Investigating Point of Care Diagnostic Strategies to Optimize the Rapid Diagnosis of COVID-19 in routine public and private health care settings in South Africa

SA COVID-19 POC STUDY

A study led by the South African Medical Research Council (SAMRC) in partnership with the National Health Laboratory Services (NHLS)

Principal investigator:

Ameena Goga, SAMRC

Co-PI:

Glenda Gray, SAMRC

Co-investigators:

- **South African Medical Research Council (SAMRC):** Elizabeth Spooner, Brodie Daniels, Kubashni Woeber, Carl Lombard (Chief Statistician), Nobubelo Ngandu, Terusha Chetty, Zakir Gaffoor, Samantha Siva, Reshmi Dassaye, Darshini Govindasamy, Trisha Ramraj, Duduzile Nsibande, Fareed Abdullah
- **National Health Laboratory services (NHLS):** Elizabeth Mayne (site PI), Kamy Chetty, Wendy Stevens, Lesley Scott, Zinhle Makatini (Charlotte Maxeke site PI)
- **CAPRISA:** Salim Abdool Karim, Kogie Naidoo (site PI)
- **Hutchinson Centre Research Institute of South Africa (HCRISA):** Simbarashe Takuva (Assistant statistician)
- **University of Cape Town (UCT) and UCT-affiliated hospital complex and community sites in Cape Town (Klipfontein and Mitchells Plain):** Keertan Dheda (site PI), Jonathan Blackburn, Wendy Burgers
- **University of Witwatersrand:** Shabir Madhi (site PI), Portia Mutevedzi (co-Investigator)
- **National Institute for Communicable Diseases (NICD):** Lynn Morris
- **Centre for Respiratory Diseases and Meningitis (CRDM), NICD:** Jinal Bhiman, Anne Von Gottberg
- **University of Pretoria / Steve Biko academic complex :** Veronica Ueckermann (site PI), Tivani Mashamba-Thompson
- **ANOVA Health Institute:** James McIntyre
- **University of Limpopo:** Tivani Mashamba-Thompson, Ruth Lekalakala (site PI)

Collaborators on the serological Luminex assay and neutralization assay:

- **Centre Hospitalier Universitaire Vaudois (CHUV): Giuseppe Pantaleo/Craig Fenwick, Luminex Serology** Assay
- University of Utrecht: Berend Jan Bosch, Neutralization assay
- Inserm: Yves Levy/Rodolphe Thiebaut, mathematical modelling

Version 5 : 31 March 2021

ADDRESS FOR CORRESPONDENCE:
Ameena Goga
HIV Prevention Research Unit
SAMRC, 1 Soutpansberg Road, Pretoria, 0001
Email: Ameena.Goga@mrc.ac.za, +2712 339 8524 / +2782 302 3168

Key words: COVID-19; SARS-CoV-2, rapid diagnosis, COVID-19 diagnostic algorithm

SUMMARY OF CHANGES:

	Version 2: 7 May 2020	Version 3.0	Version 4.0 – 20 October 2020	Version 4.0 Revision 1 13 December 2020	Version 5 – 31 March 2021
Investigators	The following investigators were added: Darshini Govindasamy, has been added under the SAMRC co-investigators. She will work on the cost-effectiveness analysis – Aim 4 Zinhle Makatini, has been added – she will coordinate the Charlotte Maxeke Site Tivani Mashamba-Thompson has been added – she will coordinate the University of Limpopo DIMAMO site Trisha Ramraj has been added as a co-investigator – she is providing intellectual inputs and assistance with the REDCap data collection system	The following investigators were added: Dr Kamy Chetty (NHLS), Jonathan Blackburn and Wendy Burgers (UCT), Ruth Lekalakala (University of Limpopo), Veronica Ueckermann (University of Pretoria) and Duduzile Nsibande). Anton Stoltz removed. Thompson added to Tivani Mashamba-Thompson's surname	unchanged	Ruth Lekalakala replaces Tivani Mashamba-Thompson as Limpopo site PI	Tivani Mashamba_Thompson added to UP site Lancet laboratories removed from protocol
Address for correspondence	None	None	Health Systems Research Unit has been removed	Unchanged	Unchanged
Aims	None	**3 aims added:** Aim 8: To compare RT-PCR / serology on saliva versus swabs. Aim 9: To investigate the ease of use of rapid POC tests for COVID-19 diagnosis. Aim 10: To investigate whether one rapid antigen test amongst persons under investigation (PUI) provides a sensitive and specific diagnostic	**Unchanged**	Unchanged	2 aims added – these aims will be answered as part of the laboratory work that is planned using specimens from the biorepository. The biorepository and ongoing serological tests were always part of the study plan

		approach in routine public and private health care sites compared to a single reverse transcriptase PCR (RT-PCR) test at initial presentation.			
Sites	Two new sites were added: Charlotte Maxeke Hospital and the University of Limpopo DIMAMO node. Umlazi Q clinic or U21 clinic added as an alternative to KwaMashu Polyclinic.	Sites are now prioritized. Data collection will start in 3 sites only (Chris Hani Baragwanath Hospital, UCT Groote Schuur and University of Limpopo). All other sites will be placed on hold.	**Unchanged**	Unchanged	A possible site in Cape Town: Kraaifontein Community Health Centre has been added
Inclusion criteria			We have delinked the asymptomatic contacts from the positive participant enrolled in the study. This means that asymptomatic contacts can be recruited if their primary positive contact is not part of the study. The inclusion criteria for asymptomatic contacts has not changed; however it is now clarified that it is not compulsory for an asymptomatic contact to be linked with a positive participant in the study	Unchanged	Unchanged
Procedures:	Rectal swab changed to saliva. Clarifications included to provide information on which rapids will be followed up- any rapid test positive will be followed up.	Blood sampling volumes increased to facilitate additional analyses to understand COVID-19 – see Table 1. Three rapid antibody tests and two/three rapid antigen tests included in the protocol. Nasal swab has been added for rapid point of care antigen testing in a limited number of participants	To allow for adequate sampling to extract peripheral blood mononuclear cells (PBMCs), we have increased blood volume to take an additional 20 mL blood	Additional funding has become available, thus Lumira antigen test will also be conducted in Western Cape	The additional swab for genome analysis has been expanded to allow additional swab taking on all consenting PUIs at baseline and positive participants at follow-up in all study sites. It has been specified that the follow-up day is the preferred day, but there is a window for follow-up

		Inclusion criteria have been clarified. Routine sample for RT-PCR is now reduced to one sample – as per revised NICD/NHLS guidelines. Swab for genomic analysis has been reduced to follow-up visits amongst any positive participants. It will not be taken at baseline or amongst asymptomatic contacts Case definition revised as per revised NICS/NHLS guidelines.			A 3, 6-month, 9 and 12 month follow-up visit has been added for all consenting PUIs who test rapid or PCR positive at baseline, if funding is available. We have removed reference to Wendy Burger's laboratory as the 20mL PBMCs at follow-up may be analysed for T and B-cell function at another laboratory, and not necessarily at Wendy's laboratory. Wendy's lab is extremely busy. We have added in 5mL SST for the Luminex assay and neutralisation assay
Sample size		Given the positivity rate, total number to be tested has been reduced to yield 361 positive participants 500 negatives will be followed up, not 10%	**unchanged**	Unchanged	Unchanged
ICFs	Consent for rectal swab amended to consent for saliva specimen	A section has been added to clarify that specimens will be stored. An additional biorepository storage form has been added A form for usability of tests has been added Sample size has been revised Blood volume has been updated to 25mL at each visit, except for the Western Cape follow up where 100 positive, negative and asymptomatic participants will be asked to give an additional 20mL blood for T-cell and B-cell function..	We have added in the 20mL for PBMCs		We have clarified that if a participant does not consent to provide blood for EDTA/SST/Heparin / ACD they can still participate in the rapid testing component of the study. Agreeing to giving blood specimens is not part of the inclusion criteria Have also added consent for access to routine PCR and blood results
CRFs	Skip patterns clarified in the case report forms, and electronic consent procedures included in the CRFs.	CRFs now include the names of the tests. ID number and date of birth of participant has been added for ease of retrieval of COVID-19 PCR results. The order of procedures have been changed so that screening occurs after the consent process. An assessment of symptom severity has been added to the	Screening has been moved to before consenting because no blood or procedures are required for screening. We made this change because the consent process takes long, and because	none	Two questions have been added on each of the following: (i) prior COVID-19 infection (ii) vaccination to document previous infection / vaccination and time of infection / vaccination The responses to the antigen tests have been clarified

		CRFs.	screening does not require any procedures, we think it is ethical to screen out participants, and only consent those who meet inclusion criteria A tool to measure time-motion for the cost effectiveness component has been added.		
Other	A flyer has been added, this will tell potential participants (PUIs coming for a COVID-19 test) about the study. A participant card has been added to explain the rapid test results and the follow-up.	Flyer has been amended to avoid any manipulation. Participant card has been amended to include return of results on the approved rapid test – Orient Gene KN95, FFP2 and any other mask similar to N95 has been added as acceptable PPE for aerosol generating procedures.	none		Two sub-studies have been linked to this protocol. Both will investigate the utlity of rapid testing in routine healthcare settings. The protocols are entitled: (i) Longitudinal surveillance of COVID-19 at five South African Medical Research Council Clinical Research Sites in eThekwini, a high HIV prevalence district South Africa. (ii) Prevalence, clinical characteristics, immunologic responses and outcomes of children with suspected or confirmed COVID-19 disease

I, Ameena Goga have read the Department of Health: *Ethics in health research: principles, processes and structures, second edition*, 2015, the *Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa,* Second Edition, 2006, Department of Health, Pretoria, South Africa (where applicable), and the Declaration of Helsinki (2013) and have prepared this proposal with due cognisance of its content. Furthermore, I will adhere to the principles expressed when conducting this proposed research project.

Ameena Goga
31 March 2021

I, Glenda Gray have read the Department of Health: *Ethics in health research: principles, processes and structures, second edition*, 2015, the *Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa,* Second Edition, 2006, Department of Health, Pretoria, South Africa (where applicable), and the Declaration of Helsinki (2013) and have prepared this proposal with due cognisance of its content. Furthermore, I will adhere to the principles expressed when conducting this proposed research project.

Glenda Gray
31 March 2021

Table of Contents

ABBREVIATIONS

Ab	Antibody
ACE2	Angiotensin-converting enzyme 2
COVID-19	Coronavirus Disease 2019
CRF	Case report form
ELISA	Enzyme-linked immunosorbent assays
Ig	Immuno-globulin
MAC	Ministerial Advisory Committee
NAAT	Nucleic acid amplification tests
NHLS	National Health Laboratory Services
NICD	National Institute of Communicable Diseases
POC	Point of care
PPE	Personal protective equipment
PUI	Persons under investigation
R_0	Basic reproductive number
RT-PCR	Reverse transcriptase polymerase chain reaction
SAHPRA	South African Health Product Regulatory Authority
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SSP	Standardized study procedures

EXECUTIVE SUMMARY: SA COVID-19 POC STUDY

AIMS:

Aim 1: To investigate whether two rapid point of care antibody COVID-19 tests, 5 to <14 days apart, amongst persons under investigation (PUI) provide a sensitive and specific diagnostic approach in routine public and private health care sites compared to a single reverse transcriptase PCR (RT-PCR) test at initial presentation. ***This is the main aim of the study.***

Aim 2: To develop a COVID-19 testing algorithm for use in community and routine health care settings in South Africa.

Aim 3: To correlate clinical symptoms/status, with rapid point of care COVID-19 test results.

- **Sub-aim 3a:** To correlate clinical symptoms (fever, cough, shortness of breath, myalgia, sore throat) at presentation and follow-up with rapid point of care COVID-19 test results amongst COVID-19 PUI.
- **Sub-aim 3b:** To correlate clinical outcome at follow-up (hospitalised but never admitted to an intensive care unit (ICU), admitted to ICU but not ventilated, ventilated, symptomatic at home, asymptomatic at home) with rapid point of care COVID-19 test results amongst COVID-19 PUI.

Aim 4: To estimate and compare the costs and effectiveness of rapid point of care testing versus RT-PCR for COVID-19 by assessing the average cost per person tested and average cost per person with a confirmed COVID-19 positive test result.

Aim 5: To describe and compare RT-PCR and rapid point of care COVID-19 test results amongst asymptomatic contacts of cases with confirmed (RT-PCR positive) COVID-19.

Aim 6: To contribute specimens to the laboratory-based validation of rapid point of care tests for COVID-19.

Aim 7: To create a biorepository of specimens from COVID-19 participants and asymptomatic contacts. These specimens will be used to understand the immune response (including neutralisation antibodies) and viral genetics of SARS-CoV-2, for the benefit of vaccine design studies.

Aim 8: To compare RT-PCR / serology on saliva versus swabs

Aim 9: To investigate the ease of use of rapid POC tests for COVID-19 diagnosis

Aim 10: To investigate whether one rapid antigen test amongst persons under investigation (PUI) provides a sensitive and specific diagnostic approach in routine public and private health care sites compared to a single reverse transcriptase PCR (RT-PCR) test at initial presentation.

Aim 11: To characterize the viral genome of SARS-CoV-2 in infected participants

Aim 12: To investigate the utility of laboratory-based serology and neutralization assays to diagnose SARS-CoV-2 and to track post infection binding and neutralizing activity of the antibody responses in order to have insights on the protective nature of the antibody responses

METHODS:

A prospective observational cohort study will be conducted, enrolling symptomatic patients with suspected COVID-19, presenting to selected health facilities or testing sites for investigation.

Study participants will be recruited from high throughput sites within three provinces, namely Gauteng, Western Cape and Limpopo. Participants will be tested for COVID-19 disease using rapid testing at the point of care. Additionally, routine swabs will be taken for COVID-19 RT-PCR, an additional swab will be taken for viral genome analysis and venipuncture blood samples will be sent to the laboratory for laboratory based COVID-19 serology, additional

testing (e.g. Vitamin D, CD4 cell count, T- and B-cell function, neutralizing antibodies) and storage for later testing. Saliva will be taken on consenting participants. All COVID-19 RT-PCR or rapid test positive participants will be followed up for repeat rapid testing and blood sampling. Approximately 500 RT-PCR and rapid test negative participants will be followed up for repeat swabs for RT-PCR, saliva collection, rapid testing and venipuncture blood sampling. One to four asymptomatic contacts of RT-PCR positive participants or asymptomatic contacts of confirmed PCR positive patients not enrolled in the study will be offered enrolment and swab collection for RT-PCR and viral genomic characterization, saliva sampling, rapid testing and venipuncture blood sampling for more indepth immunological analysis.
Furthermore, a cost-effectiveness analysis, from a health service providers' perspective will be conducted during the study period. Data on individual- and facility-level resource utilization, outcomes and costs will be collected from each site. The incremental cost per person tested and incremental cost per person with a confirmed COVID-19 positive test result using rapid point of care testing versus RT-PCR will be determined. A decision analytical Markov model will be used to estimate the incremental cost per quality-adjusted life- years gained using rapid point of care testing versus RT-PCR.

OUTCOMES

Field-based validation of rapid point of care COVID-19 tests (antibody or antigen) and development of an algorithm for point of care COVID-19 diagnosis in South Africa.
Correlations between symptoms, RT-PCR, rapid point of care (POC) antibody/antigen tests and formal laboratory-based serology tests.
Correlation of clinical characteristics and outcomes, with viral genomics and immunological and serological findings

INTENDED FEEDBACK OF THE STUDY

The study was requested by the COVID-19 Ministerial Advisory Committee (MAC). Results will be fed back to the Minister of Health, the COVID-19 MAC and National Department of Health. Results will also be published in peer-reviewed journals.

1.0 BACKGROUND

In December 2019, Wuhan city, the capital of Hubei province experienced an outbreak of pneumonia of unknown cause. By Jan 7th, 2020, Chinese scientists had isolated a novel coronavirus, initially called 2019-nCoV, renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), from these patients with viral pneumonia [1, 2]. The disease caused by SARS-CoV-2 became known as coronavirus disease 2019 (COVID-19).

Coronavirus infections

Coronavirus outbreaks in humans

Coronaviridae is a family of large RNA viruses discovered in the 1960s that primarily infect a wide range of domestic and wild animals resulting in respiratory, gastrointestinal and systemic diseases [3, 4]. Four coronaviruses are currently known to cause common colds in humans [5]. Since 2002, two strains of coronaviruses, from different zoonotic events, have caused fatal epidemics in humans. The severe acute respiratory syndrome coronavirus (SARS-CoV) caused an epidemic which started during the 2002 winter season in China and was eradicated by January 2004 after spreading to 37 countries, with a mortality rate of 9.6% (https://www.who.int/csr/sars/country/table2004_04_21/en/). The other strain emerged in 2012 in Saudi Arabia causing a short-lived epidemic followed by sporadic outbreaks in different countries [6]. In December 2019 SARS-CoV-2, a new coronavirus strain emerged, through what appears to be another zoonotic event during the Chinese winter, causing a third coronavirus epidemic in humans with a high fatality rate [7]. SARS-CoV-2 spread globally within 3 months. Transmission is mainly through respiratory droplets and fomites. [8] There is some evidence that SARS-CoV-2 may be found in faeces but no reports of faecal-oral transmission [8].

SARS-CoV-2 phylogenetics: contribution to our understanding of COVID-19 pathogenesis

Phylogenetic analysis of SARS-CoV-2 genome sequences isolated from nine Chinese patients (December 2019) shows that this strain clusters within the same clade as two bat-derived coronaviruses, thus inferring a zoonotic event from bats [9]. Among the six coronavirus strains known to have infected humans to date, SARS-CoV (from the 2002 epidemic) is the closest relative to SARS-CoV-2. High homology exists in the genomic region encoding for the receptor-binding domain of the structural 'spike' proteins of these two viruses. Therefore, they use the same mechanism to attach to and infect human host cells with angiotensin-converting enzyme 2 (ACE2) surface receptors [10, 11]. It is hypothesized that SARS-CoV-2 binds more firmly to these receptors than SARS-CoV, causing rapid and more severe disease. Previous work conducted to understand the spike protein and the receptor-binding domain of SARS-CoV (2002) has facilitated the understanding of SARS-CoV-2 pathogenesis.

SARS-CoV-2 transmission and clinical presentation of COVID-19 disease globally

Globally

The basic SARS-CoV-2 reproductive number (R_0) is approximately 2 (range 1.5-4) [3]. Herd immunity has not been confirmed yet, and, in the absence of vaccination, this R_0 could see the epidemic persisting if drastic control measures are not urgently implemented.

In humans, the SARS coronaviruses mainly infect lung cells with some cases perpetuating into cells of the immune system [4, 12]. This is an important finding, as the spread of SARS-CoV-2

will place a burden on respiratory support systems within health facilities, including an increased demand for oxygen and ventilators.
However, the clinical spectrum of SARS-CoV-2 infection is wide, hampering diagnosis and subsequent containment (Figure 1): it includes, asymptomatic infection, mild upper respiratory tract illness or mild pneumonia in approximately 80% of cases, severe viral pneumonia (≈14%), and critical disease with respiratory failure and even death in 4-20% of cases [13-17].

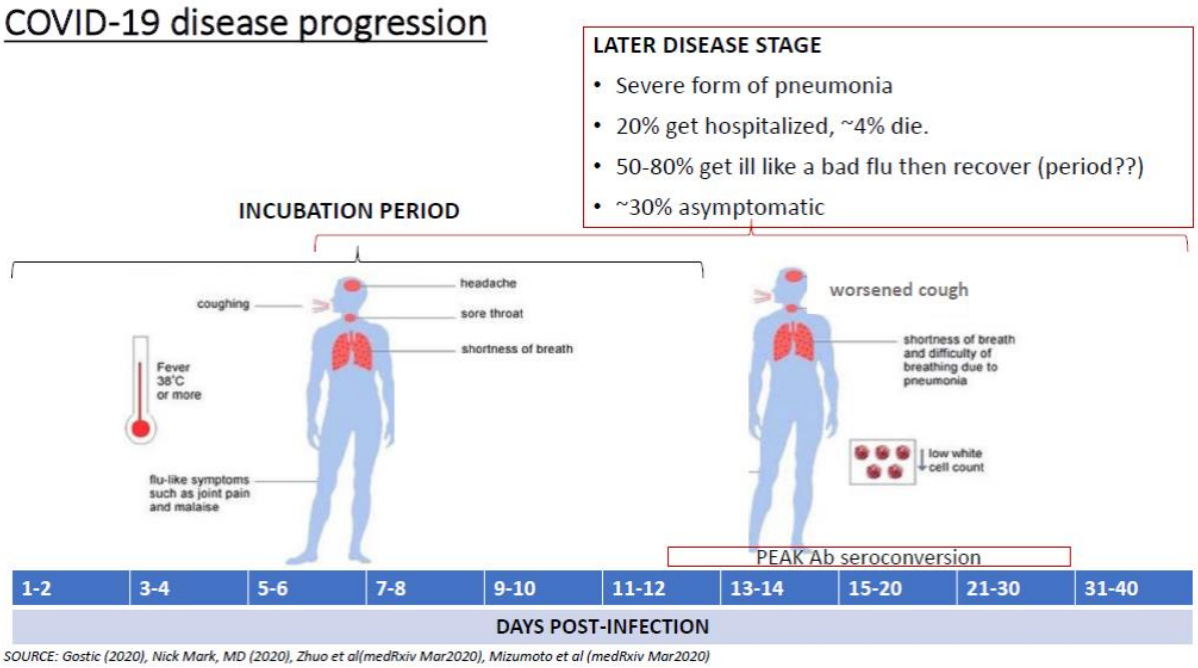


Figure 1: COVID-19 disease progression. **Figure developed by Nobubelo Ngandu, SAMRC**

Amongst hospitalized patients, viral shedding was noted for 20.0 days (IQR 17.0–24.0 days) in survivors, but SARS-CoV-2 was detectable until death in non-survivors [18]. The longest observed duration of viral shedding in survivors was 37 days.
There is no known herd immunity, vaccine or direct treatment in South Africa yet, although vaccine for health workers started in February 2021 in South Africa, as part of the Sisonke tstudy. Although SARS-CoV (2002) pre-clinical animal model studies demonstrated signals for the production of protective antibodies, challenge with live virus led to immune hypersensitivity and death [19]. Further studies indicated that progression to severe disease induces immune dysfunction leading to overproduction of immune cells and cytokines, with the destruction of healthy and infected lung tissue and acute respiratory distress syndrome [4].
Countries with delayed measures to control transmission experience higher mortality rates (www.covidvisualizer.com). Consequently, identifying and containing SARS-CoV-2 positive patients without delay, at the earliest possible stages of infection is an urgent priority.

South Africa and COVID-19 disease

In South Africa, the first laboratory-confirmed case of COVID-19 was documented on the 5th March 2020 (National Department of Health Communications (http://www.nicd.ac.za/wp-content/uploads/2020/03/COVID-19_UPDATES_18-03-2020.pdf), and occurred following international travel. Measures were subsequently instituted to contain SARS-CoV-2 spread.

Firstly a national state of disaster was declared (15th March 2020) with a partial travel ban, travel advisories discouraging the use of public transport, school closures, and prohibition of gatherings of over 100 people; subsequently, a national lockdown was declared for 21 days (26 March-16 April 2020). Furthermore, on the 30 March 2020, the South African President announced plans for the roll-out of a "screening, testing, tracing, and medical management programme on a huge scale. Around 10 000 field workers will be visiting homes in villages, towns and cities to screen residents for COVID-19 symptoms." Despite these measures, by the 6th April 2020 transmission of SARS-CoV-2 was mainly local, with 1686 positive SARS-CoV-2 infections amongst 58 098 tests conducted (detected prevalence of approximately 2.9%) and 12 deaths, yielding a case fatality rate of 0.7% (www.sacoronavirus.com – 7 April 2020).
Roll out of a 10-point plan has started, entailing community engagement; identifying symptomatic patients by mobile applications/platforms or home visits for COVID-19 testing; triage into care; managing isolation, contact tracing and mechanisms for de-isolation. The plan will also monitor test coverage and identify case clusters to target resources. In this 10-Point Plan, symptomatic patients identified on symptomatology either by home visits or via mobile platforms will be triaged into testing centres. Initially, the evaluation of a symptomatic patient will be via molecular diagnostics (Reverse transcriptase polymerase chain reaction - RT-PCR) from upper respiratory specimens such as nasopharyngeal/or oropharyngeal swabs. The 10-point plan envisages that RT-PCR will be replaced by serology tests using technologies that are currently being registered in-country. These serological tests will measure IgG/IgM and may be Point-of-Care (POC) tests.

Diagnosis of COVID-19 disease

Currently, nucleic acid amplification tests (NAAT) such as RT-PCR or deep sequencing are the gold standard for laboratory diagnosis of COVID-19, using upper or lower respiratory specimens [20]. In South Africa, the current diagnostic algorithm includes a screening RT-PCR. Screening positive tests are confirmed by a repeat RT-PCR (Figure 2) [21, 22]. Discordance between the screening and confirmatory RT-PCR necessitates the further collection of specimens and performance of a Pan-Coronavirus PCR. Negative specimens in a person under investigation necessitates the collection of further specimens and repeat tests if clinically or epidemiologically suggested, or if the initial specimen was of poor quality.

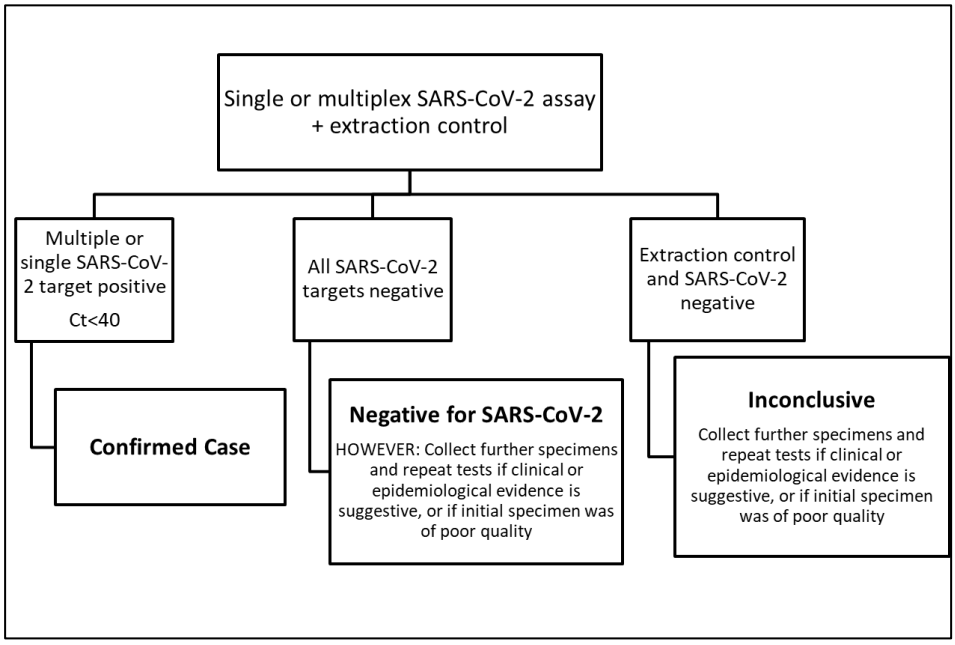


Figure 2: Current South African algorithm for SARS-CoV-2 diagnosis from NICD guidelines

However, data from China tell us that clinical specimens obtained from SARS-CoV-2 positive in-patients with COVID-19 disease (diagnosed from symptoms, radiology detection of SARS-CoV-2) demonstrate low detection rates using RT-PCR: 93% detection of SARS-CoV-2 in broncho-alveolar lavage fluid specimens, 72% in sputum, 63% in nasal swabs, 46% in fibrobronchoscope brush biopsy, 32% in pharyngeal swabs, 29% in faeces, and 1% in blood (1%) [23]. Upper respiratory swabs (naso- or oro-pharyngeal swabs) are the main specimens used for COVID-19 diagnosis and in many settings, including South Africa, RT-PCR testing is limited to laboratory settings [24].

Consequently, there are shortcomings associated with current COVID-19 diagnostic strategies [25].

(i) specimen collection for RT-PCR necessitates the handling of infectious fluids by health care personnel
(ii) detection methods depend heavily on the presence of viral genome in sufficient amounts at the site of sample collection
(iii) viral genome presence depends on the correct technique used for specimen collection
(iv) missing the time-window of viral replication can provide false-negative results
(v) there is only 32-63% positivity rate using recommended specimens in confirmed positive cases
(vi) in the absence of point of care testing, RT-PCR needs to be performed in specialized laboratories, requiring specialized staff
(vii) RT-PCR is expensive
(viii) centralized testing is associated with delayed return of results to suspected cases

These short-comings increase the risk for health care personnel, delay case isolation, contact tracing, and curbing of the pandemic and thus, facilitate COVID-19 disease spread.

New, rapid techniques based on antigen or antibody detection or both are thus urgently needed to diagnose SARS-CoV-2 infection. However, detectable antibody production is slow due to a long incubation period (ranging between 2-12 days, Figure 1) [26]. Data indicates earlier production of detectable antibody levels (by day 7) in patients with mild COVID-19 disease, with delayed antibody peaks in patients who develop severe disease [27, 28]. Given the need to scale up case identification to the community level, rapid diagnostic point of care (POC) or ELISA techniques are required. Antibody/antigen diagnostic POC techniques are an ideal option and their sensitivity and specificity, given the wide spectrum of antibody production in infected persons, need to be investigated urgently.

According to discussions with Lancet laboratories, NHLS and the Strategic Health Innovation Partnerships (Eftyxia Vardas, Elizabeth Mayne and Richard Gordon, 2-6th April 2020), 11 immunoassay tests to detect SARS-CoV-2 infection will be coming into the market soon – one of them from as soon as the 14th April 2020 (***Appendix 1***). Four of these are laboratory-based serological enzyme-linked immunosorbent assays (ELISA) or chemiluminescent immunoassays suitable for high-throughput serological testing. The remaining seven are point-of-care tests. None of these has been tested in the South African context. Several locally-produced kits are also being investigated and a Target Product Profile is currently under development, in consultation with the South African Health Product Regulatory Authority (SAHPRA). On the 2nd April FDA approved Cellex Inc. to market their qualitative IgM/IgG test under emergency use authorization providing they do their own quality assurance https://www.medpagetoday.com/infectiousdisease/covid19/85772?xid=nl_mpt_DHE_2020-04-03&eun=g1466172d0r&utm_source=Sailthru&utm_medium=email&utm_campaign=Daily%20Headlines%20Top%20Cat%20HeC%20%202020-04-03&utm_term=NL_Daily_DHE_dual-gmail-definition). There are three additional rapid tests (Maccura ®, Bioeasy ®) and Abbot®) which use serum, plasma (EDTA or citrate) or venipuncture whole blood that is under registration. The Cellex Inc. brochure notes that a positive IgM or IgG test can be used to detect acute (IgM positive) or past infections (IgM negative; IgG positive). Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. However, false-positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

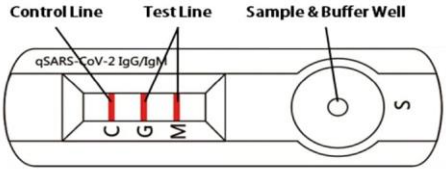


Figure 3: Appearance of the Cellex qSARS-CoV-2 IgG/IgM Rapid Test an example of a rapid on-site test for diagnosing SARVS-CoV-2 infection

Notably, all these assays are qualitative and cannot be used to detect the level of antibody titers post-symptom onset. The assays need to be validated (sensitivity, specificity, positive and negative predictive values), to justify their use to identify COVID-19 disease using human serum/plasma/whole blood/dry blood spot post symptom onset. Most importantly, these assays do not require handling of infectious virus, can be adjusted to detect different antibody types and are amendable to scaling up the diagnosis of SARS-CoV-2 infection at the community level.

Given this background, we aim to investigate the point of care rapid diagnostic strategies to optimize the rapid diagnosis of COVID-19 in Symptomatic Patients in routine health care

settings. Potential alternate diagnostic strategies are listed in ***Appendix 2***. Some of these are the rapid point of care tests – results could be available in 15 minutes to two hours. Such rapid tests depend on the collection of blood samples, which are less infectious, thus avoiding the collection of naso- and oro-pharyngeal samples at community/clinic / primary health care level. Consequently, point of care blood testing will help to reduce viral transmission at this community/clinic / primary health care level, where less specialized staff who are not comfortable with oro-/ naso-pharyngeal sampling are usually based.

As at 1st September 2020, one rapid point of care antibody test (Orient Gene) was approved for use under specific conditions in South Africa: the NHLS asked the SAMRC to undertake post-market surveillance of the Orient Gene test as part of this study. Two additional tests (Biosynex and Genrui, and possibly LumiraDx antibody) needed further validation: the NHLS asked the SAMRC to assist with the additional validation of these two tests as part of this study. Additionally the NHLS asked the SAMRC to assist with validation on two rapid antigen tests (Rapigen, and SD Biosensor and possibly LumiraDx antigen).

2.0 AIMS:

Aim 1:

To investigate whether two rapid point of care COVID-19 antibody tests, 5 to <14 days apart, amongst persons under investigation (PUI)[29] provide a sensitive and specific diagnostic approach in routine public and private health care sites compared to a single reverse transcriptase PCR (RT-PCR) test at initial presentation. ***This is the main aim of the study.***

Hypothesis:

Point of care rapid tests for COVID-19 are not inferior to RT-PCR testing for COVID-19 i.e. A rapid Ab test performed on a suspected COVID-19 person in routine conditions at two time points that are 5 to<14 days apart provides a sensitive and specific diagnostic approach compared to a single RT-PCR at initial presentation.

Significance and impact:

Standard of care PCR tests for COVID-19 diagnosis rely on obtaining naso- and oro-pharyngeal infectious samples and analyzing these in specialized laboratories. Such an approach increases the risk for health care personnel collecting samples, is expensive, delays the return of results to suspected cases for a day or more and delays case isolation, contact tracing, and curbing of the COVID-19 pandemic. It is not known whether the rapid point of care COVID-19 tests can replace RT-PCR diagnosis. Thus, we will investigate whether rapid point of care COVID-19 antibody testing performed in routine hospitals or clinics, on a COVID-19 symptomatic person under investigation (PUI) presenting for testing at a health facility at an initial and sequential (5-<14 days) visit, provide a sensitive and specific diagnostic approach compared with a single RT-PCR. The 5-< 14-day follow-up interval has been chosen as rapid antibody tests will only be useful if they can provide a diagnosis during the isolation period, which is 14 days long, and not after the isolation period. Our findings will answer the questions: Are two rapid point of care COVID-19 antibody tests one week apart not inferior to one RT-PCR test for COVID-19 diagnosis? Can we scale-up the rapid point of care COVID-19 tests for COVID-19 diagnosis in South Africa?

Aim 2:

To develop a COVID-19 testing algorithm for use in community and routine health care settings in South Africa.

Hypothesis:

If Aim 1 is true, then a rapid point of care COVID-19 testing protocol will include two serial rapid tests one week apart.

Significance and impact:

If rapid point of care COVID-19 testing is not inferior to RT-PCR then routine settings can implement a rapid point of care COVID-19 serial testing algorithm. For example, in on-site HIV rapid testing, the serial testing algorithm recommends one screening rapid test, performed on a finger prick blood sample, followed by a confirmatory test using a different finger prick blood sample, if the first test is positive [30]. If the screening test is negative, no further action is needed. If there is discordance between the screening and confirmatory test, blood is drawn and sent to the laboratory for an ELISA test. If the ELISA is negative, the result is

reported as negative. If the ELISA is positive, the result is reported as positive. Similarly, following the field-based testing of rapid point of care COVID-19 tests, we will develop an algorithm for use at facilities.

Aim 3:

To correlate clinical symptoms/status, with rapid point of care COVID-19 test results.

Sub-aim 3a: To correlate clinical symptoms (fever, cough, shortness of breath, myalgia, sore throat) at presentation and follow-up with rapid point of care COVID-19 test results amongst COVID-19 PUI.

Sub-aim 3b: To correlate clinical outcome at follow-up (hospitalised but never admitted to an intensive care unit (ICU), admitted to ICU but not ventilated, ventilated, symptomatic at home, asymptomatic at home) with rapid point of care COVID-19 test results amongst COVID-19 PUI.

Hypothesis:

Clinical symptoms and clinical outcome have a high correlation with positive rapid point of care COVID-19 test results.

Significance and impact:

The serial antibody response detected by rapid point of care COVID-19 testing, and how this correlates with symptoms is unknown. Correlating clinical symptoms and clinical outcomes will provide a better understanding of the clinical course of COVID-19 disease and how these relate to rapid test results. We will pay particular attention, during analysis to the following groups:

1. PUI who tested RT-PCR and rapid test positive at initial test.
2. PUI with discordant RT-PCR and rapid test results at initial visit: RT-PCR positive, rapid test negative or vice versa.
3. PUI who test RT-PCR and rapid test negative at initial visit.
4. PUI with co-morbidities such as TB, HIV, hypertension, diabetes, asthma

Due to the potential for a delay in the development of antibody responses, we propose to collect a third respiratory and serum sample, if funding is available at 25-30 days following the initial positive sample in a sub-sample of positive participants.

Aim 4:

To estimate and compare the costs and cost-effectiveness of rapid point of care testing versus RT-PCR for COVID-19 by assessing the average cost per person tested and the average cost per person with a confirmed COVID-19 positive test result.

Significance and impact:

If the hypothesis for Aim 1 holds true then, to guide national policy we will need to determine whether rapid point of care COVID-19 testing is cost-effective compared to RT-PCR by estimating and comparing the average cost per participant tested, and average cost per participant with a confirmed COVID-19 positive test result. This information will be essential for informing budget impact analyses examining the costs of scaling-up governments COVID-19 testing policy.

Aim 5:

To describe and compare RT-PCR and rapid point of care COVID-19 test results amongst asymptomatic contacts of cases with confirmed (RT-PCR positive) COVID-19 disease, and compare RT-PCR results with rapid point of care test results in this population.

Hypothesis:

At least 50% of asymptomatic contacts of confirmed COVID-19 cases will have positive RT-PCR test results, and at least 45% will have a positive antibody result.

Significance and impact:

Asymptomatic transmission has been described in many settings and maybe driving the COVID-19 pandemic [31, 32]. We will test asymptomatic contacts of confirmed COVID-19 cases to assess their RT-PCR results and responses to rapid point of care COVID-19 testing. These results will assist with improving the approach to COVID-19 testing. The study will use the NICD/NHLS PUI case definition to determine who should be tested for COVID-1919 (***Appendix 3***). We predict that we will find RT-PCR positive results and IgM rapid testing positive results in asymptomatic contacts, substantiating an approach that includes testing asymptomatic contacts of confirmed COVID-19 cases.

Aim 6:

To contribute specimens to the laboratory-based validation of rapid point of care tests for COVID-19.

Significance and impact:

We will contribute specimens to laboratory partners who will be conducting laboratory-based validation of rapid point of care testing platforms or formal serology tests. This will assist laboratories and the South African Health Product Regulatory Agency (SAHPRA) with developing a final Target Product Profile for rapid point of care or formal serological COVID-19 testing platforms.

Aim 7:

To create a biorepository of specimens from COVID-19 participants and asymptomatic contacts. These specimens will be used to understand the immune response (including neutralization antibodies) and viral genetics of SARS-CoV-2, for the benefit of vaccine design studies.

Significance and impact:

We will contribute COVID-19 positive specimens to laboratory and basic science collaborating researchers who will investigate whether mild disease versus severe disease; time to recovery and antibody titers are associated with specific viral genetic characteristics. These findings will assist with the development of vaccines and the design of vaccine trials.

Aim 8:

To compare RT-PCR / serology on saliva versus swabs

Significance and impact:

We will determine whether saliva can provide comparative diagnostic capabilities compared with swabs. A recent paper published by Wyllie et.al. (https://doi.org/10.1101/2020.04.16.20067835) found saliva to be more sensitive for SARS-CoV-2 diagnosis compared with nasopharyngeal swabs, and may especially pick up asymptomatic infections earlier. Such a finding will simplify and increase the safety of SARS-CoV-2 diagnosis, because saliva collection is not aerosol generating, and thus is not infectious.

Aim 9:

To investigate the ease of use of rapid POC tests for COVID-19 diagnosis

Significance and impact:

We will determine whether users (health care personnel) find the rapid tests user friendly, easy to use, feasible and safe, using the criteria put forward by Carmona et.al. [33] This information will allow us to ascertain health workers perception of the workflow, ease of use, consistency, integration and support needed when using rapid POC tests. This will inform efforts to scale up POC testing for COVID-19. Each test will be evaluated once, and separately by users in the field.

Aim 10:

To investigate whether one rapid antigen test amongst persons under investigation (PUI) provides a sensitive and specific diagnostic approach in routine public and private health care sites compared to a single reverse transcriptase PCR (RT-PCR) test at initial presentation.

Significance and impact:

We will determine whether rapid antigen tests offer a sensitive and specific diagnostics approach to SARS-CoV-2 diagnosis at initial presentation. Given that antigen testing detects the virus and that antigen tests do not rely on the development of an antibody response antigen testing may provide a more sensitivity and specific diagnostic approach to SARS-CoV-2 diagnosis than antibody testing or RT-PCR testing.

Aim 11:

To characterize the viral genome of SARS-CoV-2 in infected participants

Significance and impact:

With the December 2020 public reporting of a variant of SARS-CoV-2 in South Africa (501Y.V2) by Dr Tulio d'Oliviera, it is important to gather additional information on viral genomics. Specimens will be used to characterize the viral genome from all three provinces, correlate viral genomics with clinical characteristics and outcomes, and with immunological and serological findings. These analyses will have utility for vaccine development, monoclonal

antibody development and advice on transmissibility of SARS-CoV-2.

Aim 12:

To investigate the utility of laboratory-based serology and neutralization assays to diagnose SARS-CoV-2 and to track post infection binding and neutralizing activity of the antibody responses in order to have insights on the protective nature of the antibody responses

Significance and impact:

As stated in Table 1, specimens in the biorepository will be used to understand the immunological responses of the South African population to SARS-CoV-2 infection during acute infection and during convalesce and beyond, up to 6-months post diagnosis. These analyses will provide an understanding of the effects of SARS-CoV-2 in an African setting (South Africa), including correlates of protection, duration of protection, correlation between clinical status / outcomes and serological/imunological biomarkers. Such data are important for vaccine development, to understand re-infection and for the development of additional preventative modalities such as monoclonal or polyclonal antibodies.

Two sub-studies have been linked to this protocol. Both will investigate the utlity of rapid testing in routine healthcare settings.

The protocols are entitled:

(i) Longitudinal surveillance of COVID-19 at five South African Medical Research Council Clinical Research Sites in eThekwini, a high HIV prevalence district South Africa. (study PI: B Daniels) – approved by SAMRC HREC

(ii) Prevalence, clinical characteristics, immunologic responses and outcomes of children with suspected or confirmed COVID-19 disease (study PIs: Ameena Goga, Robin Green, Jeane Cloete – approved by SAMRC HREC on

Justification for the linking:

(i) The study will use rapid antigen and antibody testing in two longitudinal cohorts at 5 different clinical trial sites in eThekwini. The first is a cohort of healthcare workers implementing COVID-19 and HIV prevention studies, with the aim of once-weekly rapid antigen testing allowing for early isolation of infected staff to prevent COVID-19 spread among colleagues. This cohort will also allow us to monitor breakthrough infections post COVID-19 vaccination. The second cohort will be HIV prevention trial participants who will be tested for SARS-CoV-2 antigen and antibody at their monthly clinic visits. This cohort allows us to monitor for SARS-CoV-2 outbreaks and seroprevalence in the communities surrounding our clinics. Both cohorts will allow for rapid identification of reinfections and therefore possible new variants of concern.

(ii) Amongst children admitted to hospital we will assess the utility of rapid tests for paediatric COVID-19 diagnosis, including MIS-C diagnosis. Rapid test results will not be reported back to patients and not used for management unless clinically useful e.g. a positive antibody test and negative PCR test in a child with suspected

MIS-C. The gold standard test which is the PCR test for COVID-19 will always be used for COVID-19 diagnosis.

3.0 METHODS

Study design

A prospective observational cohort study will be conducted, enrolling symptomatic patients with suspected COVID-19 disease, presenting to selected health facilities or testing sites for investigation. They should meet the NICD criteria for persons under investigation (PUI).

Study sites

Study participants will be recruited from sites with high throughput for SARS-CoV-2 infections in the three provinces with the highest burden of COVID-19 disease. Participants will be informed about the study through the staff at the testing site, and through a flyer (Appendix 11). The study sites are listed below.

Activated sites:

- Gauteng Province:
  - Chris Hani Baragwanath hospital and any associated facilities, under the leadership of Shabir Madhi (site PI)
- Western Cape Province:
  - UCT-affiliated hospital complex and community sites in Cape Town (Klipfontein and Mitchells Plain) under the leadership of Keertan Dheda (site PI)
- Limpopo province:

  University of Limpopo : Capricorn and Waterberg districts under the leadership of Ruth Lekalakala: : Capricorn and Waterberg districts *site will be activated

Sites on hold (will only be activated if there is a need)

- Steve Biko Academic Complex (including Tshwane district hospital), under the leadership of Veronica Ueckermann (site PI)
- Charlotte Maxeke Hospital (if feasible)
- Addington hospital under the leadership of Brodie Daniels (site PI)
- Umlazi Polyclinic – under the leadership of Kogie Naidoo
- Alex CHC - under the leadership of James McIntyre
- possible addition of the Kraaifontein Community Health Centre under the leadership of Grant Theron

Inclusion criteria:

For the selection of participants in the prospective cohort study:

- Age 18 years and older[1]
- Has symptoms of COVID-19 and meets current NICD/NHLS case definition for testing (See ***Appendix 3***)[2][29]

1 Children may have different antibody responses compared with adults, and will thus confound the analysis. A separate paediatric protocol has been developed at SBAH and this will look at antibody responses in children. Additionally, the inclusion of children needs specialised consent forms and procedures, including assent, and staff skilled in drawing bloods from children. Consequently, children have been excluded from this protocol.

[2] Testing for COVID-19 is based on a case definition of a Person Under Investigation (PUI) developed by the National Institute for Communicable Diseases (NICD), Appendix 3. Whilst the original case definition included a travel history, given the increasing local transmission, the case definition has undergone several changes.

For selection of asymptomatic contacts:

- Has been in close contact with a SARS-CoV-2 RT-PCR positive person (>15 minutes in a poorly ventilated space or ≤1 metres apart) and
- Age 18 years and older and
- Agrees to providing paired naso-pharyngeal and blood samples and
- Self-reports not feeling sick or no symptoms of COVID-19 on initial questioning

Exclusion criteria:

- Participant does not consent to follow-up including home based follow-up

Data collection methods

At the initial / enrolment visit:

1. All prospectively enrolled participants who meet inclusion criteria will be interviewed and tested for COVID-19 using one of more rapid point of care COVID-19 tests, depending on whether the National Department of Health has selected one or more tests for implementation. This will achieve ***Aims 1, 2 and 3***.
2. Additionally, nasopharyngeal swabs and blood samples will be taken:
   a. swab for RT-PCR - in accordance with the routine NICD/NHLS protocol (see ***Appendix 4***),
   b. additional naso-pharyngeal (preferable) or nasal swab for viral genome analyses
   c. 30 mL of blood following venepuncture by a skilled nurse or phlebotomist for immunological assays/phylogenetic analysis: 10mL EDTA plasma tube, 15mL SST tube and 5mL Heparin tube. In approximately 500 participants, or more if budget allows, an additional 20mL blood will be drawn for peripheral blood mononuclear cell isolation. These blood samples will be processed or stored until they are analysed for ***Aims 6 and 7***. See Table 1 below with a list of all the tests to be conducted.
3. A saliva specimen will be collected from consenting participants. This will be used to for rapid tests, serology, RT-PCR, to identify the presence of viral genome and, if possible to assess salivary T-cells.
4. In Western Cape, Limpopo, and Gauteng province nasal swabs will be taken to validate the rapid antigen tests.
5. Each enrolled participant will complete a case report form (CRF – Appendix 5), and, if still policy, a routine NICD person under investigation form (See Appendix 6). Participant CRFs will be programmed electronically and completed electronically as far as is possible to reduce the exchange of paper - which may be contaminated with SARS-CoV-2.
6. Consent will be obtained to undertake laboratory-based antibody testing on the blood specimens and to store these specimens for additional COVID-19 immunological and phylogenetic research to understand disease acquisition and progression.
7. All participants will be consented for the home/isolation / hospital-based follow-up to maintain isolation.
8. All COVID-19 positive participants (positive on RT-PCR or any rapid testing) will be followed up at day 5 to <14. Procedures for follow up will be specified in the standardised study procedures (SSPs).

9. Date of follow-up will be confirmed on the initial visit for rapid test positive participants.
10. RT-PCR positive, rapid test negative participants will be telephoned to arrange their follow-up date.
11. If funding is available, participants who tests positive at baseline (RT-PCR or rapid test positive) will be offered follow-up at 20-25 days, 3, 6, 9 and 12 months if feasible and budget exists, to assess their clinical, serological and immunological status (***Aims 3, 11, 12***).
12. 500 participants who tested RT-PCR and all rapid tests negatives recruited consecutively will be followed up at day 5-<14.

At the first follow-up visit (day 5-<14):

1. All participants will have a repeat rapid point of care COVID-19 test.
2. 25-30 mL tubes of blood will be drawn in the Limpopo and Gauteng sites, and 40mL will be drawn in the UCT site (see Table 1 below) following venepuncture by a skilled nurse or phlebotomist, for immunological assays/phylogenetic analysis, As stipulated in Table 1 below: 5mL heparinised blood in Limpopo and Gauteng sites and 20mL heparinised blood at the UCT site, 10mL EDTA plasma tube (at UCT, Limpopo and Gauteng sites), and 10mL (15mL if PCR positive at baseline) blood in an SST tube for serum (at UCT, Limpopo and Gauteng sites). In a sub-sample an additional 20mL blood will be drawn for peripheral blood mononuclear cell isolation (see Table below). These blood samples will be processed and sent to the laboratory for processing or storage (***Aims 6 and 7)***.
3. A saliva specimen will be collected from consenting participants for rapid tests, serology, RT-PCR, to identify the presence of viral genome and, if possible to assess salivary T-cells.
5. Participants who tested RT-PCR negative at the initial visit will have a repeat swab taken for RT-PCR - in accordance with the routine NICD protocol (***Appendix 4***)).If feasible, participants who tested RT-PCR or rapid test positive at baseline will have one additional naso-pharyngeal swab (preferable) or nasal swab taken to re-characterise the viral genome.
6. Each enrolled participant will complete a follow-up case report form (CRF) to check symptoms and outcomes (***Aim 3***). Participant CRFs will be programmed electronically and completed electronically as far as is possible to reduce the exchange of paper - which may be contaminated with SARS-CoV-2.

To achieve ***Aim 5***: **At the day 5-<14 follow-up visit referred to above:**

1. Two (range 0-4) eligible asymptomatic (preferably home) contacts of RT-PCR or rapid positive patients, identified through index testing during enrolment or through laboratories or hospitals will be offered enrolment into the study.
2. They will be interviewed and swab and blood samples will be taken:
   a. one swab for RT-PCR - in accordance with the routine NICD protocol (see ***Appendix 4***),
   b. nasal swab to detect viral antigen in Western Cape, Limpopo and Gauteng provinces.
   c. 25-30 mL of blood following venepuncture by a skilled nurse or phlebotomist for immunological assays/phylogenetic analysis: 5mL blood in a heparinised tube,

10mL blood in an EDTA plasma tube, and 10mL (15mL if any rapid test is positive on this visit) in an SST tube (see Table 1 below). In approximately 200 participants, or more if budget allows, an additional 20mL blood will be drawn for peripheral blood mononuclear cell isolation. These blood samples will be processed and analysed or stored until they are analysed for ***Aims 6 and 7***.

d. additional naso-pharyngeal (preferable) or nasal swab for viral genome analyses

3. A saliva specimen will be collected from consenting participants. This will be used for serology, rapid tests, RT-PCR, to identify the presence of viral genome and, if possible to assess salivary T-cells.
4. Each enrolled asymptomatic contact will complete a patient case report form (CRF) – Appendix 5, and a routine NICD person under investigation form if these are still in use (See Appendix 6). Patient CRFs will be programmed electronically and completed electronically as far as is possible to reduce the exchange of paper - which may be contaminated with SARS-CoV-2.
5. Consent will be obtained to undertake laboratory-based antibody testing on the blood specimens, and to store these specimens for additional COVID-19 immunological and phylogenetic research to understand disease acquisition and progression.
6. If funding is available, 100 asymptomatic PCR positive participants will be re-consented for follow-up and followed up at 25-30 days, 3, 6 , 9, 12 months to understand their immunological responses (see section below).

The timing of the follow-up schedule is as follows:

Presentation day	**Preferred F-UP day**	**Number of days post initial visit**	**Preferred day – but if participant can't make that day then select another day**
Mon	9-10 days later	Thurs / Fri	Fri (±2 days)
Tues	13-14 days later	Mon/Tues	Tues (± 2 days)
Wed	7-8 days later	Wed/Thurs	Wed (±2 days)
Thus	11-12 days later	Mon/Tues	Mon (±2 days)
Fri	5-6 days later	Wed/Thurs	Thurs (±2 days)

If funding is available, a sub-sample of RT-PCR or rapid positive participants will be followed up at 25-30 days, 3, 6, 9 and 12 months(***Aim 3***). Additionally, if funding is available 100 asymptomatic PCR positive participants will be followed up at 25-30 days, 3, 6, 9 and 12 months to understand their immunological responses.

1. A nurse/phlebotomist will repeat the rapid point of care COVID-19 test.
2. 25-3O mL of blood following venepuncture by a skilled nurse or phlebotomist for immunological assays/phylogenetic analysis: 5mL blood in a heparinised tube, 10mL blood in an EDTA plasma tube, and 10mL (15mL if PCR positive at baseline) in an SST tube (see Table 1 below). If budget allows an additional 20mL or 40mL (in Cape Town participants) will be drawn for peripheral blood mononuclear cell isolation. These blood samples will be processed and analysed or stored until they are analysed for ***Aims 6 and 7***.

3. A saliva specimen will be collected from consenting participants. This will be used for serology, rapid tests, RT-PCR, to identify the presence of viral genome and, if possible to assess salivary T-cells. A patient CRF checking symptoms will also be completed to assess symptoms and outcomes.
4. A nasopharyngeal swab will be collected from consenting participants for PCR
5. An additional nasopharyngeal swab will be collected from consenting participants for genomic analysis

Table 1: Summary of procedures and samples that will be collected

	Baseline all PUIs who present for COVID-19 diagnosis at selected sites	**Follow-up of PCR positive PUIs (5-14 days) and if budget allows, follow-up of PCR positive PUIs and 100 asymptomatic PCR positive contacts at 25-30 days, 3, 6, 9 and 12 months**	**Follow-up of 500 negative PUIs**	**Asymptomatic contacts of positives n=720**	**Tests to be conducted on the specimens**
Interview at recruitment:	√	√	√	√	Not applicable
Swab for RT-PCR to diagnose COVID-19	√	N/A	√	√	COVID-19 RT-PCR
Extra swab for– viral genome analysis to be taken if participant consents	√	√		√	Genomic analysis
Saliva	0.5-1 mL	0.5-1 mL	0.5-1 mL	0.5-1 mL	rapid antibody tests, rapid antigen tests, serology, RT-PCR, to identify the presence of viral genome and, if possible to assess salivary T-cells.
SST tube	15 mL	15 mL	10 mL	10 mL	Formal serology, Vit D, serum amyloid A, transthyretin, neopterin, transferrin (or ferritin), neutralizing and serological assays including CHUV luminex assay
Heparinized tube: Limpopo, Gauteng, UCT participants not included in the row below	5mL	5mL	5mL	5mL	Serology, neutralising assays
Heparinized tube: UCT site n=100 positives, 100 negatives and 100 asymptomatic contacts	N/A	20mL	20mL	20mL	T- or B-cell function
EDTA tube	10 mL	10 mL	10 mL	10 mL	Flow cytometry, T-cell analysis, B-cell responses, HLA typing, Cytokines (IL1, TNFα, TGFβ, IL6, IL10), Endothelial markers (VCAM, adiponectin, V/P selectin) – d-dimers, platelets, CD4
ACD tube for peripheral blood mononuclear	20mL	20mL	20mL	20mL	T-cell function, B-cell function

cells					
Finger prick blood for rapid antibody tests – Orient Gene, Genrui, Biosynex	√	√	√	√	SARS-CO-v-2 IgM or IgG or both at the point of care
Nasal swab for SD Biosensor Limpopo only (n=150)	√				SARS-CoV-2 infection at the point of care when presenting for diagnosis
Nasal swab for RapiGen Limpopo only (n=150)	√				SARS-CoV-2 infection – antigen – only at baseline
Nasal swab for Lumira rapid antigen test	√	√ (if feasible)	√ (if feasible)	√(if feasible)	SARS-CoV-2 infection – antigen – only at baseline

N/A not applicable. Note: Swabs and bloods must be paired – i.e. taken at the same time

Maximum blood volumes per participant

0	Blood volumes in mL per participant for visit										Key
	EDTA		Heparin			SST		ACD			Institute
Blood volumes per participant	Baseline (BL)	Volume to be taken at each follow-up visit at 5-<14 day and if budget allows 25 30 days, 3, 6, 9, 12 month	BL	Volume to be taken at each follow-up visit at 5-<14 day and if budget allows 25-30 days, 3, 6, 9, 12 months		BL	Volume taken at each follow-up visit at 5-<14 day and if budget allows 25-30 days, 3, 6, 9, 12 months	BL	F-up at 5-<14 days only	TOTAL mL	SAMRC and NHLS samples
											PBMC-SAMRC/NHLS
UCT/Western Cape site:											
rapid positives n=110											
if in UCT study and PBMC sampling	10	10	5	20	5	15	15	20	20	120	followed-up
if NOT in UCT study and no PBMC sampling	10	10	5	5		15	15	0	0	60	
rapid negatives n = 2090 of which only 200 are followed up											
if in UCT study and PBMC sampling	10	10	5	20	5	15	10	20	20	115	followed-up
if NOT in UCT study and PBMC sampling	10	10	5	5		15	10	0	0	55	
if NOT in UCT study and no PBMC - no follow-up	10		5	0		15		0		30	not followed up
asymptomatic contacts n=220											
if in UCT study and PBMC sampling	10		5	20		15		20		70	not followed up
if NOT in UCT study and no PBMC - no follow-up	10		5			15				30	
Gauteng site:											
rapid positives n=180											
With PBMC sampling	10	10	5	5		15	15	20	20	100	followed-up
No PBMC sampling	10	10	5	5		15	15	0	0	60	
rapid negatives n=1620 of which only 180 are followed up											
With PBMC	10	10	5	5		15	10	20	20	95	followed-up
No PBMC	10	10	5	5		15	10	0	0	55	
No PBMC and no follow-up	10		5			10		0		25	not followed up
360 asymptomatic contacts											
PBMC sampling	10		5			15		20		50	not followed up
No PBMC sampling	10		5			15		0		30	
Limpopo											
rapid positives n=75											
With PBMC sampling - antibody or antigen positive	10	10	5	5		15	15	20	20	100	followed-up
No PBMC sampling	10	10	5	5		15	15	0	0	60	
rapid antibody and antigen negatives n=675 of which only 50 are followed											
With PBMC sampling - antibody or antigen positive	10	10	5	5		15	10	20	20	95	followed up
No PBMC sampling	10	10	5	5		15	10			55	

During data collection all study staff (nurses, phlebotomists, research assistants and tracing teams) will have:

1. Identification tags;
2. A copy of the Ethics approval letter;
3. A permit if lockdown is still in place;
4. Personal protective equipment to protect participants, and themselves:
   a. Face shields or goggles, disposable aprons or gowns, N95 (or similar masks such as KN95 or FFP2 masks) masks, gloves and sanitizer ***for staff taking swabs, or for staff within 2 metres of these swabs being taken***. For tracing teams: These will be changed between home visits and disposable sealable bags will be provided for such disposal.
   b. Surgical masks, gloves and sanitizer ***for staff performing interviews and drawing blood, who are not performing or close to staff performing aerosol generating procedures.*** For tracing teams: These will be changed between home visits, and disposable sealable bags will be provided for such disposal.
   c. Staff will use sanitiser before entering a home
5. Data, a phone and access to the SAMRC PI or their local PI, and the 0800 COVID-19 response number.

Each tracing team conducting home visits will travel in a designated research vehicle – they

will not use public transport. The research vehicle will be wiped down with sanitizer in between home visits. Social distancing will be maintained at all times, and as much as possible during sample collection.

Diagnostic procedures

1. For RT-PCR given the changing criteria for testing in South Arica all RT-PCR will be performed by an accredited laboratory. All results will be returned to participants, and positive results will be notified, as per national requirements.
2. For the rapid point of care COVID-19 testing, three to four rapid antibody tests will be used in all sites (Orient Gene, Biosynex, and Genrui, LumiraDx). Three or four rapid antigen tests will be used (including SD Biosensor, Rapigen, LumiraDx antigen)to assist NHLS with validating these tests.
3. Swabs will be collected for RT-PCR in accordance with routine NICD, NHLS protocols. Additional naso-pharyngeal (preferred) or nasal swabs will be collected at baseline, follow-up of positives and amongst asymptomatic contacts to characterise the viral genome if participants agree (***Aim 11)***.
4. Blood samples and saliva may be sent to the NHLS (Elizabeth Mayne's laboratory) for laboratory-based rapid/ antibody testing so that we contribute to the laboratory-based validation of tests for COVID-19, if needed. Samples may be stored at NHLS or SAMRC for additional studies (***Aim 5***).
5. Blood samples will be spun to remove plasma (EDTA and green tube) and serum (SST) and stored at recommended temperatures, as per discussion with NHLS until additional analyses.
6. Laboratories will use specimens to conduct laboratory validation of COVID-19 tests. The choice of serological assays used for this study will be determined in consultation with the COVID-19 national diagnostic and reagents working group, and the study Team will synergise testing platforms with those approved by SAHPRA and being rolled out by the National Department of Health. This will facilitate comparison between rapid tests conducted under laboratory versus field conditions. Current three formal serology tests have been approved: Abbot, Roche and Euroimmune. The laboratory-based Luminex assay will also be used to assess levels of neutralising antibody amongst participants who were rapid or PCR positive at baseline, 5-14-day follow-up, 25-30 days follow-up (if feasible) and 3, 6, 9 and 12-month follow-up (if feasible) ***(Aim 12)***.
7. More recently, the Lausanne University Hospital (CHUV) has developed and validated a novel robust and reproducible Luminex-based serological test with high throughput, sensitivity, specificity and rapid turn-around time. This beads-based assay provides a method for evaluating SARS-CoV-2-specific binding antibodies to the full length Spike trimer. The sensitivity of the assay was evaluated using sera from 93 acutely infected SARS-CoV-2 PCR-positive patients with blood sampling at 0-5 days, 6-10 days, 11-15 days and 16-33 days post-onset of symptoms (POS). As anticipated, sera collected during the early stage of the infection (0-5 days POS) had low or undetectable levels of anti-S protein IgG antibodies, with a rate of positivity of 12.5% (1 in 8 subjects; Figure 1B). Seropositivity increased to 42.1% (8/19) at 6-10 days POS and to 91.7%

(33/36) at 11-15 days POS. Almost all patients with symptoms for 16-33 days (28/29; 96.6%) displayed high antibody titers for the S protein trimer.[34] When compared to five commercial assays (Roche pan-Ig ECLIA, Epitope diagnostic ELISA IgG test, EuroImmun ELISA IgG test, Diasorin SARS-CoV-2 IgG kit, Snibe CLIA IgG test) targeting either the N and/or the S proteins, the CHUV Luminex Spike trimer assay exhibited the best accuracy with very high sensitivity in sera from both the acute and post-infection settings, and with the highest sensitivity in the sera of the post-infection phase thus providing more accurate estimates of SARS-CoV-2 infections in the general population (Fenwick 2020). It is to be highlighted that based on these results, the CHUV Luminex S protein trimer assay has been selected for a 90,000 population study in Switzerland. The pseudovirus neutralization assay is widely used to measure antibody neutralization levels for SARS-CoV-2 under biosafety level 2 facilities and was described before by UU..[35] The backbone of the pseudotyped virus comes from the vesicular stomatitis virus (VSV), in which the G gene is replaced with the firefly luciferase reporter gene, and the spike (S) protein from SARS-CoV-2 is incorporated as the membrane protein on the surface of the VSV pseudotyped virus. Inhibition of viral entry into cells by neutralizing antibodies in serum can be measured by measuring the decrease in levels of luciferase signals in the cells. The SARS2-S pseudoviral neutralization assay was tested using 16 sera from SARS-CoV-2 infected donors and 8 sera from pre-pandemic (prior to Nov 2019) adults. All the positive sera completely neutralized the pseudovirus at a dilution of 1/50, whereas pre-pandemic sera did not. The luminex based serological assay and the pseudotyping neutralization assay will be used to test the binding and neutralising antibody responses amongst participants who were rapid or PCR positive at baseline, 5-14-day follow-up, 25-30 days follow-up (if feasible) and 3, 6, 9 and 12 months follow-up (if feasible) ***(Aim 12)***.

8. Specimens that will be compared with each other will be drawn on the same day i.e. they will be paired specimens.

Aim 4: Cost-effectiveness study- data collection procedures

1. We will adopt a provider perspective to calculate economic costs for the health system. Household costs and research costs will be excluded.
2. Individual- and facility-level resource utilisation data will be extracted from participant records (e.g. test conducted), and site-level data monitoring systems (e.g. number of clients tested).
3. Financial costs will be collected from facility-level financial accounts, public documents (e.g. provincial salary scales) and project personnel interviews. Both COVID-19 test specific costs and portion of shared overheads will be determined via direct allocation methods.
4. Time motion studies will be conducted with key personnel to allocate costs to each testing and follow-up activity correctly.
5. Capital and start-up costs will be annualised over 5 years using a 3% discount rate.
6. All costs will be expressed in USD 2019 after adjusting for inflation.

Aim 9: Usability of rapid tests
Mid-way and at the end of the study, all staff performing rapid tests will be asked to complete a questionnaire (CRF: Usability Liekert scale) to obtain their views on the usability of the rapid tests. Each staff member performing rapid tests will complete one CRF for each type of rapid test.

Aim 10: extra nasal swabs will be collected to validate the rapid antigen tests, and compare the results with RT-PCR. This will be undertaken in a limited sample in one or two provinces.

Statistical considerations and data analysis

Sampling and Sample size:

For Aim 1:

The laboratory-based validation of the test kits (***Aim 6***) will be led by Elizabeth Mayne from the NHLS. Samples from this SA COVID-19 POC study and from Lancet laboratories will contribute to the laboratory-based validation. Additionally, we will conduct a field-based validation of the test kits: Phase 1 validation will include a small number of samples, allowing for a 10% error margin. Laboratory-based validation will occur in Elizabeth Mayne's laboratory, and, if needed specimens will be contributed by this study protocol, by Elizabeth Mayne's protocol for laboratory based validation - ***see Appendix 9***.

If this Phase 1 validation succeeds, increasingly stricter non-inferiority margins will then be incorporated in a stepped approach to adjust the number of required participants for the subsequent stage till accrual of up to the next 50 participants i.e. a non-inferiority margin of 7% at the start with reductions at each interim analysis stage till the minimum 3% should there be no stopping.

The sample size calculations for large-scale field-based validation of rapid point of care COVID-19 test kits is based on H. Chu and S.R. Cole (2007) and this method is implemented in R in the package MKpower in the powerdiagnostic.test program. Assuming true sensitivity (specificity) of the rapid test of 98%, where minimal acceptable level of accuracy is >90%, power of 90% and a significance level of 0.05, the estimated sample size at varying non-inferiority margins and prevalence levels is shown in Table 3 below. A 10% adjustment is made to account for loss of samples, issues with shipment etc. If necessary, the number of specimens may be augmented by those already in the database to achieve the required sample size. Given the increase in positivity rate to approximately 20% amongst PUIs tested a sample size of 1805 tested, will yield 361 COVID positive people. Assuming loss of follow-up, approximately 1985 may need to be enrolled in the study for complete data at the follow-up time point. If positivity decreases to 10% then 3971 participants will need to be tested to yield 361 positives.

Table 2 Required sample size at varying non-inferiority margins and levels of prevalence

Sensitivity	delta (non-inferiority margin)	Power	Significance level	Prevalence	Number of positive cases	Number of negatives (controls)	Total to be tested	Total to be tested (Unadjusted for losses)
0,98	0,07	0,9	0,05	0,06	100	1567	1667	1834
0,98	0,07	0,9	0,05	0,04	100	2400	2500	2750
0,98	0,07	0,9	0,05	0,02	100	4900	5000	5500
0,98	0,05	0,9	0,05	0,06	167	2616	2783	3061
0,98	0,05	0,9	0,05	0,04	167	4008	4175	4593
0,98	0,05	0,9	0,05	0,02	167	8183	8350	9185
0,98	0,03	0,9	0,05	0,06	361	5655	16	6618
0,98	0,03	0,9	0,05	0,04	361	8664	9025	9928
0,98	0,03	0,9	0,05	0,02	361	17689	18050	19855
0.98	0.03	0.9	0.05	0.2	361	1444	1805	1985
0.98	0.03	0.9	0.05	0.1	361	2888	3610	3971

For Aim 3a:

Sample size for Aim 2 is dependent on the prevalence of a new cough in the PUI population in the cases and controls. The sample size depends on the positivity rate in participants with and without at new cough (example of symptom). One would expect the positivity rate in new coughs to be higher in this population. Using 10% cases in no new cough versus 20% cases in those with new cough. When the sample size is 764, a logistic regression of a binary response variable, Y, on a binary independent variable, X_1, will have 90.02% power to detect a change in the probability that Y=1 from a value of 0.1 at baseline to 0.20, assuming the R^2 from the regression of X_1 on other Xs is 0.2, 67% of samples are in group X_1=1, and that the two-sided test is made at the 5% level.

For Aim 3b:

We simplify our clinical outcome into a binary outcome by considering hospitalized (Y=1) versus non-hospitalized (Y=0). We also assumed that 20% of underlying COVID-19 cases will required hospitalization and that the sensitivity of the rapid test is 80% When the sample size is 485, a logistic regression of a binary response variable, Y (clinical outcome), on a binary independent variable, X_1 (rapid point of care COVID-19 test), will have 90% power to detect a change in the probability that Y=1 from a value of 0.002 (false negatives that are hospitalized) in the rapid point of care negatives to 0.15 in the rapid point positives, assuming the R^2 from the regression of X_1 on other Xs is 0.2 5% of samples are in group X_1=rapid point of care positive, and that the two-sided test is made at the 5% level.

Aim 5:

720 asymptomatic contacts of RT-PCR positive patients is planned based on a matched case-control design. For this design, the study will have 96% power to detect an odds ratio of 2 assuming 10% of the asymptomatic contacts have positive on the rapid test result, at a 5% level of significance.

The sample size for each objective of this study is tabulated below.

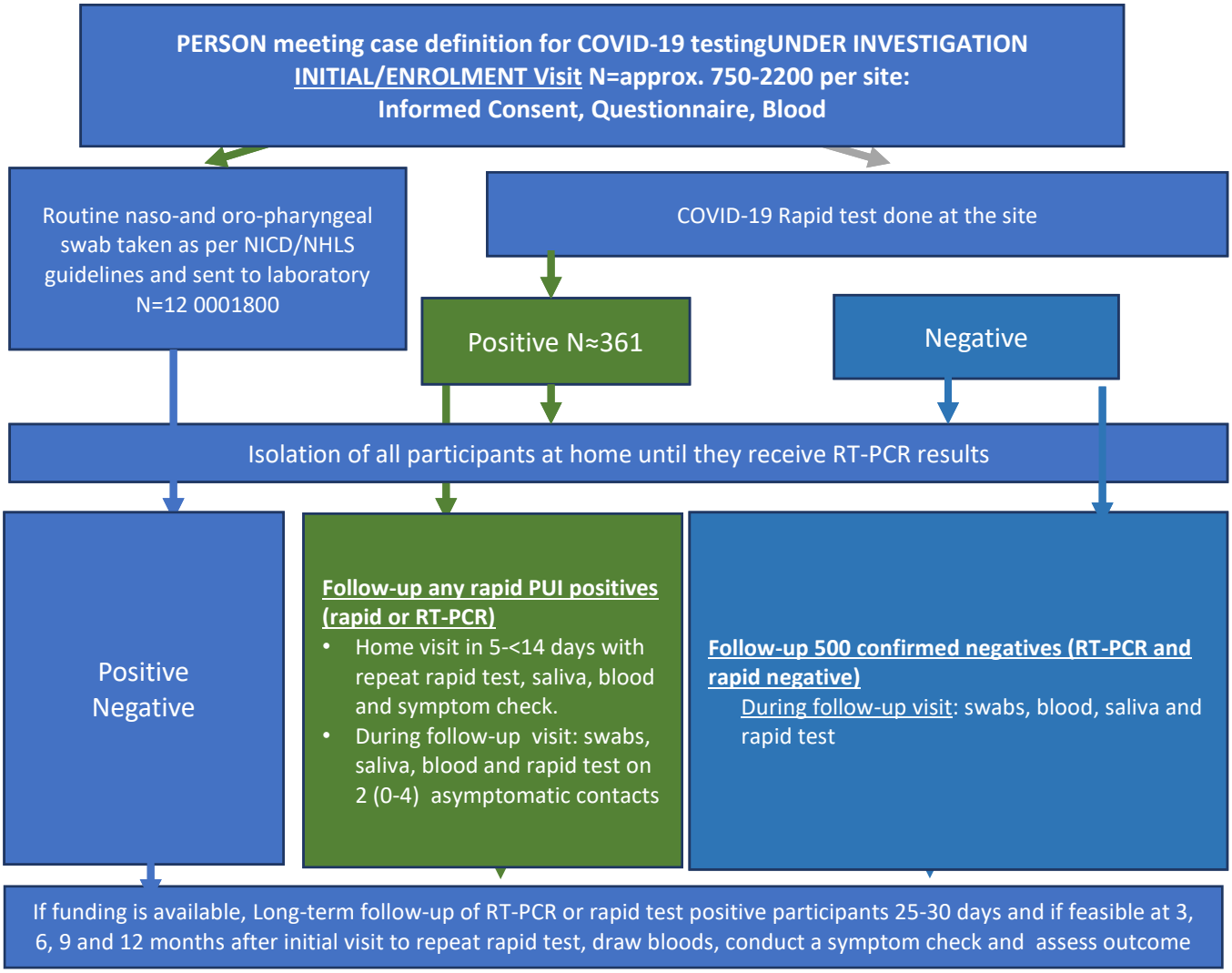
Appendix 10 – which is a separate excel spreadsheet – illustrates the specimens that will be taken at each time point.

Table 3: Sample size for each objective

Aim	Sample size (See Appendix 10)
Aim 1: To investigate whether two rapid point of care COVID-19 tests, 5 to <14 days apart, amongst persons under investigation (PUI) provide a sensitive and specific diagnostic approach in routine public and private health care sites compared to a single reverse transcriptase PCR (RT-PCR) test at initial presentation. This is the main aim of the study.	361 COVID-19 RT-PCR positive participants obtained from testing 1800-4000 PUI depending on positivity rate
Aim 2: To develop a COVID-19 testing algorithm for use in community and routine health care settings in South Africa.	
Aim 3a: To correlate clinical symptoms (fever, cough, shortness of breath, myalgia, sore throat) at presentation and follow-up with rapid point of care COVID-19 test results amongst COVID-19 PUI.	See explanation above A sample size of 764 will be needed. Thus the sample size of 1800 will be sufficient for this aim.
Aim 3b: To correlate clinical outcome at follow-up (hospitalised but never admitted to an intensive care unit (ICU), admitted to ICU but not ventilated, ventilated, symptomatic at home, asymptomatic at home) with rapid point of care COVID-19 test results amongst COVID-19 PUI.	See explanation above. A sample size of 485 will be sufficient to answer this question. Thus the total sample size of 1800 will be sufficient.
Aim 4: To estimate and compare the cost and cost-effectiveness of rapid point of care testing and RT-PCR for COVID-19 by assessing the average cost per person tested and the average cost per person with a confirmed COVID-19 positive test result.	A cost-effectiveness analysis using resource utilization and unit cost data from approximately1800approximately1800 screening tests and RT-PCR tests, and assessing the average cost per person tested, and the average cost per testing outcome for each testing strategy.
Aim 5: To describe the RT-PCR and rapid point of care COVID-19 test results amongst asymptomatic contacts of cases with confirmed (RT-PCR positive) COVID-19 disease, and compare RT-PCR results with rapid point of care test results in this population.	720 asymptomatic contacts of COVID-19 RT-PCR positive patients is planned based on a matched case-control design. For this design, the study will have 96% power to detect an odds ratio of 2 assuming 10% of the asymptomatic contacts have positive on the rapid test result, at a 5% level of significance.
Aim 6: To contribute to the laboratory-based validation of rapid point of care tests for COVID-19.	All samples will be sent to the laboratory
Aim 7: To create a biorepository and contribute to understanding the immune response and viral genetics of SARS-CoV-2	All samples will be sent to the laboratory to create a biorepository of samples
Aim 8: Comparing saliva with swabs	All positive specimens and 361 negative specimens will undergo RT-PCR on saliva versus swabs to establish the utility of saliva for COVID-19 diagnosis
Aim 9: To investigate the ease of use of rapid POC tests for COVID-19 diagnosis	All staff performing rapid tests will be asked to answer questions based on the Liekert scale (see

Aim	Sample size (See Appendix 10)
	CRF: Usability Liekert scale) to assess ease of use. One questionnaire will be completed per test that they used.
Aim 10: To investigate whether one rapid antigen test amongst persons under investigation (PUI) provides a sensitive and specific diagnostic approach in routine public and private health care sites compared to a single reverse transcriptase PCR (RT-PCR) test at initial presentation	Rapid Antigen tests will be conducted using nasal swabs from participants in one or two sites, and not from the entire study population
Aim 11: To characterize the viral genome of SARS-CoV-2 in infected participants	Convenient sample size based on number of participants who consent to an additional naso-pharyngeal (preferred) or nasal swabs at baseline, follow-up of positives and amongst asymptomatic contacts to characterise the viral genome if participants agree
Aim 12: To investigate the utility of laboratory-based serology and neutralization assays to diagnose SARS-CoV-2 and to track post infection binding and neutralizing activity of the antibody responses in order to have insights on the protective nature of the antibody responses.	All specimens from participants at baseline will be tested using laboratory-based assays. Specimens from participants who tested positive at baseline will be tested using laboratory-based serology / immunological assays including neutralization assays when they are followed up at 5-<14 days, 25-30 days, 3, 6, 9 and 12 months.

Study schema and endpoints:



Data analysis and Outcomes:

- **Aim 1:** To investigate whether two rapid point of care COVID-19 tests, 5 to <14 days apart, amongst persons under investigation (PUI) provide a sensitive and specific diagnostic approach in routine public and private health care sites compared to a single reverse transcriptase PCR (RT-PCR) test at initial presentation. ***This is the main aim of the study.***
- **Aim 2:** To develop a COVID-19 testing algorithm for routine health care sites.
- **Aim 3:** To correlate clinical symptoms/status, with rapid point of care COVID-19 test results.
- **Aim 4:** Cost and cost effectiveness analysis.
- **Aim 5:** To describe the RT-PCR and rapid point of care COVID-19 test results amongst asymptomatic contacts of cases with confirmed (RT-PCR positive) COVID-19.
- **Aim 6:** To contribute specimens to the laboratory-based validation of rapid point of care tests for COVID-19.
- **Aim 7:** To create a biorepository and contribute to understanding the immune response (including neutralisation antibodies) and viral genetics of SARS-CoV-2, for the benefit of vaccine design studies.
- **Aim 8:** Saliva versus swabs for diagnosis
- **Aim 9:** To investigate ease of use of rapid tests for COVID-19
- **Aim 10:** To assist with the validation of rapid antigen tests
- **Aim 11:** To characterize the viral genome of SARS-CoV-2 in infected participants
- **Aim 12:** To investigate the utility of laboratory-based serology and neutralization assays to diagnose SARS-CoV-2 and to track post infection binding and neutralizing activity of the antibody responses in order to have insights on the protective nature of the antibody responses

Figure 4: Summarised study schema

Table 4: Study endpoints

Aims	Endpoints	Justification for the endpoints
Aim 1: To investigate whether two rapid point of care COVID-19 tests, 5 to <14 days apart, amongst persons under investigation (PUI) provide a sensitive and specific diagnostic approach in routine public and private health care sites compared to a single reverse transcriptase PCR (RT-PCR) test at initial presentation. This is the main aim of the study.	Sensitivity, specificity, positive predictive value and negative predictive value of single antibody testing and serial antibody testing.	Will determine the extent to which rapid tests would yield false positives or negatives in field settings. Both are important in this context. A false positive would be isolated with confirmed cases and is at high risk of becoming positive. A false negative will be released into the community and will fuel transmission. The results will inform whether a rapid serological assay can be used instead of RT-PCR.
Aim 2: To develop a COVID-19 testing algorithm for use in community and routine health care settings in South Africa.	Algorithm developed to guide testing for SARS-CoV-2 at primary health care level.	Rapid, valid diagnosis of SARS-CoV-2 infection will limit community spread as contact tracing can be initiated earlier, and infected cases can be isolated earlier.
Aim 3: To correlate clinical symptoms/status, with rapid point of care COVID-19 test results.	***Outcomes:*** • Hospitalised but never admitted to an intensive care unit (ICU), • Admitted to ICU but not ventilated, • Ventilated, • Symptomatic at home, • Asymptomatic at home. ***Immunological correlates:*** • Presence or absence of viral particles in naso- / oro-/ pharyngeal samples and antibody or antigen in blood samples in symptomatic patients at initial and follow-up visits.	These results help us understand antibody development and persistence in COVID-19 disease in the South African context where high rates of TB, HIV infection, malnutrition and non-communicable diseases co-exist with poverty or affluence.
Aim 4: To estimate and compare the costs and cost-effectiveness of rapid point of care testing versus RT-PCR for COVID-19	• Average cost per person tested, • Average cost per person with a confirmed COVID-19 positive result.	If Aim 1 is true then Aim 4 will provide guidance as to whether policymakers should include rapid point of care tests in the COVID-19 diagnostic algorithm.

Aims	Endpoints	Justification for the endpoints
Aim 5: To describe the RT-PCR and rapid point of care COVID-19 test results amongst asymptomatic contacts of cases with confirmed (RT-PCR positive) COVID-19 disease and compare RT-PCR results with rapid point of care test results in this population.	***Laboratory correlates:*** RT-PCR test result and presence or absence of antibody in asymptomatic contacts at tracing.	These results will inform the community-level diagnostic algorithm for contacts, providing information on whether RT-PCR or serological assays are the preferred option.
Aim 6: To contribute to the laboratory-based validation of rapid point of care tests for COVID-19.	Laboratory-based validation conducted, and sensitivity, specificity and predictive values determined to inform the Target Product Profile in-country.	Results will inform the registration of national rapid point of care testing platforms for COVID-19.
Aim 7: To create a biorepository and contribute to understanding the immune response and viral genetics of SARS-CoV-2	Virus sequenced and correlated with the immune response	Will inform the development of vaccine products and the design of vaccine trials
Aim 8: To compare saliva with swabs for diagnosis	PCR on saliva versus swabs Serology on saliva versus swabs	Will inform specimens needed for diagnosis
Aim 9: To investigate the ease of use of rapid POC tests for COVID-19 diagnosis	5-point Liekert scale assessing ten aspects of the rapid test (see CRF: Usability Liekert scale)	This will provide information on how easily each test can be used by health care personnel
Aim 10: To assist with validation of rapid antigen tests	Sensitivity, specificity, positive predictive value and negative predictive value of single antibody testing and serial antibody testing.	Will inform validation of rapid tests
Aim 11: To characterize the viral genome of SARS-CoV-2 in infected participants	Description of viral genome	Will inform phylogenetic analyses, vaccine development and understanding of SARS-CoV-2 transmissibility
Aim 12: To investigate the utility of laboratory-based serology and neutralization assays to diagnose SARS-CoV-2 and to track post infection binding and neutralizing activity of the	Serological and immunological responses including neutralizing titres	To inform an understanding of clinical disease progression and serological/immunological response

Aims	Endpoints	Justification for the endpoints
antibody responses in order to have insights on the protective nature of the antibody responses		

Data analysis:

Data will be exported to STATA version 16 (Stata Corp., College Station, TX, USA) for analyses. P-values less than 0.05 will be considered statistically significant. For descriptive statistics frequencies and proportions will be used to summarize categorical variables and for continuous variables, the mean with standard deviation or median with interquartile range (IQR) depending on normality of the data. Distributions of categorical variables between classes will be compared using Chi-squared or Fisher's exact test. Comparisons of continuous variables between the two groups will be performed with a t-test or a Wilcoxon rank-sum test depending on their distributions.

Sensitivity and specificity of two rapid serological tests (divided by the timing of blood draw) compared to RT-PCR will be calculated including positive predictive values (PPV) and negative predictive values (NPV) with 95% confidence intervals presented for each point estimate.

The diagnostic performance of the rapid POC testing algorithms will be evaluated by the sensitivity, specificity, PPV, NPV and the diagnostic odds ratio. The predictive accuracy of the testing algorithms will be compared with receiver operating characteristics (ROC) curve analysis. Likelihood ratio test (LR) will be used to assess how good a specific rapid POC test is and to will also be used in selecting an appropriate diagnostic test(s) or sequence of tests as part of the overall algorithm. The LR have advantages over sensitivity and specificity because it is less likely to change with the prevalence of the COVID-19, and can be calculated for several levels of the symptom/sign or test and can be used to combine the results of multiple diagnostic tests and calculate the post-test probability for testing positive.

The association between the clinical symptoms (fever, cough, shortness of breath, myalgia, sore throat) at presentation and rapid point of care COVID-19 test results (algorithm) amongst COVID-19 PUI will be evaluated using logistic regression. Odds ratios for each symptom will be estimated and the model will include confounders such as age, gender and HIV status and 95% confidence intervals will be calculated.

To evaluate the association between the rapid point of care COVID-19 test results (algorithm) and disease outcome an ordinal logistic regression model will be used with age, gender and HIV status in the model as covariates. The odds ratio for the rapid test indicator will be calculated with a 95% confidence interval. The study does not have adequate power for this inference and will only be done for a hypothesis-generating purpose. Descriptive tables of the

disease outcome by rapid point of care COVID-19 test results (algorithm) overall and by subgroups will be done.

A matched case-control analysis, using conditional logistic regression, will be performed to evaluate the utility of the rapid point of care COVID-19 test results (algorithm) in asymptomatic household contacts. The regression model will be adjusted for age, gender and HIV status.

For the cost-effectiveness analysis, average cost per person tested and average cost per person with a confirmed COVID-19 positive test result will be estimated and compared per testing strategy. We will group and compare results by facility type (i.e. mobile vs. decentralised clinic). We will rank the effectiveness of each testing strategy and determine which strategy was most cost-effective (i.e. had the lowest provider cost per person with a confirmed COVID-19 positive test result). Markov modelling will be used to calculate lifetime costs, life years, incremental cost-effectiveness ratios and quality-adjusted life years. Uncertainty will be assessed through probabilistic sensitivity analysis on all outcome variables. Alternative scenarios will be constructed to enhance generalizability.

Ethical considerations

All results will be returned to patients through routine systems. All patients will receive routine care in accordance with their symptoms and NICD or institutional guidelines. Informed consent (Appendix 8) to participate in the study will be obtained from each patient, or the asymptomatic contact of a confirmed case to conduct a short interview using a CRF, sample collection via nasopharyngeal swab and finger prick, venesection and a home-based visit. Institutional approval will be obtained from the SAMRC, and reciprocal approval will be obtained by institutional IRBs / research ethics committees. Patient CRFs will be programmed electronically and completed electronically as far as is possible to reduce the exchange of paper - which may be contaminated with SARS-CoV-2. The results of all rapid tests will be discussed with participants (Appendix 12). All COVID-positive and selected negative participants will receive home visits and will not be asked to travel to facilities for repeat sampling, to avoid breaking their isolation, unless special arrangements are made to assist with transport and transport the participant to a safe clinical venue. Social distancing will be maintained as much as possible during all interactions between staff and between staff and participants. Details about protecting study staff and participants from COVID-19 are provided on page 17, in the data collection methods section.

4.0 STUDY TIMELINE

- **30 March 2020-July 2020:** Protocol development
- **10th April 2020**: Submission of version 1 to the ethics committee
- **September 2020-June 2021:** Accrual of positive cases

- **December 2020 ongoing:** Follow-up from date of first recruitment
- **June 2021:** Dissemination of first results with continued release thereafter

Also see ***Appendix 10***

5.0 BUDGET

See excel spreadsheet

6.0 REPORTING

Results will be reported to the Ministerial Advisory Committee and in peer-reviewed journals

7.0 REFERENCES:

1. Gorbalenya, AE., Baker, SC., Baric, RS., et. al., *Severe acute respiratory syndrome-related coronavirus: the species and its viruses—a statement of the Coronavirus Study Group.* bioRxiv, 2020: https://doi:10.1101/2020.02.07.937862 (preprint).
2. Phelan, A., R. Katz, and L. Gostin, *The novel coronavirus originating in Wuhan, China: challenges for global health governance.* JAMA, 2020: p. DOI:10.1001/jama.2020.1097.
3. Khan, S., et al., *The emergence of a novel coronavirus (SARS-CoV-2), their biology and therapeutic options.* J Clin Microbiol, 2020.
4. Perlman, S. and A.A. Dandekar, *Immunopathogenesis of coronavirus infections: implications for SARS.* Nat Rev Immunol, 2005. **5**(12): p. 917-27.
5. Yip, C.C., et al., *A six-year descriptive epidemiological study of human coronavirus infections in hospitalized patients in Hong Kong.* Virol Sin, 2016. **31**(1): p. 41-8.
6. Zumla, A., D.S. Hui, and S. Perlman, *Middle East respiratory syndrome.* Lancet, 2015. **386**(9997): p. 995-1007.
7. Novel Coronavirus Pneumonia Emergency Response Epidemiology, T., *[The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China].* Zhonghua Liu Xing Bing Xue Za Zhi, 2020. **41**(2): p. 145-151.
8. World Health Organization, *Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations: Scientific brief - 29 March 2020.* 2020: Geneva. Available from https://www.who.int/publications-detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations. Accessed 9 April 2020.
9. Lu, R., et al., *Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding.* Lancet, 2020. **395**(10224): p. 565-574.
10. Hoffmann, M., et al., *SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.* Cell, 2020.
11. Wan, Y., et al., *Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus.* J Virol, 2020. **94**(7).
12. Qian, Z., et al., *Innate immune response of human alveolar type II cells infected with severe acute respiratory syndrome-coronavirus.* Am J Respir Cell Mol Biol, 2013. **48**(6): p. 742-8.
13. Wang, D., B. Hu, and C. Hu, *Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. .* JAMA, 2020: p. DOI:10.1001/jama.2020.1585.
14. Chen, N., et al., *Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. .* The Lancet, 2020. **395**: p. 507-513.
15. Huang C, W.Y., Li X, et al. , *Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. .* The Lancet, 2020(395): p. 497-506.
16. Cascella, M., et al., *Features, Evaluation and Treatment Coronavirus (COVID-19)*. 2020.
17. Wu, Z. and J. McGoogan, *Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention.* JAMA, 2020.
18. Zhou, F., et al., *Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study.* The Lancet, 2020: p. https://doi.org/10.1016/S0140-6736(20)30566-3.
19. Tseng, C.T., et al., *Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus.* PLoS One, 2012. **7**(4): p. e35421.
20. Zhao, J. and et al., *Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019.* Clin Infect Dis, 2020.
21. National Institute for Communicable Diseases (NICD)/ National Health Laboratory Services (NHLS) - Centre for Respiratory Diseases and Meningitis and Outbreak Response - Division of Public Health Surveillance and Response and S.A. National Department of Health, .

Coronavirus disease 2019 (COVID-19) caused by a Novel Coronavirus (SARS-CoV-2): Guidelines for case-finding, diagnosis, management and public health response in South Africa - Version 2. 2020: Johannesburg.

22. World Health Organization, *Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: Interim guidance*. 2020: Geneva.
23. Wanga, W., et al., *Detection of SARS-CoV-2 in different types of clinical specimens.* JAMA, 2020: p. online 11 March 2020.
24. National Department of Health and National Insttute for Communicable Diseases, *Clinical management of suspected or confirmed COVID-19 disease, version 3*. 2020.
25. Guo, l., et al., *Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19).* Clinical Infectious Diseases, 2020: p. https://doi.org/10.1093/cid/ciaa310.
26. Backer, J.A., D. Klinkenberg, and J. Wallinga, *Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20-28 January 2020.* Euro Surveill, 2020. **25**(5).
27. Zhao, J., et al., *Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019.* Clin Infect Dis, 2020.
28. Thevarajan, I., et al., *Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19.* Nature Medicine, 2020.
29. National Institute for Communicable Diseases and National Department of Health, *Coronavirus disease 2019 (COVID-19) caused by a Novel Coronavirus (SARS-CoV-2): Guidelines for case-finding, diagnosis, and public health response in South Africa*. 2020.
30. National Department of Health, *National HIV testing services: policy and guidelines*, H.a. AIDS, Editor. 2015: Pretoria.
31. Rothe, C., et al., *Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany.* N Engl J Med, 2020. **382**: p. 970-971 doi: 10.1056/NEJMc2001468.
32. Song, J.-Y., J.-G. Yun, and J.-Y. Noh, *Covid-19 in South Korea — Challenges of Subclinical Manifestations.* New Engl J Med, 2020: p. doi: 10.1056/NEJMc2001801.
33. Carmona, C., et al., *Separation of plasma from whole blood using the cobas® Plasma Separation Card: a compelling alternative to dried blood spots for the quantification of HIV-1 viral load.* Journal of Clinical Microbiology, 2019. **57**(4): p. doi: 10.1128/JCM.01336-18
34. Fenwick, C., et al., *Changes in SARS-CoV-2 Spike versus Nucleoprotein Antibody Responses Impact the Estimates of 2 Infections in Population-Based Seroprevalence Studies. .* J Virol, 2020: p. doi: 10.1128/JVI.01828-20.
35. Wang, C., et al., *A human monoclonal antibody blocking SARS-CoV-2 infection. .* Nat Communication, 2020. **4**(11): p. 2251: doi: 10.1038/s41467-020-16256-y.

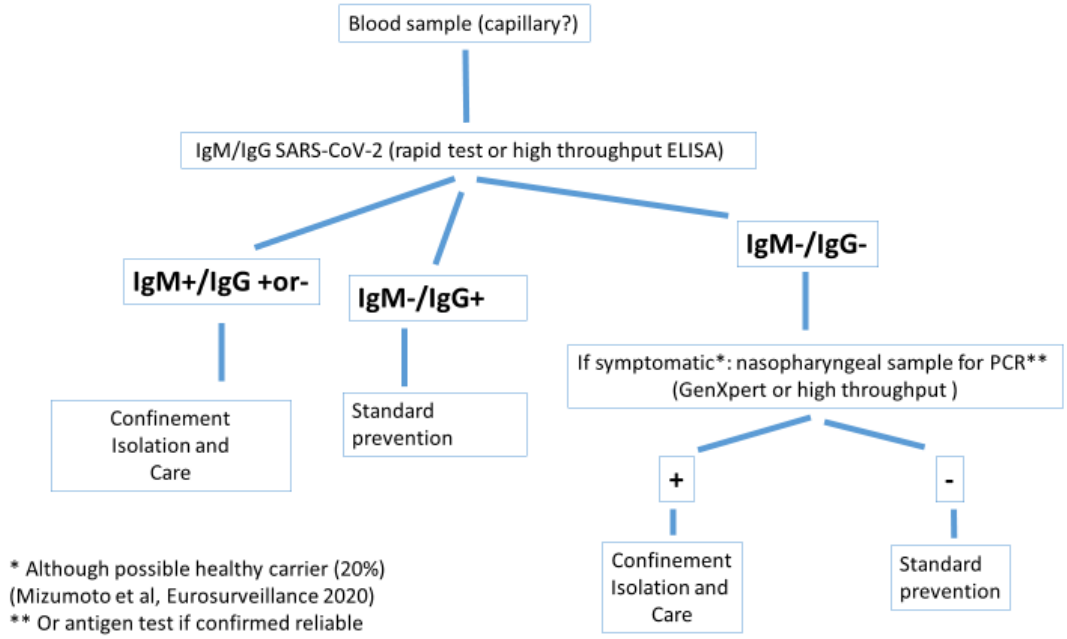
APPENDIX 1: TABLE OF TESTS PROPOSED FOR USE FOR COVID-19 TESTING

(Data from Elizabeth Mayne, NHLS)

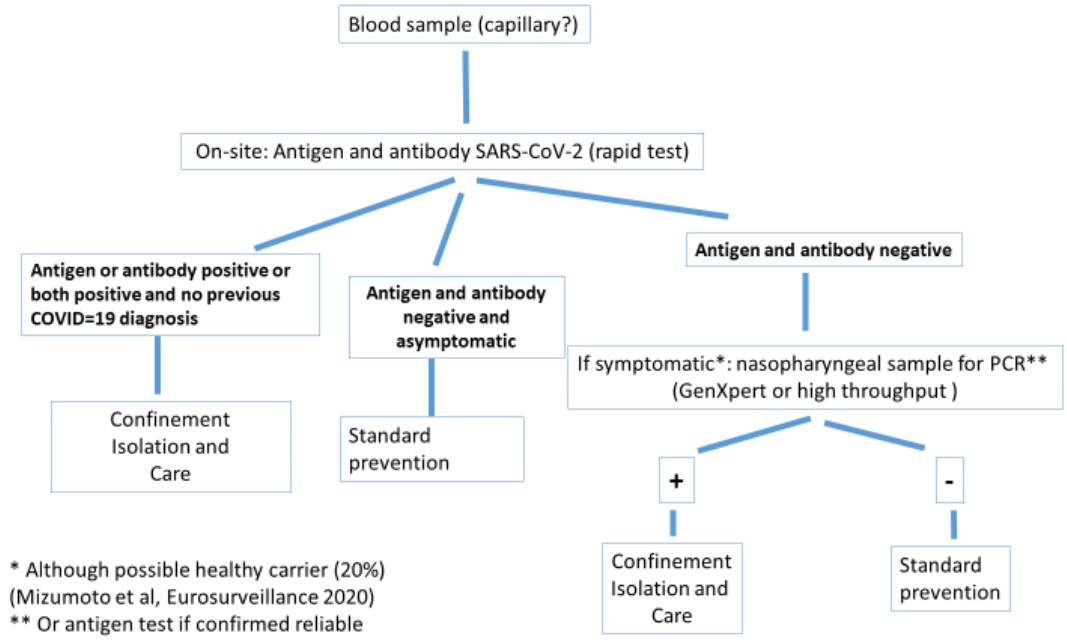
Name of assay	Sensitivity in leaflet	Specificity in leaflet	Sensitivity at NHLS	Specificity at NHLS	Comment
Innovita	87.3	100	56% IgG; 9%IgM	100	Not approved by SAHPRA
Wondfo	Not available	Not available	68-92.3%	87-100	IgG only
Dynamiker	Not available	Not available	44-53.8 (IgG); 52% (IgM)	89.2-100	Not approved by SAHPRA
CTK Biotech	96.9%	99.4%	66-75% (IgG) and 58.3 (IgM)	80.6-100	Not approved by SAHPRA
Biomedonics	88.6%	90.63%	70-95% (IgG) and 67% (IgM)	76-100	Not approved by SAHPRA
Bioeasy	Not stated	Note stated	32-61% (IgG) and 44% IgM	100	Not approved by SAHPRA
Sugentech	91%	96.7%	44-53.8% (IgG) and 64% (IgM)	89.2-100	Not approved by SAHPRA
Sinocare			56-61.5%	80-95.7	Not approved by SAHPRA
Hanzhou Alltest			56-73.3 (IgG) and 20% (IgM)	91.4-100	Not approved by SAHPRA
Genrui			68-94% (IgG) and 68% (IgM)	91.4-100	Trying to get S21 approval for SAMRC study
Biosynex			84.3-98.2% (IgG) and 64.3-69% (IgM)	92.6-100	Trying to get S21 approval for SAMRC study
Orient Gene			90.2-100% (IgM and IgG)	91-100	Approved by SAHPRA – will be used in SAMRC study

APPENDIX 2: POTENTIAL ALTERNATE DIAGNOSTIC ALGORITHMS FOR COVID-19 TESTING

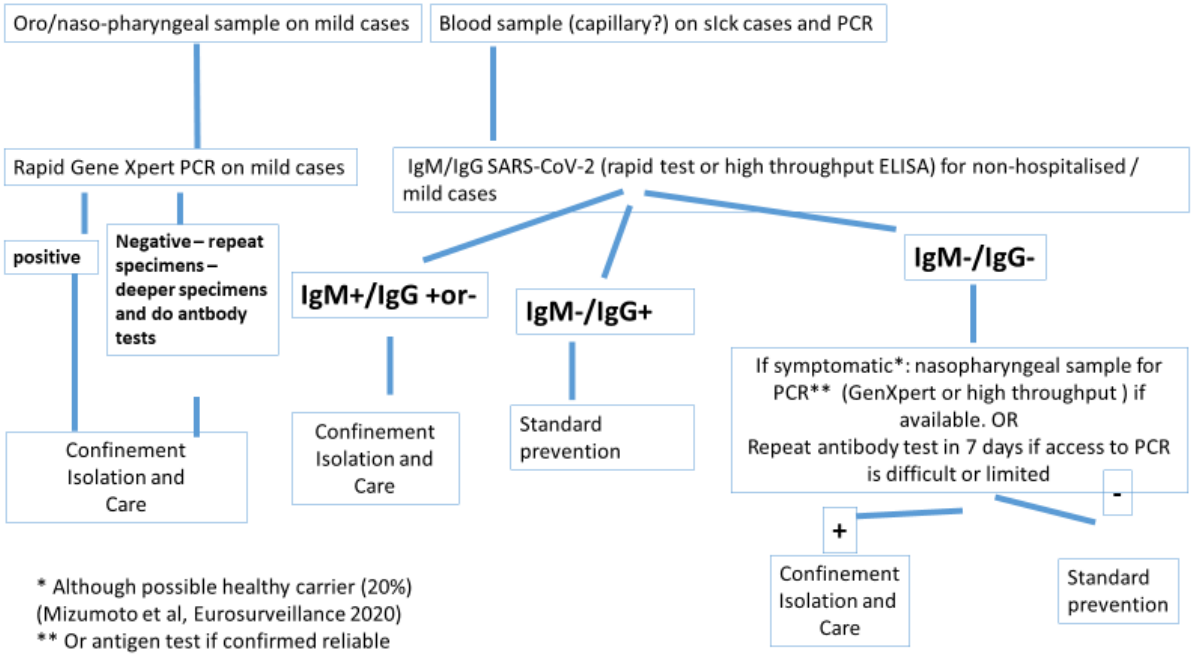
Strategy 1:



Strategy 2:



Strategy 3:


Oro/naso-pharyngeal sample on mild cases
Blood sample (capillary?) on sIck cases and PCR
Rapid Gene Xpert PCR on mild cases
IgM/IgG SARS-CoV-2 (rapid test or high throughput ELISA) for non-hospitalised / mild cases
positive
Negative – repeat specimens – deeper specimens and do antbody tests
IgM+/IgG +or-
IgM-/IgG+
IgM-/IgG-
Confinement Isolation and Care
Confinement Isolation and Care
Standard prevention
If symptomatic*: nasopharyngeal sample for PCR** (GenXpert or high throughput) if available. OR
Repeat antibody test in 7 days if access to PCR is difficult or limited
+
-
Confinement Isolation and Care
Standard prevention
* Although possible healthy carrier (20%) (Mizumoto et al, Eurosurveillance 2020)
** Or antigen test if confirmed reliable

APPENDIX 3: NATIONAL INSTITUTE OF COMMUNICABLE DISEASES SUSPECTED COVID-19 DISEASE CASE DEFINITIONS AND SYMPTOMS OF COVID-19

All participants recruited for testing at the study sites will be enrolled if they meet the current NICD / NHLS criteria for priority SARS-CoV-2 testing.

These case definitions / priority groups may change and the study will adjust enrolment to remain within the NICD/NHLS priority testing criteria. This is for 2 reasons (i) RT-PCR results are needed within 48 hours of specimen collection to arrange follow-up visits – thus priority groups will be needed for enrolment (ii) The study budget cannot afford to pay for RT-PCR testing for groups not covered by the NHLS testing criteria.

As at the 3rd July 2020 the NICD case definition was as follows:

Suspected COVID-19 case definition
Any person presenting with an acute (≤14 days) respiratory tract infection or other clinical illness compatible with COVID-19, or an asymptomatic person who is a close contact[a] of a confirmed[b] case

- Symptoms include ANY of the following respiratory symptoms: cough, sore throat, shortness of breath, anosmia (loss of sense of smell) or dysgeusia (alteration of the sense of taste), with or without other symptoms (which may include fever, weakness, myalgia, or diarrhoea)
- Note: Asymptomatic close contacts should not be routinely tested despite meeting the suspected case definition. However, testing may be indicated in certain circumstances (e.g. institutions such as care homes)

[a]**Close contact**: A person having had face-to-face contact (≤1 metre) or been in a closed space with a confirmed case for at least 15 minutes. This includes, amongst others, all persons living in the same household as a case, and people working closely in the same environment as a case. Healthcare workers or other people providing direct care for a case, while not wearing recommended personal protective equipment or PPE (e.g., gowns, gloves, N95 respirator, eye protection). A contact in an aircraft sitting within two seats (in any direction) of the case, travel companions or persons providing care, and crew members serving in the section of the aircraft where the case was seated.
[b]**Confirmed case**: A person with laboratory confirmation of SARS-CoV-2 infection (using an RT-PCR assay), irrespective of clinical signs and symptoms. Symptomatic cases are considered infectious from 2-3 days before symptom onset to 14 days after symptom onset.

Additionally, there is a proposed revised testing strategy: **REVISED PRIORITISED TESTING STRATEGY GUIDANCE**
Revised June 19th 2020

This guideline document provides a prioritised approach to enable South Africa to utilise its Covid-19 testing resources (*see testing capacity section below*) to support an integrated approach to priority needs of South Africa's Covid-19 response.

Note that testing and reporting results as rapidly as possible is a key strategy to both mitigate as well as rapidly treat patients with confirmed COVID-19 disease. Every effort must be made to ensure that those that require a test for clinical reasons receive a test and the result as rapidly as possible. The target is to ensure that those that are hospitalised and those that are supporting those that are hospitalised with COVID symptoms and or have co-morbidities are of highest priority and get a test result within 24-48 hours. Note, as test kits become more available the list of those that may be tested will increase – this includes testing for purposes of surveillance. To inform the targeted approach, the NHLS should allocate a number of tests to each province based on the burden of disease. Provincial health departments are then responsible for ensuring that tests are correctly prioritised based on the prioritisation outlined in the table below.

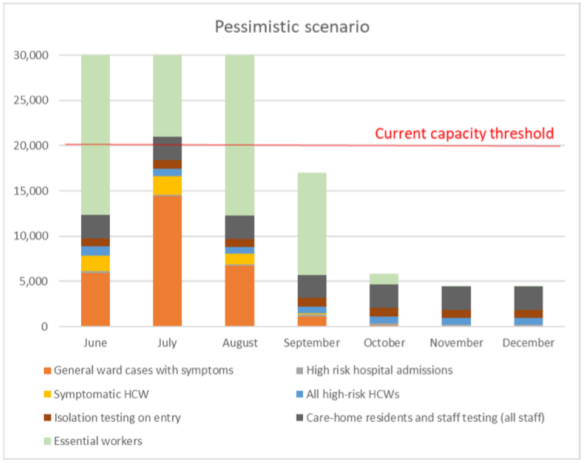
The following details guidance to clinicians, laboratories and health sector managers in both the public and private health sectors for how to prioritise targeted testing:

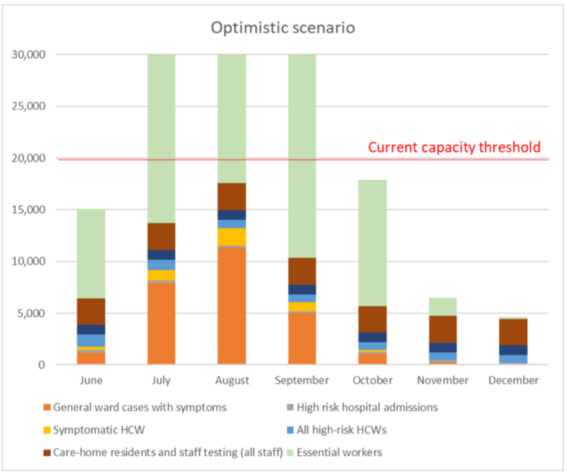
Level of Priority		Priority Groups for Testing	Rationale
High ●	Maximise clinical benefit and preserve healthcare capacity	Symptomatic hospitalised patients	Hospitalised patients with symptoms need to be tested in order to provide appropriate clinical care. Hospitalised patients are also prioritised to mitigate the risk of transmission to other vulnerable patients and health care workers in hospital settings.
		Symptomatic health care workers Asymptomatic hospital staff working with high-risk patients (i.e. immunosuppressed patients such as oncology, transplantation patients)	Provision of health care relies on the availability of adequate health care workers, and it is therefore critical that health care worker capacity is preserved (especially during the peak). Health care workers are at greater risk of exposure to the virus, and may transmit infection to both other health care workers and patients. Testing of asymptomatic health care workers working in close contact with high-risk patients is also recommended, as any transmission within these settings is likely to be associated with both high transmission and high mortality rates.
		Symptomatic care home residents and staff	Experience from other countries indicates that a high proportion of deaths have occurred in care home settings. It is therefore recommended that residents and staff in care homes are tested in order to limit transmission in these settings.

Medium	Reduce the risk of transmission	Symptomatic clients identified in designated hotspots	To stop transmission, targeted screening followed by testing should be conducted in identified hotspot areas and high-risk settings during outbreaks. The goal is to rapidly identify and manage cases to contain transmission.
		Close contacts of confirmed cases during outbreaks in high risk settings (e.g. health facilities).	
	Preserve essential services	Symptomatic essential service workers	Testing of essential service workers limits transmission and allows for early return to work, thus preserving delivery of essential services.
	Identify those most at risk of developing severe disease	Symptomatic clients with risk factors (over 55 years or with significant co-morbidities)	Identification of clients who are at high risk of developing severe disease will assist with clinical management.
		Symptomatic clients who are unable to self-isolate at home (before admission to an isolation facility)	People with mild symptoms should be advised to self-isolate at home. However, those who cannot self-isolate at home may require admission to an isolation facility – but only confirmed cases should be admitted to such facilities. Testing of such clients is therefore required in order to decide if the client should be admitted to the isolation facility, or can safely remain at home.
Low	Differentiates between suspected and confirmed cases	Symptomatic clients presenting at health facilities. Symptomatic contacts of confirmed cases. Symptomatic clients identified though workplace or other screening.	Testing is recommended, but will not affect immediate individual client management. If testing is not available, patients should self-isolate at home for 14 days or as per national protocol.
No testing	Testing is not recommended	Asymptomatic contacts of positive cases (including close contacts). Routine asymptomatic employee testing for the purposes of returning to work. Routine testing of selected groups (e.g. sportspeople) Patients who meet the de-isolation criteria as outlined in the NDOH/NICD Clinical Guidelines should not be re-tested.	Testing of these groups does not contribute towards COVID-19 containment. Asymptomatic contacts should self-monitor, and testing should be considered if they become symptomatic.
Surveillance	To be considered when antibody/serological tests are available		

When these samples are taken the health care worker should identify the sample per level of priority category on the request form by using colour coded stickers (as shown above), placed on the request form. If there are limited supplies of test kits, the laboratories will prioritise the samples according to the hierarchy of the categories. That is Priority High will be given first preference, followed by Priority Medium, etc.
Note that the implementation of the testing prioritisation strategy will require extensive communication, as well as close liaison between health workers/clinicians (who order tests) and laboratories. As noted above, NHLS has undertaken to provide stickers which will indicate the priority assigned to each specimen, but those requesting the tests will be responsible for assigning the correct priority and affixing the correct colour sticker to each specimen.
Testing Capacity *(based on data as of 1st June 2020; figures to be updated with more recent data)*

Based on recent reports, as of 23 Mary 2020 the combined daily SARS-CoV-2 testing capacity is 21,300 tests per day - 10,400 per day in the private sector, and 10,900 tests per day in thce public sector. When viewing modeled projections for testing:





Under the **pessimistic scenario**, daily tests required for the high priority testing populations tally to between 931 and 17,475 tests per day, with between 4,445 and 151,001 daily tests required for both the high and medium testing populations. The largest test volumes per testing population are required for symptomatic essential workers followed by general ward patients with symptoms and care-home residents and staff. This means that under the current testing capacity, in the Surge months (June, July and August), only the high priority testing populations plus some isolation facilities and some essential workers can be accommodated, while in the other months (September and onwards), all high and medium priority testing populations can be served.

Under the **optimistic scenario,** daily test volumes are lower than in the pessimistic scenario throughout. Volumes required for the high priority testing populations tally to between 962 and 14,037 tests per day, with between 4,635 and 123,866 daily tests required for both the high and medium testing populations. As before, the largest test volumes per testing population are required for symptomatic essential workers, followed by general ward patients with symptoms and care-home residents and staff. In the optimistic scenario, under both the current testing capacity, only the high priority testing populations plus care homes,

isolation facilities and some essential workers can be accommodated in the surge months (July and August), while in the other months (June, and September and onwards), all high and medium priority testing populations can be served.

APPENDIX 4: NATIONAL INSTITUTE OF COMMUNICABLE DISEASES GUIDELINE FOR NASO- AND ORO-PHARYNGEAL SAMPLE COLLECTION

Coronavirus disease 2019 (COVID-19) Quick Reference for Health Workers

National Institute for Communicable Diseases (NICD)

24-hour hotline number: 0800 11 1131|066 562 4021

Clinical presentation and management of suspected cases

The clinical spectrum of COVID-19 ranges from an asymptomatic or mild flu-like illness to a severe pneumonia requiring critical care. The most common clinical signs and symptoms are fever and cough with a few patients presenting with difficulty in breathing and bilateral infiltrates on chest X-rays. Treatment is supportive. The differential diagnosis for this syndrome is broad. Consider the possibility of influenza (Southern Hemisphere influenza season will begin in May or June), bacterial pneumonia, tuberculosis, *Pneumocystis jirovecii* (PCP) if immunosuppressed, and manage accordingly. Refer to NICD website https://www.nicd.ac.za/diseases-a-z-index/covid-19/covid-19-guidelines/clinical-management-of-suspected-or-confirmed-covid-19-disease/

Suspected COVID-19 case definition

Any person presenting with an acute (≤14 days) respiratory tract infection or other clinical illness compatible with COVID-19, or an asymptomatic person who is a close contact[a] of a confirmed[b] case

- Symptoms include ANY of the following respiratory symptoms: cough, sore throat, shortness of breath, anosmia (loss of sense of smell) or dysgeusia (alteration of the sense of taste), with or without other symptoms (which may include fever, weakness, myalgia, or diarrhoea)
- Note: Asymptomatic close contacts should not be routinely tested despite meeting the suspected case definition. However, testing may be indicated in certain circumstances (e.g. institutions such as care homes)

[a]**Close contact**: A person having had face-to-face contact (≤1 metre) or been in a closed space with a confirmed case for at least 15 minutes. This includes, amongst others, all persons living in the same household as a case, and people working closely in the same environment as a case. Healthcare workers or other people providing direct care for a case, while not wearing recommended personal protective equipment or PPE (e.g., gowns, gloves, N95 respirator, eye protection). A contact in an aircraft sitting within two seats (in any direction) of the case, travel companions or persons providing care, and crew members serving in the section of the aircraft where the case was seated.

[b]**Confirmed case**: A person with laboratory confirmation of SARS-CoV-2 infection (using an RT-PCR assay), irrespective of clinical signs and symptoms. Symptomatic cases are considered infectious from 2-3 days before symptom onset to 14 days after symptom onset.

Infection prevention and control (IPC) (Page 10)

1. Patients meeting the suspected case definition should be asked to wear a surgical mask once identified
2. Suspected case should be isolated and evaluated in a private room
3. Limit patient movement (e.g., portable X-ray)
4. HCWs should wear appropriate PPE:
   - Eye protection (goggles or visor)
   - Gloves
   - Apron or gown
   - Surgical mask for general patient interactions, or N95 respirator (or equivalent, e.g., FFP2 mask) for aerosol-generating procedures such as specimen collection

Specimens required for SARS-CoV-2 PCR testing (Page 10/11 & App 3/4)

Collecting a good quality specimen is vital

1. Upper respiratory tract specimen for all patients
   - A single nasopharyngeal swab is the preferred sample type. When not possible, a single nasal mid-turbinate swab, nasal or oropharyngeal swab may be collected
   - Transport and store swabs in universal/viral transport medium (UTM) or sterile saline, between 2-8°C. If UTM is not available, use dry swabs in a sterile tube. Dry swabs can be sent at ambient temperature, but must reach the laboratory within 2 days
2. Lower respiratory tract specimen when available
   - Sputum (if produced – do NOT induce), tracheal aspirates or bronchoalveolar lavage
   - Transport in standard specimen container. Does not require UTM

Note: lower respiratory tract samples may have higher sensitivity than upper respiratory tract samples and ***should additionally be collected for severe cases***

Case notification (for all confirmed cases) (Page14)

COVID-19 is classified as a Category 1 notifiable medical condition (NMC). Therefore, notification of probable and confirmed cases should be made immediately, using the NMC web portal, mobile app (preferred methods), or NMC paper-based reporting form. Contact tracing will be initiated for confirmed COVID-19 cases.

Available from https://www.nicd.ac.za/wp-content/uploads/2020/07/NICD_DoH-COVID-19-Guidelines_Final_3-Jul-2020.pdfpdfpdfpdf

APPENDIX 5: PARTICIPANT CRFS

See separate attachments for the Participants CRFs:

1. **SA COVID-19 POC STUDY-PARTICIPANT ENROLMENT CRF (PEC-1), Version 4.0, 19 Oct 2020**
2. **SA COVID-19 POC STUDY-PARTICIPANT FOLLOW-UP CRF (FV-1), Version 4.0, 19 Oct2020**
3. **SA COVID-19 POC STUDY-PARTICIPANT SCREENING AND CONSENT CRF (PSC-1), Version 4.0, 19 Oct 2020**
4. **SA COVID-19 POC STUDY-ASYMPTOMATIC AT HOME CRF (AC-1), Version 4.0, 19 OctSep 2020**
5. **SA COVID-19 POC STUDY- ASYMPTOMATIC CONTACT SCREENING AND CONSENT CRF (AC-SC-1), Version 4.0, 19 Oct 2020**

OTHER STUDY TOOLS

6. **SA COVID-19 POC STUDY – USABILITY LIEKERT SCALE CRF, Version 3.0 (first version), 7 Sep 2020 – for staff to complete to assess usability of rapid test kits**
7. **Tool for time-motion study tool for staff to complete**

APPENDIX 6: NICD PERSON UNDER INVESTIGATION FORM

See Separate attachment in pdf

APPENDIX 7: SAMPLE COLLECTION FORM FOR BLOOD AND SWABS

The routine NICD/NHLS form or a form recommended by NHLS will be used.

APPENDIX 8: INFORMATION SHEETS AND CONSENT FORMS

See separate attachment for information sheets and consent forms:

APPENDIX 9: ADDITIONAL INFORMATION ON SAMPLE SIZE

VALIDATION OF NEW TESTS IN THE LABORATORY

Stored samples from this study and Lancet laboratories will be used to conduct equivalence testing for the point of care IgG/IgM tests and ELISA/CLIA based systems identified. Potential antigen tests will also be used, and equivalence validation will be conducted, to compare with RT-PCR.

In the laboratory validation protocols compare equivalence and precision of the new test (test A) versus the existing test or "gold standard" or test B. Laboratory practice for a validation protocol is firstly to take 40 samples (mixed positive and negative) and compare the new test A to the existing test B and look at an overall agreement (number with the same result/40). If the overall agreement is 80% or more, the laboratory accepts the new test. Then we take a positive sample identified on test B and run it repeatedly on test A i.e. 20 times to test accuracy/precision.

Aim 1

July 2020 updated for 20% prevalence in testing population

power.diagnostic.test(sens = 0.98, delta = 0.03, power = 0.90, prev=.20)

```
    Diagnostic test exact power calculation

        sens = 0.98
        n = 361
        n1 = 1444
        delta = 0.03
     sig.level = 0.05
        power = 0.9
         prev = 0.2
```

NOTE: n is number of cases, n1 is number of controls

A total sample of 1805 is needed

Aim 2
This is dependent on the prevalence of a new cough in the PUI population in the cases and controls. The sample size depends on the positivity rate in participants with and without at new cough (example of symptom). One would expect the positivity rate in new coughs to be higher in this population – but what do we used
Used 10% cases in no new cough versus 20% cases in those with new cough.
When the sample size is 764, a logistic regression of a binary response variable, Y, on a binary independent variable, X_1, will have 90.02% power to detect a change in the probability that Y=1 from a value of 0.1 at baseline to 0.20, assuming the R^2 from the regression of X_1 on other Xs is 0.2, 67% of samples are in group X_1=1, and that the two-sided test is made at the 5% level.

Sample size of 1800 should therefore be fine if the positivity rates are in these ranges.

APPENDIX 10: DATA COLLECTION TIMELINE AND SAMPLES:

See excel spreadsheet

APPENDIX 11: FLYER FOR RECRUITMENT

COVID-19:
Invitation to participate in a COVID-19 study

If you are being tested for COVID-19, please consider taking part in the SA COVID-19 POC study.

(POC = point of care)

We are doing research to find a new, rapid test to diagnose / detect COVID-19.

- Many new COVID-19 rapid tests are available.
- Rapid tests can be used in communities / clinics / outpatient departments and do not need laboratories.
- Rapid test results can be available in 20-45 minutes.

But we do not know whether these rapid tests are accurate.

We are doing research to see if these rapid tests are accurate and reliable. If they are accurate and reliable South Africa can scale up testing for COVID-19 quickly

If you are interested in joining this study, please speak with the nurses and doctors at your site.

Issued by the South African Medical Research Council

APPENDIX 12: PARTICIPANT CARD

Study ID: _____ Study site: _____

Name: _____Name: _____

Address: _____Address: _____

Thank you for participating in the SA COVID-19 POC study.

Your COVID-19 rapid test result from the Orient Gene test is (write rapid result here):

_____

These are results from a rapid COVID-19 test. Your COVID-19 PCR result will come from the laboratory. Please continue to isolate (if test is positive) or quarantine.

The following applies:

1. You **will be followed up**. Your follow-up appointment is on the (date)_____
2. You may be followed up. Your tentative date is _____. Only 500 rapid test negative and RT-PCR negatives are followed up; **we will contact you once we know your RT-PCR result to confirm your follow-up** [ ]. [ ]
3. You will **not be followed up** by the study Team: you are an asymptomatic contact. We will contact you with your final COVID-19 result.

If positive you may be traced by a Department of Health / NICD/NHLS tracing team [ ].. [ ]