluations, including different types of tests (antigen- and antibody-based), sample collection methods (NP swabs and saliva), and their performance across different stages of the infection all contributed and enhanced our understanding of SARS-CoV-2 and helped to guide the South African COVID-19 response. The studies' focus on the performance of diagnostic tests in the presence of emerging SARS-CoV-2 variants, such as the Omicron variant BA.4 and BA.5, is a notable strength, providing evidence that these variants could also be detected successfully with the existing available rapid antigen kits. This information is crucial as rapid antigen testing is an important tool for managing the pandemic. The investigation into using saliva as an alternative to NP swabs addresses practical issues and could improve patient acceptability and reduce the risk of transmission.

8.2.2. Limitations

A key limitation was the limited sample sizes in some of the evaluations, such as in the Xpert® Xpress SARS-CoV-2 analysis. The studies had enrolled participants during the SARS-CoV-2 waves of infection in South Africa and only managed to enroll a limited sample of participants that were co-infected to SARS-CoV-2 and HIV or TB. Consequently, we were not able to validate the diagnostics tests evaluated in this thesis in the context of SARS-CoV-2 combined with HIV and/or TB. As the studies were undertaken in the province of KwaZulu-Natal this limits generalizability to other settings. The performance of diagnostic tests could vary based on factors such as local prevalence, healthcare infrastructure and population demographics. Furthermore, the studies were conducted during the various waves of infections and despite South Africa being signatory to the WHO Solidarity Trial for equitable access to SARS-CoV-2 vaccines including access to diagnostics, there were consistent

bottlenecks in the availability of test kits and reagents. Combined with participants' hesitancy and reluctance to test for SARS-CoV-2 due to the stringent quarantining and isolation requirements, societal stigma was pervasive, and many individuals opted not to come forward to test for SARS-CoV-2.

An important limitation was that for all the evaluations, participant vaccination status including the presence and timing of previous natural infections was not known. Thus, the impact of previous immunity on SARS-CoV-2 viral load and the performance of the diagnostic test could not be determined. While none of the evaluated tests satisfied the WHO requirements for the > 80% sensitivity in the overall sample group, they are still a valuable tool in identifying infected individuals within the first week of symptom onset and those with high viral loads and could play an important role in limiting transmission and controlling the COVID-19 pandemic.

8.3. CONCLUSIONS

Globally, COVID-19, caused by SARS-CoV-2 resulted in an unprecedented public health challenge. However, the African continent experienced an additional setback due to the disproportionately limited access to diagnostic test kits, consumables and vaccines compared to resource rich countries (Hans et al., 2021) (Duma et al., 2022). The findings of this research have provided a comprehensive understanding of the strengths and limitations of the various diagnostic tools, sample collection methods and their adaptability to emerging variants. Currently available diagnostic tests vary in levels of sensitivity and specificity, and their performance is closely related to the timing of sample collection. Extending diagnostic testing to field evaluation across populations is critical to monitor the transmission in the communities and simultaneously understand complexities of undertaking these tests in the field. Whilst NP samples had been the sample of choice, the use of saliva as an alternative sample type in our setting has shown its potential to minimising patient discomfort, reducing the risk of onward transmission while still providing reliable results.

8. 4. RECOMMENDATIONS

The following recommendations could be made based on the findings of this study:

Diversify testing strategies: To address variability in test sensitivity and specificity, it is advisable to adopt a diversified approach to testing. This can include combination of both antigen- and antibody-based tests, depending on the stage of infection and the local prevalence of the virus.

Saliva as a preferred sample collection method: Given the practicality and acceptability of saliva as an alternative to NP swabs, large scale evaluation and monitoring of saliva samples need to continue. This could improve patient compliance and the overall efficiency of testing programs.

Evaluation of tests with emerging variants: It is essential to monitor and evaluate the performance of diagnostic tests with respect to emerging variants. Ongoing surveillance and testing will be crucial as emergence of variants of concern has the potential to minimize diagnostic accuracy.

Adaption to local context: Testing strategies over time should become adaptable towards self-testing protocols to enable individuals to self-test as early as possible and limit the spread of the virus.

Enhancing training for test operators: Laboratory and field staff as key operators undertaking diagnostic testing must continue with ongoing training to minimise potential variability that may exist with testing protocols and to enhance quality assurance measures to improve the consistency and reliability of test results.

International collaboration: Networks of national and international collaboration should be established for sharing knowledge and experiences towards future pandemic preparedness.

Policy integration and translation: Recommendations from local researchers should rapidly be evaluated and integrated into public health policies.

8.5. FUTURE STUDIES

With potential epidemics and pandemics that may emerge in the future, the lessons learned in diagnostic evaluation strategies are needed to guide the scope of the diagnostic tests across different settings and populations, accounting for emerging variants and improving testing methodologies and patient uptake. Systematic comparative studies that evaluate a wide range of diagnostic tests, including newer technologies and POC tests, would provide valuable information on the strengths and limitations of different testing methodologies.

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APPENDICES

APPENDICES

APPENDIX LIST

9.1. Appendix A: Related manuscripts not included in the thesis.

9.2. Appendix B: Ethics approval letter

9.3. Appendix C: District approval

9.4. Appendix D: Informed consent forms

9.1. APPENDIX A: RELATED MANUSCRIPTS

9.1.1. Omicron BA.4/BA.5 escape neutralizing immunity elicited by BA.1 infection.

1. Khadija Khan, Farina Karim, Yashica Ganga, Mallory Berstein, Zesuliwe Jule, Kajal Reedoy, Sandile Cele, Gila Lustig, Daniel Amoako, Nicole Wolter, **Natasha Samsunder**, Aida Sivro, James Emmanual San, Jennifer Giandhari, Houriiyah Tegally, Sureshnee Pillay, Yeshnee Naidoo, Matilda Mazibuko, Yoliswa Miya, Nokuthula Ngcobo, Nitherndra Manickchund, Nombulelo Magula, Quarraisha Abdool Karim, Anne von Gottberg, Salim S. Abdool Karim, Willem Hanekom, Bernadette I. Gosnell, COMMIT-KZN Team, Richard J. Lessells, Tulio de Oliveira, Mahomed-Yunus S. Moosa & Alex Sigal. Omicron BA.4/BA.5 escape neutralizing immunity elicited by BA.1 infection. Nature Communications. August 2022 doi: 10.1038/s41467-022-32396-9.

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Omicron BA.4/BA.5 escape neutralizing immunity elicited by BA.1 infection

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SARS-CoV-2 Omicron (B.1.1.529) BA.4 and BA.5 sub-lineages, first detected in South Africa, have changes relative to Omicron BA.1 including substitutions in the spike receptor binding domain. Here we isolated live BA.4 and BA.5 viruses and measured BA.4/BA.5 neutralization elicited by BA.1 infection either in the absence or presence of previous vaccination as well as from vaccination without BA.1 infection. In BA.1-infected unvaccinated individuals, neutralization relative to BA.1 declines 7.6-fold for BA.4 and 7.5-fold for BA.5. In vaccinated individuals with subsequent BA.1 infection, neutralization relative to BA.1 decreases 3.2-fold for BA.4 and 2.6-fold for BA.5. The fold-drop versus ancestral virus neutralization in this group is 4.0-fold for BA.1, 12.9-fold for BA.4, and 10.3-fold for BA.5. In contrast, BA.4/BA.5 escape is similar to BA.1 in the absence of BA.1 elicited immunity: fold-drop relative to ancestral virus neutralization is 19.8-fold for BA.1, 19.6-fold for BA.4, and 20.9-fold for BA.5. These results show considerable escape of BA.4/BA.5 from BA.1 elicited immunity which is moderated with vaccination and may indicate that BA.4/BA.5 may have the strongest selective advantage in evading neutralization relative to BA.1 in unvaccinated, BA.1 infected individuals.

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New severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants may escape neutralizing immunity elicited by previous infection and vaccination and lead to new infection waves. Therefore, the degree to which such immune escape happens with new variants needs to be measured globally in different populations. This may be particularly informative for the region where the variant was first detected, as it may indicate the selective pressures under which the new variant evolved.

The Omicron (Pango lineage B.1.1.529) initially emerged as the BA.1 sub-lineage. BA.1 was first detected by genomic surveillance in South Africa and showed extensive immune escape[1–13]. The BA.4 and BA.5 sub-lineages, which do not differ in their spike sequence from each other, were also first detected by genomic surveillance in South Africa[14]. BA.4 and BA.5 have changes relative to the BA.1 and BA.2 sub-lineages including the L452R and F486V mutations and the R493Q reversion in the spike receptor binding domain (RBD), the domain which is likely most targeted by neutralizing antibodies[15]. BA.4 and BA.5 also differ from the BA.2 sub-lineage by a deletion of spike residues 69 and 70[16]. The L452R mutation has been reported to increase SARS-CoV-2 fusogenicity and replication in cell culture[17,18]. This mutation also occurs in the Delta variant and a mutation at spike position L452 is shared with the Omicron sub-lineage BA.2.12.1, where the substitution is L452Q[16]. The F486V mutation is located at the top of the spike receptor-binding ridge that contacts the human angiotensin converting enzyme-2 (ACE2) receptor, and is associated with escape from class 1 and class 2 RBD antibodies[19]. That is, the predominant effect of this mutation is expected to be antibody escape. Interestingly, despite its predicted ability to confer escape from neutralization, this mutation was previously rarely observed[19], possibly indicating it confers a fitness disadvantage which is compensated by other mutations in the BA.4 and BA.5 Omicron sub-lineages.

Starting in March 2022, the BA.4 and BA.5 sub-lineages led to an infection wave in South Africa which has since waned (Fig. 1a, see https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/national-covid-19-daily-report/ for source data). Excess all-cause mortality in South Africa, which was previously strongly correlated to SARS-CoV-2 infection waves, did not show a sharp increase in the BA.4/BA.5 infection wave, although excess deaths were still present (Fig. 1a, see source data at https://www.samrc.ac.za/reports/report-weekly-deaths-south-africa). While the fraction of BA.4 and BA.5 genotypes has stabilized at about three-quarters of all infections in South Africa as this is written (Fig. 1b, all data from GISAID[20]), infections with these sub-lineages are rising elsewhere, including in the US (Fig. 1b). About half of the South African population was vaccinated when BA.4 and BA.5 were first detected (Fig. 1c). Vaccination in South Africa is currently with one of two vaccines, two doses of the Pfizer BNT162b2 or one dose of the Johnson and Johnson Ad26.CoV2.S. At the time of writing about 8 million South Africans were fully vaccinated with Ad26.CoV2.S compared to about 12 million vaccinated with BNT162b2 (https://sacoronavirus.co.za/latest-vaccine-statistics/). Boosting is available in South Africa, although it was too rare in our cohort for us to investigate.

In this work, we measure the degree of escape of the BA.4 and BA.5 sub-lineages from neutralizing immunity in people previously infected with the Omicron BA.1 in South Africa and determine the effect of vaccination on immune escape using live viral isolates. We also compare immune escape of BA.1, BA.4, and BA.5 in vaccinated individuals from South Africa not infected with BA.1.

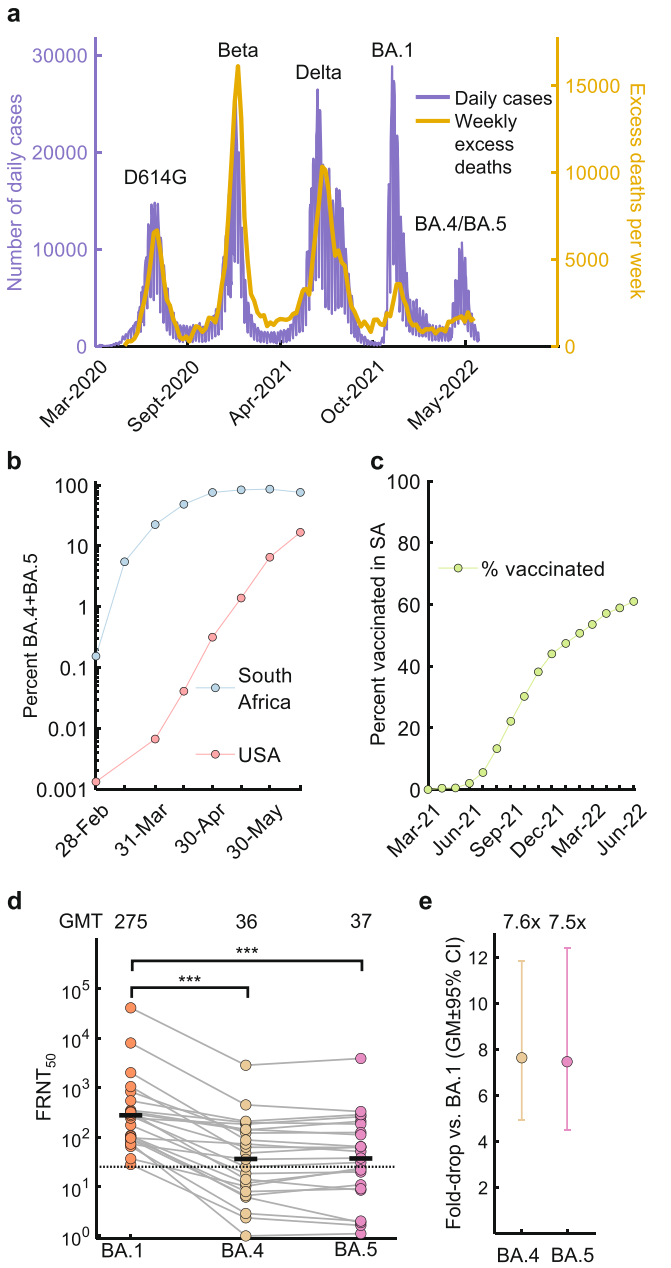

Fig. 1 | Escape of BA.4 and BA.5 from BA.1 elicited immunity in unvaccinated participants. a Daily Covid-19 cases (purple, left axis) and excess deaths (orange, right axis) in South Africa. **b** Combined fraction of BA.4 and BA.5 in South Africa and the US according to GISAID deposited sequence data. Prevalence was calculated by dividing the number of submitted BA.4 and BA.5 sequences by total submitted sequences per 2-week period starting February 15, 2022. **c** Percentage of South Africans vaccinated over time. **d** Neutralization of BA.4 and BA.5 compared to BA.1 virus by BA.1 infection elicited neutralizing immunity in $n = 24$ unvaccinated participants. Numbers are geometric mean titer (GMT) $FRNT_{50}$. Dashed line is most concentrated plasma tested. **e** Geometric mean (GM) of fold-drops in neutralization and their 95% confidence intervals for BA.4 and BA.5 relative to BA.1 calculated from (**d**). For panels (**d**) and (**e**), orange points represent BA.1, yellow BA.4, and pink BA.5. *p*-values were determined by a two-sided Wilcoxon rank sum test and represented as ***0.001-0.0001. Exact *p*-values were 4.4×10^{-4} for both BA.4 and BA.5. Source data are provided as a Source Data file.

Results

We isolated live BA.4 and BA.5 viruses from infections in South Africa to test against pre-existing immunity. This consisted of sera from unvaccinated ($n = 24$) and vaccinated ($n = 15$) people infected in the preceding infection wave which was BA.1 dominated (Fig. 1a). This cohort was previously described by us[21] and consisted of participants with mostly mild Omicron BA.1 infections who were sampled weekly from symptom onset. Samples used here were collected a median of 23 days (IQR 19–27 days) post-symptom onset, once the participants developed or increased their BA.1 neutralizing response[21]. We also

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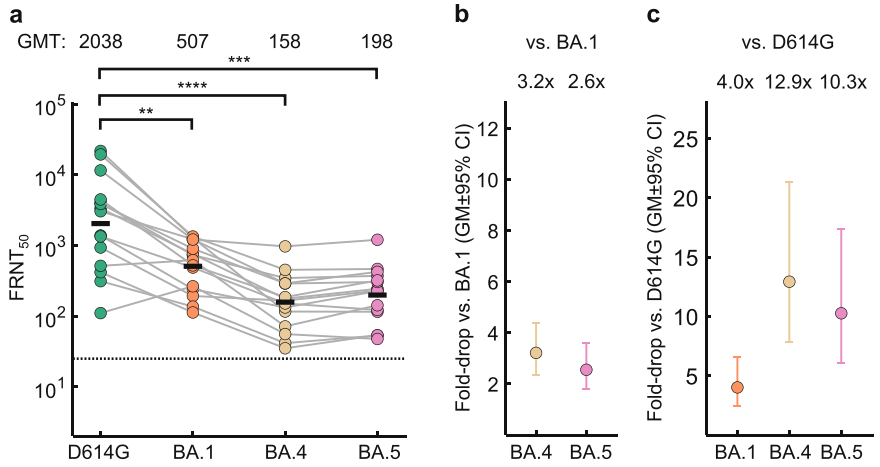

Fig. 2 | Escape of BA.4 and BA.5 from immunity elicited by vaccination combined with BA.1 breakthrough infection. a Neutralization of ancestral virus with the D614G substitution, BA.1, BA.4 and BA.5 by vaccine elicited neutralizing immunity with BA.1 breakthrough infection in $n = 15$ participants. Numbers are geometric mean titer (GMT) $FRNT_{50}$. Dashed line is most concentrated plasma tested. **b** Geometric mean (GM) of fold- drops in neutralization and their 95% confidence intervals for BA.4 and BA.5 relative to BA.1 calculated from (**a**). **c** GM of fold-drops in neutralization and their 95% confidence intervals for BA.1, BA.4 and BA.5 relative to ancestral/D614G virus calculated from (**a**). For all panels, green points are values for ancestral/D614G, orange points are BA.1, yellow points are BA.4, and pink points are BA.5. *p*-values were determined by a two-sided Wilcoxon rank sum test and represented as **0.01-0.001, ***0.001-0.0001, ****<0.0001. Exact *p*-values were 7.9×10^{-3} for BA.1, 9.7×10^{-5} for BA.4, and 1.9×10^{-4} for BA.5. Source data are provided as a Source Data file.

tested the viruses against sera from people who were vaccinated but not BA.1 infected ($n = 18$, see Supplementary Table 1 for cohort details). For study participants infected in the Omicron BA.1 infection wave, the majority (25 out of 39 infections) were confirmed Omicron/BA.1 by sequencing the infecting virus[21] (Table S1).

To quantify neutralization, we report the 50% focus reduction neutralization test value ($FRNT_{50}$), which is the inverse of the plasma dilution required for a 50% reduction in the number of infection foci relative to the no antibody control in a live virus neutralization assay[22].

We first report neutralization in the 24 unvaccinated study participants infected with BA.1. Neutralization of BA.1 was low at $FRNT_{50} = 275$. The $FRNT_{50}$ declined to 36 for BA.4 and 37 for BA.5 neutralization (Fig. 1d), 7.5 and 7.6-fold drops, respectively relative to BA.1 neutralization (Fig. 1e).

In vaccinated participants with BA.1 breakthrough infection after vaccination, BA.4 and BA.5 neutralization dropped from 507 for BA.1 to 158 for BA.4 and 198 BA.5 (Fig. 2a). The corresponding fold-drops were 3.2 for BA.4 and 2.6 for BA.5 (Fig. 2b). Given that the vaccines were designed with ancestral SARS-CoV-2 sequence, neutralization capacity against the ancestral virus with the D614G substitution may be a second benchmark to measure escape in this group. We therefore compared the neutralization of the Omicron sub-lineages to neutralization capacity against an isolate of ancestral virus from the B.1 lineage containing the D614G substitution. Neutralization of this ancestral isolate had an $FRNT_{50}$ of 2038, substantially higher than BA.1 neutralization by the same plasma (Fig. 2a). Compared to ancestral virus, neutralization dropped 4.0-fold for BA.1, 12.9-fold for BA.4, and 10.3-fold for BA.5 (Fig. 2c).

Because the cohort contained participants vaccinated with the Johnson and Johnson Ad26.CoV2.S in addition to the Pfizer BNT162b2 vaccine (Table S1) and participants who differed in their HIV-1 status (14 were people living with HIV, of whom 13 were virologically suppressed with antiretroviral therapy[21]), we examined whether HIV status and vaccine type impacted our results by comparing the fold-drop in neutralization of BA.4 and BA.5 to BA.1 in the different subgroups. Within the vaccinated group, the fold-drop with BA.4 and BA.5 was very similar when comparing neutralization of sera from participants vaccinated with Ad26.CoV2.S versus BNT162b2 (Fig. S1a). Likewise, fold-drops in neutralization did not substantially change between vaccinated people living with HIV and HIV negative participants (Fig. S1b). In contrast, there was a trend with borderline significance that showed higher BA.4 and BA.5 escape in people living with HIV who were unvaccinated (Fig. S1c).

The L452R and F486V mutations in the spike receptor binding domain could potentially mediate escape from vaccine elicited neutralization independently of BA.1 infection elicited immunity. To test this, we measured BA.1, BA.4, and BA.5 neutralization relative to ancestral D614G virus in 18 vaccinated South African participants who did not have BA.1 breakthrough infection (Table S1). Because we have previously observed that Beta variant infection may broaden vaccine elicited neutralization capacity[23], we did not include participants previously infected with a variant and restricted this group to either individuals who were vaccinated only or vaccinated and infected with ancestral/D614G. Here neutralization declined from $FRNT_{50} = 4123$ for ancestral/D614G to 208 for BA.1, 211 for BA.4 and 197 for BA.5 (Fig. 3a). BA.4 and BA.5 neutralization did not drop compared to BA.1 in this group (Fig. 3b). Fold-drops relative to ancestral virus were 19.8-fold for BA.1, 19.6-fold for BA.4 and 20.9-fold for BA.5 (Fig. 3c).

We observed that escape of BA.4 and BA.5 relative to BA.1 from neutralizing immunity was strongest in BA.1 infected unvaccinated individuals (Fig. 1e) and was moderated by vaccination in vaccinated people with BA.1 breakthrough infection (Fig. 2b). In contrast, BA.1, BA.4, and BA.5 showed similar (and extensive) escape in vaccinated people who did not have BA.1 infection elicited immunity (Fig. 3b).

BA.4 and BA.5 viruses showed very similar neutralization escape to each other, with minor differences which may be explained by experimental variation. This is expected since they share the same spike sequence, with the exception that our BA.4 isolate contained the N658S spike mutation found in a subset of BA.4 sequenced infections reported in GISAID (27% at the time of writing, see https://outbreak.info/compare-lineages?pango=BA.4&gene=S&threshold=10&nthresh=1&sub=false&dark=false) but not in BA.5. However, because we test neutralization against the live virus and not spike alone, we cannot rule out that the difference is real and occurs because of differences in the other genes (which may perhaps modulate neutralization by influencing spike surface expression or another parameter not directly related to spike sequence). In contrast to BA.4 and BA.5, we detected only minor escape of BA.2 from BA.1 elicited immunity in the same cohort of BA.1 infected individuals in a previous study[21].

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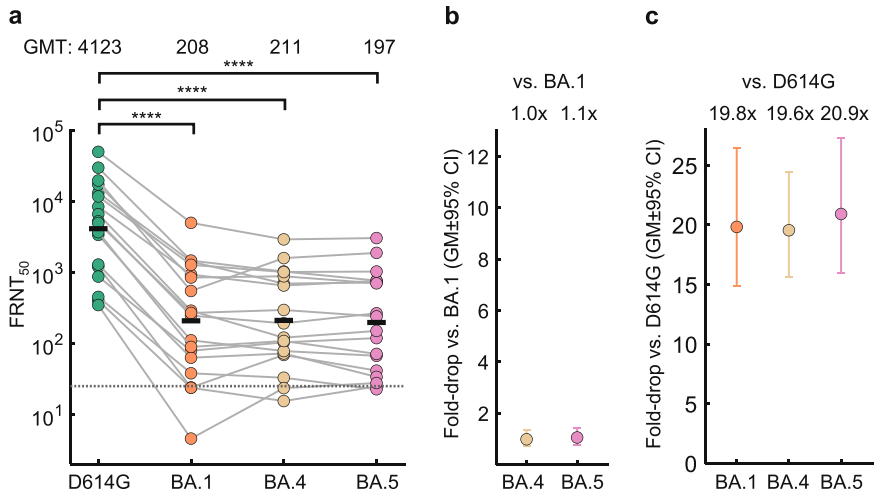

Fig. 3 | Escape of BA.4 and BA.5 from immunity elicited by vaccination combined in the absence of BA.1 infection. a Neutralization of ancestral/D614G, BA.1, BA.4 and BA.5 by vaccine elicited neutralizing immunity in $n = 18$ participants. Numbers are geometric mean titer (GMT) $FRNT_{50}$. Dashed line is most concentrated plasma tested. **b** Geometric mean (GM) of fold-drops in neutralization and their 95% confidence intervals for BA.4 and BA.5 relative to BA.1 calculated from (**a**). **c** GM of fold-drops in neutralization and their 95% confidence intervals for BA.1, BA.4 and BA.5 relative to ancestral/D614G virus calculated from (**a**). For all panels, green points are values for ancestral/D614G, orange points are BA.1, yellow points are BA.4, and pink points are BA.5. *p*-values were determined by a two-sided Wilcoxon rank sum test and represented as ****<0.0001. Exact *p*-values were 7.2×10^{-5} for BA.1, 3.2×10^{-5} for BA.4, and 2.4×10^{-5} for BA.5. Source data are provided as a Source Data file.

As we previously reported[21] and confirmed here, BA.1 elicits relatively weak neutralization in the absence of vaccination, consistent with reports showing that Omicron has reduced immunogenicity[24–26]. Even with BA.1 breakthrough infection, the $FRNT_{50}$ against ancestral virus was about half of that measured in a group composed mostly (Table S1) of people with ancestral infection and vaccination hybrid immunity (Fig. 3a). However, there are caveats to this comparison, including the order of infection and vaccination, with infection occurring first in the non-BA.1 infected group and the samples collected after vaccination.

Since our original release of the BA.4 and BA.5 neutralization results, other groups reported similar conclusions[27–31], with BA.4 and BA.5 escape from BA.1 and BA.2 elicited immunity being very similar to our measurements. These studies analyzed different cohorts from us and from each other, yet the results converged. Our cohort, which enrolls people who use the South African public health system, is generally distinguished from cohorts in other countries with active sero-surveillance of variants by the higher proportion of people who are unvaccinated, the higher proportion of people vaccinated with the Johnson and Johnson Ad26.CoV2.S vaccine, and the higher proportion of people who are living with HIV. In the vaccinated group we did not find evidence that either vaccine type or HIV status impacted the fold-drop in neutralization observed with BA.4 and BA.5 relative to BA.1. However, there was a trend to higher escape of BA.4 and BA.5 in unvaccinated individuals living with HIV. This is consistent with our previous results showing that the neutralization response elicited by a vaccine to a variant is similar between people living with HIV who are well suppressed with antiretroviral therapy and people who are HIV-negative, but that the response is attenuated by HIV in unvaccinated people[32].

A recent report showed that BA.4/BA.5 was more fusogenic in cell culture and more pathogenic relative to BA.2 in the hamster model[27]. However, despite this the BA.4/BA.5 infection wave in South Africa did not lead to a sharp increase in excess deaths associated with the other infection waves, although the association was also reduced in the BA.1 infection wave (Fig. 1a). This may indicate that, while SARS-CoV-2 pathogenicity continues to fluctuate and may evolve away from the attenuated pathogenicity observed in BA.1[33], the increased population immunity may keep disease severity relatively low[34]. Consistent with this, a recent analysis showed that neutralization capacity required to prevent severe disease is considerably lower than that required to prevent symptomatic infection[35]. In addition, there may be factors specific to the South African infection environment which reduce pathogenicity such as immunity from Beta infection combined with vaccination, which we found to broaden neutralization capacity against BA.4 and BA.5[23].

Limitations of this study include that we did not have enough participants with BA.2 infection or booster vaccination to test escape against this type of elicited immunity, which is much more common in some countries, for example those in Europe and North America. Our cohort is heterogeneous in terms of vaccination. Most participants are not vaccinated. Vaccinated participants are divided into two almost equal groups of Pfizer BNT162b2 and Johnson and Johnson Ad26.CoV2.S, though when we compared these vaccinated groups we observed that they were similar in terms of BA.4 and BA.5 escape. This may raise concerns that the heterogeneity in the relatively small vaccinated group may limit our ability to make more general conclusions about the degree of BA.4 and BA.5 immune escape in BA.1 infected vaccinated individuals.

Furthermore, the South African population differs from that of other countries where SARS-CoV-2 infection is intensively studied. South Africa has a lower fraction of vaccinated people, higher HIV prevalence, and people with previous immunity from an extensive Beta variant infection wave[22,23,36]. Every cohort is specific to the population it is drawn from, and it takes cohorts from multiple countries to get an accurate measure of immune escape of variants globally. The heterogeneity of individuals in our cohort reflects the heterogeneity in the South African population, and we chose not to limit our investigation to a specific subgroup. What may be specifically relevant in the population we study is that BA.4/BA.5, as well as BA.1, were first detected in South Africa and likely evolved in this region. Therefore, our study may indicate the selective forces at play in BA.4/BA.5 evolution. Given our observation that BA.4 and BA.5 have the strongest neutralization escape advantage in unvaccinated people, it may be important to determine whether the increasing vaccination coverage will reduce variant evolution.

Methods

Informed consent and ethical statement

Blood samples were obtained after written informed consent from adults with PCR-confirmed SARS-CoV-2 infection who were enrolled in a prospective cohort study at the Africa Health Research Institute approved by the Biomedical Research Ethics Committee at the University of KwaZulu–Natal (reference BREC/00001275/2020). The Omicron/BA.1 and BA.4 was isolated from a residual swab sample with SARS-CoV-2 isolation from the sample approved by the University of the Witwatersrand Human Research Ethics Committee (HREC) (ref. M210752). The sample to isolate Omicron/BA.5 was collected after written informed consent as part of the COVID-19 transmission and natural history in KwaZulu-Natal, South Africa: Epidemiological Investigation to Guide Prevention and Clinical Care in the Centre for the AIDS Programme of Research in South Africa (CAPRISA) study and approved by the Biomedical Research Ethics Committee at the University of KwaZulu–Natal (reference BREC/00001195/2020, BREC/00003106/2021). REDCap version 11.1.29 was used to collect participant data.

Whole-genome sequencing, genome assembly and phylogenetic analysis

RNA was extracted on an automated Chemagic 360 instrument, using the CMG-1049 kit (Perkin Elmer, Hamburg, Germany). The RNA was stored at −80 °C prior to use. Libraries for whole genome sequencing were prepared using either the Oxford Nanopore Midnight protocol with Rapid Barcoding or the Illumina COVIDseq Assay. For the Illumina COVIDseq assay, the libraries were prepared according to the manufacturer's protocol. Briefly, amplicons were fragmented, followed by indexing using the Nextera UD Indexes Set A. Sequencing libraries were pooled, normalized to 4 nM and denatured with 0.2 N sodium acetate. An 8 pM sample library was spiked with 1% PhiX (PhiX Control v3 adaptor-ligated library used as a control). We sequenced libraries on a 500-cycle v2 MiSeq Reagent Kit on the Illumina MiSeq instrument (Illumina). On the Illumina NextSeq 550 instrument, sequencing was performed using the Illumina COVIDSeq protocol (Illumina Inc, USA), an amplicon-based next-generation sequencing approach. The first strand synthesis was carried using random hexamers primers from Illumina and the synthesized cDNA underwent two separate multiplex PCR reactions. The pooled PCR amplified products were processed for tagmentation and adapter ligation using IDT for Illumina Nextera UD Indexes. Further enrichment and cleanup was performed as per protocols provided by the manufacturer (Illumina Inc). Pooled samples were quantified using Qubit 3.0 or 4.0 fluorometer (Invitrogen Inc.) using the Qubit dsDNA High Sensitivity assay according to manufacturer's instructions. The fragment sizes were analyzed using TapeStation 4200 (Invitrogen). The pooled libraries were further normalized to 4 nM concentration and 25 µL of each normalized pool containing unique index adapter sets were combined in a new tube. The final library pool was denatured and neutralized with 0.2 N sodium hydroxide and 200 mM Tris-HCL (pH7), respectively. 1.5 pM sample library was spiked with 2% PhiX. Libraries were loaded onto a 300-cycle NextSeq 500/550 HighOutput Kit v2 and run on the Illumina NextSeq 550 instrument (Illumina, San Diego, CA, USA). For Oxford Nanopore sequencing, the Midnight primer kit was used as described by Freed and Silander55. cDNA synthesis was performed on the extracted RNA using LunaScript RT mastermix (New England BioLabs) followed by gene-specific multiplex PCR using the Midnight Primer pools which produce 1200 bp amplicons which overlap to cover the 30-kb SARS-CoV-2 genome. Amplicons from each pool were pooled and used neat for barcoding with the Oxford Nanopore Rapid Barcoding kit as per the manufacturer's protocol. Barcoded samples were pooled and bead-purified. After the bead clean-up, the library was loaded on a prepared R9.4.1 flow-cell. A GridION X5 or MinION sequencing run was initiated using MinKNOW software with the base-call setting switched off. We assembled paired-end and nanopore.fastq reads using Genome Detective v2.40 (https://www.genomedetective.com) which was updated for the accurate assembly and variant calling of tiled primer amplicon Illumina or Oxford Nanopore reads, and the Coronavirus Typing Tool56. For Illumina assembly, GATK HaploTypeCaller --min-pruning 0 argument was added to increase mutation calling sensitivity near sequencing gaps. For Nanopore, low coverage regions with poor alignment quality (<85% variant homogeneity) near sequencing/amplicon ends were masked to be robust against primer drop-out experienced in the Spike gene, and the sensitivity for detecting short inserts using a region-local global alignment of reads, was increased. In addition, we also used the wf_artic (ARTIC SARS-CoV-2 pipeline, v0.3.18) as built using the nextflow workflow framework57. In some instances, mutations were confirmed visually with.bam files using Geneious software V2020.1.2 (Biomatters). The reference genome used throughout the assembly process was NC_045512.2 (numbering equivalent to MN908947.3.

Cells

Vero E6 cells (originally ATCC CRL-1586, obtained from Cellonex in South Africa) were propagated in complete growth medium consisting of Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (Hyclone) containing 10 mM of hydroxyethylpiperazine ethanesulfonic acid (HEPES), 1 mM sodium pyruvate, 2 mM L-glutamine and 0.1 mM nonessential amino acids (Sigma–Aldrich). Vero E6 cells were passaged every 3–4 days. The H1299-E3 cell line (H1299 originally from ATCC as CRL-5803) was propagated in growth medium consisting of complete Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum containing 10 mM of HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine and 0.1 mM nonessential amino acids. Cells were passaged every second day. The H1299-E3 (H1299-ACE2, clone E3) cell line was derived from H1299 as described in our previous work[1,22]. Briefly, vesicular stomatitis virus G glycoprotein (VSVG) pseudotyped lentivirus containing hACE2 was used to infect H1299 cells. ACE-2 transduced H1299 cells were subcloned at the single cell density in 96-well plates (Eppendorf) in conditioned media derived from confluent cells. After 3 weeks, wells were detached using a 0.25% trypsin-EDTA solution (Gibco) and plated in two replicate plates, where the first plate was used to determine infectivity and the second was stock. The first plate was screened for the fraction of mCherry positive cells per cell clone upon infection with a SARS-CoV-2 mCherry expressing spike pseudotyped lentiviral vector. Screening was performed using a Metamorph-controlled (Molecular Devices, Sunnyvale, CA) Nikon TiE motorized microscope (Nikon Corporation, Tokyo, Japan) with a 20x, 0.75 NA phase objective, 561 nm laser line, and 607 nm emission filter (Semrock, Rochester, NY). Images were captured using an 888 EMCCD camera (Andor). The clone with the highest fraction of mCherry expression was expanded from the stock plate and denoted H1299-E3. Cell lines have not been authenticated. The cell lines have been tested for mycoplasma contamination and are mycoplasma negative.

Virus expansion

All work with live virus was performed in Biosafety Level 3 containment using protocols for SARS-CoV-2 approved by the Africa Health Research Institute Biosafety Committee. ACE2-expressing H1299-E3 cells were seeded at 4.5×10^5 cells in a 6 well plate well and incubated for 18–20 h. After one Dulbecco's phosphate-buffered saline (DPBS) wash, the sub-confluent cell monolayer was inoculated with 500 µL universal transport medium diluted 1:1 with growth medium filtered through a 0.45-µm filter. Cells were incubated for 1 h. Wells were then filled with 3 mL complete growth medium. After 4 days of infection (completion of passage 1 (P1)), cells were trypsinized, centrifuged at $300 \times g$ for 3 min and resuspended in 4 mL growth medium. Then all infected cells were added to Vero E6 cells that had been seeded at

1.5×10^5 cells per mL, 20 mL total, 18–20 h earlier in a T75 flask for cell-to-cell infection. The coculture of ACE2-expressing H1299-E3 and Vero E6 cells was incubated for 1 h and the flask was filled with 20 mL of complete growth medium and incubated for 4 days. The viral supernatant from this culture (passage 2 (P2) stock) was used for experiments.

Live virus neutralization assay

H1299-E3 cells were plated in a 96-well plate (Corning) at 30,000 cells per well 1 day pre-infection. Plasma was separated from EDTA-anticoagulated blood by centrifugation at $500 \times g$ for 10 min and stored at −80 °C. Aliquots of plasma samples were heat-inactivated at 56 °C for 30 min and clarified by centrifugation at $10{,}000 \times g$ for 5 min. Virus stocks were used at approximately 50–100 focus-forming units per microwell and added to diluted plasma. Antibody–virus mixtures were incubated for 1 h at 37 °C, 5% CO_2. Cells were infected with 100 µL of the virus–antibody mixtures for 1 h, then 100 µL of a 1X RPMI 1640 (Sigma–Aldrich, R6504), 1.5% carboxymethylcellulose (Sigma–Aldrich, C4888) overlay was added without removing the inoculum. Cells were fixed 18 h post-infection using 4% PFA (Sigma-Aldrich) for 20 min. Foci were stained with a primary rabbit anti-spike monoclonal antibody (BS-R2B12, GenScript A02058) at 0.5 µg/mL in a permeabilization buffer containing 0.1% saponin (Sigma-Aldrich), 0.1% bovine serum albumin (BSA, Sigma–Aldrich) and 0.05% Tween-20 (Sigma–Aldrich) in phosphate-buffered saline (PBS) overnight at 4 °C, then washed with wash buffer containing 0.05% Tween-20 in PBS. Secondary goat anti-rabbit horseradish peroxidase (HRP) conjugated antibody (Abcam ab205718) was added at 1 µg/mL and incubated for 2 h at room temperature with shaking. TrueBlue peroxidase substrate (SeraCare 5510-0030) was then added at 50 µL per well and incubated for 20 min at room temperature. Plates were imaged in an ImmunoSpot Ultra-V S6-02-6140 Analyzer ELISPOT instrument with BioSpot Professional built-in image analysis (C.T.L).

Statistics and fitting

All statistics and fitting were performed using custom code in MATLAB v.2019b. Neutralization data were fit to:

$$Tx = 1/1 + (D/ID_{50}). \quad (1)$$

Here Tx is the number of foci normalized to the number of foci in the absence of plasma on the same plate at dilution D and ID_{50} is the plasma dilution giving 50% neutralization. $FRNT_{50} = 1/ID_{50}$. Values of $FRNT_{50} < 1$ are set to 1 (undiluted), the lowest measurable value. We note that the most concentrated plasma dilution was 1:25 and therefore $FRNT_{50} < 25$ were extrapolated. To calculate confidence intervals, $FRNT_{50}$ or fold-change in $FRNT_{50}$ per participant was log transformed and arithmetic mean plus 2 std and arithmetic mean minus 2 std were calculated for the log transformed values. These were exponentiated to obtain the upper and lower 95% confidence intervals on the geometric mean $FRNT_{50}$ or the fold-change in $FRNT_{50}$ geometric means.

Data availability

Sequences of outgrown Omicron sub-lineage isolates have been deposited to GenBank with accession codes as follows: Ancestral virus, B.1 lineage, with the D614G substitution, OP090658. Omicron/BA.1, OP090659. Omicron/BA.4, OP093374. Omicron/BA.5, OP093373 and have also been deposited in GISAID with accession codes and hyperlinks as follows: Ancestral virus, B.1 lineage, with the D614G substitution, EPI_ISL_602626.1 [https://www.epicov.org/epi3/frontend#357674]. Omicron/BA.1, EPI_ISL_7886688 [https://www.epicov.org/epi3/frontend#6274a9]. Omicron/BA.4, EPI_ISL_12268495.2 [https://www.epicov.org/epi3/frontend#434eae]. Omicron/BA.5, EPI_ISL_12268493.2 [https://www.epicov.org/epi3/frontend#49d7ec]. Source data are provided with this paper.

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Author contributions

A.S. and K.K. conceived the study and designed the study and experiments. A.vG., D.M., N.W., Q.A.K., S.S.A.K., G.L., A.Si, and N.S. identified and provided virus samples. M.-Y.S.M., F.K., B.I.G., M.B., K.K., N.M., N.Ma, M.M., Y.M., N.N., Z.J., K.R., and Y.G. set up and managed the cohort and cohort data. K.K., Z.J., K.R., S.C., Y.G., H.T., J.E.S., J.G., Y.N., S.P., performed experiments and sequence analysis with input from A.S., T.dO., R.J.L. A.S., K.K., and M.B. interpreted data with input from M-Y.S.M., S.S.A.K., W.H., T.dO., R.J.L. A.S. and K.K. prepared the manuscript with input from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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9.1.2. Omicron infection enhances Delta antibody immunity in vaccinated persons.

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The extent to which Omicron infection[1–9], with or without previous vaccination, elicits protection against the previously dominant Delta (B.1.617.2) variant is unclear. Here we measured the neutralization capacity against variants of severe acute respiratory syndrome coronavirus 2 in 39 individuals in South Africa infected with the Omicron sublineage BA.1 starting at a median of 6 (interquartile range 3–9) days post symptom onset and continuing until last follow-up sample available, a median of 23 (interquartile range 19–27) days post symptoms to allow BA.1-elicited neutralizing immunity time to develop. Fifteen participants were vaccinated with Pfizer's BNT162b2 or Johnson & Johnson's Ad26.CoV2.S and had BA.1 breakthrough infections, and 24 were unvaccinated. BA.1 neutralization increased from a geometric mean 50% focus reduction neutralization test titre of 42 at enrolment to 575 at the last follow-up time point (13.6-fold) in vaccinated participants and from 46 to 272 (6.0-fold) in unvaccinated participants. Delta virus neutralization also increased, from 192 to 1,091 (5.7-fold) in vaccinated participants and from 28 to 91 (3.0-fold) in unvaccinated participants. At the last time point, unvaccinated individuals infected with BA.1 had low absolute levels of neutralization for the non-BA.1 viruses and 2.2-fold lower BA.1 neutralization, 12.0-fold lower Delta neutralization, 9.6-fold lower Beta variant neutralization, 17.9-fold lower ancestral virus neutralization and 4.8-fold lower Omicron sublineage BA.2 neutralization relative to vaccinated individuals infected with BA.1. These results indicate that hybrid immunity formed by vaccination and Omicron BA.1 infection should be protective against Delta and other variants. By contrast, infection with Omicron BA.1 alone offers limited cross-protection despite moderate enhancement.

The Omicron variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified in November 2021 in South Africa and Botswana[10], has been shown by us[1] and others[2–9] to have extensive but incomplete escape from neutralizing immunity elicited by vaccines and previous infection, with boosted individuals showing better neutralization. In South Africa, Omicron infections led to a lower incidence of severe disease relative to other variants[11,12], although this can be at least partly explained by pre-existing immunity[13]. The first Omicron sublineage to appear was BA.1, which was supplanted by the BA.2 sublineage in many countries[14].

How Omicron BA.1 infection will interact with vaccination to protect against the previously dominant Delta variant, emerging variants such

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as BA.2 and other variants is still unclear. We isolated live Omicron BA.1, Omicron BA.2, ancestral, Beta and Delta viruses and neutralized viruses with plasma from participants enrolled and longitudinally sampled during the Omicron BA.1 infection wave in South Africa, with all participants having a confirmed diagnosis of SARS-CoV-2 by quantitative PCR. To quantify neutralization, we used a live virus neutralization assay and calculated the focus reduction neutralization test ($FRNT_{50}$) titre, the inverse of the plasma dilution required for 50% neutralization, as measured by the reduction in the number of infection foci. We enrolled 41 participants who reported symptoms from late November 2021 to January 2022. We successfully sequenced the infecting virus in 26 participants, and all sequences corresponded to Omicron BA.1 (Extended Data Table 1). Two participants had advanced human immunodeficiency virus (HIV) disease on the basis of a low CD4 count (<200 cells μl^{-1} throughout the study) and unsuppressed HIV infection, and we excluded these participants because of our previous data showing an atypical response to SARS-CoV-2 in advanced HIV disease[15]. Extended Data Table 2 summarizes the characteristics of the remaining 39 participants.

Of the 39 participants, 27 were admitted to hospital because of coronavirus disease 2019 symptoms. Seven required supplemental oxygen and one died. Fifteen participants were vaccinated and had a breakthrough Omicron BA.1 infection. The median time post vaccination was 139 days (interquartile range (IQR) 120–178), a time interval that would predict considerable waning of the vaccine-elicited immune response[16], which may have contributed to the breakthrough infections. Eight participants were vaccinated with two doses of Pfizer's BNT162b2 and seven were vaccinated with Johnson & Johnson's Ad26.CoV2.S (six with a single dose and one with two doses; Extended Data Table 1). The length of hospital stay was shorter in the vaccinated (3.5 days) relative to unvaccinated (8 days; Extended Data Table 2) participants. Three participants self-reported having a previous SARS-CoV-2 infection (Extended Data Table 1).

Participants were sampled at enrolment at a median of 6 days (IQR 3–9 days) after symptom onset, and again at weekly follow-up visits that were attended as practicable because of the Christmas holidays in South Africa. The last follow-up visit was a median of 23 days (IQR 19–27 days) post-symptom onset (Extended Data Table 1). Examining neutralization at all available time points per study participant showed that neutralization of the Omicron BA.1 variant increased substantially in most participants from enrolment to the time of the last follow-up (Extended Data Fig. 1), consistent with developing a neutralizing antibody response to Omicron BA.1 infection. We therefore analysed neutralization at enrolment (baseline for the study) and the last follow-up visit to quantify the increase in neutralization capacity after Omicron infection.

We observed that Omicron BA.1 neutralization increased in vaccinated individuals from a low geometric mean titre (GMT) $FRNT_{50}$ of 42 at the enrolment visit to 575 at the last follow-up visit about 2 to 3 weeks later, a 13.6-fold change (95% CI confidence interval (CI) 3.7–50.2; Fig. 1a). The samples from unvaccinated participants at the study baseline neutralized Omicron BA.1 at a similar starting level of 46 and reached a final level of 272 at the last follow-up, a 6.0-fold increase (95% CI 2.2–16.1; Fig. 1b). Neutralization of the Delta virus also increased during this period. At enrolment, neutralization capacity against the Delta virus was 192 and reached a final level of 1,091 at the last follow-up visit in vaccinated participants, a 5.7-fold increase (95% CI 1.7–18.4; Fig. 1c). Unvaccinated participants had lower Delta neutralization at baseline with Delta virus $FRNT_{50}$ = 28, and reached $FRNT_{50}$ = 91, a 3.2-fold increase (95% CI 1.3–8.1; Fig. 1d).

We next compared Omicron BA.1 to Omicron BA.2, Delta, Beta (ref. [17]) and ancestral virus neutralization at the last available follow-up visit in three sets of paired experiments, each comparing Omicron BA.2, Delta or ancestral and Beta virus neutralization to Omicron BA.1 neutralization. The range of Omicron BA.1 neutralization shown in Fig. 2a

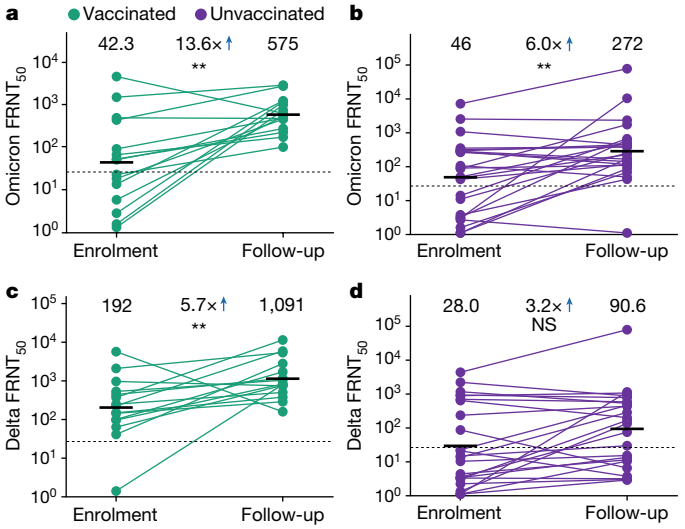

Fig. 1 | Enhancement of Delta neutralization by Omicron infection. **a**,**b**, Neutralization of the Omicron BA.1 virus over time for $n = 15$ vaccinated (**a**) and $n = 24$ unvaccinated (**b**) participants infected with Omicron BA.1. **c**,**d**, Neutralization of the Delta virus over time for the same vaccinated ($n = 15$; **c**) and unvaccinated ($n = 24$; **d**) participants as in **a**,**b**. For each participant, the sample collected at the initial enrolment visit (median 6 days post symptom onset) was compared with that collected at the last follow-up visit (median 23 days post symptom onset). The neutralization capacity per participant was determined in two independent experiments, and the numbers and horizontal bars are GMTs over all participants per group of the reciprocal plasma dilution ($FRNT_{50}$) resulting in 50% neutralization. The fold change was calculated by dividing the GMT from the follow-up by the GMT from the enrolment visit. The dashed line is the most concentrated plasma tested. The *P* values were determined by a left-sided Wilcoxon rank sum test measuring the significance of the increase; $^{**}P = 0.01–0.001$; NS, not significant. The exact *P* values are 0.0012 (**a**), 0.0081 (**b**), 0.0021 (**c**) and 0.11 (**d**).

for different experiments ($FRNT_{50}$ = 516 to 646 for vaccinated samples and 266 to 271 for unvaccinated samples) is the result of experimental variation. BA.2 neutralization was moderately and not significantly lower relative to BA.1 neutralization in both vaccinated and unvaccinated participants. Testing only participants with sequence-confirmed Omicron BA.1 infection gave a similar result (Extended Data Fig. 2). The trend for the other variants and the ancestral virus was that neutralization was higher relative to Omicron BA.1 in vaccinated participants but lower relative to Omicron BA.1 in unvaccinated participants, although the differences were mostly not significant (Fig. 2a). As a result of the relatively moderate fold change, higher participant numbers would probably be required to make the trends statistically significant.

The comparison of the other variants to Omicron BA.1 within the vaccinated or unvaccinated group does not indicate the differences in neutralization capacity elicited by Omicron BA.1 between the vaccinated and unvaccinated participants. We therefore compared neutralization of each variant between the vaccinated and unvaccinated groups at the last time point directly (Fig. 2b). The smallest difference between vaccinated and unvaccinated participants was in neutralization of Omicron BA.1, the infecting variant, with the vaccinated participants showing 2.2-fold higher neutralization. For the other variants, neutralization was higher in vaccinated participants by a factor of 4.8-fold for Omicron BA.2, 9.6-fold for Beta, 12.0-fold for Delta and 17.9-fold for ancestral (Fig. 2b). All differences were significant, and the 95% CIs for the GMT $FRNT_{50}$ of vaccinated and unvaccinated participants did not overlap for BA.2, Beta, Delta or ancestral virus neutralization (Fig. 2b). For the unvaccinated participants, the absolute neutralization capacity against the BA.2, Beta, Delta and ancestral viruses was low[18], with GMT $FRNT_{50}$ being about or below $FRNT_{50}$ = 100 (Fig. 2b).

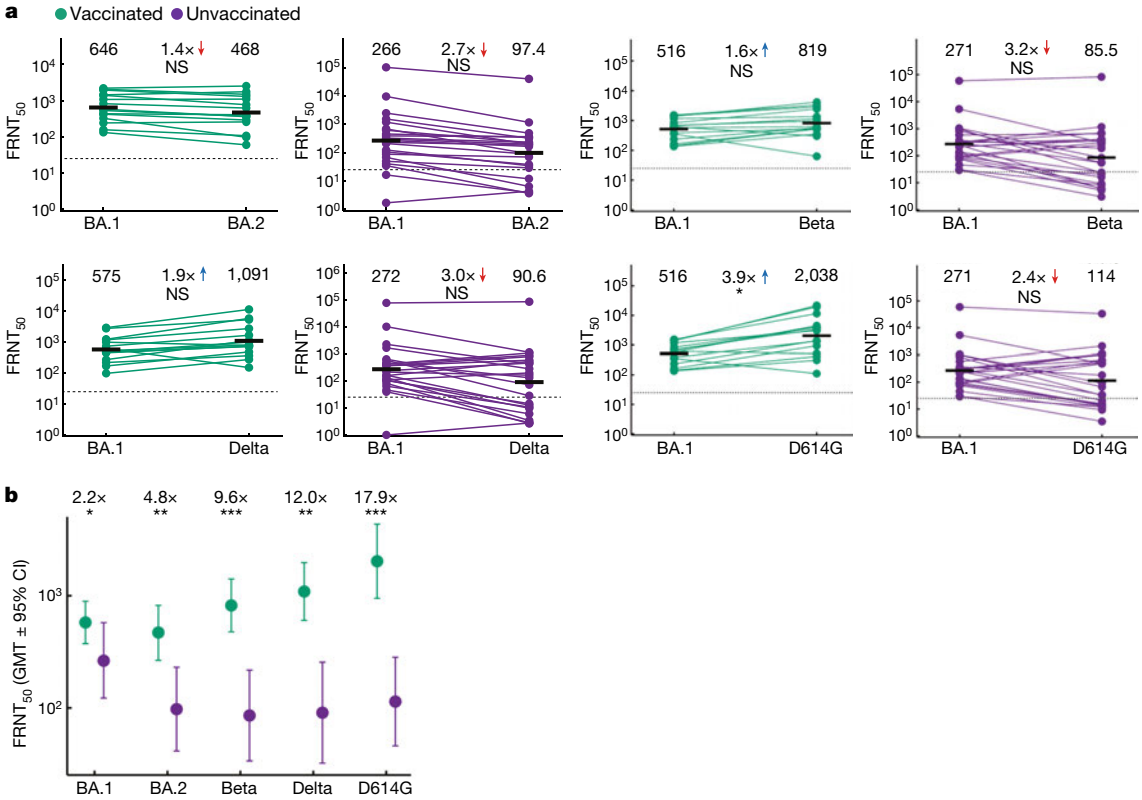

Fig. 2 | Gap in neutralizing immunity between vaccinated and unvaccinated participants infected with Omicron BA.1. a, Neutralization of Omicron BA.2, Beta, Delta and ancestral (with the D614G substitution) viruses compared to the Omicron BA.1 virus at the last available follow-up time point in $n = 15$ vaccinated or $n = 24$ unvaccinated participants infected with Omicron BA.1. The neutralization capacity per participant was determined in two independent experiments, and the numbers and horizontal bars are GMT $FRNT_{50}$. The fold change was calculated by dividing the larger by the smaller GMT. The dashed line is the most concentrated plasma tested. The *P* values were determined by a two-sided Wilcoxon rank sum test; $^{*}P = 0.05–0.01$; NS, not significant. The exact *P* values (vaccinated/unvaccinated) are: 0.22/0.087 for BA.2, 0.36/0.071 for Beta, 0.15/0.25 for Delta and 0.014/0.20 for ancestral. **b**, Comparison of the neutralization capacity against the Omicron BA.1, Omicron BA.2, Beta, Delta and ancestral (D614G) viruses in vaccinated ($n = 15$) versus unvaccinated ($n = 24$) participants infected with Omicron BA.1. The neutralization capacity per participant was determined in two independent experiments for all strains except for Omicron BA.1, for which six experiments were available and were used in the calculation. The points represent GMT $FRNT_{50}$ per group and the error bars are GMT 95% CIs. The *P* values were determined by a two-sided Wilcoxon rank sum test; $^{*}P = 0.05–0.01$; $^{**}P = 0.01–0.001$; $^{***}P = 0.001–0.0001$. The exact *P* values are 0.025 (BA.1), 0.0026 (BA.2), 4.1×10^{-4} (Beta), 0.0012 (Delta) and 3.3×10^{-4} (ancestral).

We also tested neutralization of Omicron BA.1 by Delta-variant-elicited immunity. We collected 18 plasma samples from 14 participants (including pre-vaccination and post-vaccination samples from 4 participants) previously infected in the Delta variant wave in South Africa, 8 of whom were vaccinated either before or after infection (Extended Data Table 3). Confirming previously reported results[19], we observed similar escape of Omicron BA.1 from Delta-elicited immunity across all samples tested, manifested as a 22.5-fold decrease (95% CI 14.4–35.0) in Omicron BA.1 neutralization compared to Delta virus neutralization (Fig. 3).

The large fold drop in Delta-infection-elicited neutralization capacity against Omicron BA.1 contrasts with the moderate and nonsignificant fold drops, or even fold increases, in neutralization of other variants relative to Omicron BA.1 in individuals infected with Omicron BA.1. However, in unvaccinated individuals, even though fold drops in neutralization were moderate and nonsignificant, the absolute levels of neutralization of the other variants, and of Omicron BA.1 itself, were low and on a similar scale to the cross-neutralization capacity against Omicron in Delta-infection-elicited immunity. This is consistent with other recently reported results[20], and possibly indicates that Omicron is poorly immunogenic. In agreement with recent reports[21,22], our observations show moderately and nonsignificantly lower neutralization of BA.2 by BA.1-elicited immunity. The results explain epidemiological observations showing that Omicron BA.2 reinfection is relatively rare soon after Omicron BA.1 infection[23,24].

Our results may be supportive of a scenario in which hybrid immunity formed by Omicron infection combined with vaccination protects as well or better against reinfection with variants such as Delta relative to reinfection with Omicron itself. By contrast, unvaccinated participants infected with Omicron BA.1 only, have low neutralization capacity against the Omicron BA.2, Beta, Delta and ancestral viruses.

Limitations of this study include heterogeneity in participant immune history, including two vaccination types and one boost. On the basis of the high seroprevalence observed in South Africa[25,26], some participants may also have had unreported previous infection. However, including two vaccine types did not mask the differences between vaccinated and unvaccinated participants, and the low levels of neutralization in unvaccinated participants against the ancestral, Beta and Delta viruses (the dominant strains in the preceding South African infection waves) support the notion that these participants were either not previously infected, or that immunity has waned completely. Participants were also mostly hospitalized, which may not be typical of Omicron infection[13,27]. Increased disease severity has been shown to lead to higher anti-SARS-CoV-2 antibody titres[28]. This should help in the detection of the neutralization response, but whether it would affect the trend we observed is unclear. Omicron infection is unlikely benign to the extent that hospitalization is an outlier outcome: in the USA, the number of individuals with coronavirus disease 2019 who died in the Omicron wave was similar to the number who died in the Delta

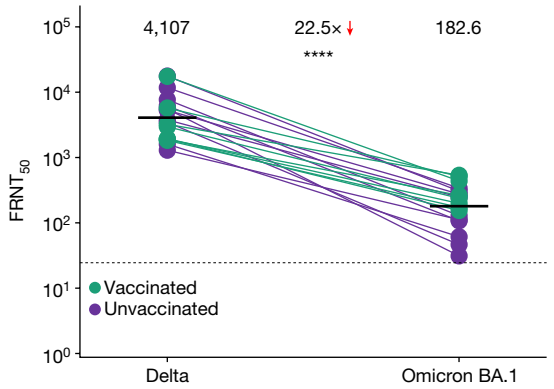

Fig. 3 | Escape of Omicron virus from Delta-infection-elicited immunity. Neutralization of Delta compared to the Omicron BA.1 virus by Delta-infection-elicited plasma immunity in vaccinated and unvaccinated participants. A total of 18 samples were tested from $n = 14$ participants infected during the Delta infection wave in South Africa. The neutralization capacity per participant was determined in two independent experiments, and the numbers and horizontal bars are GMT $FRNT_{50}$. The fold change was calculated by dividing the larger by the smaller GMT. The dashed line is the most concentrated plasma tested. $^{****}P = 3.2 \times 10^{-7}$ as determined by a two-sided Wilcoxon rank sum test.

wave[27]. Neutralizing immunity may have increased further in some participants had we sampled later: the neutralizing capacity did not plateau at the last time point in 8 of the 24 (33%) unvaccinated participants (participants 10, 14, 21, 27, 30, 31, 34 and 38; Extended Data Fig. 1) and 6 of the 15 (40%) vaccinated participants (participants 4, 6, 15, 16, 25 and 26). Therefore, the temporal dynamics give no clear indication that the immunity in the unvaccinated participants was delayed and would have reached similar levels to that of vaccinated participants if sampled later. However, the consequences of waning immunity several months post Omicron infection should be investigated.

The gap in immunity between unvaccinated individuals infected with Omicron BA.1 and vaccinated individuals with BA.1 breakthrough infection is concerning. Especially as immunity wanes, unvaccinated individuals post Omicron infection are likely to have poor cross-protection against existing and possibly emerging SARS-CoV-2 variants, despite acquiring some neutralizing immunity to the infecting Omicron BA.1 sub-lineage variant. The implication may be that Omicron BA.1 infection alone is not sufficient for protection and vaccination should be administered even in areas with a high prevalence of Omicron infection to protect against other variants.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-022-04830-x.

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Methods

Informed consent and ethics

Blood samples and the Delta isolate were obtained after written informed consent from adults with PCR-confirmed SARS-CoV-2 infection who were enrolled in a prospective cohort study at the Africa Health Research Institute approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal (reference BREC/00001275/2020). Omicron BA.1 was isolated from a residual swab sample for SARS-CoV-2 where isolation from the sample was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC; reference M210752). The sample to isolate Omicron BA.2 was collected after written informed consent as part of the study "COVID-19 transmission and natural history in KwaZulu-Natal, South Africa: Epidemiological Investigation to Guide Prevention and Clinical Care" of the Centre for the AIDS Programme of Research in South Africa (CAPRISA) and isolation from the sample approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal (reference BREC/00001195/2020, BREC/00003106/2021).

Reagent availability

Virus isolates and the cell line are available from the corresponding author. A Biosafety Level 3 facility is required for laboratories receiving live SARS-CoV-2.

Whole-genome sequencing, genome assembly and phylogenetic analysis

RNA was extracted on an automated Chemagic 360 instrument, using the CMG-1049 kit (Perkin Elmer). The RNA was stored at −80 °C before use. Libraries for whole-genome sequencing were prepared using either the Oxford Nanopore Midnight protocol with Rapid Barcoding or the Illumina COVIDseq Assay. For the Illumina COVIDseq assay, the libraries were prepared according to the manufacturer's protocol. Briefly, amplicons were tagmented, followed by indexing using the Nextera UD Indexes Set A. Sequencing libraries were pooled, normalized to 4 nM and denatured with 0.2 N sodium acetate. An 8 pM sample library was spiked with 1% PhiX (PhiX Control v3 adaptor-ligated library used as a control). We sequenced libraries on a 500-cycle v2 MiSeq Reagent Kit on the Illumina MiSeq instrument (Illumina). On the Illumina NextSeq 550 instrument, sequencing was performed using the Illumina COVIDSeq protocol (Illumina), an amplicon-based next-generation sequencing approach. The first-strand synthesis was carried using random hexamer primers from Illumina, and the synthesized cDNA underwent two separate multiplex PCR reactions. The pooled PCR-amplified products were processed for tagmentation and adaptor ligation using IDT for Illumina Nextera UD Indexes. Further enrichment and cleanup was performed as per protocols provided by the manufacturer (Illumina). Pooled samples were quantified using a Qubit 3.0 or 4.0 fluorometer (Invitrogen) through the Qubit dsDNA High Sensitivity Assay according to the manufacturer's instructions. The fragment sizes were analysed using TapeStation 4200 (Invitrogen). The pooled libraries were further normalized to 4 nM concentration and 25 µl of each normalized pool containing unique index adaptor sets was combined in a new tube. The final library pool was denatured and neutralized with 0.2 N sodium hydroxide and 200 mM Tris-HCl (pH 7), respectively. A 1.5 pM sample library was spiked with 2% PhiX. Libraries were loaded onto a 300-cycle NextSeq 500/550 HighOutput Kit v2 and run on the Illumina NextSeq 550 instrument (Illumina). For Oxford Nanopore sequencing, the Midnight primer kit was used: cDNA synthesis was performed on the extracted RNA using LunaScript RT mastermix (New England BioLabs) followed by gene-specific multiplex PCR using the Midnight Primer pools that produce 1,200-base-pair amplicons that overlap to cover the 30-kb SARS-CoV-2 genome. Amplicons from each pool were pooled and used neat for barcoding with the Oxford Nanopore Rapid Barcoding kit as per the manufacturer's protocol. Barcoded samples were pooled and bead purified. After the bead cleanup, the library was loaded on a prepared R9.4.1 flow cell. A GridION X5 or MinION sequencing run was initiated using MinKNOW software with the base-call setting switched off. We assembled paired-end and nanopore.fastq reads using Genome Detective 1.132 (https://www.genomedetective.com), which was updated for the accurate assembly and variant calling of tiled primer amplicon Illumina or Oxford Nanopore reads, and the Coronavirus Typing Tool. For Illumina assembly, the GATK HaploTypeCaller –min-pruning 0 argument was added to increase mutation calling sensitivity near sequencing gaps. For Nanopore, low-coverage regions with poor alignment quality (<85% variant homogeneity) near sequencing/amplicon ends were masked to be robust against primer drop-out experienced in the spike gene, and the sensitivity for detecting short inserts using a region-local global alignment of reads was increased. In addition, we also used the wf_artic (ARTIC SARS-CoV-2) pipeline as built using the nextflow workflow framework. In some instances, mutations were confirmed visually with .bam files using Geneious software V2020.1.2 (Biomatters). The reference genome used throughout the assembly process was NC_045512.2 (numbering equivalent to MN908947.3). For lineage classification, we used the widespread dynamic lineage classification method from the Phylogenetic Assignment of Named Global Outbreak Lineages software suite (https://github.com/hCoV-2019/pangolin).

Cells

Vero E6 cells (originally ATCC CRL-1586, obtained from Cellonex in South Africa) were propagated in complete growth medium consisting of Dulbecco's modified Eagle medium with 10% fetal bovine serum (Hyclone) containing 10 mM HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine and 0.1 mM nonessential amino acids (Sigma-Aldrich). Vero E6 cells were passaged every 3–4 days. The H1299-E3 cell line (H1299 originally from ATCC as CRL-5803) was propagated in growth medium consisting of complete Roswell Park Memorial Institute 1640 medium with 10% fetal bovine serum containing 10 mM HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine and 0.1 mM nonessential amino acids. Cells were passaged every second day. The H1299-E3 (H1299-ACE2, clone E3) cell line was derived from H1299 as described in our previous work[1,17]. Cell lines were not authenticated. Cell lines tested negative for mycoplasma contamination.

Virus expansion

All work with live virus was performed in Biosafety Level 3 containment using protocols for SARS-CoV-2 approved by the Africa Health Research Institute Biosafety Committee. ACE2-expressing H1299-E3 cells were seeded at 4.5×10^5 cells in a 6-well plate well and incubated for 18–20 h. After one Dulbecco's phosphate-buffered saline (PBS) wash, the subconfluent cell monolayer was inoculated with 500 µl universal transport medium diluted 1:1 with growth medium filtered through a 0.45-µm filter. Cells were incubated for 1 h. Wells were then filled with 3 ml complete growth medium. After 4 days of infection (completion of passage 1 (P1)), cells were trypsinized, centrifuged at 300 RCF for 3 min and resuspended in 4 ml growth medium. All cells from the P1 infection were added to Vero E6 cells that had been seeded at 2×10^5 cells ml^{-1}, 20 ml total, 18–20 h earlier in a T75 flask for cell-to-cell infection. The coculture of ACE2-expressing H1299-E3 and Vero E6 cells was incubated for 1 h, and the flask was filled with 20 ml of complete growth medium and incubated for 4 days. The viral supernatant from this culture (passage 2 (P2) stock) was used for experiments.

Live virus neutralization assay

H1299-E3 cells were plated in a 96-well plate (Corning) at 30,000 cells per well 1 day pre-infection. Plasma was separated from EDTA-anticoagulated blood by centrifugation at 500 RCF for 10 min and stored at −80 °C. Aliquots of plasma samples were heat-inactivated at 56 °C for 30 min and clarified by centrifugation at 10,000 RCF for

5 min. Virus stocks were used at approximately 50–100 focus-forming units per microwell and added to diluted plasma. Antibody–virus mixtures were incubated for 1 h at 37 °C, 5% CO_2. Cells were infected with 100 µl of the virus–antibody mixtures for 1 h, and then 100 µl of a 1× Roswell Park Memorial Institute 1640 (Sigma-Aldrich, R6504), 1.5% carboxymethylcellulose (Sigma-Aldrich, C4888) overlay was added without removing the inoculum. Cells were fixed 18 h after infection using 4% paraformaldehyde (Sigma-Aldrich) for 20 min. Foci were stained with a rabbit anti-spike monoclonal antibody (BS-R2B12, GenScript A02058) at 0.5 µg ml^{-1} in a permeabilization buffer containing 0.1% saponin (Sigma-Aldrich), 0.1% BSA (Sigma-Aldrich) and 0.05% Tween-20 (Sigma-Aldrich) in PBS. Plates were incubated with primary antibody overnight at 4 °C, and then washed with wash buffer containing 0.05% Tween-20 in PBS. Secondary goat anti-rabbit antibody conjugated to horseradish peroxidase (Abcam ab205718) was added at 1 µg ml^{-1} and incubated for 2 h at room temperature with shaking. TrueBlue peroxidase substrate (SeraCare 5510-0030) was then added at 50 µl per well and incubated for 20 min at room temperature. Plates were imaged in an ImmunoSpot Ultra-V S6-02-6140 Analyzer ELISPOT instrument with BioSpot Professional built-in image analysis (Cellular Technology Limited).

Statistics and fitting

Statistical methods were not used to predetermine sample size, and blinding and randomization were not used. All statistics and fitting were performed using custom code in MATLAB v.2019b. Neutralization data were fitted to:

$$\mathrm{Tx} = 1/1 + (D/\mathrm{ID}_{50}).$$

Here Tx is the number of foci normalized to the number of foci in the absence of plasma on the same plate at dilution D, and ID_{50} is the plasma dilution giving 50% neutralization. $FRNT_{50} = 1/ID_{50}$. Values of $FRNT_{50} < 1$ are set to 1 (undiluted), the lowest measurable value. We note that the most concentrated plasma dilution was 1:25 and therefore $FRNT_{50} < 25$ data were extrapolated. To calculate CIs, $FRNT_{50}$ or fold change in $FRNT_{50}$ per participant was log transformed and the arithmetic mean plus 2 s.d. and the arithmetic mean minus 2 s.d. were calculated for the log-transformed values. These were exponentiated to obtain the upper and lower 95% CIs on the geometric mean $FRNT_{50}$ or the fold change in $FRNT_{50}$ geometric means.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Sequences of outgrown Omicron sublineages have been deposited in GISAID (https://www.gisaid.org/) with accessions EPI_ISL_7886688 (Omicron BA.1), EPI_ISL_9082893 (Omicron BA.2) and EPI_ISL_602626.1 (ancestral/D614G). Delta (EPI_ISL_3118687) and Beta (EPI_ISL_678615) isolates have been described previously[15]. Raw images of the data are available upon reasonable request. Source data are provided with this paper.

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Author contributions A.Sigal., K.K. and F.K. conceived the study and designed the study and experiments. A.v.G., Q.A.K., S.S.A.K., G.L., A.Sivro and N.S. identified and provided virus samples. M.-Y.S.M., F.K., B.I.G., M.B., K.K., T.N., M.M., N. Mthabela, Z.M., N.N., Y.M., N. Mbatha, N. Manickchund, N. Magula, Z.J., K.R. and Y.G. set up and managed the cohort and cohort data. K.K., Z.J., K.R., S.C., H.T., J.E.S., Y.G., J.G., Y. Ramphal, A.B.M.K., D.A. and J.N.B. performed experiments and sequence analysis with input from A.Sigal, T.d.O., R.J.L. and J.N.B., A.Sigal, K.K., F.K., R.M. and Y. Rosenberg interpreted data with input from M.-Y.S.M., G.G., S.S.A.K., W.H., T.d.O., N. Magula, R.J.L. and P.L.M., A.Sigal, K.K., G.L., F.K. and M.B. prepared the manuscript with input from all authors.

Competing interests The authors declare no competing interests.

Additional information

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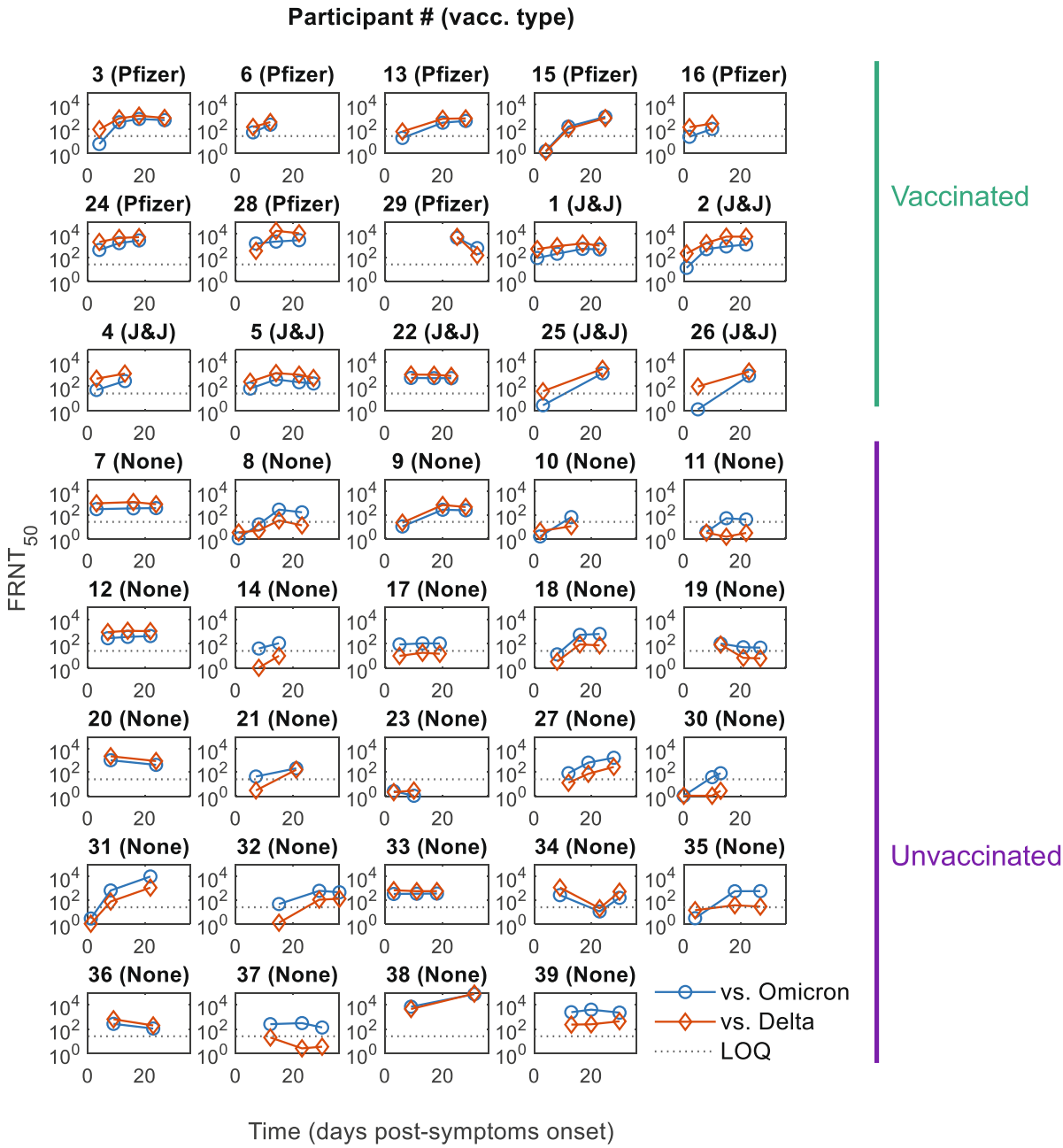
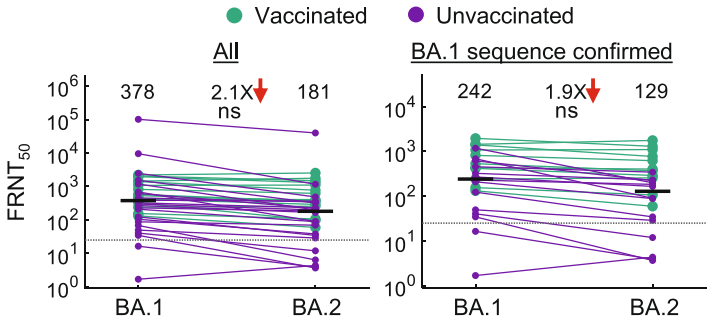


Extended Data Fig. 1 | Longitudinal Omicron/BA.1 and Delta neutralization capacity in Omicron/BA.1 infected participants. Neutralization of Omicron (blue) and Delta (red) at all study visits. Participant number is as in Extended Data Table 1. Top three rows are participants vaccinated with Pfizer BNT162b2 (n = 8) or Johnson and Johnson Ad26.CoV2.S (n = 7) and bottom five rows are unvaccinated participants (n = 24). X-axis is the time post-symptom onset when sample was collected, and y-axis is neutralization as $FRNT_{50}$. Dashed line is the most concentrated plasma tested (LOQ, limit of quantification below which $FRNT_{50}$ values are extrapolated). All participants recovered except participant 29, who died.



Extended Data Fig. 2 | Fold-drop in BA.2 versus BA.1 neutralization in all and sequence confirmed samples. Neutralization of Omicron BA.2 compared to BA.1 in participants described in Extended Data Table 1, excluding participant 14 for technical reasons and participants 40 and 41 because of advanced HIV disease. Left panel shows neutralization capacity in all n = 38 participants and right panel shows neutralization capacity for n = 25 participants where infection was successfully sequenced and determined to be BA.1. Dashed line is the most concentrated plasma tested. p-values were 0.077 for all and 0.15 for BA.1 sequence confirmed participants as determined by a two-sided Wilcoxon rank sum test. ns, not significant.

Extended Data Table 1 | Characteristics of Omicron/BA.1 infected participants

#	Age	Sex	Vacc. type	Date of vacc.	Vacc. to enrol. (days)	Date symp. onset	Ct at enrol.	Symp. to enrol. (days)	Symp. to last follow-up (days)	Max CD4	Sub-lineage	Seq. GISAID ID	Supp. O_2	Hosp.
1	30-39	M	AD26.COV2	MAR-2021	274	DEC-2021*	24.9	1	23	1071	BA.1	EPI_ISL_9967759	No	No
2	30-39	M	AD26.COV2* *	NOV-2021	14	NOV-2021	14.5	1	22	789	BA.1	EPI_ISL_9967761	No	No
3	50-59	F	BNT162b2	JUL-2021	138	DEC-2021	16.8	4	27	777	BA.1	EPI_ISL_8604915	No	No
4	30-39	F	AD26.COV2	MAY-2021	210	DEC-2021	30.7	3	13	1169	BA.1	EPI_ISL_8604910	No	No
5	20-29	F	AD26.COV2	SEP-2021	89	DEC-2021	23.9	5	27	1220	BA.1	EPI_ISL_9967760	No	Yes
6	10-19	F	BNT162b2	JUL-2021	157	DEC-2021	23.1	6	12	732	BA.1	EPI_ISL_8604906	No	Yes
7	20-29	F				NOV-2021	UND	3	24	712	N/A	N/A	No	Yes
8	30-39	M				DEC-2021	18.2	1	23	847	BA.1	EPI_ISL_8604919	No	Yes
9	40-49	F				DEC-2021	32.3	6	28	1032	BA.1	EPI_ISL_8604901	No	Yes
10	20-29	M				DEC-2021	30.4	2	13	1197	BA.1	EPI_ISL_8604908	No	Yes
11	20-29	F				DEC-2021	28.3	8	22	863	BA.1	EPI_ISL_8604913	No	No
12	20-29	F				DEC-2021*	UND	7	22	1259	BA.1	EPI_ISL_8604912	No	Yes
13	30-39	M	BNT162b2	JUL-2021	129	NOV-2021	31.6	6	28	1069	BA.1	EPI_ISL_8604916	No	Yes
14	20-29	M				NOV-2021	30.8	8	15	1225	N/A	N/A	No	Yes
15	60-69	F	BNT162b2	JUL-2021	139	DEC-2021	24.6	4	25	345	BA.1	EPI_ISL_8604920	No	Yes
16	60-69	M	BNT162b2	DEC-2021	15	DEC-2021	24.6	2	10	904	BA.1	EPI_ISL_8578311	No	No
17	30-39	M				DEC-2021	37.0	5	19	1008	BA.1	EPI_ISL_8604923	No	No
18	60-69	F				DEC-2021	26.8	8	23	1111	BA.1	EPI_ISL_8578312	Yes	Yes
19	30-39	M				DEC-2021*	30.7	13	27	1077	BA.1	EPI_ISL_8604924	No	Yes
20	20-29	F				DEC-2021	35.9	8	24	533	BA.1	EPI_ISL_8604911	No	Yes
21	20-29	M				DEC-2021	29.1	7	21	225	BA.1	EPI_ISL_8604922	No	No
22	30-39	F	AD26.COV2	AUG-2021	120	DEC-2021	33.4	9	23	777	BA.1	EPI_ISL_8693907	No	Yes
23	20-29	F				DEC-2021	35.8	3	10	1167	BA.1	EPI_ISL_8604902	No	No
24	50-59	M	BNT162b2	AUG-2021	128	DEC-2021	36.6	4	18	605	N/A	N/A	No	Yes
25	30-39	F	AD26.COV2	APR-2021	237	DEC-2021	23.5	3	24	640	BA.1	EPI_ISL_8604914	No	No
26	50-59	F	AD26.COV2	JUL-2021	150	DEC-2021	UND	5	23	716	N/A	N/A	No	No
27	50-59	F				DEC-2021	32.4	12	28	625	N/A	N/A	Yes	Yes
28	80-89	F	BNT162b2	JUL-2021	177	JAN-2022	30.8	7	22	407	N/A	N/A	Yes	Yes
29	60-69	M	BNT162b2	JUL-2021	178	DEC-2021[$]	UND	25	32	351	N/A	N/A	Yes	Yes
30	40-49	M				DEC-2021	20.2	0	13	844	BA.1	EPI_ISL_8604909	No	No
31	30-39	F				DEC-2021	34.8	1	22	647	N/A	N/A	Yes	Yes
32	50-59	F				DEC-2021	28.2	15	36	620	BA.1	EPI_ISL_8578347	No	Yes
33	20-29	F				DEC-2021	UND	3	18	902	N/A	N/A	No	Yes
34	30-39	F				DEC-2021	34.8	9	30	1363	N/A	N/A	No	Yes
35	50-59	F				DEC-2021	26.6	4	27	766	BA.1	EPI_ISL_8578342	Yes	Yes
36	20-29	F				DEC-2021	UND	9	23	1212	N/A	N/A	No	Yes
37	50-59	F				DEC-2021	UND	12	30	995	N/A	N/A	No	Yes
38	30-39	M				DEC-2021	UND	9	31	746	N/A	N/A	No	Yes
39	50-59	F				DEC-2021	UND	13	30	840	N/A	N/A	Yes	Yes
40	30-39	F				DEC-2021	22.5	5	19	61***	BA.1	EPI_ISL_8578314	Yes	Yes
41	40-49	F				NOV-2021	29.8	17	24	53***	N/A	N/A	No	Yes

Ct enrol.: qPCR cycle threshold for SARS-CoV-2 at enrollment. Symptoms to enrol.: time between symptoms onset and study enrolment. Symp. to last follow-up: time between symptoms onset and last follow-up visit. Max CD4: maximum CD4 count per microliter blood across all study visits. Supp O_2: participant required supplemental oxygen during the study. Hosp.: participant hospitalized during the study. UND: Undetectable Ct. N/A: Not available; sequencing failed, usually due to insufficient virus substrate. *Reported previous infection. **Boosted with Ad26.CoV2.S in Nov-2021. ***Participants with persistent low CD4 count and uncontrolled HIV viremia indicative of advanced HIV disease and immune suppression. Excluded from analysis. [$]Deceased.

Extended Data Table 2 | Summary characteristics of Omicron/BA.1 infected participants

	All 39	Vaccinated 15 (38%)	Unvaccinated 24 (62%)
Age	35 (27-55)	37 (32-60)	31.5 (26-49)
Female	25 (64%)	9 (60%)	16 (67%)
Vaccination to enrollment (days)	-	139 (120-178)	-
Symptom onset to enrolment (days)	6 (3-9)	4 (3-6)	7.5 (3-9)
Symptom onset to last follow-up (days)	23 (19-27)	23 (18-27)	23 (20-28)
Maximum CD4 count (cell/μL)	844 (647-1077)	777 (605-1069)	882.5 (729-1139)
Required supp. O_2	7 (18%)	2 (13%)	5 (21%)
Hospitalized	27 (69%)	8 (53%)	19 (79%)
Duration of hospitalization (days)	7 (3-11)	3.5 (2.5-14.5)	8 (3-11)

Values are median (IQR). Hospital stay calculated to last inpatient study visit.

Extended Data Table 3 | Characteristics of Delta infected participants

#	Age	Sex	Vacc. type	Date of vacc.	Vacc. to enrol. (days)	Date symp. onset	Ct at enrol.	Symp. to collection (days)	Seq. GISAID ID
1	40-49	F				JUL-2021	26	26	EPI_ISL_3722338
2	40-49	M				JUL-2021	31	23*	EPI_ISL_3722335
3	50-59	M				JUL-2021	30	31	N/A
4	50-59	M				JUN-2021	27	37	N/A
5	40-49	M				JUL-2021	35	44	N/A
6	30-39	M				JUL-2021	37	32	N/A
7	70-79	M	BNT162b2	JUN-2021	37	JUL-2021	37	15	N/A
8	60-69	F	BNT162b2	NOV-2021	14	AUG-2021	UND	116	N/A
9	40-49	F	AD26.COV	MAY-2021	117	JUL-2021	UND	31	N/A
10	50-59	F	AD26.COV	APR-2021	147	JUL-2021	UND	57	N/A
11 Pre	40-49	M				AUG-2021*	35	13*	N/A
11 Post	40-49	M	BNT162b2	OCT-2021	18	AUG-2021	UND	83	N/A
12 Pre	40-49	M				JUL-2021	23	24	EPI_ISL_3939068
12 Post	40-49	M	AD26.COV	SEP-2021	32	JUL-2021	UND	92	N/A
13 Pre	30-39	M				JUL-2021	27	24	EPI_ISL_3939088
13 Post	30-39	M	AD26.COV	SEP-2021	32	JUL-2021	UND	94	N/A
14 Pre	50-59	F				JUL-2021*	27	23*	EPI_ISL_3447779
14 Post	50-59	F	BNT162b2	OCT-2021	22	JUL-2021	UND	93	N/A

*Asymptomatic, date of diagnostic swab used instead of symptoms onset. Ct enrol: qPCR cycle threshold for SARS-CoV-2 at enrollment. UND: undetectable. Pre: sample taken pre-vaccination. Post: sample taken post-vaccination for participants with a pre-vaccination sample. N/A: not available.

9.1.3. Beta infection combined with Pfizer BNT162b2 vaccination leads to broadened neutralizing immunity against Omicron.

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Beta infection combined with Pfizer BNT162b2 vaccination leads to broadened neutralizing immunity against Omicron

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Abstract

Omicron (B.1.1.529) shows extensive escape from vaccine immunity, although vaccination reduces severe disease and death[1]. Boosting with vaccines incorporating variant spike sequences could possibly broaden immunity[2]. One approach to choose the variant may be to measure immunity elicited by vaccination combined with variant infection. Here we investigated Omicron neutralization in people infected with the Beta (B.1.351) variant and subsequently vaccinated with Pfizer BNT162b2. We observed that Beta infection alone elicited poor Omicron cross-neutralization, similar to what we previously found[3] with BNT162b2 vaccination alone or in combination with ancestral or Delta virus infection. In contrast, Beta infection combined with BNT162b2 vaccination elicited neutralization with substantially lower Omicron escape.

Results and Discussion

South Africa had a Beta dominated infection wave November 2020 to May 2021[4]. We enrolled 18 Beta infected participants based on infection date who were later vaccinated with Pfizer BNT162b2 (Table S1). A pre-vaccine sample was taken a median of 29 days post-symptom onset, and a post-vaccine sample 31 days post-vaccination. Five participants received one vaccine dose and the others two doses. None were boosted.

We measured plasma neutralization capacity as $FRNT_{50}$ using live virus neutralization. Post-vaccination, Beta virus neutralization increased 21.3-fold (95% CI 9.7-46.8, Fig 1a) from geometric mean titer (GMT) $FRNT_{50}$ of 112 to 2385. Omicron/BA.1 $FRNT_{50}$ pre-vaccination was 6, rising post-vaccination 69.3-fold (95% CI 32.0-150, Fig 1b) to 447. Pre-vaccination, Omicron/BA.1 neutralization was 17.3-fold lower than Beta virus neutralization (95% CI 11.2-26.1, Fig 1c), confirming previous results[3]. Because many pre-vaccination samples did not reach 50% BA.1 neutralization with the most concentrated plasma tested, we tested additional samples from Beta infected individuals and obtained similar results (19.2-fold escape, Fig S1). In contrast, the fold-drop between Beta and Omicron/BA.1 neutralization post-vaccination was 5.3-fold (95% CI 4.4-6.4, Fig 1d). Hence, Omicron/BA.1 escape was reduced more than 3-fold. Similarly to BA.1, cross-neutralization of Omicron/BA.2 post-

vaccination was only 4.2-fold lower (95% CI 3.4-5.2, Fig 1e). Ancestral virus neutralization showed a 1.5-fold decline relative to Beta (95% CI 1.2-1.8, Fig 1f).

These results contrast with our previous findings for BNT162b2 vaccination alone or in combination with ancestral or Delta virus infection, where we observed approximately 20-fold drops in Omicron neutralization[3]. The limitations are that here we tested immunity generated by Beta virus infection followed by vaccination. Whether boosting with Beta sequence post-primary vaccination would be similar is unclear. However, it may be one line of evidence which argues for testing this approach.

References

1 Sigal, A. Milder disease with Omicron: is it the virus or the pre-existing immunity? *Nature Reviews Immunology* **22**, 69-71, doi:10.1038/s41577-022-00678-4 (2022).

2 Corbett, K. S. *et al.* Protection against SARS-CoV-2 Beta variant in mRNA-1273 vaccine-boosted nonhuman primates. *Science* **374**, 1343-1353, doi:10.1126/science.abl8912 (2021).

3 Cele, S. *et al.* Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature*, doi:10.1038/s41586-021-04387-1 (2021).

4 Cele, S. *et al.* Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. *Nature* **593**, 142-146, doi:10.1038/s41586-021-03471-w (2021).

5 Rössler, A., Riepler, L., Bante, D., von Laer, D. & Kimpel, J. SARS-CoV-2 Omicron Variant Neutralization in Serum from Vaccinated and Convalescent Persons. *N Engl J Med*, doi:10.1056/NEJMc2119236 (2022).

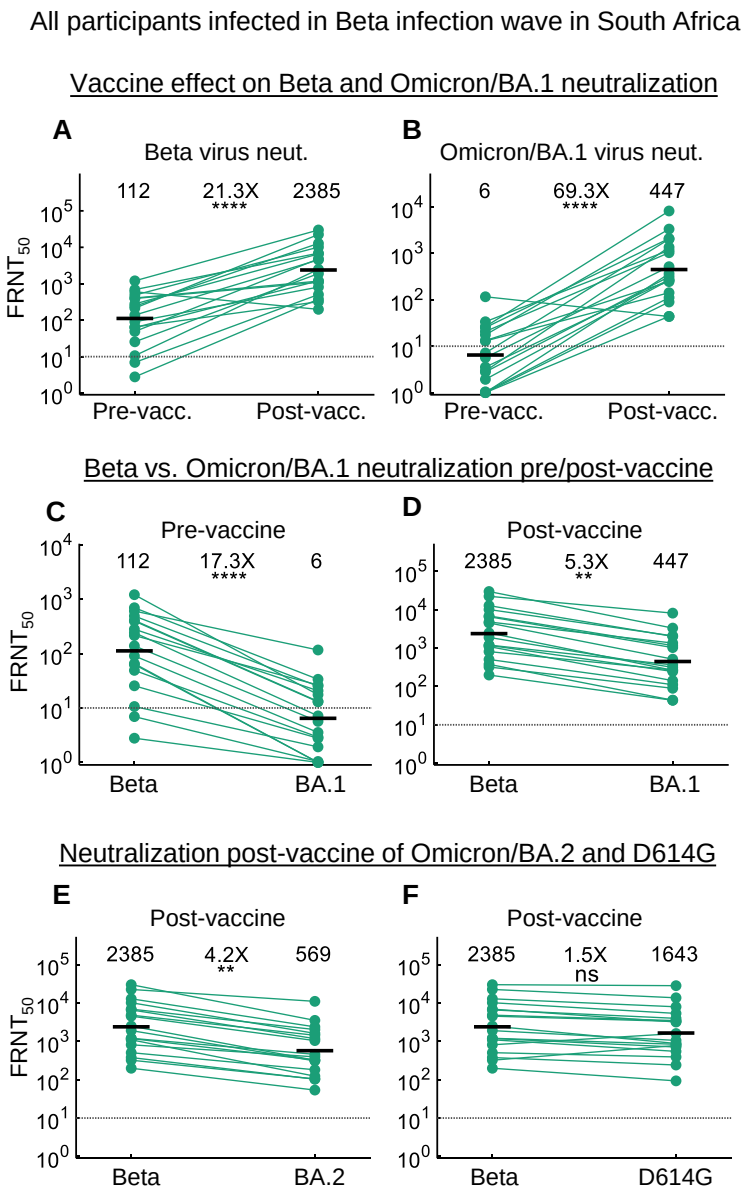

Figure 1: Hybrid immunity elicited by Beta variant infection combined with Pfizer BNT162b2 vaccination reduces Omicron escape. (A) Neutralization of Beta virus by plasma from n=18 convalescent participants infected during the Beta infection wave (December 2020 - May 2021) in South Africa, before (left) and after vaccination with BNT162b2 (right). Participants were sampled a median of 29 days post-symptom onset (pre-vaccine), and 31 days post-vaccine (Table S1). The same participant samples are used in the subsequent panels. Numbers are geometric mean titers (GMT) of the reciprocal plasma dilution ($FRNT_{50}$) resulting in 50% neutralization. Fold-change is calculated by dividing the larger GMT value by the smaller value. Dashed line is most concentrated plasma tested. (B) Neutralization of Omicron/BA.1 virus before and after vaccination. (C) Neutralization of Beta versus Omicron/BA.1 virus pre-vaccination. (D) Neutralization of Beta versus Omicron/BA.1 virus post-vaccination. (E) Neutralization of Beta versus Omicron/BA.2 virus post-vaccination. (F) Neutralization of Beta versus ancestral/D614G virus post-vaccination. Data points are geometric means of $FRNT_{50}$ measurements from two (BA.1 pre- and post-vaccine, Beta pre-vaccine, and BA.2 post-vaccine conditions) or four (Beta and D614G post-vaccine conditions) independent experiments performed on different days. p-values were: (A) 1.2×10^{-5}, (B) 3.1×10^{-7}, (C) 4.1×10^{-5}, (D) 0.0021, (E) 0.0050, (F) 0.10 as determined by the Wilcoxon rank sum test.

Supplementary appendix to: Beta infection combined with Pfizer BNT162b2 vaccination leads to broadened neutralizing immunity against Omicron

Cele et al.

Contents

Table S1: Summary participant characteristics

	Number (%) or median (IQR)
Female (number, percentage)	11 (61%)
Age (years)	45.5 (35-63)
Symptom onset to pre-vaccine sample (days)	28.5 (25-38)
Symptom onset to vaccination (days)	201 (162-240)
Vaccination to post-vaccine sample (days)	31 (18-65)
Symptom onset to post-vaccine sample (days)	265 (192-283)

Supplementary materials and methods

Informed consent and ethical statement

Blood samples used for plasma isolation and neutralization experiments and swabs for isolation of the ancestral/D614G and Delta viruses were obtained after written informed consent from adults with PCR-confirmed SARS-CoV-2 infection who were enrolled in a prospective cohort study at the Africa Health Research Institute approved by the Biomedical Research Ethics Committee at the University of KwaZulu–Natal (reference BREC/00001275/2020). The Beta virus was obtained from residual swab samples used for diagnostic testing by the National Health Laboratory Service (BREC approval reference BREC/00001510/2020). The Omicron/BA.1 virus was isolated from a residual swab sample with SARS-CoV-2 isolation from the sample approved by the University of the Witwatersrand Human Research Ethics Committee (HREC) (ref. M210752). The sample to isolate Omicron/BA.2 was collected after written informed consent as part of the "COVID-19 transmission and natural history in KwaZulu-Natal, South Africa: Epidemiological Investigation to Guide Prevention and Clinical Care" Centre for the AIDS Programme of Research in South Africa (CAPRISA) study and approved by the Biomedical Research Ethics Committee at the University of KwaZulu–Natal (reference BREC/00001195/2020, BREC/00003106/2021).

Data and sequence availability statement

Sequence of Omicron sub-lineage viruses have been deposited in GISAID with accession EPI_ISL_7886688 (Omicron/BA.1), EPI_ISL_9082893 (Omicron/BA.2), EPI_ISL_678615 (Beta), and EPI_ISL_602622 (ancestral D614G). Raw images of the data are available upon reasonable request.

Cells

H1299 cell lines were propagated in growth medium consisting of complete Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum containing 10mM of HEPES, 1mM sodium pyruvate, 2mM L-glutamine and 0.1mM nonessential amino acids. H1299 cells were passaged every second day. The H1299-E3 (H1299-ACE2, clone E3) cell line was derived from H1299 (CRL-5803) as described in our previous work[1,2].

Live virus neutralization assay

H1299-E3 cells were plated in a 96-well plate (Corning) at 30,000 cells per well 1 day pre-infection. Plasma was separated from EDTA-anticoagulated blood by centrifugation at 500 rcf for 10 min and stored at −80 °C. Aliquots of plasma samples were heat-inactivated at 56 °C for 30 min and clarified by centrifugation at 10,000 rcf for 5 min. Virus stocks were used at approximately 50-100 focus-forming units per microwell and added to diluted plasma.

Antibody–virus mixtures were incubated for 1 h at 37 °C, 5% CO_2. Cells were infected with 100 µL of the virus–antibody mixtures for 1 h, then 100 µL of a 1X RPMI 1640 (Sigma-Aldrich, R6504), 1.5% carboxymethylcellulose (Sigma-Aldrich, C4888) overlay was added without removing the inoculum. Cells were fixed 18 h post-infection using 4% PFA (Sigma-Aldrich) for 20 min. Foci were stained with a rabbit anti-spike monoclonal antibody (BS-R2B12, GenScript A02058) at 0.5 µg/mL in a permeabilization buffer containing 0.1% saponin (Sigma-Aldrich), 0.1% BSA (Sigma-Aldrich) and 0.05% Tween-20 (Sigma-Aldrich) in PBS. Plates were incubated with primary antibody overnight at 4 °C, then washed with wash buffer containing 0.05% Tween-20 in PBS. Secondary goat anti-rabbit HRP conjugated antibody (Abcam ab205718) was added at 1 µg/mL and incubated for 2 h at room temperature with shaking. TrueBlue peroxidase substrate (SeraCare 5510-0030) was then added at 50 µL per well and incubated for 20 min at room temperature. Plates were imaged in an ImmunoSpot Ultra-V S6-02-6140 Analyzer ELISPOT instrument with BioSpot Professional built-in image analysis (C.T.L).

Statistics and fitting

All statistics and fitting were performed using custom code in MATLAB v.2019b. Neutralization data were fit to:

$Tx=1/1+(D/ID_{50})$.

Here Tx is the number of foci normalized to the number of foci in the absence of plasma on the same plate at dilution D and ID_{50} is the plasma dilution giving 50% neutralization. $FRNT_{50} = 1/ID_{50}$. Values of $FRNT_{50} <1$ are set to 1 (undiluted), the lowest measurable value. We note that the most concentrated plasma dilution was 1:10 for experiments with weak neutralization (pre-vaccine samples) and therefore $FRNT_{50} < 10$ were extrapolated. To calculate confidence intervals, $FRNT_{50}$ or fold-change in $FRNT_{50}$ per participant was log transformed and arithmetic mean + 2 std and arithmetic mean - 2 std were calculated for the log transformed values. These were exponentiated to obtain the upper and lower 95% confidence intervals on the geometric mean $FRNT_{50}$ or the fold-change in $FRNT_{50}$ geometric means.

Acknowledgements

This study was supported by the Bill and Melinda Gates award INV-018944 (AS), National Institutes of Health award R01 AI138546 (AS), and South African Medical Research Council award 6084COAP2020 (AS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1 Cele, S. *et al.* Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. *Nature* **593**, 142-146, doi:10.1038/s41586-021-03471-w (2021).

2 Cele, S. *et al.* Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature*, doi:10.1038/s41586-021-04387-1 (2021).

Supplementary Figure 1

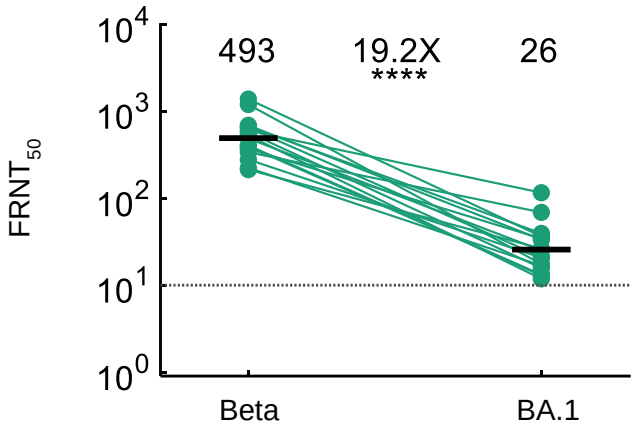

Figure S1: Omicron escape in participants with Omicron neutralization greater than $FRNT_{50}$=10. For this analysis, participant samples pre-vaccination (n=8, participant numbers 1, 5, 6, 12, 13, 14, 17, 18 according to Table S2) were selected based at least 50% Omicron/BA.1 neutralization at the most concentrated plasma used (1:10 dilution, corresponding to $FRNT_{50}$=10 shown by dashed horizontal line). In addition, n=8 samples from unvaccinated participants infected in the Beta infection wave in South Africa were selected solely on $FRNT_{50}$>10 for Omicron/BA.1 neutralization (participants 19-26 listed on Table S3). Data points are geometric means of $FRNT_{50}$ measurements from two independent experiments performed on different days. p-value was $7.7x10^{-7}$ as determined by the Wilcoxon rank sum test and fold-change 95% confidence intervals were 14.1 to 28.1-fold.

9.2. APPENDIX B: ETHICS APPROVAL LETTER

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

21 March 2022

Mrs Natasha Samsunder (891101429)
School of Lab Med & Medical Sc
Medical School

Dear Mrs Samsunder,

Protocol reference number: BREC/00003902/2022
Project title: Evaluation of Laboratory Tests for COVID-19 in South Africa
Degree: PhD

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 21 March 2022. Please ensure that any outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Amended_Lockdown_Level_1_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 21 March 2022. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 12 April 2022.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

28 March 2023

Mrs Natasha Samsunder (891101429)
School of Laboratory Medicine & Medical Science
Medical School

Dear Mrs Samsunder,

Protocol reference number: BREC/00003902/2022
Project title: Evaluation of Laboratory Tests for COVID-19 in South Africa
Degree: PhD

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	**21 March 2023**
Expiration of Ethical Approval:	**20 March 2024**

I wish to advise you that your application for recertification received on 27 March 2023 for the above study has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee (BREC). The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 09 May 2023.

Yours sincerely

[redacted]

……..
Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

31 March 2020

Prof Salim Safurdeen Abdool Karim (93336)
CAPRISA
MEDICAL SCHOOL

Dear Prof Salim Safurdeen Abdool Karim,

Protocol reference number: BREC/00001195/2020
Project title: COVID-19 transmission and natural history in KwaZulu-Natal, South Africa: Epidemiological Investigation to Guide Prevention and Clinical Care
Non-Degree purposes

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given **full ethics approval** and may begin as from 31 March 2020. **Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.**

This approval is subject to national and UKZN lockdown regulations and the general BREC circular emailed by the Research Office on 23rd March 2020 and repeatedly since.

This approval is valid for one year from **31 March 2020. T**o ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **noted** by a full Committee at its next meeting taking place on **12 May 2020.**

Yours sincerely

[redacted]

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc: DVC College of Health Sciences

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

06 October 2021

Dr Ayesha Bibi Mahomed Kharsany (34327)
School of Lab Med & Medical Sc
Medical School

Dear Dr Kharsany,

Protocol reference number: BREC/00003106/2021
Project title: Natural History and Laboratory Tests for COVID-19 in South Africa HALT_COVID_19 Non-Degree Purposes

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 06 October 2021. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Amended_Lockdown_Level_1_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 06 October 2021. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 09 November 2021.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

9.3. APPENDIX C: DISTRICT APPROVAL

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

DIRECTORATE:
Health Research & Knowledge Management

Physical Address: 330 Langalibalele Street, Pietermaritzburg
Postal Address: Private Bag X9051
Tel: 033 395 2805/ 3186/ 3123 Fax: 033 394 3782
Email: [redacted]
www.kznhealth.gov.za

Dear Prof Salim Abdool Karim

Re: Approval of research

The research proposal entitled:

"COVID-19 transmission and natural history in KwaZulu-Natal, South Africa: Epidemiological Investigation to Guide Prevention and Clinical Care", as approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (reference BREC/00001195/2020)

was reviewed by the Chair of the KZN Provincial Health Research and Ethics Committee, in terms of the amended process for the approval of Covid-19 related research, as noted by the Head of the KZN Department of Health on 24/03/2020.

The proposal is hereby **approved** for research to be undertaken at King Dinizulu Hospital and KwaMashu Polyclinic.

1. You are requested to take note of the following:
   a. *Kindly liaise with the facility managers BEFORE your research begins in order to ensure that conditions in the facility are conducive to the conduct of your research.*
   b. *Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.*
   c. *Provide an interim progress report and final report (electronic and hard copies) when your research is complete to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za*
   d. *Please note that the Department of Health shall not be held liable for any injury that occurs as a result of this study.*

For any additional information please contact Dr E Lutge on 0834158778.

Yours Sincerely

[redacted]

Dr E Lutge
Chairperson, Health Research Committee
Date: 09/04/2020

9.4. APPENDIX D: INFORMED CONSENT FORMS

CAPRISA
CENTRE FOR THE AIDS PROGRAMME OF RESEARCH IN SOUTH AFRICA
Generating Knowledge - Impacting Health

SCREENING INFORMED CONSENT DOCUMENT

COVID-19 transmission and natural history in KwaZulu-Natal, South Africa: Epidemiological Investigation to Guide Prevention and Clinical Care

Version 2.0, 5 March 2021
(corresponds with Protocol Version 2.0, 30 September 2020)

PRINCIPAL INVESTIGATOR: Prof Salim S. Abdool Karim

PHONE: 031 260 4550 / 4555 (08:00am – 05:00pm)

If the volunteer cannot read, this form must be read to the volunteer exactly as written, in the volunteer's language of choice, and a witness must sign this form to confirm that the correct information was given to the volunteer and that the volunteer freely consents to be in this study.

BACKGROUND
COVID-19 is an infectious disease caused by a newly discovered coronavirus. Since the time it was identified in December 2019, COVID-19 has spread throughout the world and caused over 1 million deaths. The South African government have implemented several ways to try and slow the spread of COVID-19. These include social distancing and limiting the gathering of large groups. Another essential way to control COVID-19 is increase testing so that the people with COVID-19 can be isolated and prevent further spread. We are assisting the government efforts to control COVID-19 by offering screening and testing for suspected cases in two communities in KwaZulu-Natal.

You are being asked to take part in this study to help establish how the COVID-19 coronavirus is spreading and to provide information on people you may have been in contact with over the past 2 weeks to help us trace other cases of COVID-19.

Before you decide if you want to participate in this study, we want you to learn more about the study. This consent form gives you information about this study. Study staff will talk with you and answer any questions you may have. Once you read and understand its requirements, you can decide if you want to take part in the study or not. If you do decide to participate in this study, you will sign your name or make your mark on this form. A copy of this document will be offered to you.

It is important to know that your participation in this research is your decision and taking part in this study is completely voluntary (see Your Rights as a Research Participant/Volunteer for more information).

WHAT IS THE PURPOSE OF THIS STUDY?
The overall goal of this project is to establish how the SARS-COV-2 coronavirus is spreading in urban and rural KwaZulu-Natal in order to guide locally appropriate prevention and clinical care.

STUDY PRODUCTS
There are no study products (investigational drugs or other products) involved in this research study.

STUDY PROCEDURES
You will be asked to provide us with up to three swabs from your nose and/or throat to test for COVID-19. The results of this test will be provided to you as soon as they are available. If the test is positive for COVID-19, you will be provided with the results with appropriate post-test counselling about the finding and will be advised to remain in isolation with no physical contact with others (even those living in the same house as

you) for 14 days. One swab will be used for PCR testing, and the other swabs will be used to validate new COVID-19 tests.

You may also be asked to provide a teaspoon of blood that will be used to assess immunity to COVID-19. The results of the antibody test performed on site will be provided to you and findings explained. Positive antibody test indicates previous or ongoing exposure to COVID-19 and does not mean you are immune to infection and further not everyone with Covid-19 infection develop antibodies.

As Covid-19 is a notifiable condition and can be spread to others who you have been in contact with we will ask you to identify people who you have been in contact with over the past 2 weeks with their contact details so that if you test positive we can contact them for screening. If you have already tested positive for COVID 19 then we would like to offer your family members and friends that you have been in contacted with in the past 2- 3 weeks screening for COVID-19. We will also ask you a few questions about your where you live; age, and occupation and your health that will include (but is not limited to) questions about co-infections with HIV or tuberculosis, any other chronic conditions (including hypertension and diabetes), and any medications you are currently using.

RISKS AND/OR DISCOMFORTS
During the interview, you may be asked some questions you may find embarrassing or make you uncomfortable. You can choose not to answer questions during the interview if you do not wish to.

Another possible risk of participation in this study is loss of confidentiality of the information you give as health authorities need to be informed if you test positive for Covid-19. Every effort will be made to protect your information, but this cannot be guaranteed.

You may feel discomfort or pain during the blood collection. You may feel dizzy or faint. You may have a bruise, swelling, small clot, or infection where the needle is inserted in your arm.

BENEFITS
Information and education will be provided to you to ensure that you are well-informed about COVID-19 and its mechanisms of transmission.

People you have been in immediate contact with will be traced and screened / tested. They will also learn whether they are carrying the virus. This approach may help prevent further spread of COVID-19 in the community.

Participants in this study may also appreciate the opportunity to contribute to the deeper understanding of the COVID-19 virus, as well as prevention and clinical care.

Medical care for other health conditions will not be part of this study. This study cannot provide you with general medical care, but study staff will refer you to other available sources of care, if needed.

NEW INFORMATION
You will be told of any new information learned during this study that might affect your willingness to participate in the study. You will also be told when study results may be available, and how to learn about them.

WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT
You may be removed from the study early without your permission if:

- The study is cancelled by the local government or the Research Ethics Committee (EC). The Research Ethics Committee is a committee that watches over the safety and rights of research participants.
- You are unwilling or unable to comply with required study procedures
- Other reasons that may prevent you from completing the study successfully

COSTS TO YOU
There is no cost to you.

REIMBURSEMENT
There is no reimbursement for the screening procedures. If you are eligible to participate in any follow-up studies you will provide separate consent for those studies that will include information on reimbursement. You will receive COVID-19 antigen and antibody test free of charge and the results will be reported to you as soon as they are available.

CONFIDENTIALITY
Efforts will be made to keep the information gained confidential. However, it is not possible to guarantee confidentiality. The researchers will do everything they can to protect your privacy. However, your personal information may be disclosed if required by law. Any publication of this study will not use your name or identify you personally.

Your records may be reviewed by

- Research Ethics Committee
- Study staff
- The South African government.

DATA STORAGE AND DESTRUCTION
Your personal information collected in this study will be archived in accordance with applicable guidelines and laws, including the Protection of Personal Information Act, 2013.

RESEARCH-RELATED INJURY
It is unlikely that you will be injured because of screening procedures. If you are injured, CAPRISA will give you immediate necessary treatment for your injuries. You will not have to pay for this treatment. You will be told where you can receive additional treatment for your injuries.

YOUR RIGHTS AS A RESEARCH PARTICIPANT/VOLUNTEER
You do not give up any legal rights by signing this consent form. Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. If you choose not to participate or to leave the study, you will not lose the benefit of services to which you would otherwise be entitled. If you want the results of the study after the study is over, let the study staff members know.

PROBLEMS OR QUESTIONS
If you ever have any questions about the study, or if you have a research-related injury, you should contact Prof Salim S. Abdool Karim at 031 260-4550 / 4555.

If you have questions about your rights as a research participant, you should contact the Biomedical Research Ethics Committee, University of KwaZulu-Natal, Research Office, Westville campus, Govan Mbeki Building, Private Bag X54001, Durban, 4000, KwaZulu-Natal, South Africa. Tel: 031 260 2486 Fax: 031 260 4609, Email: BREC@ukzn.ac.za.

SIGNATURES – VOLUNTARY CONSENT

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to participate in this study, please sign your name or make your mark below.

Participant Name (print)	Participant Signature	Date
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
Witness Name	Witness Signature	Date

CONSENT FOR STORAGE AND FUTURE TESTING OF SPECIMENS

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IFOMU LEMVUMO UNOLWAZI

Ukudluliselwa kwe-COVID-19 kanye nomlando wemvelo KwaZulu-Natali eSouth Afrika: Uphenyo lwezifo ukuze Kuqondiswe Ukuvimbela Nokunakekelwa Emtholampilo

Protocol Version 1.0, 26 March 2020

UMPHENYI OMKHULU: Prof Salim S. Abdool Karim
UCINGO: 031 260 4550 / 4555 (08:00am – 05:00pm)

Uma ivolontiya lingakwazi ukufunda, lelifomu kumele lifundelwe njengoba libhaliwe, ngolwimi olukhethwe ivolontiya, futhi ufakazi kumele asayine lelifomu ukuqinisekisa ukuthi ivolontiya linikiwe ulwazi oluyilo nokuthi ivolontiya linekezile imvumo yokubasocwaningweni ngokukhululeka.

ISIZINDA/UMSUSA
Ngo-Novemba 2019, isigameko/umbiko wokuqala ngesifo esisha, okuthe ngokuhamba kwesikhathi umnyango wezempilo kazwelonke wasetha igama lokuthi i-COVID-19, abikwa ngabasebenzi bezempilo basezweni laseChina. Kusukela lapho lesisifo esisha sesisabalale kumhlaba wonke. Ezinyangeni nje ezimbalwa, sekubikwe izigameko/imbiko engaphezulu kuka 300 000 e-COVID-19 emazweni angu-69 emhlabeni wonke. Eningizimu Afrika umbiko wokuqala nge-COVID-19 wabikwa ngomhlaka 5 Mbasa 2020 futhi izinombolo zalembiko ziyaqhubeka nokukhula usuku nosuku. Imbiko eminingi ngeyabantu abasanda kuhambela ezweni lase Europe noma e-US, kodwa sekunobufakazi obucacile ngokuthelelana komphakathi futhi inani lemibiko qobolwayo mhlawumbe ingaphezulu kakhulu kwale esibikiwe.

Uhulumeni wase Ningizimu Afrika useqalise izindlela ezimbalwa ukuzama ukukwehlisa ukubhebhetheka kwe-COVID-19. Lapho kubalwa ukuqhelelana kwabantu kanye nokukhawula imibuthano yabantu abaningi. Enye yezindlela ebalulekile yokulawula i-COVID-19 ukuhlola abantu abaningi ukuze abantu abane COVID -19, bazohlukaniswa futhi kuvikelwe ukuqhubeka ukuyisabalalisa. Sisiza uhulumeni emizwamweni yokulawula i-COVID-19 ngokunikezela ngokuhlolwa kwabasolwa ukuba nezimpawu emiphakathini emibili yaKwa-Zulu Natali.

Uyacelwa ukuba ubambe iqhaza kulolucwaningo ukusiza nokuhlonza ukuthi igciwane lecorona COVID-19 libhebhetheka kanjani nokunikezela ngolwazi lwabantu okungenzeka uke wahlanganyela nabo emasontweni amabili adlulile ukusiza ukuthola umkhondo ngeminye imibiko/izigameko nge-COVID-19.

Ngaphambi kokuba uthathe isinqumo umangabe uyathanda ukubamba iqhaza kulolucwaningo, sifisa ukuba wazi kabanzi ngalolucwaningo. Lelifomu lemvumo unolwazi likunikeza ulwazi mayelana nocwaningo. Umsebenzi wocwaningo uzoxoxisana nawe aphinde aphendule noma imiphi imibuzo ongaba nayo. Uma usufundile waqonda okudingekayo, unganquma ukuthi uyathanda ukubamba iqhaza noma cha. Uma unquma ukubamaba iqhaza ocwaningweni, uzosayina igama lakho noma wenze uphawu kulelifomu. Ikhophi yalelifomu izonikezelwa kuwe.

Kubalulekile wazi ukuthi ukuzibandakanya kulolucwaningo kuyisinqumo sakho kanye nokubamba iqhaza kulokucwaningo kungukuzinikela ngokuphelele (bheka Amalungelo akho njengombambiqhaza/ivolontiya ocwaningweni ukuthola ulwazi olugcwele).

IYINI INHLOSO YALOLUCWANINGO?
Inhloso ephelele yalomsebenzi ukuthola ukuthi i-SARS-COV-2 coronavirus isakazeka kanjani emadolobheni kanye nasemaphandleni KwaZulu-Natali ukuze kuqondisiseke indlela efanelekile yokuvimbela kanye nokunakekelwa ngokwezempilo.

IMIKHIQIZO YOCWANINGO
Ayikho imikhiqizo ecwaningwayo (imithi ephenywayo noma eminye imikhiqizo) ehlangene nalolucwaningo.

IZINQUBO ZOCWANINGO
Usanda kusinikezela ngamaswabhu akho eyasekhaleni kanye neyomphimbo ukuhlolela i-COVID-19 noma kungenzeka usuvele uhloliwe futhi uyayazi imiphumela yakho. Imiphumela yalokukuhlolwa uzonikezwa yona ngokushesha uma isikhona. Uma ukuhlolwa kubonisa ukuthi uthelelekile nge-COVID-19, uzonikezwa imiphumela nokululekwa okufanele mayelana nokutholakele, futhi uzolulekwa ukuba uhlale ngawedwana ngaphandle kokuxhumana ngokuthintana nabanye (ngisho nalabo ohlala nabo endlini) izinsuku ezingu14. Kungenzeka usuvele uhlolile futhi uyayazi imiphumela yakho. Sidinga amasampula akho angeziwe ukubheka ukuthi umzimba wakho ngabe ulwisana kanjani negciwane i-COVID-19. Sizocela ukuba uphinde usiphe amanye amaswabhu kanye namasampula egazi (cishe ngangamathisipuni ayishumi). Iswabhu izosetshenziswa ukuqinisekisa ukuhlolwa ngenkathi amasampula egazi esetshenziselwa ukuhlola ukhuthi amasosha akho omzimba alwisana kanjani negciwane. Uzothintwa okungenani kanye ngocingo emasontweni amabili ukubuza ngezimpawu okungengezeka ukuba unazo, okuhlanganisa imfiva (ukushisa komzimba okweqile kokulindelekile), ukukhwehlela okomile okuhlanganisa nokuphelelwa umoya Kanye nokucinana kwesifuba. Uma unezimpawu eziqinile zesifo i-COVID-19 uzodluliselwa esibhedlela ukuze uyonakekelwa njengomyalolelo wokunakekelwa. Sizophinda sikuthinte futhi ezinyangeni ezimbili ngemva kokuhlolwa kwakho okubonisa ukuthi uthelelekile nge-COVID-19, sikucele ukuba usihlinzeke ngenye isampula yegazi (cishe ngangamathisipuni ayishumi) Lesampula izogcinelwa ucwaningo lwangomuso ukuqinisekisa indatshana entsha nge COVID-19 kanye nokuqonda ukuthi i-COVID-19 ihlasela kanjani amasosha omzimba.

Kulolucwaningo sizokubuza imibuzo embalwa ukuhlonza ukuthi ngobani okewaxhumana nabo emasontweni amabili adlule ukuze sihlonze futhi siphinde sibahlole. Uma usuvele usunemiphumela ebonisa ukuthi unayo i-COVID-19 sizobe sesicela ukuthi usihlinzeke ngemininingwane yamalunga omndeni wakho kanye nabangani okewaxhumana nabo emasontweni amabili kuya kwamathathu adlule ukuhlolelwa i-COVID-19. Sizobuye futhi sikubuze ngomphakathi wakho kanye nangezempilo. Lokhu kuzohlanganisa (kodwa kungagcini lapho) imibuzo ngokutheleleka nge HIV noma isifo sofuba, nanoma ngezinye izifo ezingamahlakhona (okuhlanganisa iphika kanye noshukela), futh nanoma imiphi eminye imishanguzo okungenzeka ukuba uyayisebenzisa.

Sizophinda sisebenzise amaswabu lawa owawusihlinzeke ngawo ukuhlolela i-COVID-19 ukulandelela igciwane sizame ukuthola ukuthi lifanakanjani leligciwane lakho kwamanye asabalele emphakathini.

UBUNGOZI KANYE/NOMA UKUNGAKHULULEKI
Phakathi nengxoxo, ungabuzwa eminye yemibuzo engakwenza uzizwe uhlazekile noma ungakhululekile. Abahloli bocwaningo abaqeqeshiwe bazokusiza ukubhekana nanoma imiphi imizwa noma imibuzo onayo. Noma ngasiphi isikhathi ungakhetha ukungaphenduli imibuzo phakathi nengxoxo.

Obunye ubungozi obungenzeka ngalolucwaningo ukulahlekelwa ubumfihlo ngolwazi olunikezayo. Kuzokwenziwa konke okusemandleni ukuvikela ulwazi lwakho oluyimfihlo, kodwa lokhu ngeke kuqinisekiswe.

Ungezwa ukungakhululeki noma ubuhlungu uma kudonswa igazi. Ungazizwa unesiyezi noma ubuthaka. Ungaba nokulimala, ukuvuvuka, isibazi esincane, noma ukuvunda lakungena khona inalithi esandleni noma engalweni.

IZINZUZO
Ulwazi nokufundiswa kuzohlinzekwa kuwe ukuze kuqinisekiswe ukuthi unolwazi oluphelele mayelana ne-COVID-19 kanye nangendlela ethelelana ngayo.

Abantu osanda kuxhumana nabo bazolandelelwa bahlolwe. Bazobe sebeyathola ukuthi banalo yini igciwane. Lendlela ingasiza ukunqanda ukubhebhetheka kwe-COVID-19 emphakathini.

Ababambiqhaza kulolucwaningo bangathokozela ithuba lokuba nesandla ekuqondisiseni kabanzi ngegciwane i-COVID-19, nokunqanda kanye nokunakekelwa ngokwezempilo.

IMVUMO YOKUGCINWA NOKUHLOLWA KWANGOMUSO KWAMASAMPULA

Sicela imvumo yakho ukugcina izinsalela zakho zegazi kanye nolwazi olumayelana nezempilo ukukusebenzisa kucwaningo lwangomuso. Lolulwazi lwezempilo lungahlanganisa amaqiniso ngawe siqu njengobuhlanga bakho, ubuzwe, ubulili, izimo zezempilo kanye nesilinganiso seminyaka yakho kodwa hhayi igama lakho. Uma uvuma, amasampula akho kanye nolwazi olumayelana nezempilo kuzogcinwa endaweni ephephile futhi ngendlela ephephile ezikhungweni ezihlonzelwe ukwenza lokho ukuze kube ngabacwaningi abavumelekile kuphela abakwazi ukufinyelela kuwo amasampula. Abanye babasebenzi bakulendawo bazodinga ukuba bafinyelele kumasampula akho ukuwagcina nokulandelela ukuthi akuphi, kodwa labantu angeke babenalo ulwazi olukuvezayo ngqo ukuthi ungubani. Ungaqhubeka ubhalise kulolucwaningo noma ngabe ukhetha ukuthi amasampula akho asalile angagcinela ucwaningo lwangomuso. Asikho isikhathi esikaliwe sokuthi amasampula akho nolwazi olumayeyelana nezempilo kuyogcinwa kuze kubenini noma ukuthi lamasampula angahlolwa nini. Uma ungafisi izinsalela zamasampula egazi zigcinwe, sizozilahla lezonsalela zamasampula. Noma iluphi olunye ucwaningo lwangomuso olungenziwa kuyomele futhi luvunyelwe ikomidi lezamalungelo ocwaningo. Ungahoxisa imvumo yakho yokugcinwa kwamasampula kanye nokuhlolwa kwangomuso kwamasampula noma ngasiphi isikhathi ngokuthi unikezele ngesicelo esibhaliwe noma ngomlomo kumuntu ophethe ucwaningo.

_____ Inishiyela no Suku

Mina **NGIYAVUMA** ukuthi amasampula egazi, ama swabhu kanye nolwazi lwezempilo lubekelwe ucwaningo lwangomuso.

_____ Inishiyela no Suku

Mina **ANGIVUMI** ukuthi amasampula egazi, ama swabhu amu kaye nolwazi lwezempilo kubekelwe ucwaningo lwangomuso.

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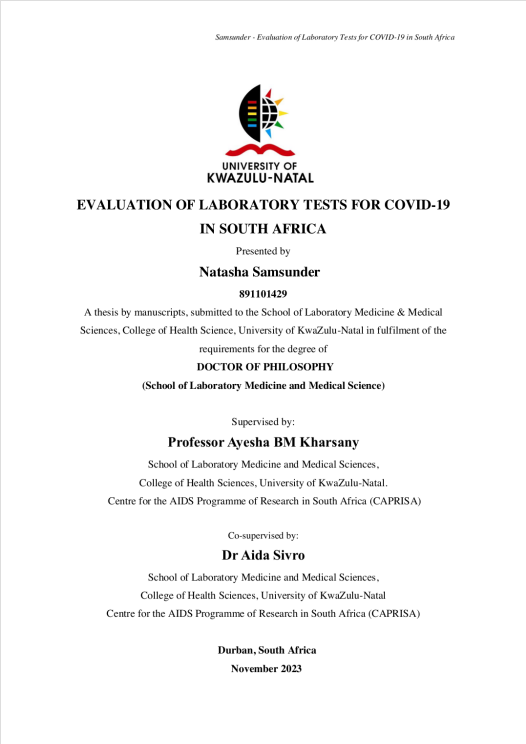

Samsunder - Evaluation of Laboratory Tests for COVID-19 in South Africa

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EVALUATION OF LABORATORY TESTS FOR COVID-19 IN SOUTH AFRICA

Presented by

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