COMPARISON OF SARS-COV-2 RAPID TESTS AND FORMAL SEROLOGICAL TESTING ON DECEASED PERSONS IN CAPE TOWN METRO

By

Tayna Carlisle

CRLTAY002

Minor Research Dissertation

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

in partial fulfilment of the requirements for the degree of

MPhil in Biomedical Forensic Science

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

August 2022

Supervisors:

Dr Laura Heathfield

(Division of Forensic Medicine and Toxicology, University of Cape Town)

Prof Lorna Martin

(Division of Forensic Medicine and Toxicology, University of Cape Town)

Declaration

I, Tayna Leigh Carlisle, declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

This dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisors in any manner whatsoever.

Signature: ……. Signed by candidate

(Tayna Carlisle)

Date: 26/07/2022

Word count: 15064

Acknowledgements

There are so many people to thank for their contribution to this study. First and foremost, I must thank my supervisor Dr Laura Heathfield. She dedicated a lot to this project, and I am so grateful for her help and guidance through this project. Special thanks must also go to my co-supervisor Professor Lorna Martin for her constant support.

I would like to thank Dr Laura Taylor and Dr Itumeleng Molefe for being involved with this project from the beginning and for always taking time out of their busy schedules to help. I would also like to thank Mr Wayne Mitten, Mr Richard Pietersen and Mr Ron Ackerbeg for showing me the Salt River Mortuary processes and for helping me set up an efficient system there. Thank you to Dr Janette Verster and Dr Candice Wilscott-Davids for facilitating the study at Tygerberg mortuary. I would like to thank Prof Johan Dempers for his assistance in the early stages of this project. My condolences to his family.

Dr Stefan Opperman deserves special praise for assisting me at the NHLS and for always being so willing to help me with the sample testing and Dr Chun Yat Chu for assisting me when Dr Opperman moved facilities.

I would like to acknowledge a few others for their assistance in this study: Prof Adrian Brink and Sabrina Hendricks from the NHLS, Carl Gordon, Andrew White, and Arnolene Campbell from Tygerberg mortuary, Michael Vismer from the Forensic Pathology Service, Beverly Grose and Marlon Ohlsson from BARC.

Appreciation must go to the valuable staff at Salt River Mortuary, Tygerberg Mortuary, BARC and the NHLS for all their help. I would also like to thank the MPhil Biomedical Forensic Science class of 2019 for supporting me constantly over the years.

Regarding funding, I want to thank the University of Cape Town, the Division of Forensic Medicine and Toxicology, the National Research Foundation and thank you to SureScreen Diagnostics for the donation of tests for this study. The views of this study do not necessarily reflect the views of the National Research Foundation.

I am so grateful to all the people involved – which includes my friends and family for the emotional support. This thesis would not exist if they had not encouraged me to continue.

Abstract

The COVID-19 disease was declared a global pandemic in 2020 and since, it is unclear how many people have truly been infected. Additionally, there is a paucity of research into post-mortem antibody testing. An antibody screening tool that is suitable for use in the mortuary setting would go a long way to better document previous COVID-19 infections in deceased persons for surveillance purposes, which would add value to public health systems. This pilot study aimed to explore the use of the SureScreen COVID- 19 IgG/IgM Rapid Test Cassette in a deceased population, and to compare it to the gold- standard antibody tests in South Africa, to determine the most suitable form of antibody testing for post-mortem samples. Thirty cases, with suspected COVID-19 infection in their lifetime, were recruited from Salt River and Tygerberg mortuaries following informed consent from next-of-kin. Positive COVID-19 PCR (PCP) test confirmation for SARS-CoV-2 was located for 19 of the participants. Blood was collected at autopsy into serum separator tubes which, were found to separate better when centrifuged immediately after sample collection. SureScreen testing was carried out alongside Roche Diagnostics Elecsys Anti-SARS-CoV-2 and Abbott Architect SARS-CoV-2 IgG Assay. For the confirmed PCP cases, Elecsys' sensitivity was the highest at 94.74%, followed by SureScreen IgG (78.95%). There was only one case with PCP confirmation with a negative Elecsys result and, in this instance, there was a longer interval between death and autopsy (8 days). No variables relating to time intervals between PCP, death and antibody testing were found to significantly influence the antibody test results. Overall Roche's Elecsys performed the best on our cohort of post-mortem serum samples, followed by SureScreen, and lastly, Abbott's Architect assay. Based on these results alone, the SureScreen test demonstrates potential as a screening tool in the mortuary setting, which should be followed up with Roche's Elecsys assay for diagnostic confirmation. However, it is recommended that the sample size be expanded to add weight to this preliminary conclusion.

Table of Contents

DECLARATION 2

ACKNOWLEDGEMENTS 3

ABSTRACT 4

ABBREVIATIONS 8

LIST OF TABLES 10

LIST OF FIGURES 11

CHAPTER 1: LITERATURE REVIEW 12

1.1. BACKGROUND 12

1.2. SARS-COV-2 13

1.2.1. Virus and structure 13

1.2.2 Transmission and replication 14

1.2.3 SARS-CoV-2 variants 15

1.3. ANTI-SARS-COV-2 ANTIBODIES 17

1.3.1 Vaccines 18

1.4 SARS-COV-2 DETECTION METHODS 20

1.4.1 Diagnostic testing for current infections 20

1.4.1.1 Nucleic acid-based detection of SARS-CoV-2 20

1.4.1.2 SARS-CoV-2 antigen testing 21

1.4.1.3 Limitations of diagnostic testing 21

1.4.2 Diagnostic testing for previous infections 22

1.4.2.1 Lateral flow immunoassays 24

1.4.2.2 Limitations of anti-SARS-CoV-2 antibody testing 26

1.5. ANTIBODY DETECTION IN THE DECEASED 26

1.6. RATIONALE 39

1.7. AIM 39

1.8. OBJECTIVES 39

CHAPTER 2: METHODOLOGY 40

2.1 STUDY DESIGN AND APPROVALS 40

2. 2. POPULATION COHORT 40

2.3. SAMPLE COLLECTION 41
2.4. ANTIBODY TESTING 42
2.5 DATA ANALYSIS 43
CHAPTER 3: RESULTS 46
3.1 RESULTS 46
3.1.1 Overview *46*
3.1.1.1 SureScreen test results 48
3.1.1.2 Elecsys test results 49
3.1.1.3 Architect test results 49
3.1.1.4 Test comparisons 50
3.1.2 Assessment of variables that may affect the tests' results *52*
CHAPTER 4: DISCUSSION 54
4.1 OVERVIEW 54
4.2 SURESCREEN RAPID TEST FOR POST-MORTEM SAMPLES 54
4.3 RESULTS OF THE ANTIBODY TESTS IN COMPARISON TO EXISTING DATA 56
4.4 LIMITATIONS AND FUTURE WORK 58
4.5 CONCLUSION 59
5. REFERENCES 60
6. APPENDICES 77
APPENDIX 1: APPROVALS 77
A. The University of Cape Town Human Research Ethics committee approval *77*
B. The University of Stellenbosch Human Research Ethics committee approval *79*
C. Approval from SAHPRA *81*
D. Transport of biological samples permit *84*
APPENDIX 2: IFUS 85
A. IFU of the SureScreen COVID-19 IgG/IgM Rapid test cassette *85*
APPENDIX 3: INFORMATION AND INFORMED CONSENT FORM 86
A. Information form *86*
B. Consent form *89*
APPENDIX 4: LITERATURE REVIEW 90
A. Table describing the different SARS-CoV-2 variants (Adapted from O'Toole et al., 2021; CDC, 2022b; WHO, 2022c) *90*

B. List of companies and SARS CoV-2 ELISA products currently approved by SAHPRA for IgM/IgG/IgA detection in COVID-19 patients (Adapted from SAHPRA, 2022a). *91*
C. List of companies and antibody coronavirus LFI kits currently approved by SAHPRA for antibody detection in COVID-19 patient (Adapted from (SAHPRA, 2022a). *92*
APPENDIX 5: RESULTS 95
A. Table summarising variables and the results of the three types of COVID-19 antibody testing. *95*
B. Comparison of results from Roche's Elecsys Anti-SARS-CoV-2 assay and the SureScreen rapid test cassette IgM *95*
C. Comparison of results from Roche's Elecsys Anti-SARS-CoV-2 assay and the SureScreen rapid test cassette IgG *96*
D. Comparison of results from Abbott Architect's SARS-CoV-2 IgG Assay and the SureScreen rapid test cassette IgM *96*
E. Comparison of results from Abbott Architect's SARS-CoV-2 IgG Assay and the SureScreen rapid test cassette IgG *96*
F. Comparison of results from Abbott Architect's SARS-CoV-2 IgG Assay and Roche's Elecsys Anti-SARS-CoV-2 assay *96*

Abbreviations

ACE-2	Angiotensin Converting Enzyme 2
AIC	Akaike Information Criterion
Ag-RDT	Antigen Detecting Rapid Diagnostic Tests
ANVISA	National Health Surveillance Agency
Architect	Abbott Architect SARS-Cov-2 Igg Assay
BARC	Bioanalytical Research Corporation South Africa
CDC	Centre For Disease Control and Prevention
cDNA	Complementary Deoxyribonucleic Acid
CDSCO	Central Drugs Standard Control Organisation
CE	Conformitè Europëenne
CMIA	Chemiluminescent Microparticle Immunoassay
COD	Cause Of Death
COI	Cut Off Index
CoV	Coronavirus
COVID-19	Coronavirus Disease 2019
DNA	Deoxyribonucleic Acid
DHRSA	Department Of Health: Republic of South Africa
E	Envelope
ED	Early Detection
EDTA	Ethylenediamine Tetra Acetic Acid
Elecsys	Roche's SAHPRA-Approved Elecsys Anti-SARS-Cov-2
ELISA	Enzyme Linked Immunosorbent Assay
EUA	Emergency Use Authorisation
Fc	Fragment Crystallisable
FDA	U.S Food and Drug Administration
FN	False Negative
FPS	Forensic Pathology Service
GISAID	Global Initiative on Sharing Avian Influenza Data
h	Hour
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
HSA	Health Science Authority
IFA	Immunofluorescence Assay
IFU	Instructions For Use
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IVD	In-Vitro Diagnostic Medical Device
LD	Late Detection
LFI	Lateral Flow Immunoassay
LTD	Limited Company

M	Membrane
MERS	Middle East Respiratory Syndrome
N	Nucleocapsid
NAbs	Neutralising Antibodies
NGS-SA	Network For Genomics Surveillance in South Africa
NHLS	National Health Laboratory Service
NK	Natural Killer
NMPA	National Medical Products Administration
p.s.o	Post Symptom Onset
PANGOLIN	Phylogenetic Assignment of Named Global Outbreak Lineages
PCP	Positive COVID-19 Polymerase Chain Reaction
PCR	Polymerase Chain Reaction
PM	Post-Mortem
POA	Percent Overall Agreement
PTY	Proprietary Company
R	Statistical program
RBD	Receptor Binding Domain
RNA	Ribonucleic Acid
RT	Reverse Transcription
RUO	Research Use Only
S	Spike
SAHPRA	South African Health Products Regulation Association
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOP	Standard Operating Procedure
SRM	Salt River Mortuary
SST	Serum Separator Tube
SUDA	Sudden Unexpected Death of An Adult
SureScreen	Surescreen COVID-19 IgG/IgM Rapid Test Cassette
TGA	Therapeutic Goods Administration
TM	Tygerberg Mortuary
TP	True Positive
UCT	University Of Cape Town
VOC	Variant Of Concern
VOHC	Variant Of High Concern
VOI	Variant Of Interest
VUM	Variant Under Monitoring
WB	Western Blot
WHO	World Health Organisation
α-CoV	Alpha Coronavirus
β-CoV	Beta Coronavirus
γ-CoV	Gamma Coronavirus
δ-CoV	Delta Coronavirus

List of tables

TABLE 1.1: Table displaying the COVID-19 waves and the causing variants during the SARS-COV-2 pandemic. 16

TABLE 1.2: Table outlining the SAHPRA registered COVID-19 vaccinations (adapted from: World health organization, 2021A; SAHPRA, 2022; South African Government news agency, 2022) 19

TABLE 1.3: Table detailing the specificities and sensitivities of the various forms of testing used in this study. 23

TABLE 1.4: Overview of literature found regarding SARS-COV-2 antibody detection using LFI's from clinical key and sciencedirect 30

TABLE 2.1: Samples obtained during autopsy for the various tests. 42

TABLE 3.1: Table detailing the variables and the results of the three types of covid-19 antibody testing. 47

TABLE 3.2: Table detailing the different sensitivities of the various tests at different intervals of time between PCP and death with intervals outlined by Elecsys IFU 51

TABLE 3.3: Table detailing the different sensitivities of the various tests at different intervals of time between PCP and death based on time intervals present in this study 51

TABLE 3.4: Table summarising the percent overall agreement and p-values generated by the comparisons of different testing 52

TABLE 3.5: Table summarising the linear regression data of the comparison of all the types of testing 52

TABLE 3.6: Table summarising the linear regression data of the comparison of Surescreen IgM and IgG and Architect to Elecsys. 53

List of figures

FIGURE 1.1: Pie chart displaying the various countries at which the studies, included in the literature review table, were performed. 28

FIGURE 2.1: Box and whisker plot describing the age range of the study participants 41

FIGURE 3.1: Chart visualising the median number of days between PCP, death, autopsy, and antibody testing for all three forms of testing. 50

Chapter 1: Literature review

1.1. Background

In March 2020, the World Health Organisation (WHO) stated that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) triggered a global pandemic (Yang *et al.*, 2020; World Health Organization [WHO], 2020a). The presence of the virus, which causes coronavirus disease (COVID-19), was first reported in December 2019, when multiple cases of pneumonia, of unknown aetiology, arose in Wuhan, Hubei Province, China (Lu *et al.*, 2020). Since then the pandemic spread worldwide and has subsequently devasted global health and medical care facilities (Lu *et al.*, 2020). As of 5 July 2022, a total of 3 995 784 confirmed COVID-19 cases in South Africa were recorded with the total number of deaths rising to 101 847 (Department of Health: Republic of South Africa [DHRSA], 2022).

Reverse transcription polymerase chain reaction (RT-PCR) is the most used method for SARS-CoV-2 detection, however, serological testing has become a viable alternative for detecting either antigens or antibodies (Wang *et al.*, 2020). Through the development of serological testing, lateral flow immunoassays (LFI) have been introduced for screening purposes. However, LFIs have yet to be used in a mortuary setting and studies on antibody testing in the deceased population using this method are non-existent. The introduction of antibody testing on deceased individuals, who have been suspected of having COVID-19 in their lifetime, could improve the awareness of the number of people who have been previously infected. It is also important to investigate which form of antibody testing is the most suitable for post-mortem blood samples, as most assays have been developed and tested on ante-mortem samples. This literature review will present a brief introduction to SARS-CoV-2 (structure, transmission, replication, and variants), describe anti-SARS-CoV-2 antibodies and vaccines developed against SARS-CoV-2, describe the methods of testing, as well as discuss the lack of knowledge on COVID-19 testing in the deceased.

1.2. SARS-CoV-2

1.2.1. Virus and structure

SARS-CoV-2 is classified as a coronavirus (CoV) which is a large family of enveloped, non-segmented, single-stranded ribonucleic acid (RNA) viruses, which cause a respiratory infection (Sun *et al.*, 2020; WHO, 2020a). It is the third zoonotic disease by CoVs to affect humans, following SARS-CoV and Middle-East Respiratory Syndrome (MERS) (Costela-Ruiz *et al.*, 2020).

CoVs are categorised under the order Nidovirales, family Coronaviridae, and subfamily Orthocoronavirinae (Malik, 2020). The CoV subfamily is separated into four genera: alpha CoV (α-CoV), beta CoV (β-CoV), gamma CoV (γ-CoV) and delta CoV (δ-CoV) according to specific phenotypic and genotypic characteristics (Malik, 2020; Sun *et al.*, 2020). The γ and δ-CoV are primarily found in birds, while α and β-CoV are known to infect mammals (Binnicker, 2020; Malik, 2020). The β-CoV genus comprises of MERS-CoV, SARS-CoV and SARS CoV-2 which all have high mutation rates which increase the transmissibility of the viruses (Ortiz-Prado *et al.*, 2020).

The SARS-CoV-2 virus particle has a roughly spherical, or moderately pleomorphic morphology (Sun *et al.*, 2020; Vasireddy *et al.*, 2021). The envelope of the SARS-CoV-2 virion consists of two lipid layers where the membrane (M), envelope (E), and spike (S) structural proteins are attached (Fernández-Rodríguez *et al.*, 2020; Sidiq *et al.*, 2020; Vasireddy *et al.*, 2021). The S glycoprotein is a transmembrane protein that projects outwards on the virion surface, providing the virus with a protective barrier (Fernández-Rodríguez *et al.*, 2020; Ortiz-Prado *et al.*, 2020). The S glycoprotein is comprised of two subunits S1 and S2. S1 houses a receptor binding domain (RBD) which binds to the host cell receptor, and S2 contains components required for viral fusion (Sidiq *et al.*, 2020).

The interior of the virion is comprised of a helical nucleocapsid (N) protein bound to a single positive-strand RNA genome (Ortiz-Prado *et al.*, 2020). The N proteins are thought to act together with the M proteins throughout virion assembly and play a critical role in boosting the efficiency of viral transcription and assembly (Sun *et al.*, 2020). The E, M and N proteins also provide protection to the virus when it is outside the host cell (Sidiq *et al.*, 2020).

1.2.2 Transmission and replication

The Centers for Disease Control and Prevention (CDC) (2021) and WHO (2020d) stated the three main forms of SARS-CoV-2 transmission are : (i) close contact and droplet transmission, (ii) airborne transmission and (iii) transmission from contaminated surfaces (fomite transmission) (WHO, 2020d; Centers for Disease Control and Prevention [CDC], 2021a).

Once a person is infected with SARS-CoV-2, the virus travels from the back of the throat to the lungs and finally enters the blood system (Kumar *et al.*, 2020). The time between exposure and onset of symptoms is typically 2 days - 14 days due to the virus' incubation period (Ortiz-Prado *et al.*, 2020; WHO, 2022a). During the disease, individuals may experience some of the following symptoms, determined by the variant of SARS-CoV-2 they are infected with: fever, cough, body aches, shortness of breath, headaches, loss of taste and smell, sore throat, congestions, nausea, vomiting, diarrhoea and fatigue (Dheda *et al.*, 2020; Kumar *et al.*, 2020; WHO, 2022a). Often individuals with certain risk factors (*i.e.* age above 50, underlying illness, tobacco smoking and comorbidities) will exhibit worse symptoms such as difficulty breathing, or chest pain (Dheda *et al.*, 2020; Drain, 2022). Patients with the more severe symptoms require hospitalisation whilst individuals with mild to moderate symptoms have been encouraged to manage their symptoms at home (Dheda *et al.*, 2020; CDC, 2022d; Drain, 2022).

Once the SARS-CoV-2 virus enters a host cell it immediately begins replicating by exploiting the host cell machinery. The entry of the virus into the host is mediated by the S glycoprotein binding with a cell-surface receptor called angiotensin converting enzyme 2 (ACE-2) present on the surface of respiratory cells (Kumar *et al.*, 2020; Ortiz-Prado *et al.*, 2020; Vasireddy *et al.*, 2021).

The virion enters the cell using receptor-mediated endocytosis and the fusion of the virus leads to the viral nucleocapsid being released into the cytosol of the infected cell (Ortiz-Prado *et al.*, 2020; V'kovski *et al.*, 2020). Once the viral RNA is released, it translates into viral polyproteins using the translation machinery of the host cell protein (Sidiq *et al.*, 2020). The assembly of virions is quickly followed by the creation of new genomic RNA and structural elements. Once

assembled, the newly formed virions bud off the host cell surface via exocytosis (Ortiz-Prado *et al.*, 2020; V'kovski *et al.*, 2020). Variants are formed when errors occur during the replication of the viral genome (Vasireddy *et al.*, 2021).

1.2.3 SARS-CoV-2 variants

The rise of mutations of SARS-CoV-2 has contributed to consecutive waves of COVID-19. The SARS-CoV-2 virus is known to mutate at a rate of, approximately, 1.1×10^{-3} substitutions per site, per year (Fernandes *et al.*, 2022). This makes the pandemic very difficult to manage, especially in low- and middle-income countries who are unable to provide vaccines effectively to the public (Mabuka *et al.*, 2021).

In late 2020, WHO developed a system for naming and classifying new SARS-CoV-2 variants. The variants can be classified as: variant of interest (VOI), variant of concern (VOC), variant under monitoring (VUM) and variant of high consequence (VOHC) (CDC, 2022c; WHO, 2022b). The variants categorised as a VUM include variants with the potential of more severe COVID-19 disease or increased transmissibility that are no longer circulating at high levels (WHO, 2022b). A VOC and a VOI are determined based on increased transmissibility, increased hospitalisations and deaths, reduced effectiveness of vaccinations and other treatments, and greater risk to public health (CDC, 2022c; WHO, 2022b). A VOC is determined to be a greater risk than a VOI if there is strong evidence of a decreased response to treatments and vaccines (Fernandes *et al.*, 2022; WHO, 2022b). A VOHC is a variant that has data to suggest that prevention measures have significantly reduced effectiveness compared to previously circulating variants (CDC, 2022c; WHO, 2022b).

Sequenced variant genomes are uploaded to the Global Initiative on Sharing Avian Influenza Data (GISAID) database and an algorithm called The Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN) then maps links between the genomes (Mabuka *et al.*, 2021; O'Toole *et al.*, 2021). In June 2020, the Network for Genomics Surveillance in South Africa (NGS-SA) was formed to monitor the spread of SARS-CoV-2 (Mabuka *et al.*, 2021). Since July 2022, South Africa has been affected by five waves of COVID-19 infection (Cohen *et al.*, 2022; Johns Hopkins University, 2022)(Table 1.1). A full list of the COVID-19

variants and their status (as of 19 July 2022) can be found in Appendix 4A (O'Toole *et al.*, 2021; CDC, 2022c).

Table 1.1: Table displaying the COVID-19 waves and the causing variants during the SARS-CoV-2 pandemic.

Wave number	Month and year of wave peak in South Africa	Causing variant
1	July 2020	Wuhan-Hu-1 (Alpha) strain
2	January 2021	Beta variant
3	July 2021	Delta variant
4	December 2021	Omicron variant
5	May 2022	Omicron variant

In October 2020 the Beta variant was detected. The Beta variant consists of three RBD mutations and it was found to have resistance to pre-existing neutralising antibodies (Mabuka *et al.*, 2021). The Alpha, Beta, Eta, and Delta SARS-CoV variants all contributed to the third wave, however, by June 2021, the Delta variant was present in 66% of the sequenced genomes of infected individuals (Mabuka *et al.*, 2021).

The fourth and fifth waves in South Africa were caused by the Omicron variant. The Omicron variant was first detected in Botswana on the 24 November 2021 and was reported to WHO, by South African scientists, on the 24 November 2021, when cases in South Africa began to rise (WHO, 2022c). As of the 15 July, the Omicron variant is the only VOC, and since its categorisation, there has been an increasing discovery of other Omicron variants that have caused global widespread waves (WHO, 2022c). Omicron is characterized by many mutations, including many in the S protein which increases transmissibility and allows the variant to avoid the human immune response. This has provided Omicron a transmission advantage over other variants (European Centre for Disease Prevention and Control, 2022; WHO, 2022c).

1.3. Anti-SARS-CoV-2 antibodies

Once a person is infected with the virus, the production of protective antiviral antibodies is initiated. The human antibody responses against SARS-CoV-2 are comprised of immunoglobulin (Ig) M, IgG3, IgG1 and IgA antibodies to the viral E and N and S glycoproteins (French and Moodley, 2020; Sun *et al.*, 2020).

IgA and IgM are produced during the early stages of an infection, followed by production of IgG (Tollånes *et al.*, 2020). IgA and IgM neutralise respiratory viruses by preventing their attachment to ACE2 and therefore, play a crucial role in protecting mucosal surfaces against pathogens (French and Moodley, 2020; Sterlin *et al.*, 2021). IgG antibodies possess the more important functional characteristics that neutralise the virus. Viral neutralisation by IgG occurs through i) binding antibody regions to viral antigens and preventing the virus from binding to cell receptors, and ii) the binding of the fragment crystallisable (Fc) regions on IgG to activate natural killer (NK) cells and induce antibody-dependent cellular cytotoxicity of infected cells (French and Moodley, 2020; Sun *et al.*, 2020).

In one study, once infection had occurred and post-symptoms, it took approximately five days for IgM and IgA to appear with the subsequent appearance of IgG at approximately 14 days (Fernández-Rodríguez *et al.*, 2020). Nakano *et al.* (2021) reported that the median IgM and IgG seroconversion period is 10 days or more after symptom onset (Nakano *et al.*, 2021). Another study showed that two weeks after symptom onset, 95% - 100% of infected patients had IgM or IgG, or both, antibodies to the S protein of the SARS-CoV-2 envelope in their serum (French and Moodley, 2020). Zhao *et al.* (2020) stated that seroconversion can be induced as early as 4 days with the median time of positivity for IgM being 11 days, and IgG 14 days after disease onset (Zhao *et al.*, 2020).

How long anti-SARS-CoV-2 antibodies remain detectable, following natural infection, is also currently uncertain and has been widely debated (Liu *et al.*, 2020; Ripperger *et al.*, 2020; Z. Li *et al.*, 2020; De Giorgi *et al.*, 2021). Studies based on the human immune response to natural SARS-CoV, determined that IgG antibodies lasted for two years after infection while IgM was undetectable after 12 weeks (Liu, *et al.*, 2019; Li *et al.*, 2020). Data focussed on SARS-CoV-2 infection indicates that neutralising antibodies can last for numerous months in infected

patients but do decrease over time (Ripperger *et al.*, 2020). Ripperger *et al.* (2020) published that of 5 882 COVID-19 recovered individuals' antibodies were still present in their blood five to seven months after the disease (Ripperger *et al.*, 2020). A more recent study performed by De Giorgi *et al.* (2022) found that in follow-up analyses of 228 donors, 91.4% had detectable IgG levels up to 11 months post-symptom recovery (De Giorgi *et al.*, 2021).

Long *et al.* (2020) found that IgG levels and neutralising antibodies, in a high proportion of recovered COVID-19 individuals, started to decrease within two to three months after infection (Long *et al.*, 2020). Crawford *et al.* (2020) similarly stated that neutralising antibodies fell fourfold from 30 days - 90 days after symptom onset but did remain detectable up to >90 days post symptom onset (Crawford *et al.*, 2020). Regarding immunity post vaccination, research suggests that protection from the Pfizer and Moderna COVID-19 vaccines starts to fade around four months after a booster dose (Ferdinands *et al.*, 2022). However, some infected individuals do not generate detectable IgG/IgM antibodies following infection, therefore, a negative antibody test result does not rule out previous infection (CDC, 2020).

All of the previous information was generated pre-Omicron with variants such as Alpha and Delta, who possess limited immune escape characteristics (WHO, 2022c). Through mutations, the new variants of SARS-CoV-2 have mutated to evade naturally induced immunity in order to better their chances of survival (Alefishat *et al.*, 2022). There is no evidence to suggest that the variants affect the time of seroconversion, however, the duration of protection, post natural infection, depends on a range of factors but especially the severity of the variant infection (Hall *et al.*, 2022). According to WHO (1 June 2022), data post-Omicron is still being collected in prospective cohort studies before conclusions regarding this can be drawn (WHO, 2022c).

1.3.1 Vaccines

In order to combat COVID-19, vaccinations were developed less than 12 months from the start of the pandemic (Ritchie *et al.*, 2022). As of the 29 April 2022, 34.7 million doses have been administered and 18.3 million (30.8%) South Africans were fully vaccinated (Ritchie *et al.*, 2022). In July 2022, the vaccines registered by the South African Health Products Regulation

Association (SAHPRA) are listed in Table 1.2 (WHO, 2021a; SAHPRA, 2022b; South African Government News Agency, 2022).

Table 1.2: Table outlining the SAHPRA registered COVID-19 vaccinations (Adapted from: World Health Organization, 2021a; SAHPRA, 2022; South African Government News Agency, 2022)

Name of vaccine	Manufacturer	Date registered	Type of vaccine
Janssen COVID-19 vaccine	Janssen Pharmaceutica (PTY) LTD	30/03/2021	Viral vector/adenovirus
Comirnaty	Pfizer laboratories	25/01/2022	Genetic / mRNA
COVID-19 vaccine MC Pharma (Sinopharm)	MC Pharma (PTY) LTD	31/01/2022	Inactivated
COVID-19 Vaccine LHS	Umsebe Healthcare	24/05/2022	Inactivated

*PTY - proprietary company, LTD – limited company, mRNA – messenger ribonucleic acid,

Currently, the two vaccines being administered to the public are Pfizer's Comirnaty and Janssen's vaccine. On the 23 August, 2021, the Pfizer-BioNTech vaccine was approved by the U.S. Food and Drug Administration (FDA)(U.S. Food & Drug Administration, 2022). The Pfizer-BioNTech vaccine is a messenger ribonucleic acid (mRNA) vaccine and is available as a two-dose vaccine with at least 5 months between each dose (U.S. Food & Drug Administration, 2022). Once the vaccine enters the human system, it instructs the cells to produce an identical protein to the SARS-CoV-2 viral S protein and the human immune system elicits a response to it and, therefore, produces antibodies to recognise the viral S protein when infected (The Immunisation Advisory Centre, 2022). The Pfizer BioNTech vaccine has an efficacy of 95% against symptomatic SARS-CoV-2 infection pre-Omicron (WHO, 2021b).

The Janssen vaccine is a single-dose vaccine that is adenovirus-based, which means the vaccine consists of a weakened version of a virus encoding a stabilised variant of the SARS-CoV-2 S protein. The genetic material of the virus does not get replicated in the patient's system but the human immune response will recognise the viral S protein when reinfected (International

Medical Aid, 2022). The Janssen vaccine was 66.3% effective in clinical trials (pre-Omicron) at preventing laboratory-confirmed COVID-19 infection (CDC, 2022a).

The WHO reported that vaccinations produce lower levels of protection against severe disease for Omicron when compared to earlier VOCs (WHO, 2021a). However, WHO states that a booster dose of the registered COVID-19 vaccines appears to restore protection against severe disease and death against currently the currently circulating variants at levels that remain acceptable (WHO, 2021a).

1.4 SARS-CoV-2 detection methods

Due to the continuous emergence of new SARS-CoV-2 variants, there is an evolving need for newer detection methods for SARS-CoV-2. Current testing technologies either target specific viral nucleic acids, viral proteins or anti-SARS-CoV-2 antibodies (CDC, 2022b; Fernandes *et al.*, 2022). The choice of test depends on whether the aim is diagnosis of current infection (RT-PCR or antigen) or determination of previous infection (antibody) (Fernandes *et al.*, 2022). The need to evaluate the latter method as a means to identify previous COVID-19 in a deceased population will be discussed.

1.4.1 Diagnostic testing for current infections

1.4.1.1 Nucleic acid-based detection of SARS-CoV-2

The nucleic acid-based detection of SARS-CoV-2 is performed using a PCR technique which is a highly sensitive and specific method that involves the amplification and detection of deoxyribonucleic acid (DNA) (Bustin and Nolan, 2020; Ortiz-Prado *et al.*, 2020). This technique is often used in molecular biology as a diagnostic test for a variety of microorganisms and pathogens (Bustin and Nolan, 2020). However, diagnostics for SARS-CoV-2, which replicates using viral RNA, requires a variation of the PCR technique referred to as RT-PCR (Bustin and Nolan, 2020). Using a RNA-dependent DNA polymerase, RT allows the conversion of RNA into complementary DNA (cDNA), which is then targeted using primers and amplified using DNA polymerase (Bustin and Nolan, 2020). This technique is able to detect the genetic material of SARS-CoV-2 with many RT-PCR kits targeting different genes

(Dheda *et al.*, 2020). The majority of the RT-PCR kits target the N protein gene, with other alternatives being the E gene (viral envelope), the S gene, and the helicase gene (RNA polymerase) (Li *et al.*, 2020; Lippi *et al.*, 2020).

1.4.1.2 SARS-CoV-2 antigen testing

SARS-CoV-2 antigen detecting rapid diagnostic tests (Ag-RDT) focus on the detection of specific viral proteins (antigens) of SARS-CoV-2 virus (DHRSA, 2021; CDC, 2022b). This form of testing was approved by SAHPRA in October 2020 for use in South Africa and has since become a more preferred form of diagnostic testing due to its lower turnaround time compared to RT-PCR with results being available in less than 30 minutes (DHRSA, 2021). This allows for flexibility of testing which can be performed at-home testing (self-testing), at the point of care, or in a laboratory (DHRSA, 2021; CDC, 2022b). Ag-RDTs can be used one to three days pre-symptom onset and during the first few days of symptom onset when patients have high viral loads and are most infectious (DHRSA, 2021).

1.4.1.3 Limitations of diagnostic testing

Whilst the nucleic acid-based detection is the most preferred form of diagnostic testing for SARS-CoV-2, the RT-PCR technique itself has several limitations. Most importantly, the tests have prolonged turnaround times and are required to be performed in certified laboratories, using expensive equipment, and trained technicians (Li *et al.*, 2020). Another major limitation to this detection method is the close similarity of genomes between SARS-CoV-2 and SARS-CoV (82%) which could lead to cross reaction and false positives (Ortiz-Prado *et al.*, 2020).

The diagnosis of COVID-19 requires the detection of two or more SARS-CoV-2 genes. Repeated RT-PCR testing would potentially be required to confirm a clinical diagnosis (Li *et al.*, 2020; Lippi *et al.*, 2020). It had been observed that, in some cases, the initial RT-PCR test had a negative result and later was found to be positive after subsequent testing (Wang *et al.*, 2020). In mild disease, the RT-PCR false negative rate is approximately 30 % – 40 % (Wang *et al.*, 2020; Pan *et al.*, 2020; Yang *et al.*, 2020).

Whilst Ag-RDT testing is more affordable and the results are obtained quicker, there are also many limitations regarding Ag-RDTs. The main issues being that there is a smaller detection window and Ag-RDTs generally have similar specificity but are less sensitive than RT-PCR (DHRSA, 2021). Therefore, it is recommended that Ag-RDT results be followed up by RT-PCR results (CDC, 2022b).

Overall the three major challenges of this technique are: the detection of small amounts of viral RNA, ensuring the positive result means detection of SARS-CoV-2 genome and not that of similar species, as well as the testing of large numbers of samples effectively while avoiding false negatives and false positives (Caruana *et al.*, 2020). Additionally, these forms of testing only provide a positive result if the virus is currently infecting the patient. Once the patient has recovered from the virus it can no longer be detected with this type of molecular test. With sufficient regularity of testing proving to be difficult in low income areas due to lack of access to reagents and equipment and reluctance to be tested due to high test prices, the true prevalence of the virus is unknown (Ortiz-Prado *et al.*, 2020). It is therefore necessary to explore different types of testing that can retrospectively determine if an individual previously had COVID-19 in their lifetime.

1.4.2 Diagnostic testing for previous infections

Anti-SARS-CoV-2 antibody testing has gained more attention in the scientific community and has been incorporated as a screening technique for COVID-19. As previously mentioned, the initial antibody response to SARS-CoV-2 is from IgM and IgA antibodies with IgG antibodies being produced later in the infection cycle (Liu *et al.*, 2020). By developing techniques that can detect anti-SARS-CoV-2 antibodies, more patients who have been infected can be identified. Since the emergence of the COVID-19 pandemic, many different antibody testing techniques have been developed, such as: immunofluorescence assays (IFA), enzyme linked immunosorbent assays (ELISA), and Western blot (WB) analysis (Sidiq *et al.*, 2020). Majority of these techniques focus on IgM and IgG detection from serum/plasma, with few incorporating other anti-SARS-CoV-2 antibodies. Liu *et al.* (2020) stated that during the first few days of infection, the IgM component of the test provided a sensitivity of only 70% (Liu *et al.*, 2020). As the infection progressed past the first two weeks, this figure rose to 92.3% (Liu *et al.*, 2020).

IgM antibodies therefore indicated a recent or even current infection (Liu *et al.*, 2020). Generally, by this time, the IgG antibodies would have been produced and this part of the test presents a sensitivity of 98.6% (Liu *et al.*, 2020). The detection of IgG is, therefore, an indication that there was a prior infection (Liu *et al.*, 2020). As of May 2022, SAHPRA has approved 15 different ELISA instruments for the use of IgM/IgG/IgA detection in COVID-19 patients (SAHPRA, 2022a) (Appendix 4B).

Anti-SARS-CoV-2 antibody testing could be used to trace contacts, improve surveillance, and identify those who have already had contact with the virus (Nuccetelli *et al.*, 2020). Antibody tests, therefore, could be used to identify patients that were previously infected with SARS-CoV-2 and can provide insight into the stages of infection in the patient.

The Abbott Architect SARS-CoV-2 IgG Assay (hereon referred to as Architect) is used standardly in the private sector and the Roche Elecsys Anti-SARS-CoV-2 (hereon referred to as Elecsys) is used standardly in the public sector. The sensitivities and details of the testing are documented in Table 1.3 (information received from the manufacturers).

Table 1.3: Table detailing the specificities and sensitivities of the various forms of testing used in this study.

Type of testing	**Method of testing**	**SARS-CoV-2 protein used**	**Antibody targets**	**Sensitivity (%)**			**Specificity (%)**
				7-13 days pPCR	**>14 days pPCR**	**Total**	
COVID-19 IgG/IgM Rapid Test Cassette (SureScreen Diagnostics)	LFI	N antigen	IgM and IgG			97.4 (IgG) : 86.8 (IgM)	99.3 (IgG); 98.6 (IgM)
Elecsys Anti-SARS-CoV-2 (Roche) (NHLS)	ELISA	N antigen	All antibodies (including IgA, IgM and IgG)	85.3	99.5		99.8
Architect SARS-CoV-2 IgG Assay (Abbott) (BARC)	Chemiluminescent microparticle immunoassay (CMIA)	N antigen	IgG	86.36	100	98.1	99.6

*pPCR – post PCR confirmation (Muench *et al.*, 2020; Roche, 2020; Surescreen Diagnostics, 2020; Jugwanth *et al.*, 2022).

1.4.2.1 Lateral flow immunoassays

Anti-SARS-CoV-2 antibody testing has progressed and in turn resulted in the introduction of lateral flow immunoassays (LFI). The LFIs used for COVID-19 antibody testing are a type of rapid test designed for screening of anti-SARS-CoV-2 IgM and IgG in human plasma, serum or whole blood (Autobio, 2020; Ortiz-Prado *et al.*, 2020).

LFIs are a more cost effective and versatile form of antibody testing. The test can be performed by the patient or a healthcare professional, in a variety of settings including the laboratory, clinic or home (Koczula and Gallotta, 2016). The SureScreen COVID-19 IgG/IgM Rapid Test Cassette (hereon referred to as the SureScreen IgG/IgM test) is one of many lateral flow immunoassays developed for IgG and IgM detection. The test has been CE marked, and has also been previously validated against a large panel of negative controls, and in serum from confirmed PCP cases (Pickering *et al.*, 2020). The test has been approved in the United Kingdom as well as by other companies such as: Invima (Colombia), Instituto Conmemorativo Gorgas (Panama) and AGEMED (Brazil) (SureScreen Diagnostics, 2020). The SureScreen IgM/IgG test has also been validated in South Africa and was approved by the South African Health Products Regulation Association on the 30 October 2020 (Surescreen Diagnostics, 2020; SAHPRA, 2022a).

Many LFIs use varying sample types based on how the tests are designed. Most LFIs can be performed with capillary whole blood collected by a fingerstick procedure, but many have also been developed for use of venous whole blood, serum or plasma (CDC, 2020). A study investigating the detection of Zika virus RNA in different sample types reported that there was a higher detection rate of the RNA in plasma specimens during the first 5 days after symptom onset (Rossini *et al.*, 2017). However, another study indicated that serum and plasma samples from both human immunodeficiency virus (HIV)-infected and non-HIV-infected subjects can be used interchangeably to test for antibody responses (Siev *et al.*, 2011). The use of two samples for testing can be deemed wasteful, particularly when there is a limited amount of blood available, such as in deceased persons.

LFIs could prove to be useful in surveillance studies to determine the number of people in the population who were exposed to the virus. Surveillance studies can be used to monitor viral

movements in community transmission, understand co-circulation of respiratory viruses, and provide insight to the upgrading of diagnostic tests (World Health Organization, 2020). A study by Fusco *et al.* (2020) highlights the importance of surveillance studies, especially when tracking transmissions from health-care workers in order to reduce nosocomial spread (Fusco *et al.*, 2020). These studies are critical to conduct, not only in clinical cases but in forensic post-mortem (PM) ones as well. Determining the cause of death (COD) of individuals is imperative. If it can be determined that there was a prior infection of SARS-CoV-2 in a deceased individual, it can provide insight, to forensic medical practitioners, into whether that was the cause or contributed to death.

Initially when the SureScreen test was developed, the assumption was that it was unable to differentiate antibodies developed by natural infection or SARS-CoV-2 vaccination. However, a study performed by Suhandynata *et al.* (2021) determined that The Elecsys S antibody and Diazyme neutralising antibodies (NAbs) assays identified adaptive immune response in vaccinated patients, whilst the Elecsys N antibody assay and Diazyme IgG assay did not (Suhandynata *et al.*, 2021). The CDC (2021) suggests that reactivity of S and N specific antibodies could help differentiate natural SARS-CoV-2 infection from vaccination in antibody testing, particularly for anti-SARS-CoV-2 S protein vaccines (CDC, 2021a). The Surescreen test targets IgM and IgG antibodies generated against SARS-CoV-2 N protein (Table 1.3). The vaccines currently being administered in South Africa contain the S protein; this results in the formation of anti-S protein antibodies. It is assumed that if an anti-SARS-CoV-2 antibody test targets anti-SARS-CoV-2 N protein antibodies then the result is consistent with natural infection. Therefore, the SureScreen test should detect antibodies generated by natural infection.

As of May 2022, SAHPRA has approved 38 different LFIs for the use of IgG/IgA/IgM detection in COVID-19 patients (SAHPRA, 2022a)(Appendix 4C). There is still little data relating to the use of LFIs in a forensic setting, however in a clinical setting SureScreen IgG/IgM had a reported sensitivities of 97.4% (IgG) and 86.8% (IgM), and specificities of 99.3% (IgG) and 98.6% (IgM), in comparison to the gold-standard tests in South Africa, Elecsys and Architect, which have sensitivities of 99.5% and 100%, and specificity of 99.8%

and 99.6%, respectively (Table 1.3). Therefore, it is important to explore LFIs in parallel to the gold-standard tests used in South Africa.

1.4.2.2 Limitations of anti-SARS-CoV-2 antibody testing

The results of this form of testing relies on the seroconversion of the patient and the interpretation of the results may not be straightforward (Mayne *et al.*, 2020). If the level of antibodies present in a sample are below the detection limit of the assay, then that may result in a false negative result (Autobio, 2020). Many studies have debated the adequate amount of time needed after the onset of symptoms to be able to successfully detect the anti-SARS-CoV-2 antibodies. Antibodies in some individuals can be detected during the first week of illness or they may arise later, 2 weeks to 3 weeks post-symptom onset (CDC, 2020; Zhao *et al.*, 2020). Thus, detection of IgM without IgG is uncommon if the test is performed soon after the onset of the disease.

It is important to note that this form of testing cannot be used for diagnostics as it is limited by the unreliability of human immune response. Many patients might experience a delayed immune response, or if the patient is asymptomatic, they might not produce antibodies at all (Mayne *et al.*, 2020; Yongchen *et al.*, 2020). Therefore, the presence of antibodies cannot mean the patient is currently infected with SARS-CoV-2 (Mayne *et al.*, 2020). Other challenges with anti-SARS-CoV-2 antibody testing are: cross reactivity of antibodies developed from previous infection with other human CoVs and uncertainty regarding the durability of the antibody response (Mayne *et al.*, 2020).

1.5. Antibody detection in the deceased

There is no current information regarding anti-SARS-CoV-2 antibody testing in the deceased using LFIs, ELISA or CMIA, using blood samples. LFI testing in the deceased population could improve the health and safety procedures in the mortuaries as well as provide another tool to aid in the investigation of COD. The information can be used to determine if COVID-19 contributed to the persons death (in hospital or sudden unexplained deaths in adults [SUDA]

cases) and if the test is positive then this information can be used for contact tracing of the family.

There are many issues regarding PM antibody testing. PM blood can be altered by haemolysis, autolysis and bacterial contamination which interfere with infectious markers in the blood (Wilkemeyer *et al.*, 2012; Victer *et al.*, 2016). These could lead to false antibody results and would negatively affect the test sensitivity (Wilkemeyer *et al.*, 2012; Victer *et al.*, 2016). Although false negative antibody results of PM blood samples do occur, they are infrequent (<1 %), unlike false positive results which occur more often at about 3.5 % (Wilkemeyer *et al.*, 2012).

A study performed by Challine *et al.* (2006) analysed 565 deceased individuals for serological markers of HIV type 1 and 2, human T cell leukaemia virus type 1, hepatitis B and hepatitis C viruses. Of these 565, 19.1 % showed positive results, which were proven to be false positives as confirmation testing results were not in agreement, and no false negatives were reported (Challine *et al.*, 2006). Wilkemeyer *et al.* performed a similar serological testing study where only 4% of samples showed different results for pre-mortmem and PM blood (Wilkemeyer *et al.*, 2012). However, contrary to the findings of Challine *et al.* (2006) and Heim *et al.* (1999), Edler *et al.* (2011), who looked into HIV, hepatitis B virus and hepatitis C virus, encountered neither false-positive nor false negative results on PM blood up to 48 hours after death (Heim *et al.*, 1999; Challine *et al.*, 2006; Edler *et al.*, 2011).

It is also unknown how long SARS-CoV-2 antibodies can remain in a deceased individual. As previously mentioned, Edler *et al.* (2011) reported that serological detection of antibodies, for HIV, hepatitis B virus and hepatitis C virus, could readily be demonstrated up to 48 hours PM (Edler *et al.*, 2011). They also noted that the serum samples showed minimal haemolysis at 36 hours PM and a moderate to severe haemolysis at 48 hours PM (Edler *et al.*, 2011). Therefore, ensuring sample collection happens before 48 hours PM is critical.

A review of the literature was conducted using ScienceDirect and Clinical Key to collate the literature available on LFIs/rapid tests and determine: if the tests are validated, the specificity and sensitivity of the tests, and in which countries these studies occur (Table 1.4). The key words used were lateral flow, rapid test, COVID-19, SARS-CoV-2, IgM and IgG. A total of

149 and 24 articles were found from ScienceDirect and Clinical Key, respectively. Articles were excluded if they did not incorporate a LFI or if they involved viruses other than SARS-CoV-2. Only original research articles were included, while reviews, case reports and letters to the editor were excluded.

After studies were excluded, there were a total of 23 applicable studies. A second updated search was performed on ScienceDirect in August 2021. The keywords used were the same as the first set of searches. The total number of results were 387, however, only the papers showing 2 or more of the keywords were downloaded (n=38). Of the 38 papers, only nine were added to the table due to relevancy.

The 32 studies used 45 different LFIs. Of the total amount of different LFIs, 23 were repeated in different studies with the Wondfo SARS-CoV-2 Antibody Test, Dynamiker 2019-nCoV IgG/IgM Rapid Test and Orient COVID-19 IgG/IgM Rapid Test Cassette (Orient Gene Biotech) most frequently appearing. The studies spanned over 15 different countries with the highest number of studies being performed in France (n=6) (Figure 1.1).

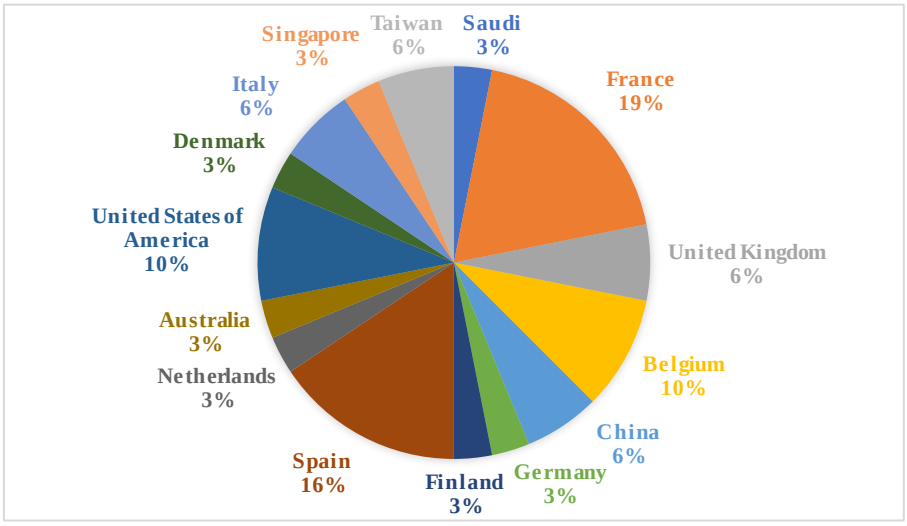


Figure 1.1: Pie chart displaying the various countries at which the studies, included in the literature review table, were performed.

The percentage of validated tests was 69.13% (Table 1.4). It was determined if the tests were validated through the Foundation for Innovative New Diagnostics (www.finddx.org), a global non-

profit organisation focussed in the development and delivery of diagnostics to combat major diseases affecting the world's poorest populations (FIND, 2020). What is also notable is that the sensitivities of the tests all increase with a longer duration post symptom onset.

However, none of these studies have assessed LFIs on post-mortem samples, with all samples analysed for clinical purposes. This highlights that there is a knowledge gap regarding the use of this technique on samples from deceased individuals. It is also noticeable that many of the studies use serum for their testing, with only 9.37% using plasma only.

Table 1.4: Overview of literature found regarding SARS-CoV-2 antibody detection using LFI test kits using Clinical Key and ScienceDirect.

	Authors	Country and city	LFI test kit	C/D	Sample type (S/P/W)	Sample size	Validated test (y/n)	Authorisations	Detection times (E/L/U)	Sensitivity for ED (%)	Time test is performed p.s.o (days)	Sensitivity for LD (%)	Time test is performed p.s.o (days)	Overall specificity (%)
1	(Awaji *et al.*, 2021)	Saudi Arabia	COVID-19 IgG/IgM Rapid Test (Prima)	C	W, P & S	82	y	CE-IDV	E & L	19.35	7-16	64.2	16-48	95
			2019-nCov Antibody Test (Innovita Biological Technology Co. Ltd)				y	China NMPA EUA, Australia TGA, Brazil ANVISA, Singapore HAS, CE-IVD		12.9		85.71		95
2	(Batra *et al.*, 2020)	United Kingdom	Abbott Panbio™ COVID-19 IgG/IgM rapid test device	C	S & P	272	y	CE-IVD	L	N/A	N/A	99.1	>14	100
3	(Black *et al.*, 2021)	New York, United States of America	2019-nCoV IgG/IgM Antibody Detection Kit (Biolidics Ltd)	C	S, P & W	40	y	Singapore HAS, CE-IVD	E & L	29	<7	92	>7	92
4	(Cavalera *et al.*, 2020)	Torino, Italy	Developed in-house	C	S	147	n	_	L	N/A	N/A	94.6	> 7	100
5	(Charpentier *et al.*, 2020)	Paris, France	COVID-Presto® rapid test (AAZ-LMB)	C	S	262	y	CE-IVD	E & L	83	≤9	100	>14	IgM - 100; IgG - 98.3
			NG-Test® IgM-IgG COVID-19 (NG Biotech)				y	CE-IVD		83		97		IgM - 86.5; IgG - 96.2

Table 1.4: Overview of literature found regarding SARS-CoV-2 antibody detection using LFI test kits using Clinical Key and ScienceDirect (Continued)

6	(Chen *et al.*, 2020)	Taipei, Taiwan	Wondfo SARS-CoV-2 Antibody Test (Guangzhou Wondfo Biotech Co., Ltd.)	C	S	540	n	N/A	L	N/A	N/A	91.4	> 21	100
			ASK COVID-19 IgG/IgM Rapid Test (TONYAR Biotech Inc.)				n	N/A		N/A		97.4		100
			Dynamiker 2019-nCoV IgG/IgM Rapid Test (Dynamiker Biotechnology Co., Ltd.)				y	CE-IVD		N/A		90.1		100
7	(Demey *et al.*, 2020)	Amiens, France	Anti-SARS-CoV-2 Rapid Test (Autobio Diagnostics Co., Ltd)	C	S	22	y	China NMPA EUA, US FDA EUA, Brazil ANVISA, CE-IVD	E & L	IgM - 36.36; IgG - 45.45	7	IgM - 100; IgG - 100	24	N/A
			SARS-CoV-2 IgG/IgM (Xiamen Biotime Biotechnology)				y	US FDA EUA, CE-IVD		IgM - 41.18; IgG - 41.18		IgM - 100; IgG - 100		N/A
			2019-nCoV IgM/IgG Diagnostic Test Kit (ISIA BIO-Technology Co)				n	N/A		IgM - 22.73; IgG - 22.73		IgM - 86.36; IgG - 100		N/A
			2019-nCoV IgG/IgM Antibody Detection Kit (Biolidics Ltd)				y	Singapore HAS, CE-IVD		IgM - 40; IgG - 40		IgM - 86.36; IgG - 100		N/A
8	(Van Elslande *et al.*, 2020)	Leuven, Belgium	COVID-19 IgG/IgM Rapid Test Cassette (Orient Gene Biotech)	C	S	270	y	South Africa SAHPRA, Australia TGA, CE-IVD	E & L	35.1	0 - 6	92.1	14 - 25	97.1
			IgM/IgG Serologic Rapid Test (Multi-G B.V.)				y	CE-IVD		13.5		57.9		100

Table 1.4: Overview of literature found regarding SARS-CoV-2 antibody detection using LFI test kits using Clinical Key and ScienceDirect (Continued)

			COVID-19 IgG/IgM Rapid Test (Prima)				y	CE-IDV		27		68.4		98.1
			Clungene COVID-19 IgM/IgG Rapid Test Cassette				n	N/A		10.8		55.3		99
			VivaDiag SARS-CoV-2 IgM/IgG Rapid test				y	Australia TGA, India CDSCO, Singapore HSA, CE-IVD		35.1		94.7		100
			StrongStep® COVID-19 IgG/IgM Combo Test				n	N/A		8.1		50		100
			Dynamiker 2019-nCoV IgG/IgM Rapid Test (Dynamiker Biotechnology Co., Ltd.)				y	CE-IVD		27		94.7		99
9	(Guedez-López *et al.*, 2020)	Madrid, Spain	Wondfo SARS-CoV-2 Antibody Test (Guangzhou Wondfo Biotech Co., Ltd.)	C	S	145	n	N/A	E & L	18.8	< 7	83.3	~ 21	81.8
			Sienna, 2019-nCoV IgG/IgM Rapid Test Cassette (T&D Diagnostics)				n	N/A		36.6		100		75
			Prometheus® Bio Inc., 2019-nCoV IgG/IgM Test Cassette				y	CE-IVD		68.6		100		12.5
10	(Hoste *et al.*, 2020)	Madrid, Spain	Developed in-house	C	S	1065	n	N/A	U	91.2	N/A	N/A	N/A	100
11	(de la Iglesia *et al.*, 2020)	León, Spain	Wondfo SARS-CoV-2 Antibody Test (Guangzhou Wondfo Biotech Co., Ltd.)	C	W	110	n	N/A	E	63	>7	N/A	N/A	100

Table 1.4: Overview of literature found regarding SARS-CoV-2 antibody detection using LFI test kits using Clinical Key and ScienceDirect (Continued)

			2019-nCoV IgG/IgM Rapid Test Cassette (AllTest Biotech Co., Ltd)				y	Brazil ANVISA, Australia TGA, CE-IVD		56.5		N/A	N/A	100
12	(Jääskeläinen *et al.*, 2020)	Helsinki, Finland	2019-nCoV IgG/IgM (Acro Biotech)	C	S	100	y	Brazil ANVISA	E & L	IgG - 56.1; IgM - 46.3	3 - 51	N/A	N/A	IgG - 74.4; IgM - 69.5
			SARS-CoV-2 IgG/IgM (Xiamen Biotime Biotechnology)				y	US FDA EUA, CE-IVD		IgG - 71.9; IgM - 81.3		N/A	N/A	IgG - 97.5; IgM - 88.8
13	(Jones *et al.*, 2021)	Bristol, United Kingdom	AbC-19™ Rapid Test (Rapid Test Consortium)	C	P	4842	n	N/A	L	N/A	N/A	92.5	52-75	97.9
			COVID-19 IgG/IgM Rapid Test Cassette (Orient Gene Biotech)				y	SAHPRA - Australia TGA - CE-IVD				94		95.8
			COVID-19 Raid Test Cassette (SureScreen Diagnostics)				y	SAHPRA				94		97
			COVID-19 IgG/IgM Rapid test (Biomerica)				n	N/A				95.1		92
14	(Kohmer *et al.*, 2020)	Frankfurt, Germany	COVID-19 IgG/IgM Rapid Test Device (Assure Tech Co., Ltd)	C	S & P	33	y	Brazil ANVISA, US FDA EUA, CE-IVD	E & L	62.5	5-9	93.8	10-18	100
15	(Lau *et al.*, 2021)	Singapore	Panbio COVID-19 IgG/IgM Rapid Test device (Abbott Diagnostics)	C	S	133	y	CE-IVD	E & L	10.8	0-6	97.2	>14	98.7
			SARS-CoV-2 Rapid Antibody (POCT) test (Roche)				n	N/A		18.5		97.2		100

Table 1.4: Overview of literature found regarding SARS-CoV-2 antibody detection using LFI test kits using Clinical Key and ScienceDirect (Continued)

16	(Z. Li *et al.*, 2020)	China	SARS-CoV-2 rapid IgG-IgM combined antibody test kit (Jiangsu Medomics Medical Technologies)	C	S & P	8525	n	N/A	U	88.66	N/A	N/A	N/A	90.63
17	(McAulay *et al.*, 2020)	United States of America	2 iterations of the Rapid Response™ COVID-19 Test Cassette (BTNX Inc.)	C	W, S & P	352	y	CE-IVD	L	N/A	N/A	Kit 1 - 95; Kit 2 - 91	> 14	Kit 1 - 98; Kit 2 - 100
			SARS-COV-2 IgG/IgM Rapid Test (ACON Laboratories)				y	CE-IVD				95		98
			Standard Q COVID-19 IgM/IgG Duo (SD BIOSENSOR)				y	Brazil ANVISA, CE-IVD		N/A		92		100
18	(Montesinos *et al.*, 2020)	Brussels, Belgium	2019-n-CoV IgG/IgM rapid test cassette (LabOnTime, Bio Marketing Diagnostics)	C	S	200	n	N/A	E & L	37.9	0-7	93.93	>15	100
			Coronavirus (2019-n-CoV) antibody IgG/IgM assay (Avioq, Biotech)				y	Brazil ANVISA, RUO		27.58		93.93		95.8
			QuickZen COVID-19 IgM/IgG Kit (QuickZen) (ZenTech, Angleur)				y	RUO		34.48		90.9		100
19	(Nicol *et al.*, 2020)	Angers, France	Prometheus® Bio Inc., 2019-nCoV IgG/IgM Test Cassette	C	S	~225	y	CE-IVD	E & L	43.8	0 - 7	100	> 15	95.3
20	(Nicol *et al.*, 2020)	Angers, France	NG-Test® IgM-IgG COVID-19 (NG Biotech)	C	S	293	y	CE-IVD	E & L	43.8	0-7	100	>15	95.3

Table 1.4: Overview of literature found regarding SARS-CoV-2 antibody detection using LFI test kits using Clinical Key and ScienceDirect (Continued)

21	(Nilsson *et al.*, 2021)	Odense, Denmark	2019-nCoV IgG/IgM Rapid Test Cassette (Acro Biotech)	C	P	57	n	N/A	E & L	67	1-7	96	>28	92.8
			Diagnostic Kit for IgM/ IgG Antibody to Corona Virus (Zhuhai Livzon Diagnostics)				y	Brazil ANVISA - CE-IVD		33		61		98.5
			OnSite™ COVID-19 IgG/IgM (CTK Bitotech, Inc)				y	Australia TGA - Brazil ANVISA - India CDSCO		33		86		93.5
22	(Ong *et al.*, 2020)	Netherlands	Rapid 2019-nCoV IgG/IgM Combo Test Card (Xiamen Boson Biotech Co. Ltd)	C	P	25	y	Singapore HAS, CE-IVD	E & L	50	4-14		N/A	100
			qSARS-CoV-2 IgG/IgM Cassette Rapid Test (Cellex Inc.)				y	US FDA EUA, Australia TGA, Brazil ANVISA, CE-IVD		20		N/A		100
			Dynamiker 2019-nCoV IgG/IgM Rapid Test (Dynamiker Biotechnology Co., Ltd.)				y	CE-IVD		10		N/A		100
			COVID-19 IgG/IgM Rapid Test Cassette (Orient Gene Biotech)				y	South Africa SAHPRA, Australia TGA, CE-IVD		55		N/A		100
			Prometheus® Bio Inc., 2019-nCoV IgG/IgM Test Cassette				y	CE-IVD		20		N/A		100

Table 1.4: Overview of literature found regarding SARS-CoV-2 antibody detection using LFI test kits using Clinical Key and ScienceDirect (Continued)

			Wantai SARS-CoV-2 Ab Rapid test (Wantai Biological Pharmacy Enterprise Co., Ltd)				y	Australia TGA		50				100
23	(Pallett *et al.*, 2020)	United Kingdom	OnSite™ COVID-19 IgG/IgM (CTK Bitotech, Inc)	C	S	400	y	Australia TGA, Brazil ANVISA, India CDSCO	L	N/A	N/A	90	> 14	94
			Encode SARS-CoV-2 split IgM/IgG One Step Rapid Test Device (Zhuhai Encode Medical Engineering)				n	N/A		N/A	N/A	92.9		98
24	(Péré *et al.*, 2020)	Paris, France	COVID-19 BSS (IgG/IgM) (Biosynex Swiss)	C	S	100	y	Brazil ANVISA - Singapore HSA - CE-IVD	L	N/A	N/A	95.8	>28	98.1
			COVID-19 IgG/IgM Test (Humasis Co., Ltd)				y	Korea MFDS EUA - Brazil ANVISA - CE-IVD		N/A		91.6		86.5
			LYHER COVID-19 IgM/IgG Rapid Test (Medakit Ltd)				n	N/A		N/A		92.3		100
			SIENNA™ COVID-19 (IgG/IgM) Rapid Test Cassette (Salofa Oy)				n	N/A		N/A		97.9		98.1
			NG-Test® IgM-IgG COVID-19 (NG Biotech)				y	CE-IVD				91.4		84.6
25	(Pérez-García *et al.*, 2020)	Spain	2019-nCoV IgG/IgM Rapid Test Cassette (AllTest Biotech Co., Ltd)	C	S	190	y	Brazil ANVISA, Australia TGA, CE-IVD	E & L	64.4	< 14 days	88	> 14 days	100

Table 1.4: Overview of literature found regarding SARS-CoV-2 antibody detection using LFI test kits using Clinical Key and ScienceDirect (Continued)

26	(Robosa *et al.*, 2020)	New South Wales, Australia	OnSite™ COVID-19 IgG/IgM (CTK Biotech, Inc)	C	S	60	y	Australia TGA, Brazil ANVISA, India CDSCO	L	N/A	N/A	85.7	>14	98.4
			2019-nCov Antibody Test (Innovita Biological Technology Co. Ltd)				y	China NMPA EUA, Australia TGA, Brazil ANVISA, Singapore HAS, CE-IVD		N/A		62.1		93.6
			SARSCoV-2 Antibody Test Strip (Changsha Sinocare Inc.)				y	CE-IVD		N/A		65.5		85.7
			Standard Q COVID-19 IgM/IgG Duo (SD BIOSENSOR)				y	Brazil ANVISA, CE-IVD		N/A		68		97.2
			Standard Q COVID-19 IgM/IgG Combo Test (SD BIOSENSOR, Inc)				y	Brazil ANVISA, CE-IVD		N/A				
27	(Sotgiu *et al.*, 2020)	Sassari, Italy	COVID-19 IgM-IgG Dual Antibody Rapid Test (BioMedomics, Inc.)	C	S	202	y	India CDSCO, CE-IVD	U	88.7				90.6
28	(Triest, Geebelen and Pauw, 2020)	Brussels, Belgium	QuickZen COVID-19 IgM/IgG Kit (QuickZen) (ZenTech, Angleur)	C	S	197	y	IVD-D	U	70.4	N/A	N/A	N/A	91
			COVID-19 IgG/IgM Rapid Test Cassette (Orient Gene Biotech)				y	SAHPRA - Australia TGA - CE-IVD		100		N/A		94.4
			Wantai SARS-CoV-2 Ab Rapid test (Wantai Biological Pharmacy Enterprise Co., Ltd)				y	Australia TGA		38.9		N/A		100

Table 1.4: Overview of literature found regarding SARS-CoV-2 antibody detection using LFI test kits using Clinical Key and ScienceDirect (Continued)

			COVID-Presto® rapid test (AAZ-LMB)				y	CE-IVD		98.1		N/A		97.8
			IgM/IgG Serologic Rapid Test (Multi-G B.V.)				y	CE-IVD		49.1		N/A		100
29	(Velay *et al.*, 2020)	Strasbourg, France	COVID-19 BSS (IgG/IgM) (Biosynex Swiss)	C	S & P	325	y	Brazil ANVISA Singapore HSA CE-IVD	E & L	55	< 15	95	> 28	99
			COVID-19 Sign IgM/IgG (Servibio/VEDALAB)				n	N/A		56		69		83
30	(Wu *et al.*, 2020)	Taipei, Taiwan	2019-nCoV IgG/IgM Rapid Test Cassette (AllTest Biotech Co., Ltd)	C	S	74	y	Brazil ANVISA, Australia TGA, CE-IVD	E & L	50	< 14	100	> 21	100
			Dynamiker 2019-nCoV IgG/IgM Rapid Test (Dynamiker Biotechnology Co., Ltd.)				y	CE-IVD		41.3		100		100
			ASK COVID-19 IgG/IgM Rapid Test				n	N/A		47.8		100		100
			Wondfo SARS-CoV-2 Antibody Test (Guangzhou Wondfo Biotech Co., Ltd.)				n	N/A		52.2		100		100
31	(Xie *et al.*, 2020)	Texas, United States of America	2019-Novel Coronavirus IgG/IgM Detection Kit (Nanjing Vazyme Medical Technology Co., Ltd)	C	S & P	100	y	China NMPA EUA - Brazil ANVISA - CE-IVD	E & L	60	0-5	100	>6	96.1
32	(C. Zhang *et al.*, 2020)	China	Developed in-house	C	S	1722	n	N/A	E & L	IgM - 22.22; IgG - 55.56	< 15	IgM - 62.2; IgG - 88.32	> 21	87.28

* C – clinical, D – deceased, S – serum, P – plasma, W – whole blood, L – late, E – early, U – unknown, ED- early detection, LD – late detection, p.s.o – post symptom onset, CE – IVD - CE Marked in-vitro diagnostic medical device, NMPA – national medical products administration, EUA – emergency use authorisation, US FDA – United States Food and Drug Administration, ANVISA – national health surveillance agency, HSA – heath science authority, SAHPRA – South African health products regulation association, TGA – therapeutic good administration, CDSCO – central drugs standard control organisation, RUO – research use only.

1.6. Rationale

In the mortuary setting, there is a need to monitor previous SARS-CoV-2 infections in the deceased to improve surveillance and public health response. It would potentially assist in determination of COD and development of health and safety protocols. Currently, the investigation of anti-SARS-CoV-2 antibody testing in deceased individuals is extremely limited. ELISA is the gold standard for anti-SARS-CoV-2 antibody testing, however, it has yet to be tested on post-mortem samples, it is expensive, and it cannot be performed at mortuaries. LFIs have gained momentum due to their low cost and low turn-around time when obtaining results, but LFIs have also yet to be assessed on post-mortem samples. This data could result in improved protocols for understanding which testing assay is most suited for detecting antibodies in post-mortem blood. This study does not intend to do further validation tests, but to rather explore the potential use of LFIs and gold standard anti-SARS-CoV-2 antibody testing in the private and public health sector in South Africa.

1.7. Aim

The aim of this pilot study was to explore the use of the SureScreen COVID-19 IgG/IgM Rapid Test Cassette in a deceased population as well as compare the results to the ELISA assays routinely in the private and public health sectors.

1.8. Objectives

- To perform anti-SARS-CoV-2 antibody testing on a pilot cohort of deceased individuals
- To determine if the SureScreen rapid test and its protocol are suitable for post-mortem samples
- To compare the results of the SureScreen IgG/IgM rapid test to the two antibody tests in the public and private health sectors in South Africa; namely Roche Diagnostics' Elecsys Anti-SARS-CoV-2, and Abbott's Architect SARS-CoV-2 IgG Assay, and to existing data.
- To explore the effect of certain variables such as time intervals between PCP, death, autopsy, and testing, as well as the effects of vaccinations on the test results.

Chapter 2: Methodology

2.1 Study design and approvals

This study was a quantitative pilot study performed to assess the feasibility of anti-SARS-CoV-2 antibodies in the deceased population, before recruiting a large cohort, as the deceased population is a vulnerable population. It was conducted as a prospective, observational, cohort study, in the deceased population. This study received approval from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC REF: 637/2020), Stellenbosch University Human Research Ethics Committee (HREC REF: M21/03/002_RECIP_UCT_637/2020_COVID-19) and the SAHPRA (MD20201201) (Appendix 1A, 1B, 1C, respectively). The study was also logged on the National Health Research Database (NHRD) and was approved by the NHLS. Transport of the biological samples was approved by the inspector of anatomy (Appendix 1D).

2. 2. Population cohort

This pilot study focused on recruiting deceased individuals that had COVID-19 in their lifetime, irrespective of when the infection took place. Cases were included if:

- The next-of-kin reported that the deceased had COVID-19 in their lifetime
- The deceased was over the age of 18 years old

Cases were excluded if:

- The body showed macroscopic signs of decomposition
- The deceased individuals remained unidentified and if there was no contact information for next-of-kin

When this study began, vaccinations against SARS-CoV-2 were not yet offered in South Africa. When vaccinations become available in South Africa, it was decided to include cases where the deceased was vaccinated against SARS-CoV-2. However, the vaccination status of the individuals could not be verified using independent and official health records, so the information received was based on an oral report from the next-of-kin.

Next of kin of the deceased individuals who met the inclusion criteria were approached for informed consent when the family came to the mortuary to legally identify their family member

(Appendix 3A and 3B) This involved following a published ethical framework for requesting consent from grieving next of kin (Heathfield *et al.*, 2017)

A total of 30 participants were obtained for this study over the course of one year. Five cases were from Tygerberg Mortuary and 25 cases were from Salt River Mortuary. The study participants were mainly male which represented 66.66% of total participants. The ages of the participants ranged from 18 years – 82 years with the mean age being 45.8 years (Figure 2.1).

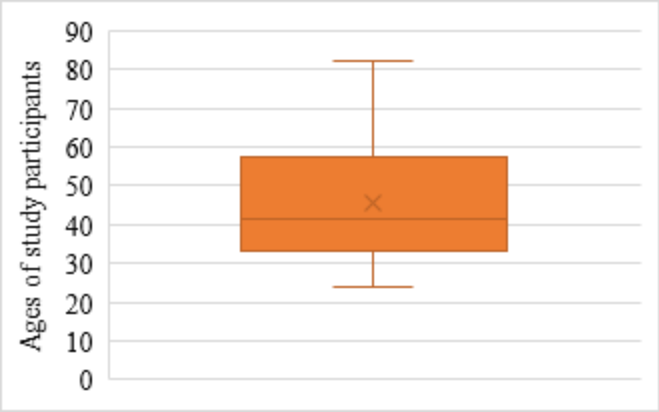


Figure 2.1: Box and whisker plot describing the age range of the study participants

Although all families reported that the deceased had COVID-19 infection during their lifetime, this could only be confirmed with PCR results for nineteen of the 30 cases. For the cases without PCP confirmation, the month and year as reported by next of kin (where available) were used in data analyses.

The following information was collected for each participant in the study: age, sex, date of COVID-19 infection (if known), status of COVID-19 vaccination, and date of death. This information was collected from the post-mortem records. The data was managed according to a data management plan (available on request). Each deceased individual was deidentified by being allocated a unique study number and only this appeared on samples. This was done to ensure the confidentiality of the participants. Access to this data was restricted by means of password protection and limited access to listed investigators only.

2.3. Sample collection

After informed consent was received from the next-of-kin of the deceased individuals (Appendix 3B), two blood samples were collected at autopsy from each included case by the

assigned forensic pathologist. A volume of 4 mL of blood was collected into two serum separator tubes (SSTs) according to the standard operating procedures of the mortuary. The time and date of sampling as well as the name of the pathologist taking the sample were recorded. Samples were stored at 4 °C for no more than 12 hours, before transportation to the respective laboratories: one SST of blood was sent to BARC while the other tube was sent to the NHLS.

Table 2.1: Samples obtained during autopsy for the various tests.

Type of testing	Autopsy	Test used	Location of test
Rapid testing	1x SST (serum)	COVID-19 IgG/IgM Rapid Test Cassette (SureScreen Diagnostics)	NHLS
SAHPRA-approved testing in public sector		Elecsys Anti-SARS-CoV-2 (Roche)	NHLS
SAHPRA-approved testing in private sector	1x SST (serum)	Architect SARS-CoV-2 IgG Assay (Abbott)	BARC

*SST: Serum separator tube, IgG - Immunoglobulin G, IgM – Immunoglobulin M, SAHPRA – south African Health Products Regulation Association, NHLS – National Health Laboratory Service, BARC – Bio Analytical Research Corporation. SARS – severe acute respiratory syndrome, CoV – coronavirus.

2.4. Antibody testing

Upon arrival in the NHLS laboratory, the samples were centrifuged at 4000 *x g* for 10 minutes and were stored at 2-8 °C until testing. Prior to SureScreen COVID-19 IgG/IgM Rapid Test testing, the blood sample, the test cassette, and reagents were equilibrated to room temperature. The testing was then performed according to the manufacturer's instructions (Appendix 2A). After 10 minutes the rapid test results were interpreted. A coloured line at the control line region (C) was a valid test and if there was no coloured line present then the test was invalid. A coloured line present at the IgG region meant the sample was positive for IgG, while a coloured line present at the IgM region meant the sample was positive for IgM. If no coloured lines appeared other than the control line, then the test was negative for both antibodies (Appendix 2A). The intensity of the colours in the IgG and/or IgM test line region varied depending on the concentration of SARS-COV-2 antibodies in the specimen. Any shade of

colour in the IgG and/or IgM test line region(s) was considered positive. The results were documented using contemporaneous notes and by photography.

The remaining serum samples were subsequently tested using the Elecsys assay at the NHLS according to the manufacturer's instructions with no deviations (Roche Diagnostics, 2021). Whilst majority of the samples were tested using Elecsys Anti-SARS-CoV-2 N Antibody test, four of the cases were testing using Elecsys Anti-SARS-CoV-2 S Total Antibody test. This was due to a national shortage of reagents for the N antibody test. An interpretation of a positive result for the N antibody test is a cut off index (COI ≥ 1.0 whilst a COI of < 1.0 indicates a negative or non-reactive result (Roche, 2020). A positive result for the Elecsys Anti-SARS-CoV-2 S Total will be given if the volume of antibodies detected is ≥0.8 U/mL, if the result is lower, then the result will be non-reactive (Roche Diagnostics, 2022). According to the NHLS, higher titers are likely to indicate natural infection as opposed to vaccination (Diana Hardie, personal communication, July 2022).

The second SST was tested using the Architect Assay at BARC following the methods outlined in the package insert with no deviations (Abbott Laboratories, 2021). The cutoff index for this form of testing is 1.40 Index (S/C). if the result is below the cut off then the result is negative, if the result is above the cut off then it is positive (Abbott Laboratories, 2021).

2.5 Data analysis

The number of days between: PCP and death, PCP and autopsy, death and testing, PCP and testing and death and autopsy were calculated using the dates of each occurrence. The medians were calculated for each of these variables.

For statistical analysis, contingency tables were created comparing i) the SureScreen rapid IgM and IgG test results, to the Elecsys results ii) the SureScreen rapid IgM and IgG test, to the Architect results and iii) the Elecsys and the Architect results, for all 30 cases. Fischer's exact test was performed on these contingency tables using the statistical program R (level of significance = 0.05). The contingency tables were also used to calculate the percent overall agreement (POA) using Equation 2.1.

$$POA = (a + d)/(a + b + c + d)$$

Where in a contingency table:

	Test 1 positive results	**Test 1 negative results**
Test 2 positive results	a	b
Test 2 negative results	c	d

Equation 2.1: Formula to calculate POA

The 19 cases with PCP confirmation were used to calculate the sensitivity of each antibody test using Equation 2.2, as they were the only cases determined to be true negatives or false positives, but further statistical analyses were limited for these cases due to the low sample size. Specificity and accuracy were not able to be calculated as there were no cases in the study cohort where it was certain that the individual did not have COVID-19 in their lifetime.

$$Sensitivity = TP/(TP+FN)$$

Where:

TP = True positives

FN = False negatives

Equation 2.2: Formula to calculate sensitivity

The sensitivities were calculated at certain intervals. First, they were calculated on the intervals outlined by Elecsys' instruction for use (IFU) as majority of the data points collected would fit into the categories (0-6 days, 7-13 days, > 14 days) (Roche Diagnostics, 2021). The intervals outlined by Abbott Architect's IFU did not allow for more than one data point to fit into the intervals (< 3 days, 3-7 days, 8-14 days, > 14 days) (Abbott Laboratories, 2021). Further intervals were analysed based on the number of days between PCP and death found in this study. This was performed to further the understanding of the results and identify the difference between sensitivities of the higher number of days between PCP and death and the lower number of days. The intervals chosen were: < 1 days, 6-27 days, 90-200 days, 200-600 days and 600+ days.

A logistic regression analysis was performed on R to compare the following variables against the test results: PCP confirmation, and the number of days between; PCP and death, PCP and autopsy, death and testing, PCP and testing and death and autopsy. Vaccination status was not included as it was a non-binary variable (yes, no or unknown). The two cases that did not have estimations for COVID-19 infection dates were excluded from the model. Collinearity amongst the variables pertaining to the number of days was assessed using a Pearson correlation test. The number of days between PCP and death, number of days between PCP and autopsy and number of days between PCP and testing were found to be collinear to each other (correlation > 0.8), thus only one needed to be included in the logistic regression. The variables therefore chosen for the logistic regression were: (i) PCP confirmation, and number of days between (ii) PCP and death, (iii) death and testing, and (iv) death and autopsy.

Chapter 3: Results

3.1 Results

3.1.1 Overview

The results of each test alongside case demographics, PCP confirmation, vaccination status, and time intervals between infection, death, autopsy, and antibody testing are tabulated for each case (Table 3.1). The time that occurred between (i) PCP and death was 0-702 days (median = 81 days), (ii) death and autopsy was 1-8 days (median = 4 days), and (iii) autopsy and antibody testing was 0-13 days (median = 4 days). Six participants had received vaccinations against SARS-CoV-2 during their lifetime, thirteen were not vaccinated, and the remaining eleven had unknown vaccination status. The results of each antibody test will be presented in sub-sections below.

Table 3.1: Table detailing the variables and the results of the three types of COVID-19 antibody testing.

Case number	Sex	Age	Vaccinated	Days between PCP and death	Days between PCP and autopsy	Days between death and autopsy	SureScreen			Elecsys			Architect			Days between SureScreen testing and Elecsys testing	Days between SureScreen testing and Architect testing	SureScreen results		Elecsys results	Architect results
							Days between death and testing	Days between PCP and testing	Days between autopsy and testing	Days between death and testing	Days between PCP and testing	Days between autopsy and testing	Days between death and testing	Days between PCP testing	Days between autopsy and testing			IgM	IgG	Ab	IgG
1♦	F	63	n	127	131	4	5	132	1	6	138	7	6	133	2	6	1	-	+	+	+
2	M	55	n	136	140	4	4	140	0	3	143	3	5	141	1	3	1	+	+	+	+
3♦	F	58	n	146	149	3	4	150	1	4	154	5	5	151	2	4	1	-	+	+	-
4♦	M	60	n	19	21	2	2	21	0	5	26	5	3	22	1	5	1	-	-	+	-
5	F	52	n	41	43	2	4	45	2	1	46	3	5	46	3	1	1	-	+	+	+
6	M	26	n	334	336	2	8	342	6	1	343	7	9	343	7	1	1	-	-	+	-
7♦	F	82	unknown	352	355	3	3	355	0	3	358	3	4	356	1	3	1	-	+	+	-
8	F	41	n	7	13	6	10	17	4	4	21	8	11	18	5	4	1	-	-	-	-
9°	M	34	unknown	347	353	6	13	360	7	10	370	17	14	361	8	10	1	-	-	-	-
10°♦	F	68	y	702	706	4	7	709	3	6	715	9	8	710	4	6	1	+	+	+	-
11°♦	M	36	n	8	12	4	6	14	2	6	20	8	7	15	3	6	1	+	+	+	+
12°	M	28	n	n/a	n/a	3	4	n/a	1	6	n/a	7	5	n/a	2	6	1	-	+	+	+
13	M	29	y	n/a	n/a	4	6	n/a	2	3	n/a	5	7	n/a	3	3	1	-	+	+	-
14♦	M	42	n	0	6	6	10	10	4	22	32	26	12	12	6	22	2	+	+	+	+
15	M	52	n	62	64	2	6	68	4	22	90	26	8	70	6	22	2	-	+	+	+
16	M	24	y	0	2	2	6	6	4	1	7	5	7	7	5	1	1	-	+	+	+
17♦	M	77	y	0	3	3	7	7	4	1	8	5	8	8	5	1	1	+	+	+	-
18	F	37	unknown	81	82	1	8	89	7	2	91	9	9	90	8	2	1	-	-	+	-
19	M	37	unknown	81	82	1	8	89	7	1	90	8	9	90	8	1	1	-	-	+	-
20♦	M	47	unknown	625	630	5	18	643	13	6	649	19	19	644	14	6	1	-	-	+	-
21♦	F	57	n	12	17	5	13	25	8	6	31	14	14	26	9	6	1	-	+	+	+
22♦	M	38	unknown	699	703	4	8	707	4	6	713	10	9	708	5	6	1	+	+	+	-
23♦	M	66	y	6	14	8	15	21	7	5	26	12	16	22	8	5	1	+	+	-	-
24♦	M	25	unknown	97	102	5	18	115	13	5	120	18	19	116	14	5	1	-	+	+	-
25♦	F	35	n	1	5	4	10	11	6	5	16	11	11	12	7	5	1	-	-	+	-
26♦	F	41	unknown	575	580	5	8	583	3	5	588	8	9	584	4	5	1	-	+	+	-
27♦	M	56	unknown	273	275	2	15	288	13	0	288	13	15	288	13	0	0	-	+	+	+
28♦	M	30	unknown	1	5	4	11	12	7	3	15	10	12	13	8	3	1	-	-	+	-
29♦	M	30	y	27	31	4	12	39	8	3	42	11	13	40	9	3	1	-	+	+	-
30♦	M	48	unknown	695	700	5	6	701	1	3	704	4	8	703	3	3	2	-	+	+	-
Medians				**81**	**82**	**4**	**8**	**89**	**4**	**12.5**	**90.5**	**8**	**4.5**	**90**	**5**	**4.5**	**1**				

*F – female, M – Male, PCP – positive COVID-19 PCR, y – yes, n - no, + - positive, - - negative. ° - cases analysed using Elecsys Anti-SARS-CoV-2 S Total Antibody test. ♦ - cases with PCP confirmation

3.1.1.1 SureScreen test results

Of the 30 cases, 21 had positive SureScreen rapid results as denoted by the IgG indicator (n = 21) and IgM indicator (n = 7). All seven positive IgM results were also positive for the IgG marker, and there were no instances of an IgM positive and IgG negative test result. There were four instances where IgM and IgG were both negative but COVID-19 infection during life was confirmed by PCP (cases 4, 20, 25, 28) (Table 3.1). In two of these cases (case 25 and 28), there was only one day between PCP and death, thus limiting the time for seroconversion. There were however, three other cases where the days between PCP and death were all zero (case 14, 16 and 17), yet these cases all had a positive IgG result and two also had a positive IgM result. Case 20 had 625 days between PCP and death - although this time interval seemed particularly long, there were other cases with similar long-time intervals that produced positive SureScreen results (e.g., cases 10, 22 and 30). The number of days between death and testing ranged from 2 days to 18 days for the four negative cases, which was also similar to other cases which yielded positive IgG results (e.g., case 24).

Table 3.1 shows that SureScreen IgM yielded a positive result when the number of days between PCP and death were low (case 11, 14, 23). If the number of days was high between PCP and death, positive results were only present if the case was confirmed to be vaccinated (case 10, 17, 23). Whilst only 50% of the six vaccinated cases were positive for IgM, these cases were all positive for IgG (cases 10, 13, 16, 17, 23, 29). The IgG negative test results did not have a single case with positive COVID-19 vaccination. The days between death and testing ranged from 2-18 days, the days between PCP and testing ranged from 6-709 days, and the days autopsy and testing ranged from 0-13 days (Table 3.1)

The median days between death and sample testing were all relatively similar (Table 3.1). The IgG positive results had mostly higher medians for the days between (i) PCP and testing, (ii) PCP and autopsy and (iii) PCP and death.

3.1.1.2 Elecsys test results

Elecsys' assay produced the fewest number of negative results (3/30), as documented in Table 3.1 (case 8, 9 and 23). Although case 23 had PCP and vaccination confirmation and it was expected for this result to be positive, this case had highest number of days between admission and autopsy (8 days) (Table 3.1). The second period between death and autopsy was six days (case 14) which yielded a positive result, suggesting a potential cut off for the Elecsys test to only be used when the period between death and autopsy is six days or less.

The postive Elecsys results had higher medians for number of days between (i) PCP and testing, (ii) PCP and autopsy and (iii) PCP and death than the negative results (Table 1.3). The days between death and testing ranged from 5-32 days, the days between PCP and testing ranged from 7-715 days, and the days autopsy and testing ranged from 3-26 days (Table 3.1). The four cases that were analysed using Elecsys Anti-SARS-CoV-2 S Total Antibody test all had a volume of >250 U/mL. The high antibody titre is reflective of previous natural SARS-CoV-2 infection as antibodies produced from vaccinations are generally not as high. As the rest of the cases were analysed using the N antibody test the results are also indicative of previous natural SARS-CoV-2 infection and the vaccinations available in South Africa for SARS-CoV-2 only generate immune response to the S protein.

3.1.1.3 Architect test results

Architect had the highest number of negative results (66.67%) (Table 3.1) with many of the negative results occurring in cases with PCP confirmation (15 out of 19 cases with PCP confirmation: cases 3, 4, 7, 10, 17, 20, 22-30). The days between death and testing ranged from 3-19 days, the days between PCP and testing ranged from 7-710 days, and the days between autopsy and testing ranged from 1-14 days (Table 3.1). The highest number of days between PCP and death was case 10 at 702 days -while SureScreen and Elecsys both produced positive results for this case, the Architect result yielded a negative result.

From Appendix 5A, the median days between (i) PCP and autopsy as well as between (ii) PCP and death are much higher for the negative results compared to the positive results. Architect's negative results also had the higher percentage of PCP confirmation and vaccinated cases compared to the positive results. As the Architect antibody test detects IgM antibodies to the SARS-CoV-2 N protein, it can be assumed that these results are also indicative of previous

natural SARS-CoV-2 infection and the vaccinations available in South Africa for SARS-CoV-2 only generate immune response to the S protein.

3.1.1.4 Test comparisons

SureScreen and Architect tests had a higher median number of days between PCP and death for their positive results than their negatives and their median values were the same (Figure 3.1). Roche's Elecsys test had a lower median number of days between PCP and death. The medians for the positive results for number of days between death and autopsy, and autopsy and antibody testing were relatively similar for the SureScreen and Architect test. However, the median number of days between autopsy and antibody testing was higher for the Elecsys assay negative results compared to the other forms of testing. The negative results, for all three tests, had slightly higher medians for number of days between death and autopsy (4-6 days), and autopsy and antibody testing (6-12 days).

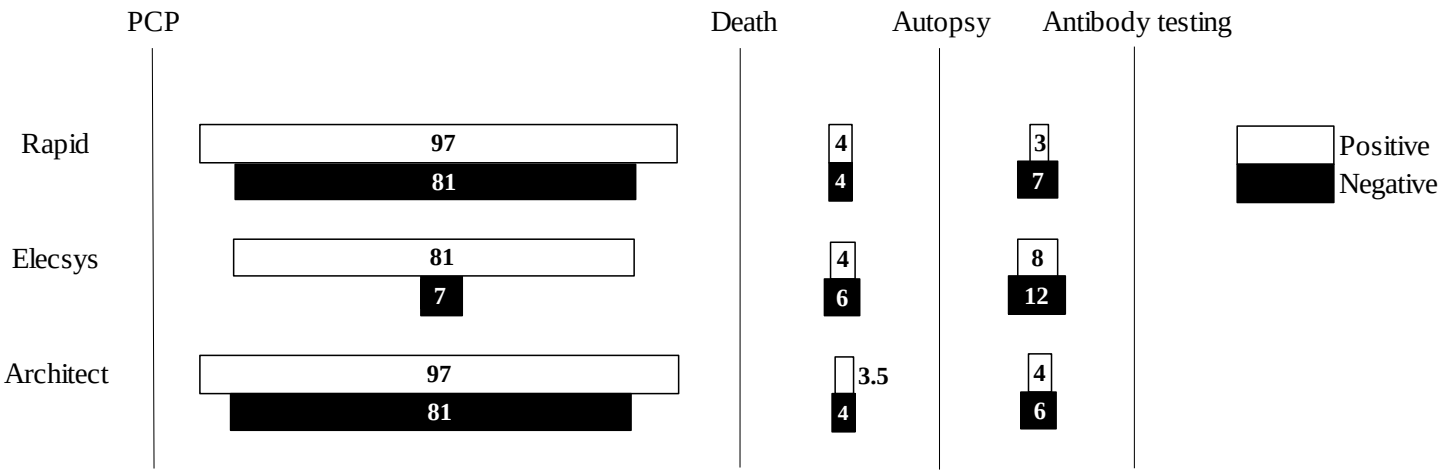


Figure 3.1: Chart visualising the median number of days between PCP, death, autopsy, and antibody testing for all three forms of testing.

The overall sensitivities of the antibody tests of the 19 cases with PCP confirmation is depicted in Table 3.2. Elecsys had the highest sensitivity at 94.74% with SureScreen IgG following at 78.95%. Architect and SureScreen IgM had similar sensitivities at 26.32% and 31.58% respectively.

The sensitivities were then calculated for each test based on time intervals (from Elecsys IFU) between PCP and death for the 19 cases with PCP confirmation (Table 3.2). For SureScreen IgM and Architect, the highest sensitivities were recorded for cases that had 7-13 days between

PCP confirmation and death. SureScreen IgG had the highest sensitivity at above 14 days between PCP confirmation and death. Elecsys had 100% sensitivity at 7-13 days and above 14 days. The lowest sensitivities were recorded for SureScreen IgM and Architect at 0-6 days and SureScreen IgM at >14 days.

Table 3.2: Table detailing the different sensitivities of the various tests at different intervals of time between PCP and death with intervals outlined by Elecsys IFU

Days between PCP and death	Number of cases	Sensitivity			
		SureScreen IgM	SureScreen IgG	Elecsys	Architect
0-6	5	60%	60%	80%	25%
7-13	2	100%	50%	100%	100%
>14	12	20%	83.33%	100%	20%
Total	19	31.58%	78.95%	94.74%	26.32%

When the sensitivities were further assessed in terms of other relevant intervals, it is notable that Roche's Elecsys assay performed consistently for all interval ranges, with the lowest sensitivity occurring at 6-27 days (Table 3.3). SureScreen IgM's sensitivity decreased between the 6–600 days (between PCP and death) with no positive results occurring between 90-600 days. Surprisingly, the sensitivity for SureScreen IgM jumped from 0% to 50% in the 601+ days category. SureScreen IgG performed the best in the two intervals that SureScreen IgM's sensitivities were 0%. Abbot's Architect had low sensitivities for all the intervals with the highest and lowest intervals having 0% and 25% sensitivities, respectively (Table 3.3).

Table 3.3: Table detailing the different sensitivities of the various tests at different intervals of time between PCP and death based on time intervals present in this study

Days between PCP and death	**Number of cases**	**Sensitivity**			
		SureScreen IgM	**SureScreen IgG**	**Elecsys**	**Architect**
< 1	4	50%	50%	100%	25%
6-27	5	40%	80%	80%	40%
28-89	0	-	-	-	-
90-200	3	0%	100%	100%	33.33%
201-600	3	0%	100%	100%	33.33%
601+	4	50%	75%	100%	0%

The percentage agreement of the results between each pairwise test was calculated, and the Fischer exact test was performed to assess if there were any significant differences between the results of the three different tests (Table 3.4). Out of all pairwise tests, the results from the Elecsys and SureScreen IgG tests were in the most agreement with each other (73.33%) while Elecsys and SureScreen IgM tests results were in the least agreement with each other (26.66%). Further, the SureScreen IgG results were significantly different from those obtained from the results obtained from Architect test ($p = 0.01$) (Table 3.4).

Table 3.4: Table summarising the percent overall agreement and p-values generated by the comparisons of different testing

	Test Type	**p-value**			
		SureScreen IgM	**SureScreen IgG**	**Elecsys**	**Architect**
Percent overall agreement (%)	**SureScreen IgM**		0.07	0.56	0.66
	SureScreen IgG	53.33		0.21	0.01*
	Elecsys	26.66	73.33		0.53
	Architect	63.33	63.33	43.33	

3.1.2 Assessment of variables that may affect the tests' results

A logistic regression showed that none of the time intervals were significantly associated with the antibody test results for any of the three tests (Table 3.5, $p > 0.05$). There was also no notable association between the test results and age and sex. Of note however, the relationship between the number of days between death and autopsy and the Elecsys results could not be calculated as the algorithm did not converge. Overall, the Elecsys assay had the lowest Akaike Information Criterion (AIC), suggesting it was the better model.

Table 3.5: Table summarising the linear regression data of the comparison of all the types of testing

		Test types							
		SureScreen IgM		SureScreen IgG		Elecsys		Architect	
		p-value	AIC	p-value	AIC	p-value	AIC	p-value	AIC
Variables	PCP confirmation (yes)	0.39	39.46	0.09	39.26	0.25	25.23	0.62	39.87
	Days between PCP and death	0.87		0.91		0.72		0.13	
	Days between death and testing	0.13		0.12		0.98		0.51	
	Days between death and autopsy	0.06		0.45		n/a		0.95	

As Elecsys produced the better model and from the previous results it had the higher sensitivity when compared to the PCP confirmed cases, the other test results were compared to the Elecsys results using logistic regression analysis, to assess if any of the other tests would have a significant relationship with the Elecsys results, however, there were no p-values below 0.05 (Table 3.6).

Table 3.6: Table summarising the linear regression data of the comparison of SureScreen IgM and IgG and Architect to Elecsys.

Test type	**Elecsys**	
	p-value	**AIC**
SureScreen IgM	0.73	22.95
SureScreen IgG	0.21	21.37
Architect	1	20.57

Chapter 4: Discussion

4.1 Overview

This pilot study focussed on COVID-19 antibody testing in the deceased population using the SureScreen Diagnostics COVID-19 IgM/IgG rapid test cassette. The purpose of this pilot study was to assess if the SureScreen test could potentially be implemented as a screening tool in a mortuary setting so that previous COVID-19 infections could be more accurately documented for public health reasons. Being a pilot study, this study focused on generating novel preliminary data which would inform further testing or future studies. The importance of antibody testing for surveillance purposes was highlighted in chapter 1, and the use of LFI's in a resource-constrained and highly busy forensic mortuary is motivated by the ease, low-cost and fast turnaround time for results when using LFIs.

This chapter will discuss the (i) the protocol of the SureScreen test for post-mortem samples, (ii) the results of the SureScreen test in comparison to Roche Diagnostics' Elecsys Anti-SARS-CoV-2, and Abbott Architect SARS-CoV-2 IgG Assay and existing data for the assays, and (iii) the effect of certain variables such as time intervals between PCP, death, autopsy, and testing, as well as the effects of vaccinations on the test results. Lastly, this chapter will describe the limitations of this pilot study and present future recommendations based on the data generated in this study.

4.2 SureScreen rapid test for post-mortem samples

As COVID-19 antibody testing is not often performed on post-mortem samples, this study was interested in the parameters around testing and the development of an optimum procedure. This study found that positive COVID-19 antibody results could be detected after six days from death and obtaining post-mortem samples for the Elecsys and Architect tests, and eight days for the SureScreen test (Table 3.1). This result is meaningful in a forensic mortuary setting because the average time between death and autopsy was 3.77 days in this study, and is typically three days in Western Cape mortuaries (Reid *et al.* 2020; Thompson *et al.*, 2013). These time frames between death and autopsy were considerably longer than those reported by Edler and colleagues (2011) who suggested that post-mortem serological testing in HIV-, HBV- or HCV-infected deceased persons should be on blood samples collected within two days post-mortem (Edler *et al.*, 2011).

Regarding when the separation of serum is to take place after sample collection, SureScreen's recommendations are that the serum/plasma must be separated immediately after sample collection to avoid haemolysis (Surescreen Diagnostics, 2020). These recommendations were echoed by other studies (Edler *et al.*, 2011; Kalus *et al.*, 2011). Due to centrifuges not being readily available at forensic mortuaries, separation of serum could not be performed immediately in this study, but rather, samples were transported to the laboratory for separation. This occurred within 12 hours of sample collection and based on observational data gained whilst performing this study, it was found that the longer the samples were left, the less likely they were to produce serum. Samples that were unable to produce serum had to be excluded from the study as then only whole blood would be available for testing and would detriment the integrity of the protocol. Although whole blood can be used on the SureScreen test, once submitted to the formal antibody testing at the NHLS and BARC there needs to be a minimum amount of serum. If a sample is submitted without serum, then testing could not be performed. This highlights the ease of LFIs as they can be used for point of care testing, compared to the formal antibody testing that requires the whole blood samples to be separated into serum/plasma and have to be performed at a laboratory (Roche, 2020; Abbott Laboratories, 2021).

The recommendations of the storage of the serum samples varied for each test. SureScreen states that serum samples may be stored at 2-8°C for up to 3 days after collection, whereas Elecsys and Architect state that serum can be stored at 2-8°C for 7 days after collection (Surescreen Diagnostics, 2020; Abbott Laboratories, 2021; Roche Diagnostics, 2021). This study did not restrict the time between sample collection and testing so that the workflow was authentic and as realistic as possible, to increase the translatability of these results into practice. For example, the NHLS only conducted the Elecsys testing on Mondays, whereas testing at BARC required the transport of samples first to their Cape Town branch, followed by couriering to Johannesburg and then batching according to their internal SOPs. These were the realities of testing samples with approved serological tests in accredited laboratories in South Africa. When analysing the results in Table 3.1, the cases where the number of days between autopsy and testing for the tests were above the manufacturers recommendations still had positive results. These time delays, therefore, did not seem to affect the results and this was confirmed by the results of the logistic regression which showed no significant association between the number of days between antibody test results and the number of days between

autopsy and testing. Indeed, the logistic regression analysis (Table 3.5) showed there were no strong relationships between any of the time intervals and the test results.

4.3 Results of the antibody tests in comparison to existing data

Table 3.2 shows that the Elecsys test has the highest overall sensitivity from the data obtained in this study and its sensitivity outlined by the manufacturers for > 14 days post PCP was one of the best sensitivities documented by the manufacturers for these tests. This would be why even with the high number of days between autopsy and testing, positive results were still obtained.

Another possible reason as to why Roche's Elecsys assay may have a higher sensitivity is because it detects a larger array of COVID-19 antibodies such as: IgM, IgG and IgA (Roche Diagnostics, 2021). The reports for Elecsys do not specify which antibody was detected but provides quantitative results of antibodies generated against SARS-CoV-2 N protein whilst SureScreen detects only IgM and IgG and Architect specifically detect IgG.

SureScreen IgM had a high sensitivity at 7-13 days post PCP. This correlates with literature stating that IgM appears approximately five days after infection (Liu *et al.*, 2020; Ripperger *et al.*, 2020; Z. Li *et al.*, 2020). This is also a reason for the higher sensitivity for SureScreen IgM at 0-6 days post PCP compared to the IgG tests. However, SureScreen IgM's sensitivity in this study jumped from 0% for 90-600 days between PCP and death to 50% for the cases with over 601 days between PCP and death. This finding does not correlate with results obtained from Liu et al. (2019) and Li et al. (2020) who state that IgM was undetectable in patients after 12 weeks after initial infection (Liu, *et al.*, 2019; Li *et al.*, 2020).

This study's findings reported lower sensitivities for Architect's assay compared to the manufacturers' reporting (Abbott Laboratories, 2021). As Architect's assay tests for IgG, it was hypothesised that the Architect results would correlate with SureScreen IgG's. However, the results from these two tests were found to significantly differ from each other ($p = 0.01$) (Table 3.4). The majority of the publications that reported sensitivities for the Architect test focussed on cohorts of hospitalised patients with severe COVID-19, therefore having very high immune system activity (Johnston *et al.*, 2013; Abbott Laboratories, 2021). This study had a more extended time period between PCP and death which may have resulted in a decreased sensitivity (Abbott Laboratories, 2021).

This leads to another interesting factor, namely how long COVID-19 antibodies could be detectable post infection. The number of days between PCP and death ranged from 0-702 days. A study performed by De Giorgi *et al* (2021) found that of 116 cases, 91.4% had detectable IgG levels up to 11 months (+- 330 days) after symptom recovery (De Giorgi *et al.*, 2021). In this study, the sensitivities were 100% for SureScreen IgG and Elecsys at 200-600 days between PCP and death and 75% and 100%, respectively, for 600+ days (Table 3.3). This demonstrates that positive results can be obtained in higher time intervals between PCP and death.

The POA results showed the best agreement between Elecsys and SureScreen IgG at 73.33% and the lowest was Elecsys and SureScreen IgM at 26.66%. The POA for Elecsys and Architect was also low, at 43.33% (Table 3.4). A study performed by Tan and colleagues (2021) compared the sensitivities of Elecsys and Architect and determined that Elecsys slightly outperformed Architect at critical time points of 14 days and 21 days and stated it could be due to Roche's Elecsys assay measuring total antibodies and the Architect assay specifically detecting IgG (Tan *et al.*, 2021).

According to the next-of-kin of the 30 cases, 20% of the cases were vaccinated, 43.33% were not vaccinated and 36.67% had unknown vaccination status (Table 3.1). As previously mentioned, the SureScreen, Elecsys and architect assays have the ability to detect antibodies generated from natural infection as the vaccines being rolled out in South Africa elicit immunological response to the S protein (MU Health Care, 2021; The Immunisation Advisory Centre, 2022).

Suhandynata *et al.* (2021) reported that antibody tests that exclusively detects N protein antibodies would most likely not detect individuals vaccinated against the S protein (Suhandynata *et al.*, 2021). The tests used in this study (SureScreen, Elecsys and Architect) were developed to detect antibodies to the N protein (Surescreen Diagnostics, 2020; Abbott Laboratories, 2021; Roche Diagnostics, 2021). In four instances, however, a different variation of the Elecsys assay was performed that not only targeted SARS-CoV-2 N antibodies, but also S antibodies (cases 9, 10, 11 and 12). Only one of these cases were confirmed to have been vaccinated against SARS-CoV-2 (case 10). The reason for the change in the test was due to a reagent shortage for the original Elecsys testing performed for the other cases.

It is still uncertain when the vaccines were administered for the cases with confirmed vaccination status, therefore, a positive test for those four cases could indicate either prior infection or prior vaccination. However, the volume of antibodies detected was high (>250 U/ml). Due to this and since the main forms of testing have been N protein based, and the two vaccinations being rolled out in South Africa are S protein based, it can be assumed that it is more likely that the positive results are from natural infection and not vaccination. Therefore, the vaccination status of the study participants did not affect the test results. There were also no data indicating an effect on test results due to sex and age.

4.4 Limitations and future work

This pilot study was performed to assess the Surescreen test in the deceased population for the first time, and in doing so, to gain insight into developing an optimised workflow for COVID-19 antibody detection post-mortem as well as to identify issues with sampling, feasibility of the mortuary setting to conduct antibody tests, preliminary insight into how the test performs on PM samples and if data is comparable to the manufacturer and other studies. As it was a pilot study the cohort was small. A limitation was that of our 30 cases, only 19 had PCP confirmation. Therefore, only 19 cases were able to be used to determine sensitivity. This was due to incomplete/non-existent medical records for many of the deceased individuals being admitted to Salt River and Tygerberg mortuary. In South Africa during the study period, there appeared to be undertesting for COVID-19, due to the low socio-economic status, low access to laboratory testing and the expense of tests (Cohen *et al.*, 2022).

The second limitation of this study was that negative controls were not able to be used in this study. This is because it was impossible to recruit a case during the study period who definitely did not have COVID-19 in their lifetime. If they did not have symptoms nor did a PCR test, these was no way to be certain that the individual was not infected during their lifetime. Even if the deceased had record of a negative COVID-19 PCR test, the infection may have occurred and not been tested earlier on in the individual's lifetime. Further, blood samples taken from deceased individuals before the pandemic could not be used as (i) there was no consent in place for this and (ii) the time between autopsy and testing would have long surpassed the published guidelines, thus a negative test result may be due to these time delays as opposed to the person never having had COVID-19 before. However, for complete evaluations of tests, both the sensitivity *and specificity* are required – but to calculate specificity, the results from known negative cases would need to be tested. Specificity is important as it would indicate false

positive results, which is also an important factor in determining the suitability of antibody tests. Therefore, this limitation of not being able to recruit true negative controls prevented holistic analysis - thus, recommendations from this study are only based on sensitivity of the test.

4.5 Conclusion

To conclude it was noted that Roche Elecsys Anti-SARS-CoV-2 performed the best on the cohort of post-mortem serum samples. The SureScreen IgM/IgG rapid test cassette had adequate performance for IgG detection but had a lower sensitivity than the Elecsys assay. Abbott Architect SARS-CoV-2 IgG Assay performed the worst in this study with a low positivity rate for the PCP confirmed cases. From this study, it is recommended that the Elecsys assay is most suitable for diagnosis of past infection over SureScreen and Architect. However, to reach a conclusion about SureScreen test's suitability or role in the mortuary setting as a screening tool, the study requires more cases with PCP confirmation to draw more robust statistical conclusions.

This conclusion is based on whether other types of SARS-CoV-2 vaccines are eventually provided to the South African public. If the vaccines remain to be the types containing SARS-CoV-2 S protein, then these tests will still be able to detect natural infection. However, if vaccines containing the N protein are administered then there may be implications to the use of these tests.

Overall, these results represent the first empirical data for the use of SureScreen IgM/IgG rapid test cassette, Roche Elecsys Anti-SARS-CoV-2 assay, and Abbott Architect SARS-CoV-2 IgG assay on post-mortem samples. This pilot study can guide further research which would inevitably assist with COVID-19 surveillance in the mortuary setting, using anti-SARS-CoV-2 antibody testing.

5. References

Abbott Laboratories. 2021. *SARS-CoV-2 IgG for use with Architect. FDA.gov*. Available at: https://www.fda.gov/media/137383/download (Accessed: 30 May 2022).

Ai, T., Yang, Z., Hou, H., Zhan, C., Chen, C., Lv, W. *et al.* 2020. Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. *Radiology*. 296(2): E32–E40. Available at: https://doi.org/10.1148/radiol.2020200642.

Alefishat, E., Jelinek, H.F., Mousa, M., Tay, G.K. and Alsafar, H.S. 2022. Immune response to SARS-CoV-2 variants: A focus on severity, susceptibility, and preexisting immunity. *Journal of Infection and Public Health*. 15(2):277–288. Available at: https://doi.org/10.1016/j.jiph.2022.01.007.

Autobio. 2020. *Anti-SARS-CoV-2 Rapid Test - Instructions for Use*.

Al Awaji, N.N., Ahmedah, H.T., Alsaady, I.M., Bawaked, R.A., Alraey, M.A., Alasiri, A.A. *et al.* 2021. Validation and performance comparison of two SARS-CoV-2 IgG/IgM rapid tests. *Saudi Journal of Biological Sciences*. 28(6):3433–3437. Available at: https://doi.org/10.1016/j.sjbs.2021.03.007.

Batra, R., Olivieri, L.G., Rubin, D., Vallari, A., Pearce, S., Olivo, A., *et al.* 2020. A comparative evaluation between the Abbott Panbio™ COVID-19 IgG/IgM rapid test device and Abbott Architect™ SARS CoV-2 IgG assay. *Journal of Clinical Virology*. 132:104645. Available at: https://doi.org/10.1016/j.jcv.2020.104645.

Binnicker, M.J. 2020. From the Common Cold to a Chaotic Contagion: the Potential for Coronaviruses To Cause Outbreaks of Severe Respiratory Disease Representing a Global Health Threat. *Clinical Microbiology Newsletter*. 42(12):95–103. Available at: https://doi.org/10.1016/j.clinmicnews.2020.05.004.

Black, M.A., Shen, G., Feng, X., Garcia Beltran, W.F., Feng, Y., Vasudevaraja, V. *et al.* 2021. Analytical performance of lateral flow immunoassay for SARS-CoV- 2 exposure screening on venous and capillary blood samples. *Journal of immunological methods*. 489:112909. Available at: https://doi.org/10.1016/j.jim.2020.112909.

Bustin, S.A. and Nolan, T. 2020. RT-qPCR Testing of SARS-CoV-2: A Primer. *International Journal of Molecular Sciences*. 21(8):3004. Available at: https://doi.org/

10.3390/ijms21083004.

Caruana, G., Croxatto, A., Coste, A.T., Opota, O., Lamoth, F., Jaton, K. *et al.* 2020. Diagnostic strategies for SARS-CoV-2 infection and interpretation of microbiological results. *Clinical Microbiology and Infection*. 26(9):1178–1182. Available at: https://doi.org/10.1016/j.cmi.2020.06.019.

Cavalera, S., Colitti, B., Rosati, S., Ferrara, G., Bertolotti, L., Nogarol, C. *et al.* 2020. A multi-target lateral flow immunoassay enabling the specific and sensitive detection of total antibodies to SARS COV-2. *Talanta* [Preprint]. 1:(223):121737 Available at: https://doi.org/10.1016/j.talanta.2020.121737.

Centers for Disease Control and Prevention. 2020. *Interim Guidelines for COVID-19 Antibody Testing*. Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html (Accessed: 13 August 2020).

Centers for Disease Control and Prevention. 2021a. *Science Brief: SARS-CoV-2 Infection-induced and Vaccine-induced Immunity*. Available at: https://www.cdc.gov/coronavirus/2019- ncov/science/science-briefs/vaccine-induced-immunity.html (Accessed: 27 July 2022).

Centers for Disease Control and Prevention. 2021b. *Scientific Brief: SARS-CoV-2 Transmission*. Available at: https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/sars-cov-2-transmission.html (Accessed: 26 July 2022).

Centers for Disease Control and Prevention. 2022a. *Johnson & Johnson's Janssen COVID-19 Vaccine: Overview and Safety*. Available at: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/janssen.html (Accessed: 29 April 2022).

Centers for Disease Control and Prevention. 2022b. *Overview of Testing for SARS-CoV-2, the virus that causes COVID-19*. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html (Accessed: 27 July 2022).

Centers for Disease Control and Prevention. 2022c. *SARS-CoV-2 Variant Classifications and Definitions*. Available at: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fvariants%2Fvariant-info.html (Accessed: 26 July 2022).

Centers for Disease Control and Prevention. 2022d. *Symptoms of COVID-19*. Available at: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html (Accessed: 24 July 2022).

Challine, D., Roudot-Thoraval, F., Sabatier, P., Dubernet, F., Larderie, P., Rigot, P. *et al.* 2006. Serological viral testing of cadaveric cornea donors. *Transplantation*. 82(6):788–793. Available at: https://doi.org/10.1097/01.tp.0000236572.27197.08.

Charpentier, C., Ichou, H., Damond, F., Bouvet, E., Chaix, M.L., Ferré, V. *et al.* 2020. Performance evaluation of two SARS-CoV-2 IgG/IgM rapid tests (Covid-Presto and NG-Test) and one IgG automated immunoassay (Abbott). *Journal of Clinical Virology*. 132:10–13. Available at: https://doi.org/10.1016/j.jcv.2020.104618.

Chen, S.Y, Lee, Y.L., Lin, Y.C., Lee, N.Y., Liao, C.H., Hung, Y.P. *et al.* 2020. Multicenter evaluation of two chemiluminescence and three lateral flow immunoassays for the diagnosis of COVID-19 and assessment of antibody dynamic responses to SARS-CoV-2 in Taiwan. *Emerging Microbes and Infections*. 9(1):2157–2168. Available at: https://doi.org/10.1080/22221751.2020.1825016.

Cheng, P., Luo, K., Xiao, S., Yang, H., Hang, J. and Ou, C. 2021. Predominant airborne transmission and insignificant fomite transmission of SARS-CoV-2 in a two-bus COVID-19 outbreak originating from the same pre-symptomatic index case. *Journal of Hazardous Materials*. 425:128051. Available at: https://doi.org/10.1016/j.jhazmat.2021.128051.

Chia, P.Y., Coleman, K.K., Tan, Y.K., Ong, S.W.X., Gum, M., Lau, S.K., *et al.* 2020. Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. *Nature Communications*. 11(1):2800. Available at: https://doi.org/10.1038/s41467-020-16670-2.

Cohen, C., Kleynhans, J., von Gottberg, A., McMorrow, M.L., Wolter, N., Bhiman, JN., *et al.* 2022. SARS-CoV-2 incidence, transmission, and reinfection in a rural and an urban setting: results of the PHIRST-C cohort study, South Africa, 2020-21. *The Lancet. Infectious diseases*. 22(6)821–834. Available at: https://doi.org/10.1016/S1473-3099(22)00069-X.

Costela-Ruiz, V.J., Illescas-Montes, R., Puerta-Puerta, J.M., Ruiz, C. and Melguizo-Rodríguez, L. 2020 SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. *Cytokine and Growth Factor Reviews*. 54:62-75. Available at:

https://doi.org/10.1016/j.cytogfr.2020.06.001.

Crawford, K., Dingens, A., Equia, R., Wolf, C., Wilcox, N., Logue, J. *et al.* 2020. Dynamics of neutralizing antibody titers in the months after SARS-CoV-2 infection. *Journal of Infectious Diseases.* 223(2):197-205. Available at: https://doi.org/10.1093/infdis/jiaa618.

Demey, B., Daher, N., François, C., Lanoix, J.P., Duverlie, G., Castelain, S. *et al.* 2020. Dynamic profile for the detection of anti-SARS-CoV-2 antibodies using four immunochromatographic assays. *Journal of Infection*. 81(2):e6–e10. Available at: https://doi.org/10.1016/j.jinf.2020.04.033.

Department of Health: Republic of South Africa. 2021. *Guide to Antigen Testing For SARS-CoV-2 in South Africa*. Available at: https://www.health.gov.za/wp-content/uploads/2021/08/GUIDE-TO-ANTIGEN-TESTING-FOR-SARS-COV-2-IN-SOUTH-AFRICA_V4_06.07.2021.pdf (Accessed: 12 July 2022).

Department of Health: Republic of South Africa. 2022. *Update on Covid-19 (5 July 2022).* Available at: https://www.health.gov.za/covid19/ (Accessed: 12 July 2022).

Dheda, K., Davids, M., Chang, J.-W., Gina, P., Pooran, A., Makambwa, E. *et al.* 2020. Diagnosis of COVID-19: Considerations, Controversies and Challenges in South Africa. *Wits Journal of Clinical Medicine*. 2:3. Available at: https://doi.org/10.18772/26180197.2020.v2nsia1.

van Doremalen, N., Morris, D., Holbrook, M., Gamble, A., Williamson, B., Tamin, A. *et al.* 2020. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *Wits Journal of Clinical Medicine.* 2:(SI):0–3. Available at: http://doi.org/10.1056/NEJMc20049731

Drain, P.K. 2022. Rapid Diagnostic Testing for SARS-CoV-2. *New England Journal of Medicine*. 386(3):264–272. Available at: https://doi.org/10.1056/nejmcp2117115.

Edler, C., Wulff, B., Schröder, A.S., Wilkemeyer, I., Polywka, S., Meyer, T. *et al.* 2011. A prospective time-course study on serological testing for human immunodeficiency virus, hepatitis B virus and hepatitis C virus with blood samples taken up to 48 h after death. *Journal of Medical Microbiology*. 60(7):920–926. Available at: https://doi.org/10.1099/jmm.0.027763-0.

van Elslande, J., Houben, E., Depypere, M., Brackenier, A., Desmet, S., André, E. *et al.* 2020.

Diagnostic performance of seven rapid IgG/IgM antibody tests and the Euroimmun IgA/IgG ELISA in COVID-19 patients. *Clinical Microbiology and Infection*. 26(8):1082–1087. Available at: https://doi.org/10.1016/j.cmi.2020.05.023.

European Centre for Disease Prevention and Control. 2022. *SARS-CoV-2 variants of concern as of 15 July 2022*. Available at: https://www.ecdc.europa.eu/en/covid-19/variants-concern (Accessed: 26 July 2022).

Ferdinands, J.M. Rao, S., Dixon, B.E., Mitchell, P.K., De Silva, M.B., Irving, S.A. *et al*. 2022. Waning 2-Dose and 3-Dose Effectiveness of mRNA Vaccines Against COVID-19–Associated Emergency Department and Urgent Care Encounters and Hospitalizations Among Adults During Periods of Delta and Omicron Variant Predominance. *MMWR Recommendations and Reports*. 71(7):255–263. Available at: https://doi.org/10.15585/mmwr.mm7107e2.

Fernandes, Q., Inchakalody, V.P., Merhi, M., Mestiri, S., Taib, N., Moustafa Abo El-Ella, D. *et al*. 2022. Emerging COVID-19 variants and their impact on SARS-CoV-2 diagnosis, therapeutics and vaccines. *Annals of Medicine*. 54(1):524–540. Available at: https://doi.org/10.1080/07853890.2022.2031274.

Fernández-Rodríguez, A., Casas, I., Culebras, E., Morilla, E., Cohen, M.C. and Alberola, J. 2020. COVID-19 and post-mortem microbiological studies. *Spanish Journal of Legal Medicine*. 46(3):127–138. Available at: https://doi.org/10.1016/j.remle.2020.05.007.

FIND. 2020. *Sars-CoV-2 Diagnostic Pipeline*. Available at: https://www.finddx.org/covid-19/pipeline/?avance=Commercialized&type=Rapid+diagnostic+tests&test_target=Antibody&status=all&section=immunoassays&action=default#diag_tab (Accessed: 30 November 2020).

French, M.A. and Moodley, Y. 2020. The role of SARS-CoV-2 antibodies in COVID-19: Healing in most, harm at times. *Respirology*. 25(7):680–682. Available at: https://doi.org/10.1111/resp.13852.

Fusco, F.M., Pisaturo, M., Iodice, V., Bellopede, R., Tambaro, O., Flumeri, G.P. *et al*. 2020. COVID-19 among healthcare workers in a specialist infectious diseases setting in Naples, Southern Italy: results of a cross-sectional surveillance study. *Journal of Hospital Infection*. 105:596–600. Available at: https://doi.org/10.1016/j.jhin.2020.06.021.

De Giorgi, V., West, K.A., Henning, A.N., Chen, L.N., Holbrook, M.R., Gross, R. *et al.* 2021. Naturally Acquired SARS-CoV-2 Immunity Persists for up to 11 Months following Infection. *Journal of Infectious Diseases*. 224(8):1294–1304. Available at: https://doi.org/10.1093/infdis/jiab295.

Guedez-López, G.V. Alguacil-Guillén, M., González-Donapetry, P., Bloise, I., Tornero-Marin, C., González-García, J. *et al.* 2020. Evaluation of three immunochromatographic tests for rapid detection of antibodies against SARS-CoV-2. *European Journal of Clinical Microbiology and Infectious Diseases*. 39(12):2289-2297. Available at: https://doi.org/10.1007/s10096-020- 04010-7.

Guo, Z.D. Wang, Zhong Y., Zhang, S.F., Li, X., Li, L., *et al.* 2020. Aerosol and Surface Distribution of Severe Acute Respiratory Syndrome Coronavirus 2 in Hospital Wards, Wuhan, China, 2020. *Emerging Infectious Diseases*. 26(7):1586–1591. Available at: https://doi.org/10.3201/eid2607.200885.

Hall, V. Foulkes, S., Insalata, F., Kirwan, P., Saei, A., Atti, A. *et al.* 2022. Protection against SARS- CoV-2 after Covid-19 Vaccination and Previous Infection. *New England Journal of Medicine*. 386(13):1207–1220. Available at: https://doi.org/10.1056/nejmoa2118691.

Heathfield, L.J., Maistry, S., Martin, L.J., Ramesar, R. and De Vries, J. 2017. Ethical considerations in forensic genetics research on tissue samples collected post-mortem in Cape Town, South Africa. *BMC Medical Ethics*. 18(1):1–8. Available at: https://doi.org/10.1186/s12910-017-0225-6.

Heim, A., Wagner, D., Rothämel, T., Hartmann, U., Flik, J. and Verhagen, W. 1999. Evaluation of serological screening of cadaveric sera for donor selection for cornea transplantation. *Journal of Medical Virology*. 58(3):291–295. Available at: https://doi.org/10.1002/(SICI)1096-9071(199907)58:3<291::AID-JMV16>3.0.CO;2-D.

Hoste, A.C.R., Venteo, A., Fresco-Taboada, A., Tapia, I., Monedero, A., López, L., *et al.* 2020. Two serological approaches for detection of antibodies to SARS-CoV-2 in different scenarios: a screening tool and a point-of-care test. *Diagnostic Microbiology and Infectious Disease*. 98(4):115167. Available at: https://doi.org/10.1016/j.diagmicrobio.2020.115167.

International Medical Aid. 2022. *Johnson & Johnson COVID-19 Vaccine: What You Need To Know*. Available at: https://medicalaid.org/johnson-johnson-covid-19-vaccine-what-

you- need-to-know/?gclid=Cj0KCQjwma6TBhDIARIsAOKuANwSlZ60vhmkSVqqCLe5avAWO_OVpP yMDVPgPzF_1UkyTtAg0S6yg9caAn6bEALw_wcB (Accessed: 29 April 2022).

Jääskeläinen, A.J., Kuivanen, S., Kekäläinen, E., Ahava, M.J., Loginov, R., Kallio-Kokko, H., *et al.* 2020. Performance of six SARS- CoV-2 immunoassays in comparison with microneutralisation. *Journal of Clinical Virology*. 129:104512. Available at: https://doi.org/10.1016/j.jcv.2020.104512.

Johns Hopkins University. 2022. *COVID-19 cases: South Africa*. Available at: https://news.google.com/covid19/map?hl=en-ZA&mid=%2Fm%2F0hzlz&gl=ZA&ceid=ZA%3Aen (Accessed: 26 July 2022).

Johnston, S., McKay, P., Kebadze, T., Hu, K., Samnuan, K., Aniscenko, J., *et al.* 2013. Evaluation of the Abbott Architect, Roche Elecsys and Virtus S1 SARS-CoV-2 antibody tests in community-managed COVID-19 cases. *medRxiv*.1–31. Available at: https://doi.org/10.1101/2020.10.27.20220509.

Jones, H.E., Mulchandani, R., Taylor-Phillips, S., Ades, A.E., Shute, J., Perry, K.R. *et al.* 2021. Accuracy of four lateral flow immunoassays for anti SARS-CoV-2 antibodies: a head-to-head comparative study. *EBioMedicine*. 68:103414. Available at: https://doi.org/10.1016/j.ebiom.2021.103414.

Jugwanth, S., Gededzha, M.P., Mampeule, N., Zwane, N., David, A., Burgers, W.A. *et al.* 2022. Performance of the Abbott SARS-CoV-2 IgG serological assay in South African 2 patients. *PLoS ONE*. 17:1–11. Available at: https://doi.org/10.1371/journal.pone.0262442.

Kalus, U., Wilkemeyer, I., Caspari, G., Schroeter, J. and Pruss, A. 2011. Validation of the serological testing for anti-HIV-1/2, anti-HCV, HBsAg, and anti-HBc from post-mortem blood on the Siemens-BEP-III automatic system. *Transfusion Medicine and Hemotherapy*. 38(6):365–372. Available at: https://doi.org/10.1159/000334481.

Koczula, K.M. and Gallotta, A. 2016. Lateral flow assays. *Essays in Biochemistry*. 60(1):111–120. Available at: https://doi.org/10.1042/EBC20150012.

Kohmer, N., Westhaus, S., Rühl, C., Ciesek, S. and Rabenau, H.F. 2020. Clinical performance of different SARS-CoV-2 IgG antibody tests. *Journal of Medical Virology*. 92(10):2243–2247. Available at: https://doi.org/10.1002/jmv.26145.

Kumar, M., Taki, K., Gahlot, R., Sharma, A. and Dhangar, K. 2020. A chronicle of SARS-CoV-2: Part-I - Epidemiology, diagnosis, prognosis, transmission and treatment. *Science of the Total Environment*. 734(336):139278. Available at: https://doi.org/10.1016/j.scitotenv.2020.139278.

de la Iglesia, J., Fernández-Villa, T., Fegeneda-Grandes, J.M., Gómez-García, M., Majo García, R., López Sanz, S. *et al.* 2020. Concordance between two rapid diagnostic tests for the detection of antibodies against SARS-CoV-2. *Semergen*. 46(S1):28–32. Available at: https://doi.org/10.1016/j.semerg.2020.06.009.

Lau, C.S., Hoo, S.P., Liang, Y.L., Phua, S.K. and Aw, T.C. 2021. Performance of two rapid point of care SARS-COV-2 antibody assays against laboratory-based automated chemiluminescent immunoassays for SARS-COV-2 IG-G, IG-M and total antibodies. *Practical Laboratory Medicine*. 24:e00201. Available at: https://doi.org/10.1016/j.plabm.2021.e00201.

Li, D., Wang, D., Dong, J., Wang, N., Huang, H., Xu, H. *et al.* 2020. False-negative results of real-time reverse-transcriptase polymerase chain reaction for severe acute respiratory syndrome coronavirus 2: Role of deep-learning-based ct diagnosis and insights from two cases. *Korean Journal of Radiology*. 21(4):505–508. Available at: https://doi.org/10.3348/kjr.2020.0146.

Li, Z. Yi, Y., Luo, X., Xiong, N., Liu, Y., Li, S. *et al.* 2020. Development and clinical application of a rapid IgM- IgG combined antibody test for SARS-CoV-2 infection diagnosis. *Journal of Medical Virology*. 92(9):1518–1524. Available at: https://doi.org/10.1002/jmv.25727.

Lippi, G., Simundic, A.M. and Plebani, M. 2020. Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). *Clinical Chemistry and Laboratory Medicine*. 58(7):1070–1076. Available at: https://doi.org/10.1515/cclm-2020-0285.

Liu, L., Yuen, K. and Chen, Z. 2019. Anti–spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. *JCI insight*. 4(4):e123158. Available at: https://doi.org/10.5603/fm.a2020.0011.

Liu, R., Liu, X., Yuan, L., Han, H., Shereen, M.A., Zhen, J. *et al* .2020. Analysis of adjunctive serological detection to nucleic acid test for severe acute respiratory syndrome

coronavirus 2 (SARS-CoV-2) infection diagnosis. *International Immunopharmacology*. 86:106746. Available at: https://doi.org/10.1016/j.intimp.2020.106746.

Long, Q.X., Tang, X., Shi, Q., Li, Q., Deng, H., Yuan, J. *et al.* 2020. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nature Medicine*. 26(8):1200–1204. Available at: https://doi.org/10.1038/s41591-020-0965-6.

Lu, L., Zhong, W., Bian, Z., Li, Z., Zhang, K., Liang, B. *et al.* 2020. A Comparison of Mortality-related Risk Factors of COVID-19, SARS, and MERS: A Systematic Review and Meta-analysis. *The Journal of infection.* 81(4):e18-e25. Available at: https://doi.org/10.1016/j.jinf.2020.07.002.

Mabuka, T., Naidoo, N., Ncube, N., Yiga, T., Ross, M., Kurehwa, K. *et al.* 2021. The Impact of SARS-CoV-2 Lineages (Variants) on the COVID-19 Epidemic in South Africa. *medRxiv*. Available at: https://doi.org/10.1101/2021.10.22.21265316.

Malik, Y.A. 2020. Properties of coronavirus and SARS-CoV-2. *Malaysian Journal of Pathology*. 42(1):3–11. Available at: PMID: 32342926.

Mayne, E.S., Scott, H.L., Semete, B., Julsing, A., Jugwanth, S., Mampeule, N., David, A., Gededzha, M.P., Goga, A., Hardie, D., Preiser, W., Chetty, K., Rees, H., Sanne, I., Mlisana, K., George, J.A. and Stevens, W. 2020. The role of serological testing in the SARS-CoV-2 outbreak. *South African Medical Journal.* 110(9):842–845. Available at: https://doi.org/10.7196/SAMJ.2020.v110i9.15098.

McAulay, K., Bryan, A., Greninger, A.L., Grill, F., Lake, D., Kaleta, E.J. *et al.* 2020. Retrospective clinical evaluation of 4 lateral flow assays for the detection of SARS-CoV-2 IgG. *Diagnostic Microbiology and Infectious Disease*. 98(3):115161. Available at: https://doi.org/10.1016/j.diagmicrobio.2020.115161.

Montesinos, I., Gruson, D., Kabamba, B., Dahma, H., Van den Wijngaert, S., Reza, S. *et al.* 2020. Evaluation of two automated and three rapid lateral flow immunoassays for the detection of anti-SARS-CoV-2 antibodies. *Journal of Clinical Virology*. 128:104413. Available at: https://doi.org/10.1016/j.jcv.2020.104413.

MU Health Care. 2021. *What You Need to Know About the Johnson & Johnson COVID-19 Vaccine*. *University of Missouri.* Available at: https://www.muhealth.org/our-stories/what-you-need-know-about-johnson-johnson-covid-19-vaccine#:~:text=The

scientists behind the Johnson,t replicate or cause illness. (Accessed: 10 July 2022).

Muench, P., Jochum, S., Wenderoth, V., Ofenloch-Haehnle, B., Hombach, M., Strobl, M. *et al*. 2020. Development and validation of the Elecsys anti-SARS-CoV-2 immunoassay as a highly specific tool for determining past exposure to SARS-CoV-2. *Journal of Clinical Microbiology*. 58(10). Available at: https://doi.org/10.1128/JCM.01694-20.

Nakano, Y., Kurano, M., Morita, Y., Shimura, T., Yokoyama, R., Qian, C. *et al.* 2021. Time course of the sensitivity and specificity of anti-SARS-CoV-2 IgM and IgG antibodies for symptomatic COVID-19 in Japan. *Scientific Reports*. 11(1):1–10. Available at: https://doi.org/10.1038/s41598-021-82428-5.

Nicol, T., Lefeuvre, C., Serri, O., Pivert, A., Joubaud, F., Dubée, V. *et al.* 2020. Assessment of SARS-CoV-2 serological tests for the diagnosis of COVID-19 through the evaluation of three immunoassays: Two automated immunoassays (Euroimmun and Abbott) and one rapid lateral flow immunoassay (NG Biotech). *Journal of Clinical Virology*. 129:104511. Available at: https://doi.org/10.1016/j.jcv.2020.104511.

Nilsson, A., Holm, D., Justese, U., Gorm-Jensen, T., Andersen, N., Ovrehus, A. *et al.* 2021. Comparison of six commercially available SARS-CoV-2 antibody assays—Choice of assay depends on intended use. *International Journal of Infectious Diseases*. 103:381–388. Available at: https://doi.org/10.1016/j.ijid.2020.12.017.

Nuccetelli, M., Pieri, M., Grelli, S., Ciotti, M., Miano, R., Andreoni, M. *et al.* 2020. SARS-CoV-2 infection serology: a useful tool to overcome lockdown? *Cell Death Discovery*. 6(1):38. Available at: https://doi.org/10.1038/s41420-020-0275-2.

O'Toole, Á. Scher, E., Underwood, A., Jackson, B., Hill, V., McCrone, J. *et al.* 2021. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. *Virus Evolution*. 7(2). Available at: https://doi.org/10.1093/ve/veab064.

Ong, D.S.Y., Man, S.J. De Lindeboom, F.A. and Koeleman, J.G.M. 2020. Comparison of diagnostic accuracies of rapid serological tests and ELISA to molecular diagnostics in patients with suspected coronavirus disease 2019 presenting to the hospital. *Clinical Microbiology and Infection*. 26:1094.e7-1094.e10. Available at: https://doi.org/10.1016/j.cmi.2020.05.028.

Ortiz-Prado, E., Simbaña-Rivera, K., Gómez-Barreno, L., Rubio-Neira, M., Guaman, L.P., Kyriakidis, N.C. *et al.* 2020. Clinical, molecular and epidemiological characterization of

the SARS-CoV2 virus and the Coronavirus disease 2019 (COVID-19), a comprehensive literature review. *Diagnostic Microbiology and Infectious Disease*. 98(1):115094. Available at: https://doi.org/10.1016/j.diagmicrobio.2020.115094.

Pallett, S.J.C., Rayment, M., Patel, A., Fitzgerald-Smith, S.A.M., Denny, S.J., Charani, E. *et al*. 2020. Point-of-care serological assays for delayed SARS-CoV-2 case identification among health-care workers in the UK: a prospective multicenter cohort study. *The Lancet Respiratory Medicine*. 8(9)885–894. Available at: https://doi.org/10.1016/S2213-2600(20)30315-5.

Pan, Y., Long, L., Zhang, D., Yuan, T., Cui, S., Yang, P. *et al*. 2020. Potential False-Negative Nucleic Acid Testing Results for Severe Acute Respiratory Syndrome Coronavirus 2 from Thermal Inactivation of Samples with Low Viral Loads. *Clinical chemistry*. 66(6):794–801. Available at: https://doi.org/10.1093/clinchem/hvaa091.

Péré, H., Boubassa, R.S., Tonen-Wolyec, S., Podglajen, I., Veyer, D. and Belec, L. 2020. Analytical performances of five SARS-CoV-2 whole-blood finger-stick IgG-IgM combined antibody rapid tests. *Journal of Virological Methods*. 290:114067. Available at: https://doi: 10.1016/j.jviromet.2021.114067.

Pérez-García, F., Pérez-Tanoira, R., Romanyk, J., Arroyo, T., Gómez-Herruz, P. and Cuadros-González, J. 2020. Alltest rapid lateral flow immunoassays is reliable in diagnosing SARS-CoV-2 infection from 14 days after symptom onset: A prospective single-center study. *Journal of Clinical Virology*. 129:104473. Available at: https://doi.org/10.1016/j.jcv.2020.104473.

Pickering, S., Betancor, G., Pedro Galao, R., Merrick, B., Signell, A.W., Wilson, H.D. *et al.* 2020. Comparative assessment of multiple COVID-19 serological technologies supports continued evaluation of point-of-care lateral flow assays in hospital and community healthcare settings. *PLOS Pathogens.* 16(9): e1008817. Available at: https://doi.org/10.1371/journal.ppat.1008817.

Ripperger, T.J. Uhrlaub, J.L., Watanabe, M., Wong, R., Castaneda, Y., Pizzato, H.A. *et al.* 2020. Orthogonal SARS-CoV-2 Serological Assays Enable Surveillance of Low-Prevalence Communities and Reveal Durable Humoral Immunity. *Immunity*. 53(5):925-933.e4. Available at: https://doi.org/10.1016/j.immuni.2020.10.004.

Ritchie, H., Mathieu, E., Rodés-Guirao, L., Appel, C., Giattino, C., Ortiz-Ospina, E. *et al*. 2022. Coronavirus Pandemic (COVID-19). *Our World in Data*. Available at: https://ourworldindata.org/coronavirus. (Accessed: 28 May 2022).

Robosa, R.S., Sandaradura, I., Dwyer, D.E. and O'Sullivan, M.N. 2020. Clinical evaluation of SARS-CoV-2 point-of-care tests. *Pathology*. 52(7):783–789. Available at: https://doi.org/10.1016/j.pathol.2020.09.002.

Roche Diagnostics. 2020. *Elecsys® Anti-SARS-CoV-2 Immunoassay for the qualitative detection of antibodies against SARS-CoV-2*. Available at: https://diagnostics.roche.com/us/en/products/params/elecsys-anti-sars-cov-2.html. (Accessed: 11 August 2021).

Roche Diagnostics. 2021. *Elecsys Anti-SARS-CoV-2 S*. *FDA.gov*. Available at: https://www.fda.gov/media/144037/download (Accessed: 11 August 2021).

Roche Diagnostics. 2022. *Elecsys® Anti-SARS-CoV-2 S*. Available at: https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2-s.html#productSpecs (Accessed: 28 July 2022).

Rossini, G., Gaibani, P., Vocale, C., Cagarelli, R. and Landini, M.P. 2017. Comparison of Zika virus (ZIKV) RNA detection in plasma, whole blood and urine – Case series of travel- associated ZIKV infection imported to Italy, 2016. *Journal of Infection*. 75(3):242–245. Available at: https://doi.org/10.1016/j.jinf.2017.05.021.

South African Health Products Regulation Association. 2022a. *List of Authorised Serological Test Kits May 2022*. Available at: https://www.sahpra.org.za/medical-devices/medical-devices-and-in-vitro-diagnostics-test-kits/ (Accessed: 12 July 2022).

South African Health Products Regulation Association. 2022b. *SAHPRA registered health products*. Available at: https://www.sahpra.org.za/registered-health-products/ (Accessed: 24 July 2022).

Sia, S.F., Yan, L.M., Chin, A.W.H., Fung, K., Choy, K.T., Wong, A.Y.L. *et al*. 2020. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature*. 583(7818):834–838. Available at: https://doi.org/10.1038/s41586-020-2342-5.

Sidiq, Z., Hanif, M., Dwivedi, K.K. and Chopra, K.K. 2020. Benefits and limitations of serological assays in COVID-19 infection. *Indian Journal of Tuberculosis*. 67(4S):S163-

S166. Available at: https://doi.org/10.1016/j.ijtb.2020.07.034.

Siev, M., Yu, X., Prados-Rosales, R., Martiniuk, F.T., Casadevall, A. and Achkar, J.M. 2011. Correlation between serum and plasma antibody titers to mycobacterial antigens. *Clinical and Vaccine Immunology*. 18(1):173–175. Available at: https://doi.org/10.1128/CVI.00325-10.

Sotgiu, G., Barassi, A., Miozzo, M., Saderi, L., Piana, A., Orfeo, N. *et al.* 2020. SARS-CoV-2 specific serological pattern in healthcare workers of an Italian COVID-19 forefront hospital. *BMC Pulmonary Medicine*. 20(1):1–6. Available at: https://doi.org/10.1186/s12890-020-01237-0.

South African Government News Agency (2022) SAHPRA registers Chinese Sinopharm COVID-19 vaccine. 7 February. Available at: https://www.sanews.gov.za/south-africa/sahpra- registers-chinese-sinopharm-covid-19-vaccine (Accessed: 31 June 2022).

Sterlin, D., Malaussena, A. and Gorochov, G. 2021. IgA dominates the early neutralizing antibody response to SARS-CoV-2 virus. *Medecine/Sciences*. 37(11):968–970. Available at: https://doi.org/10.1051/medsci/2021154.

Suhandynata, R.T., Bevins, N.J., Tran, J.T., Huang, D., Hoffman, M.A., Lund, K. *et al.* 2021. SARS-CoV-2 Serology Status Detected by Commercialized Platforms Distinguishes Previous Infection and Vaccination Adaptive Immune Responses. *The journal of applied laboratory medicine*. 6(5):1109–1122. Available at: https://doi.org/10.1093/jalm/jfab080.

Sun, B. Feng, Y., Mo, X., Zheng, P., Wang, Q., Li, P. *et al.* 2020. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. *Emerging Microbes and Infections*. 9(1):940–948. Available at: https://doi.org/10.1080/22221751.2020.1762515.

Surescreen Diagnostics. 2020. *COVID-19 Coronavirus Rapid Test Cassette*. Available at: https://www.surescreen.com/products/covid-19-coronavirus-rapid-test-cassette (Accessed: 12 August 2020).

Tan, S.S., Saw, S., Chew, K.L., Wang, C., Pajarillaga, A., Khoo, C. *et al.* 2021. Comparative clinical evaluation of the roche elecsys and abbott severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serology assays for coronavirus disease 2019 (covid-19). *Archives of Pathology and Laboratory Medicine*. 145(1):32–38. Available at: https://doi.org/10.5858/arpa.2020-0499-SA.

The Immunisation Advisory Centre. 2022. *How Cominarty is made and what it contains*. Available at: https://covid.immune.org.nz/how-comirnaty-made-and-what-it-contains (Accessed: 10 July 2022).

Thompson, V. 2013. *Statement of Vonita Thompson*. Available at: https://www.westerncape.gov.za/assets/departments/health/h41_2020_covid-19_management_of_decedents.pdf (Accessed: 14 March 2022).

Tollånes, M.C., Bakken Kran, A.M., Abildsnes, E., Jenum, P.A., Breivik, A.C. and Sandberg, S. 2020. Evaluation of eleven rapid tests for detection of antibodies against SARS-CoV-2. *Clinical Chemistry and Laboratory Medicine*. 58(9):1595–1600. Available at: https://doi.org/10.1515/cclm-2020-0628.

Triest, D., Geebelen, L. and Pauw, R. 2020. Performance of five rapid serological tests in mild-diseased subjects using finger prick blood for exposure assessment to SARS-CoV-2. *Journal of Clinical Virology*. 142:104897. Available at: https://doi.org/10.1016/j.jcv.2021.104897

U.S. Food & Drug Administration. 2021. *Antibody Testing Is Not Currently Recommended to Assess Immunity After COVID-19 Vaccination: FDA Safety Communication*. Available at: https://www.fda.gov/medical-devices/safety-communications/antibody-testing-not-currently- recommended-assess-immunity-after-covid-19-vaccination-fda-safety (Accessed: 14 March 2022).

U.S. Food & Drug Administration. 2022. *Comirnaty and Pfizer-BioNTech COVID-19 Vaccine*. Available at: https://www.fda.gov/emergency-preparedness-and-response/coronavirus- disease-2019-covid-19/comirnaty-and-pfizer-biontech-covid-19-vaccine (Accessed: 29 April 2022).

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H. and Thiel, V. 2020. Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Reviews Microbiology*. 19(3):155–170. Available at: https://doi.org/10.1038/s41579-020-00468-6.

Vasireddy, D., Vanaparthy, R., Mohan, G., Malayala, S.V. and Atluri, P. 2021. Review of COVID-19 Variants and COVID-19 Vaccine Efficacy: What the Clinician Should Know? *Journal of Clinical Medicine Research*. 13(6):317–325. Available at: https://doi.org/10.14740/jocmr4518.

Velay, A., Gallais, F., Benotmane, I., Wendling, M.J., Danion, F., Collange, O. *et al.* 2020.

Evaluation of the performance of SARS-CoV-2 serological tools and their positioning in COVID-19 diagnostic strategies. *Diagnostic Microbiology and Infectious Disease*. 98(4):115181. Available at: https://doi.org/10.1016/j.diagmicrobio.2020.115181.

Victer, T.N., Dos Santos, C.S.R., Báo, S.N. and Sampaio, T.L. 2016. Deceased tissue donor serology and molecular testing for HIV, hepatitis B and hepatitis C viruses: a lack of cadaveric validated tests. *Cell and Tissue Banking*. 17(4):543–553. Available at: https://doi.org/10.1007/s10561-016-9564-7.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J. *et al.* 2020. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA - Journal of the American Medical Association*. 323(11):1061–1069. Available at: https://doi.org/10.1001/jama.2020.1585.

Wang, X., Tan, L., Wang, Xu, Liu, W., Lu, Y., Cheng, L. *et al.* 2020. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. *International Journal of Infectious Diseases*. 94:107–109. Available at: https://doi.org/10.1016/j.ijid.2020.04.023.

Wilkemeyer, I., Pruss, A., Kalus, U. and Schroeter, J. 2012. Comparative infectious serology testing of pre- and postmortem blood samples from cornea donors. *Cell and Tissue Banking*. 13(3):447–452. Available at: https://doi.org/10.1007/s10561-012-9326-0.

World Health Organisation. 2020a. *Modes of Transmission of Virus Causing COVID-19: Implications for IPC precaution recommendations: Scientific Brief 29 March 2020*. Available at: https://apps.who.int/iris/bitstream/handle/10665/331616/WHO-2019-nCoV-Sci_Brief-Transmission_modes-2020.2-eng.pdf?sequence=1&isAllowed=y (Accessed: 26 July 2022).

World Health Organisation. 2020b. *Naming the coronavirus disease (COVID-19) and the virus that causes it*. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it (Accessed: 20 July 2020).

World Health Organization. 2020c. *Surveillance strategies for COVID-19 human infection*. Available at: https://www.who.int/docs/default-source/coronaviruse/risk-comms-updates/update-29-surveillance-strategies-for-covid-19-human-infection.pdf?sfvrsn=3c2cab92_2 (Accessed: 14 August 2020).

World Health Organisation. 2020d. *Transmission of SARS-CoV-2: Implecations for Infection Prevention Precautions: Scientific Brief 09 July 2020*. Available at: https://apps.who.int/iris/bitstream/handle/10665/333114/WHO-2019-nCoV-Sci_Brief-Transmission_modes-2020.3-eng.pdf?sequence=1&isAllowed=y (Accessed: 26 July 2022).

World Health Organisation. 2021a. *COVID-19 Vaccine (Vero Cell), Inactivated (Sinopharm)*. Available at: https://www.who.int/publications/m/item/sinopharm-vero-cell---inactivated - covid-19-vaccine (Accessed: 24 July 2022).

World Health Organisation. 2021b. Interim recommendations for use of the Pfizer – BioNTech COVID-19 vaccine. *World Health Organization*. 19(January):1–7. Available at: https://www.who.int/publications/i/item/WHO-2019-nCoV-vaccines-SAGE_recommendation-BNT162b2-2021.1 (Accessed: 24 July 2022).

World Health Organisation. 2022a. *Coronavirus disease (COVID-19)*. Available at: https://www.who.int/health-topics/coronavirus#tab=tab_3 (Accessed: 24 July 2022).

World Health Organisation. 2022b. *Interim statement on hybrid immunity and increasing population seroprevalence rates*. Available at: https://www.who.int/news/item/01-06-2022-interim-statement-on-hybrid-immunity-and-increasing-population-seroprevalence-rates (Accessed: 27 July 2022).

World Health Organisation. 2022c. *Tracking SARS-CoV-2 variants*. Available at: https://www.who.int/activities/tracking-SARS-CoV-2-variants (Accessed: 26 July 2022).

Wu, J.L., Tseng, W.P., Lin, C.H., Lee, T.F., Chung, M.Y., Huang, C.H., *et al.* 2020. Four point-of-care lateral flow immunoassays for diagnosis of COVID-19 and for assessing dynamics of antibody responses to SARS-CoV-2. *Journal of Infection*. 81(3):435–442. Available at: https://doi.org/10.1016/j.jinf.2020.06.023.

Xie, X., Nielsen, M.C., Muruato, A.E., Fontes-gar, C.R. and Ren, P. 2020. Evaluation of a SARS-CoV-2 lateral flow assay using the plaque reduction neutralization test. *Diagnostic Microbiology and Infectious Disease*. 99(2):115248. Available at: https://doi: 10.1016/j.diagmicrobio.2020.115248.

Yang, M., Chen, S., Huang, B., Zhong, J.M., Su, H., Chen, Y.J. *et al.* 2020. Pathological Findings in the Testes of COVID-19 Patients: Clinical Implications. *European Urology Focus*. 6(5):1124-1129. Available at: https://doi.org/10.1016/j.euf.2020.05.009.

Yang, Y. Yang, M., Shen, C., Wang, F., Yuan, J., Li, J. *et al.* 2020. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. *medRxiv*. Available at: https://doi.org/10.1101/2020.02.11.20021493.

Yongchen, Z., Shen, H., Wang, X., Shi, X., Li, Y., Yan, J. *et al.* 2020. Different longitudinal patterns of nucleic acid and serology testing results based on disease severity of COVID-19 patients. *Emerging Microbes and Infections*. 9(1):833–836. Available at: https://doi.org/10.1080/22221751.2020.1756699.

Zhang, C., Zhou, L., Liu, H., Zhang, S., Tian, Y., Huo, J. *et al.* 2020. Establishing a high sensitivity detection method for SARS-CoV-2 IgM/IgG and developing a clinical application of this method. *Emerging Microbes and Infections*. 9(1):2020–2029. Available at: https://doi.org/10.1080/22221751.2020.1811161.

Zhang, X.Y., Huang, H.J., Zhuang, D.L., Nasser, M.I., Yang, M.H., Zhu, P. *et al.* 2020. Biological, clinical and epidemiological features of COVID-19, SARS and MERS and AutoDock simulation of ACE2. *Infectious Diseases of Poverty*. 9(1):1–11. Available at: https://doi.org/10.1186/s40249-020-00691-6.

Zhao, J., Yuan, Q., Wang, H., Liu, W., Liao, X., Su, Y. *et al.* 2020. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*. 71(16):2027-2034. Available at: https://doi.org/10.1093/cid/ciaa344.

6. Appendices

Appendix 1: Approvals

A. The University of Cape Town Human Research Ethics committee approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

12 November 2020

HREC REF: 637/2020

Dr L Heathfield
Division of Forensic Medicine & Toxicology
FHS
Email: laura.heathfield@uct.ac.za
Student: critay002@myuct.ac.za

Dear Dr Heathfield

PROJECT TITLE: INVESTIGATION INTO THE DETECTION OF SARS-COV-2 ANTIBODIES IN DECEASED PERSONS IN CAPE TOWN-MPHIL CANDIDATE-MS TANYA CARLISLE

Thank you for your response letter addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 November 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Ms Tanya Carlisle will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

B. The University of Stellenbosch Human Research Ethics committee approval

UNIVERSITEIT
STELLENBOSCH
UNIVERSITY

Approval Notice

New Application

04/03/2021

Project ID :21772

HREC Reference No: M21/03/002_RECIP_UCT_637/2020_COVID-19

Project Title: Investigation into the detection of SARS-CoV-2 antibodies in deceased persons in Cape Town

Dear Dr. Janette Verster

The **Response** received on 04/03/2021 was reviewed by members of **Health Research Ethics Committee** via **expedited** review procedures on 04/03/2021 and you have been granted **full approval**.

Please note the following information about your approved research protocol:

Protocol Approval Date: 04 March 2021

Protocol Expiry Date: 03 March 2022

Please note:

- Before starting the project, the HREC of Record, in this instance the UCT HREC, to provide in writing to the Head, Health Research Office at SU:
  1. Their willingness to accept the role HREC of Record (Primary HREC).
  2. That the Primary Investigator situated at the HREC of Record will provide the oversight to the project, inclusive of reporting to SU HREC any events (across sites and/ or individual and/ or local site) and progress reports in co-operation with the local PI.
- Similarly, SU HREC also commits to raise any concerns or provide feedback, if indicated, in consultation with the local Investigator to the PI and the HREC of Record".

Please remember to use your **Project ID 21772** and Ethics Reference Number **M21/03/002_RECIP_UCT_637/2020_COVID-19** on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review

Translation of the informed consent document(s) to the language(s) applicable to your study participants should now be submitted to the HREC.

Please note you can submit your progress report through the online ethics application process, available at: Links Application Form Direct Link and the application should be submitted to the HREC before the year has expired. Please see *Forms and Instructions* on our HREC website (www.sun.ac.za/healthresearchethics) for guidance on how to submit a progress report.

The HREC will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.

Please note that for studies involving the use of questionnaires, the final copy should be uploaded on Infonetica.

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility, permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Please consult the Western Cape Government website for access to the online Health Research Approval Process, see: https://www.westerncape.gov.za/general-publication/health-research-approval-process. Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.

For standard HREC forms and instructions, please visit: Forms and Instructions on our HREC website https://applyethics.sun.ac.za/ProjectView/Index/21772

If you have any questions or need further assistance, please contact the HREC office at 021 938 9677.

Yours sincerely,

Mrs. Melody Shana

Coordinator

HREC1

National Health Research Ethics Council (NHREC) Registration Number:

REC-130408-012 (HREC1)•REC-230208-010 (HREC2)

Federal Wide Assurance Number: 00001372
Office of Human Research Protections (OHRP) Institutional Review Board (IRB) Number:
IRB0005240 (HREC1)•IRB0005239 (HREC2)

The Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical norms and principles for research, established by theWorld Medical Association (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects;the South African Department of Health (2006). Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition); as well as the Department of Health (2015). Ethics in Health Research: Principles, Processes andStructures (2nd edition).

The Health Research Ethics Committee reviews research involving human subjects conducted or supported by the Department of Health and Human Services, or other federal departments or agencies that apply the Federal Policy for the Protection of Human Subjects to such research (United States Code of Federal Regulations Title 45 Part 46); and/or clinical investigations regulated by the Food and Drug Administration (FDA) of the Department of Health and Human Services.

C. Approval from SAHPRA

SAHPRA
South African
Health Products
Regulatory Authority

SAHPRA Head Office
Building A
Loftus Park
2nd Floor
Kirkness Road
Arcadia
0083

Division of Forensic Medicine and Toxicology, Falmouth building
Faculty of Health Science
University of Cape Town
Anzio Road
Observatory

Enquiries: Momeena Omarjee
Tel: N/A
Email: Momeena.omarjee@sahpra.org.za
Ref: MD20201201

Dear Dr Laura Heathfield,

RE: APPLICATION TO CONDUCT A MEDICAL DEVICE CLINICAL TRIAL

Protocol Number:	Version 1
Protocol Title:	Investigation into the detection of SARS-CoV-2 antibodies in deceased persons in Cape Town
Reference Number:	MD20201201
Applicant :	Dr Laura Heathfield
Investigational Product:	SureScreen COVID-19 IgG/IgM Rapid Test Cassette

In view of the promulgation of the Medicines and Related Substances Act, 1965 (Act 101 of 1965) on the 1 June 2017 and the publication of the Medical Device Regulations on the 9 December 2016, approval of this clinical trial, by the South African Health Products Regulatory Authority, as well as the relevant Ethics approval, must be obtained, prior to commencement of the clinical trial.

The application for a medical device clinical trial, dated December 2020 received by the Medical device Unit refers:

SAHPRA
South African
Health Products
Regulatory Authority

SAHPRA Head Office
Building A
Loftus Park
2nd Floor
Kirkness Road
Arcadia
0083

1.RESOLUTION AND APPROVAL

The South African Health Products Regulatory Authority (SAHPRA) resolved that the application for the clinical trial protocol, noted above, be approved.

2.BEFORE COMMENCEMENT OF TRIAL

Please Note: Copies of written Ethics Committee approval/(s) must be submitted to SAHPRA before the commencement of the trial.

3.AUTHORISATION

Authorisation is hereby granted for the importation and use of a sufficient quantity of the unregistered medical device, for the duration of the trial, **solely for the purpose of a clinical trial to be conducted by:**

1. **National Principal Investigator:**
   Dr Laura Heathfield (BSc in Genetics and Physiology, BSc (Med), Msc forensic science, PhD in Human Genetics)

2. **Sub-investigator**
   -**Prof Lorna Martin** (MB. BCh Dip for Med, M Med Path (Forensics .)
   -**Miss Tayna Carlisle** (BSc Biochemistry and Microbiology, BSc Hons Biochemistry, MSc Bioinformatics)
   -**Dr Laura Taylor** (MB ChB, Dip for Med (SA) Path, MMed (Forensics), FC for Path. Forensic Pathologist at FPS)
   - **Dr Itumeleng Molefe** MB ChB, Dip for Med Clin/Path , FC for Path , PG Dip HPE , MMEd)
   - **Dr Janette Verster** MBChB ; Dip for Med (Path)(SA); FC for Path (SA); MMed (Forensic Pathology)

   Address: Division of Forensic Medicine and Toxicology, Falmouth building

   Faculty of Health Science, University of Cape Town, Anzio Road, Observatory

   Authorized Quantity to be imported:
   120 test cassettes

4.THE AUTHORISATION OF THIS CLINICAL TRIAL IS SUBJECT TO THE FOLLOWING PROVISOS:

4.1 The Clinical Trial shall be conducted in accordance with the Protocol submitted to and approved by the Authority;

4.2 The Authority must be notified of any proposed amendment(s) to the Protocol and any amendment(s) to the Protocol must be approved by the Authority prior to implementation;

SAHPRA
South African
Health Products
Regulatory Authority

SAHPRA Head Office
Building A
Loftus Park
2nd Floor
Kirkness Road
Arcadia
0083

4.3 All Clinical trial must be conducted in accordance with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines, and the South African Clinical Trial (SACT) Guidelines;

4.4 The Authority shall be informed immediately of any toxic effects or death, which may occur during the Clinical Trial and of any data received which, might cast doubt on the validity of the continuation of the Clinical Trial

4.5 The Authority shall be notified of any decision to discontinue the Clinical Trial and the reason for such cancellation shall be stated;

4.6 The medical device(s), authorized for use in trial, may only be used for the purpose of this trial;

4.7 The medical device(s) shall be only be used by or under the direction of the authorized Trialist. In the events that the Trialist permits another Medical Practitioners to use the medical device(s), the Trialist shall remain responsible for any eventuality arising from such usage;

4.8 Approval by the Authority must be sought prior to the addition of a new Trialist. In the event that a Trialist, who was not authorised in the initial Clinical Trial Protocol Authorisation, is requested to participate in the Clinical Trial, the Curriculum Vitae (CV) of the additional Trialist must be provided to the

Authority. The CV of the additional Trialist must be prepared using the relevant SAHPRA Curriculumn Vitae Format and must include the full names, address and qualification of the proposed Trialist; and

4.9 In the event that an authorized Trialist ceases to participate in the Clinical Trial, the Authority shall be informed and the reason for such cessation shall be given

5. PROGRESS REPORTS

The Authority must be furnished with signed, six-monthly Progress Reports, from each Trialist, including a report of the final results of the trial.

6. INFORMED CONSENT

In line with the relevant regulatory requirement, all Clinical Trials must adhere to the 'Principles of Informed Consent'. These requirements apply to Trial Volunteers, as well as Participants (Patients) (Reference: Section 4.8 of ICH GCP guidelines and Section 3.5 of SACT Guidelines).

DocuSigned by:
momeena omarjee
CF9564DC56FA4D3...

Ms Momeena Omarjee

Acting Deputy Director : Medical Devices

Date: 24 February 2021

D. Transport of biological samples permit

Western Cape Government
Health

INSPECTORATE OF ANATOMY
IOA@westerncape.gov.za
Tel: +27 21 826 5730
Francie Van Zyl Drive Tygerberg 7505

REFERENCE: **TRA|4|46|0321|1**
ENQUIRIES: MS. N. PARKER

DIVISION OF FORENSIC MEDICINE AND TOXICOLOGY

DIVISION OF PATHOLOGY

FACULTY OF HEALTH SCIENCES

UNIVERSITY OF CAPE TOWN

WESTERN CAPE GOVERNMENT: HEALTH
RYAN CLAYTON
PROVINCIAL HEALTH OFFICER

Attention: MS TANYA CARLISLE

Dear Ms Carlisle

RE: PERMISSION FOR THE TRANSPORT AND USE OF SPECIMENS

Approval is granted for the request to acquire, transport and use of specimens from Salt River and Tygerberg Mortuary, to NHLS and Lancet laboratories, for a pilot study. The samples should be used for the purpose for which the Health Research Ethics Committee (HREC) provided approval. Our office should be notified of any fundamental changes relating to the use and transport of these samples.

Please notify us when the study is completed.

Yours faithfully

MR RYAN CLAYTON
Health Officer
Provincial Inspector of Anatomy
Provincial Inspector of Blood Transfusion Services
Provincial Transplant Co-ordinator
Date: 08th March 2021

PROVINCIAL HEALTH OFFICER
RYAN CLAYTON
08 MAR 2021
WESTERN CAPE GOVERNMENT: HEALTH
P.O. Box 19071, Tygerberg, 7505

Appendix 2: IFUs

A. IFU of the SureScreen COVID-19 IgG/IgM Rapid test cassette

SureScreen Diagnostics

COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/ Serum/ Plasma) Package Insert

A rapid test for the qualitative detection of antibodies (IgG and IgM) to SARS-CoV-2 in whole blood, serum, or plasma.
For professional in vitro diagnostic use only.

【INTENDED USE】
The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to SARS-CoV-2 in human whole blood, serum, or plasma as an aid in the diagnosis of primary and secondary SARS-COV-2 infections.

【SUMMARY】
COVID-19(Corona Virus Disease) is the infectious disease caused by the most recently discovered coronavirus. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019. The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but don't develop any symptoms and don't feel unwell. Most people (about 80%) recover from the disease without needing special treatment. Around 1 out of every 6 people who gets COVID-19 becomes seriously ill and develops difficulty breathing. Older people, and those with underlying medical problems like high blood pressure, heart problems or diabetes, are more likely to develop serious illness. About 2% of people with the disease have died. People with fever, cough and difficulty breathing should seek medical attention. People can catch COVID-19 from others who have the virus. The disease can spread from person to person through small droplets from the nose or mouth which are spread when a person with COVID-19 coughs or exhales. These droplets land on objects and surfaces around the person. Other people then catch COVID-19 by touching these objects or surfaces, then touching their eyes, nose or mouth. People can also catch COVID-19 if they breathe in droplets from a person with COVID-19 who coughs out or exhales droplets. Most estimates of the incubation period for COVID-19 range from 1-14 days.
The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid test that utilizes a combination of SARS-COV-2 antigen coated colored particles for the detection of IgG and IgM antibodies to SARS-COV-2 in human whole blood, serum, or plasma.

【PRINCIPLE】
The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a qualitative membrane-based immunoassay for the detection of SARS-CoV-2 antibodies in whole blood, serum, or plasma. This test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in IgG test line region. During testing, the specimen reacts with SARS-CoV-2 antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in IgG test line region. If the specimen contains IgG antibodies to SARS-CoV-2, a colored line will appear in IgG test line region. In the IgM component, anti-human IgM is coated in IgM test line region. During testing, the specimen reacts with anti-human IgM. IgM antibodies to SARS-CoV-2, if present in the specimen, reacts with the anti-human IgM and the SARS-CoV-2 antigen-coated particles in the test cassette, and this complex is captured by the anti-human IgM, forming a colored line in IgM test line region.
Therefore, if the specimen contains IgG antibodies to SARS-CoV-2, a colored line will appear in IgG test line region. If the specimen contains IgM antibodies to SARS-CoV-2, a colored line will appear in IgM test line region. If the specimen does not contain antibodies to SARS-CoV-2, no colored line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

【REAGENTS】
The test cassette contains to specific antigen conjugated gold colloid particles and anti-human IgM, anti-human IgG coated on the membrane.

【PRECAUTIONS】
- For professional in vitro diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used tests, specimens and potentially contaminated material should be discarded according to the local regulations.
- Humidity and temperature can adversely affect results.

【STORAGE AND STABILITY】
The kit can be stored at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

【SPECIMEN COLLECTION AND PREPARATION】
- The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) can be performed using whole blood, serum, or plasma.
- To collect Fingerstick Whole Blood Specimens:
- Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
- Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
- Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
- Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
- Add the Fingerstick Whole Blood specimen to the test cassette by using a dropper or micropipette measuring 10ul. The dropper provided with the test dispenses approximately 10µl in one drop even if more blood is aspirated in the dropper.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long-term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with federal regulations for transportation of etiologic agents.

【MATERIALS 】

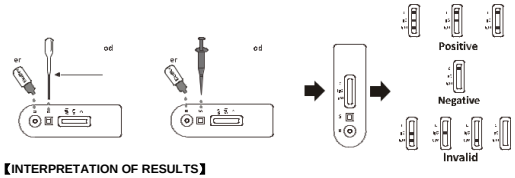
Materials provided

Test cassettes	Droppers
Buffer	Package insert

Materials required but not provided

Specimen collection containers	Centrifuge (for plasma only)
Micropipette	Timer
Lancets (for fingerstick whole blood only)	

【DIRECTIONS FOR USE】
Allow the test cassette, specimen, buffer, and/or controls to reach room temperature (15-30°C) prior to testing.
1. Bring the pouch to room temperature before opening. Remove the test cassette from the sealed pouch and use it within one hour.
2. Place the test cassette on a clean and level surface.
   - For Serum or Plasma or Whole Blood Specimens:
     - To use a dropper: Hold the dropper vertically, draw the specimen up to the Fill Line (approximately 10µl), and transfer the specimen to the specimen well (S) of the test cassette, then add 2 drops of buffer (approximately 80µl) to the buffer well (B) and start the timer. Avoid trapping air bubbles in the specimen well.
     - To use a micropipette: Pipette and dispense 10µl of specimen to the specimen well (S) of the test cassette, then add 2 drops of buffer (approximately 80µl) to the buffer well (B) and start the timer.
3. Wait for the colored line(s) to appear. The test result should be read at 10 minutes. Do not interpret the result after 20 minutes.



【INTERPRETATION OF RESULTS】
(Please refer to the illustration above)
IgG and IgM POSITIVE:* **Three lines appear**. One colored line should be in the control line region (C), and two colored lines should appear in IgG test line region and IgM test line region. The color intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies and is indicative of secondary SARS-COV-2 infection.
IgG POSITIVE:* **Two lines appear**. One colored line should be in the control line region (C), and a colored line appears in IgG test line region. The result is positive for SARS-COV-2 virus specific-IgG and is probably indicative of secondary SARS-COV-2 infection.
IgM POSITIVE:* **Two lines appear**. One colored line should be in the control line region (C), and a colored line appears in IgM test line region. The result is positive for SARS-COV-2 virus specific-IgM antibodies and is indicative of primary SARS-COV-2 infection.
*NOTE: The intensity of the color in the IgG and/or IgM test line region(s) will vary depending on the concentration of SARS-COV-2 antibodies in the specimen. Therefore, any shade of color in the IgG and/or IgM test line region(s) should be considered positive.
NEGATIVE: **One colored line should be in the control line region (C)**. No line appears in IgG and IgM test line region(s).
INVALID: **Control line fails to appear**. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

【QUALITY CONTROL】
An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal valid procedural control, it confirming adequate membrane wicking. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

【LIMITATIONS】
1. The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is for in vitro diagnostic use only. The test should be used for the detection of SARS-COV-2 antibodies in whole blood, serum or plasma specimens only. Neither the quantitative value nor the rate of increase in SARS-COV-2 antibody concentration can be determined by this qualitative test.
2. The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) will only indicate the presence of SARS-COV-2 antibodies in the specimen and should not be used as the sole criteria for the diagnosis of SARS-COV-2.
3. In the early onset of fever, anti-SARS-COV-2 IgM concentrations may be below detectable levels.
4. The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
5. Results from immunosuppressed patients should be interpreted with caution.
6. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
7. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of SARS-COV-2 infection.

【EXPECTED VALUES】
Primary SARS-COV-2 infection is characterized by the presence of detectable IgM antibodies 3-7 days after the onset of infection. Secondary SARS-COV-2 infection is characterized by the elevation of SARS-COV-2-specific IgG. In the majority of the cases, this is accompanied by elevated levels of IgM.

【PERFORMANCE CHARACTERISTICS】

Sensitivity and Specificity
The COVID-19 IgG/IgM Rapid Test Cassette was compared with a leading commercial PCR. The study included 181 specimens for IgG and IgM.

IgG Results

Method		*PCR*		Total Results
COVID-19 IgG/IgM Rapid Test Cassette for IgG	Results	Positive	Negative	
	Positive	37	1	38
	Negative	1	142	143
Total Results		38	143	181

Sensitivity: 97.4% (95%CI: 86.2%~99.9%)* Specificity: 99.3% (95%CI: 96.2%~99.9%)*
Accuracy: 98.9 %(95%CI: 96.1%~99.9%)* *Confidence Interval

IgM Results

Method		*PCR*		Total Results
COVID-19 IgG/IgM Rapid Test Cassette for IgM	Results	Positive	Negative	
	Positive	33	2	35
	Negative	5	141	146
Total Results		38	143	181

Sensitivity: 86.8 %(95%CI: 71.9%-95.6%)* Specificity: 98.6 %(95%CI: 95.0%~99.8%)*
Accuracy: 96.1 %(95%CI: 92.2%~98.4%)* *Confidence interval

Cross-reactivity
The COVID-19 IgG/IgM Rapid Test Cassette (whole blood/Serum/Plasma) has been tested for anti-influenza A virus, anti-influenza B virus, anti-RSV, anti-Adenovirus, HBsAg, anti-Syphilis, anti-H. Pylori, anti-HIV and anti-HCV positive specimens. The results showed no cross-reactivity.

Interfering Substances
The following potentially interfering substances were added to SARS-CoV-2 negative and positive specimens.

Acetaminophen: 20 mg/dL	Caffeine: 20 mg/dL	Albumin: 2 g/dL
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL	Ethanol: 1%
Ascorbic Acid: 2g/dL	Creatine: 200mg/dl	Bilirubin: 1g/dL
Hemoglobin: 1000mg/dl	Oxalic Acid: 60mg/dL	Uric acid: 20mg/ml

None of the substances at the concentration tested interfered in the assay.

【BIBLIOGRAPHY】

1. World Health Organization (WHO). WHO Statement Regarding Cluster of Pneumonia Cases in Wuhan, China. Beijing: WHO; 9 Jan 2020.
2. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. Adv Virus Res 2011;81:85-164.
3. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019; 17:181-192.
4. Su S, Wong G, Shi W, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. TrendsMicrobiol 2016;24:490-502.

Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
	Store between 2-30°C	LOT	Lot Number	REF	Catalog #

Manufacturer
SureScreen Diagnostics Ltd
1 Prime Parkway
Prime Enterprise Park
Derby: DE1 3QB
United Kingdom

CE

Number: RP5327100
Effective date:

Appendix 3: Information and informed consent form

A. Information form

INFORMATION FORM

Study title: Comparison of Sars-CoV-2 Rapid Tests and Formal Serological Testing on Deceased Persons in Cape Town Metro

Principal investigator: Dr Laura Heathfield
Co-investigators: Tayna Carlisle, Prof Lorna Martin, Dr Tumi Molefe, Dr Laura Taylor, Prof Adrian Brink, Dr Stefan Opperman, Prof Johan Dempers and Dr Janette Verster.

Introduction to the study

We are sorry to hear that someone in your family has passed away recently. We would like to ask for your permission to allow your deceased family member to participate in a research study to understand more about COVID-19. This study is led by the University of Cape Town, and it also forms part of Tayna Carlisle's Master's degree.

The purpose of the study is to try and determine if we can find SARS-CoV-2 antibodies in people who have passed away. This will help us to know if the person was sick when they were alive. This form explains what will happen if you do allow your deceased family member to participate. It is completely your choice if you want to allow your family member to participate in this study. Please read it carefully and feel free to ask any questions before you decide to participate or not.

Background

The COVID-19 disease is caused by the virus called SARS-CoV-2. The virus can cause people to get sick, for example, they might have a sore throat, a high temperature or might find it hard to breathe. **When people get sick with a virus, their body tries to fight off the virus by making antibodies. We do not know how many people have been sick with COVID-19 and what the best way to test for this is. We want to test for antibodies in deceased people with different types of tests to answer these questions.**

Procedure

You will be asked to give permission to allow blood from your deceased family member to be tested for SARS-CoV-2 antibodies. The blood will only be taken if you agree. In total, 8 mL of blood will be taken, which is the same amount as less than 2 teaspoons.

If you agree to allow them to participate, then the blood samples will be tested using three different antibody tests: COVID-19 IgG/IgM Rapid Test Cassette (SureScreen), SARS-CoV-2 IgG Assay ARCHITECT i Systems (Abbott Laboratories) and the Elecsys® Anti-SARS-CoV-2 (Roche Diagnostics). If there is any leftover blood after any of the tests, it will not be kept. If you choose to not participate, then no blood samples will be collected at all. You do not need to pay for these tests.

There are several things you need to know before allowing blood samples to be taken from your deceased family member:

1. You will not be paid to allow your deceased family member to participate in this study.
2. If you choose to allow your deceased family member to participate, your name and the name of your family member will not be made known. We need to note how old they were, if they were male or female, the date they had their positive COVID-19 test, and the date they started getting sick from COVID-19. The privacy of the samples and this information will be kept confidential.
3. When the study is finished, the results from all the participants will be combined and published so that other mortuaries can benefit from this information. No names will be included in this.
4. Most importantly, it is completely your choice if you want to allow your deceased family member to participate.
    - If you say no, there will be no consequences to you, or your family member, and it will not affect the medical exam at the mortuary.
    - If you say yes, it will also not affect the medical exam at the mortuary, by agreeing, there will be no delays at the mortuary. You may also change your mind and withdraw at any time up until study is finished.

If you may have any questions want grief centre or psychological support, please ask the researcher. If you have any questions about the rights and welfare of your deceased family member in the study, please contact the Chairperson of the University of Cape Town Faculty

of Health Science Human Research Ethics Committee, **Professor Marc Blockman** on (021) 406 6496. If you require any further information about this study, please contact Dr Laura Heathfield at 021 406 6569 or laura.heathfield@uct.ac.za.

Making your choice

Please carefully read each sentence below and decide on your choice. After reading each sentence, please **tick the Yes or No box**. Whatever you decide, it will not hurt you or your deceased family member in any way. **Please note that the information and consent forms will be translated into the family member's language of choice.**

- Thank you for your time –

Participant number: _____

WC/11/_____/202

B. Consent form

I, _____ (full name),

the spouse/partner/major child/parent/guardian/major brother/major sister (circle relationship) of the deceased

I confirm that I have:	**Yes**	**No**
a) Read and understood contents of this form and agree to be a part of the research study.		
b) Was told about this study's purpose, procedures, possible benefits, and risks.		
I give consent and agree that:	**Yes**	**No**
a) The blood samples that have been taken from my deceased family member can tested for SARS-CoV-2 antibodies using three different tests		
b) The following information about my deceased family member can be used: age, male/female, date and result of COVID-19 test before death, date they started getting sick from COVID-19 and their vaccination status.		
I further understand that:	**Yes**	**No**
a) This study is will not benefit me or my family directly.		
b) I can at any time change my mind about saying yes or no to the study and I must tell the primary investigator of my decision.		
c) The research may be published but neither the deceased nor the family of the deceased will be identified.		

_____

Full name of person obtaining consenting

_____

Signature of person obtaining consenting Date

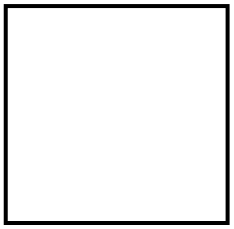
_____

Full name of person authorising consent

_____

Signature of person authorising consent Date

If the person authorising consent cannot write, then a thumb print can be obtained



_____

Full name of witness

_____

Signature of witness Date

Appendix 4: Literature review

A. Table describing the different SARS-CoV-2 variants (Adapted from O'Toole *et al.*, 2021; CDC, 2022b; WHO, 2022c)

WHO label	Pango Lineage	Country where first detected	Date of designation		
			VOC	VOI	VUM
Alpha	B.1.1.7 and Q lineages	United Kingdom	December 2020		September 2021
Beta	B.1.351 and descendant lineages	South Africa	December 2020		September 2021
Gamma	P.1 and descendant lineages	Brazil	December 2020		September 2021
Delta	B.1.617.2 and AY lineages	India	June 2021		April 2022
Epsilon	B.1.427	United States of America	March 2021	February 2021	September 2021
	B.1.429			June 2021	
Eta	B.1.525	United Kingdom and Nigeria		February 2021	September 2021
Iota	B.1.526	United States of America		February 2021	September 2021
Kappa	B.1.617.1	India		May 2021	September 2021
N/A	B.1.617.3	N/A		May 2021	September 2021
Zeta	P.2	Brazil			September 2021
Mu	B.1.621,	Colombia			September 2021
	B.1.621.1				
Omicron	B.1.1.529	South Africa and Botswana	November 2022		November 2022
	BA.1				
	BA.1.1				
	BA.2				
	BA.3				
	BA.4 and BA.5 lineages				

B. List of companies and SARS CoV-2 ELISA products currently approved by SAHPRA for IgM/IgG/IgA detection in COVID-19 patients (Adapted from SAHPRA, 2022a).

SAHPRA Licence Holder	Product Name	Product Description	Original Manufacturer	Date Authorised
Roche Diagnostics (Pty) Ltd	Elecsys Anti-SARS-COV-2 (E1G)	SARS CoV-2 ELISA IgG	Roche Diagnostics GmbH	24/08/2020
Abbott Laboratories South Africa (Pty) Ltd	Architect SARS-CoV-2 IgG Control / Assay / Calibrator	SARS CoV-2 ELISA IgG	Abbott Ireland, Diagnostics Division, Finisklin Business Park, Sligo, Ireland	24/08/2020
Euroimmun Medical Laboratory Diagnostics South Africa (Pty) Ltd	Anti-SARS-CoV-2 ELISA IgA	SARS CoV-2 ELISA IgA	Medizinische Labordiagnostika AG, Deutschland, Germany	24/08/2020
Abbott Laboratories South Africa (Pty) Ltd	Alinity SARS-CoV-2 IgG Control / Assay / Calibrator	SARS CoV-2 ELISA IgG	Abbott Ireland, Diagnostics Division, Finisklin Business Park, Sligo, Ireland	24/08/2020
Euroimmun Medical Laboratory Diagnostics South Africa (Pty) Ltd	Anti-SARS-CoV-2 ELISA IgG	SARS CoV-2 ELISA IgG	Medizinische Labordiagnostika AG, Deutschland, Germany	24/08/2020
Beckman Coulter (Pty) Ltd- Cape Town	Beckman Coulter SARS-CoV-2 immunoglobulin G (IgG) antibody IVD, kit, chemiluminescent immunoassay	SARS CoV-2 ELISA IgG	Beckman Coulter Inc Ireland	13/04/2021
Roche Diagnostics (Pty) Ltd	Elecsys Anti-SARS-COV-2 (E2G)	SARS CoV-2 ELISA IgG	Roche Diagnostics GmbH	13/04/2021
Beckman Coulter (Pty) Ltd- Johannesburg	Beckman Coulter SARS-CoV-2 immunoglobulin G (IgG) antibody IVD, kit, chemiluminescent immunoassay	SARS CoV-2 ELISA IgG	Beckman Coulter Inc Ireland	23/04/2021
Abbott Laboratories South Africa (Pty) Ltd	Architect SARS-CoV-2 IgG II Quant Assay	SARS CoV-2 ELISA IgG	Abbott Ireland Diagnostics Division	11/06/2021
Abbott Laboratories South Africa (Pty) Ltd	Architect SARS-CoV-2 IgM II Quant Assay	SARS CoV-2 ELISA IgM	Abbott Ireland Diagnostics Division	18/06/2021
The Scientific Group (Pty) Ltd	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Control, Reagent, Calibrator (COV2G & COV2T)	SARS CoV-2 ELISA IgG	Ortho Clinical Diagnostics	03/08/2021
Roche Diagnostics (Pty) Ltd	Roche Elecsys ® Anti-SARS-CoV-2 S immunoassay (E1G)	SARS CoV-2 ELISA IgG	Roche Diagnostics GmbH	17/09/2021

Abbott Laboratories South Africa (Pty) Ltd	SARS-CoV-2 IgM ALINITY	SARS CoV-2 ELISA IgM	Abbott Ireland Diagnostics Division	08/10/2021
Abbott Laboratories South Africa (Pty) Ltd	Alinity SARS CoV2 IgG Quant Assay	SARS CoV-2 ELISA IgG	Abbott Ireland Diagnostics Division	26/11/2021
Roche Diagnostics (Pty) Ltd	Roche Elecsys ® Anti-SARS-CoV-2 S immunoassay (E2G)	SARS CoV-2 ELISA IgG	Roche Diagnostics GmbH	01/03/2022

*Pty – proprietary company, SARS – severe acute respiratory syndrome, CoV – coronavirus, IgG – immunoglobulin G, ELISA – enzyme-linked immunosorbent assay, IgA – immunoglobulin A, IVD – in vitro diagnostics.

C. List of companies and antibody coronavirus LFI kits currently approved by SAHPRA for antibody detection in COVID-19 patient (Adapted from (SAHPRA, 2022a).

SAHPRA Licence Holder	Product Name	Product Description	Original Manufacturer
Tiptoptrade (Pty) Ltd	SARS CoV-2 IgG/IgM test	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Link Medical Solutions (Pty) Ltd	SARS CoV-2 IgG/IgM test	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Direct Retail Goods (Pty) Ltd	SARS CoV-2 IgG/IgM test	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Nu-world Industries (Pty) Ltd	SARS CoV-2 IgG/IgM test	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Inqaba Health Solutions (Pty) Ltd	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Sinosa Trading (Pty) Ltd	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Patient Focus Africa (Pty) Ltd	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Smart Medical	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Sobham Resources (Pty) Ltd	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Timhuti Medical Supply (Pty) Ltd	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
SUA Medical Supplies	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH

Vita Aid (Pty) Ltd	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Mmed distributors (Pty) Ltd	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
SMT Diagnostics	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
IDT Diagnostics t/a ICT International	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Novex Pharmaceuticals	Sienna COVID-19 IgG/IgM Rapid Test Cassette	Serological Corona virus IgG/ IgM Rapid Test Kit	T&D Diagnostics Canada Pvt. Ltd.
FAMKA SERVICES INTERNATIONAL	Istoc COVID-19 IgG/IgM Rapid Test Cassette	Serological Corona virus IgG/ IgM Rapid Test Kit	CORE TECHNOLOGY CO LTD
Agera Health (Pty) Ltd	Sure Biotech COVID-19 IgG/IgM rapid lateral flow assay	Serological Corona virus IgG/ IgM Rapid Test Kit	QINGDAO HIGHTOP BIOTECH
Life Assay Diagnostics (Pty) Ltd	Test-it CoV-2 Rapid Test Cassette	Serological Corona virus IgG/ IgM Rapid Test Kit	LIFE ASSAY DIAGNOSTICS (PTY) LTD
Doctor Four You Practice Managers (Pty) Ltd	SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Pro Med Diagnostics (Pty) Ltd	Lambra SARS CoV-2 IgG/IgM test Rapid	Serological Corona virus IgG/ IgM Rapid Test Kit	ZHEJIANG ORIENT GENE BIOTECH
Afrivet Business Management (Pty) Ltd	Nowcheck Covid 19 IgG/ IgM	Serological Corona virus IgG/ IgM Rapid Test Kit	BIONOTE
ECM Technologies (Pty) Ltd	NOVA Test COVID-19 IgG/IgM Rapid Test	Serological Corona virus IgG/ IgM Rapid Test Kit	ATLASLINK (BEIJING) TECHNOLOGY CO. LTD.
Global Strategic Advisory Group (Pty) Ltd	SureScreen COVID-19 IgG/IgM rapid Test	Serological Corona virus IgG/ IgM Rapid Test Kit	SURESCREEN DIAGNOSTICS LTD
Msi Empire Group (Pty) Ltd	Kewei COVID-19 IgG/IgM rapid Test	Serological Corona virus IgG/ IgM Rapid Test Kit	BEIJING KEWEI CLINICAL DIAGNOSTIC REAGENT INC
BICE Properties (Pty) Ltd.	Spring Healthcare COVID-19 IgM/IgG rapid Test	Serological Corona virus IgG/ IgM Rapid Test Kit	SPRING HEALTHCARE SERVICES AG
Mbabala Consulting (Pty) Ltd	Humasis COVID-19 lgG/lgM Antibody Test Kit	Serological Corona virus IgG/ IgM Rapid Test Kit	HUMASIS CO., LTD
Tasosol (Pty) Ltd	Humasis COVID-19 lgG/lgM Antibody Test Kit	Serological Corona virus IgG/ IgM Rapid Test Kit	HUMASIS CO., LTD
THACALI DISTRIBUTORS & MARKETING	ACCU-TELL® COVID-19 IgG/IgM Cassette (Whole Blood/Serum/Plasma)	Serological Corona virus IgG/ IgM Rapid Test Kit	ACCUBIOTECH CO., LTD.

Homemed (Pty) Ltd	Ecotest COVID-19 IgG/IgM Rapid Test	Serological Corona virus IgG/ IgM Rapid Test Kit	ASSURE TECH (HANGZHOU) CO LTD
Jomara International cc	Xiamen Boson Rapid 2019-nCoV IgG/IgM Combo test card	Serological Corona virus IgG/ IgM Rapid Test Kit	XIAMEN BOSON BIOTECH CO., LTD.
MNANDI Pharma Solutions Pty. Ltd.	Nanjing Norman COVID-19 IgM-IgG rapid test kit	Serological Corona virus IgG/ IgM Rapid Test Kit	NANJING NORMAN BIOLOGICAL TECHNOLOGY CO. LTD
Tasosol (Pty) Ltd	Accurate Rapid COVID-19 IgM/IgG Combo test	Serological Corona virus IgG/ IgM Rapid Test Kit	HUMEDIX
TGM Innovations Pty Ltd T/A AFRICAN DIAGNOSTIC EXCELLENCE	Biotime SARS-CoV-2 IgG/IgM Rapid Qualitative Test	Serological Corona virus IgG/ IgM Rapid Test Kit	XIAMEN BIOTIME BIOTECHNOLOGY CO LTD
Mélánge Healthcare Trading	Sienna COVID-19 IgG/IgM Rapid Test Cassette	Serological Corona virus IgG/ IgM Rapid Test Kit	SALOFA OY
Patient Focus Africa (Pty) Ltd	Chembio DPP SARS-CoV-2 IgM/IgG rapid lateral flow assay	Serological Corona virus IgG/ IgM Rapid Test Kit	CHEMBIO DIAGNOSTIC SYSTEMS INC
Medical Diagnostech (Pty) Ltd	MD COVID-19 IgG/IgM Rapid Test	Serological Corona virus IgG/ IgM Rapid Test Kit	MEDICAL DIAGNOSTECH (PTY) LTD
Bio-Rad Laboratories (Pty) Ltd	Platelia SARS-CoV-2 Total Ab assay	Serological Corona virus IgG/ IgM Rapid Test Kit	BIO-RAD FRANCE

*Pty – proprietary company, SARS – severe acute respiratory syndrome, CoV – coronavirus, IgA – immunoglobulin A, IgG – immunoglobulin G, IgM – immunoglobulin M

Appendix 5: Results

A. Table summarising variables and the results of the three types of COVID-19 antibody testing.

	Total cases	Percentage of cases with PCP confirmation	Percentage of cases vaccinated	Median days between PCP and death	Median days between PCP and autopsy	Median days between death and autopsy	Median days between death and testing	Median days between autopsy and testing	Median days between PCP and testing
SS IgM positives	7	85.71%	42.86%	8	14	4	7	4	21
SS IgG positives	21	71.43%	28.57%	97	102	4	6	3	115
SS IgM negatives	23	56.52%	13.04%	81	82	4	8	4	89
SS IgG negatives	9	44.44%	0%	81	82	4	10	7	8
Elecsys positives	27	78.26%	21.74%	81	82	4	11	8	91
Elecsys negatives	3	33.33%	33.33%	7	14	6	20	12	26
Architect positives	10	50%	10%	41	43	3.5	7	4	46
Architect negatives	20	75%	25%	97	102	4	9	6	116

*SS – SureScreen, PCP – positive COVID-19 PCR

B. Comparison of results from Roche's Elecsys Anti-SARS-CoV-2 assay and the SureScreen rapid test cassette IgM

Roche's Elecsys Anti-SARS-CoV-2 results	**SureScreen IgM rapid test cassette results**		
	Positive	Negative	Total
Positive	6	21	27
Negative	1	2	3
Total	7	23	30

C. Comparison of results from Roche's Elecsys Anti-SARS-CoV-2 assay and the SureScreen rapid test cassette IgG

Roche's Elecsys Anti-SARS-CoV-2 results	**SureScreen IgG rapid test cassette results**		
	Positive	Negative	Total
Positive	20	7	27
Negative	1	2	3
Total	21	9	30

D. Comparison of results from Abbott Architect's SARS-CoV-2 IgG Assay and the SureScreen rapid test cassette IgM

Abbott Architect's SARS-CoV-2 IgG Assay results	**SureScreen IgM rapid test cassette results**		
	Positive	Negative	Total
Positive	3	7	10
Negative	4	16	20
Total	7	20	30

E. Comparison of results from Abbott Architect's SARS-CoV-2 IgG Assay and the SureScreen rapid test cassette IgG

Abbott Architect's SARS-CoV-2 IgG Assay results	**SureScreen IgG rapid test cassette results**		
	Positive	Negative	Total
Positive	10	0	10
Negative	11	9	20
Total	21	9	

F. Comparison of results from Abbott Architect's SARS-CoV-2 IgG Assay and Roche's Elecsys Anti-SARS-CoV-2 assay

Roche's Elecsys Anti-SARS-CoV-2 results	**Abbott Architect's SARS-CoV-2 IgG Assay results**		
	Positive	Negative	Total
Positive	10	17	27
Negative	0	3	3
Total	10	20	30