Characterization of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Shedding and the Novel Coronavirus Disease (COVID-19) Clinical Features among the First Confirmed Cases in South Africa

Contents

1. Executive summary 4
2. Investigators 6
3. Introduction 8
3.1. Clinical presentation 9
3.2. Duration of viral shedding and antibody response 11
4. Justification 11
5. Study aim and objectives 12
5.1. Aim 12
5.2. Objectives 12
5.2.1. Primary objectives 12
5.2.2. Secondary objectives 12
6. Methods 13
6.1. Case definition and identification of cases 13
6.2. Isolation of COVID-19 confirmed cases 15
6.3. Eligibility criteria 16
6.3.1. Inclusion criteria 16
6.3.2. Exclusion criteria 16
6.4. Sample size 16
6.5. Enrollment and study procedures 16
6.5.1. Enrollment of COVID-19 confirmed cases 16
6.5.2. Follow-up of COVID-19 confirmed cases 17
6.5.2.1. Sequential specimens collection 18
6.5.2.2. Sequential clinical data collection 20
6.6. Laboratory procedures 20
6.6.1. Molecular detection 21
6.6.2. Virus isolation 21
6.6.3. Antibody detection 21
6.7. Study timelines 22
6.8. Ethical Considerations 22
6.8.1. Privacy 22
6.8.2. Institutional Review Board 22

6.8.3. Informed Consent 22
6.9. Risk associated with study participation 23
6.9.1. HIV testing 23
6.9.2. Nasopharyngeal and oropharyngeal specimen collection 23
6.9.3. Stool specimen collection 23
6.9.4. Blood collection 24
6.10. Study Discontinuation 24
6.11. Anticipated Benefits 24
***6.12.* Compensation 24**
7. Data management 25
8. Reporting and data analysis 25
9. References 27
10. Appendices 28
10.1. Appendix I: Suspected case – case investigation form 28
10.2. Appendix II: Suspected case – specimen submission form 30
10.3. Appendix III: Confirmed case – case record form (on enrollment) 31
10.4. Appendix IV: Confirmed case – daily assessment form 34
10.5. Appendix V: Confirmed case – final outcome form 36
10.6. Appendix VI: Confirmed case – follow-up laboratory slip 40
10.7. Appendix VII: Confirmed case – follow-up calendars 41
10.8. Appendix VIII: Participant information leaflet and informed consent 44
10.9. Appendix IX: Infection control measures 51
10.10. Appendix X: Procedures for the collection of oropharyngeal (throat) and nasopharyngeal swabs 52
10.11. Appendix XI: Procedures for the collection of stool specimens 54
10.12. Appendix XII: Procedures for the collection of blood specimens 56
10.13. Appendix XIV: Procedures for packaging and submission of specimens 58

1. Executive summary

Introduction

On 31st December 2019, the World Health Organization (WHO) was alerted to a cluster of pneumonia of unknown etiology in patients in Wuhan City, Hubei Province of China, which, one week later, was attributed to a novel coronavirus (severe acute respiratory syndrome coronavirus 2: SARS-CoV-2). The WHO assessment of the risk of this event is very high in China and at the regional and global level. Gaining an early understanding of the transmissibility potential and clinical features of illness associated with SARS-CoV-2 infection are key to guide containment and mitigation measures in local settings and at global level. This protocol forms part of the South Africa preparedness plan for the investigation of SARS-CoV-2 infections following virus introduction in the country.

Aim

To assess the magnitude (viral load) and duration of SARS-CoV-2 shedding and the clinical features associated with SARS-CoV-2 infection among HIV-infected and HIV-uninfected hospitalized, confirmed cases of COVID-19 (**co**rona**vi**rus **d**isease 2019; i.e., patients testing positive for SARS-CoV-2 on rRT-PCR) in South Africa.

Objectives

Primary objectives

i. To determine the time period (range and median) in days that individuals continue to shed SARS-CoV-2 from respiratory and stool specimens after symptoms onset using real-time reverse transcription polymerase chain reaction (rRT-PCR) and viral culture.
ii. To determine the temporal variations of SARS-CoV-2 viral-load in respiratory and stool specimens using reverse transcriptase polymerase chain reaction (rRT-PCR).
iii. To determine the viremia of SARS-CoV-2 during infection.
iv. To determine the antibody response to SARS-CoV-2 during and early-post infection and whether there is an association with patient outcome.
v. To determine the clinical features of COVID-19 over time, including signs and symptoms, hematological changes, severity, and progression of illness.

vi. To correlate the duration and magnitude of SARS-CoV-2 shedding detected by rRT-PCR and culture and the antibody response to the clinical features of illness.

Secondary objectives

i. To assess factors that may affect SARS-CoV-2 shedding duration.
ii. To assess factors that may affect SARS-CoV-2 viral load.
iii. To assess factors that may affect SARS-CoV-2 duration and severity of illness.
iv. To determine the genetic characteristics of SARS-CoV-2 of infected patients and assess the impact of prolonged shedding on within-host SARS-CoV-2 evolution through whole genome sequencing.
v. To compare validity of using nasal swab versus nasopharyngeal swab specimens for detection of SARS-CoV-2 by rRT-PCR

Study procedures

The identification of confirmed COVID-19 cases will rely on surveillance and case finding as described by the national guidelines. As per national guidelines confirmed COVID-19 cases will be isolated in selected facilities. Following consenting and enrollment into the study, sequential respiratory and stool samples will be collected every two days until cessation of shedding (test negative for SARS-CoV-2 on rRT-PCR on two consecutive respiratory samples) or death of the patient. Blood samples will be collected at pre-defined intervals from symptoms onset during and early-post infection to assess viremia and the antibody response to the virus. The progression of illness will be documented in detail through the use of standard data collection forms administered at enrollment and daily thereafter until cessation of shedding or death of the patient.

2. Investigators

Table 1: Novel Coronavirus (COVID-19) viral shedding and clinical characterization study investigators

Organization	Investigator	Qualifications	Contact details	Study Position
Centre for Respiratory Diseases and Meningitis (CRDM), National Institute for Communicable Diseases (NICD)	Cheryl Cohen	MBBCh, FCPath (SA) Micro, DTM&H, MSc (Epidemiology), PhD (Epidemiology)	+27 11 386 6593 cherylc@nicd.ac.za	Principal investigator
CRDM, NICD	Sibongile Walaza	MBBCh, MSc (Epidemiology), PhD (Epidemiology)	+27 11 386 6410 sibongilew@nicd.ac.za	Co-investigator
CRDM, NICD	Jocelyn Moyes	MBBch, DTM&H, MSc (Med) Epidemiology and Biostatistics	+27 82 883 2044 jocelynm@nicd.ac.za	Co-investigator
CRDM, NICD	Jackie Kleynhans	MSc	+27 11 555 0383 jackiel@nicd.ac.za	Co-investigator
CRDM, NICD	Anne von Gottberg	MBBCh, FCPath (SA) Micro, DTM&H, PhD (Microbiology)	+27 11 555 0352 nicolew@nicd.ac.za	Co-investigator
CRDM, NICD	Amelia Buys	ND Medical Technology (Virology)	+27 11 386 6373 ameliab@nicd.ac.za	Co-investigator
CRDM, NICD	Jinal Bhiman	MSc (Medicine), PhD (Virology)	+27 11 386 6390 jinalb@nicd.ac.za	Co-investigator
CRDM, NICD	Nicole Wolter	MSc (Epidemiology), PhD (Molecular microbiology)	+27 11 555 0352 nicolew@nicd.ac.za	Co-investigator
MassGenics and Influenza Division, Centers for Disease Control and Prevention (ID-CDC)	Stefano Tempia	DVM, MSc (Epidemiology), PhD (Epidemiology)	+27 11 555 0543 wlu4@cdc.gov	Co-investigator
ID-CDC	Meredith McMorrow	MD, MPH, FAAP	+27 12 424 9081 bwe3@cdc.gov	Co-investigator
Division of Public Health Surveillance and Response, NICD	Susan Meiring	MBChB, DTM&H	+27 21 404 5540 Susan.meiring@nhls.ac.za	Co-investigator
Division of Public Health Surveillance and Response, NICD	Vanessa Quan	MBBCh, DCH, DTM&H, MPH	+27 11 386 6012 vanessaq@nicd.ac.za	Co-investigator

Table 2: GERMS-SA Syndromic and Enhanced laboratory confirmed surveillance hospitals and their site coordinators

Province	Hospital(s)	Site Coordinator
Eastern Cape	Port Elizabeth, Livingstone and Dora Nginza Hospitals	John Black; Vanessa Pearce
Free State	Universitas and Pelonomi Hospitals	Masego Moncho, Malotji Maloba, Nickie Goedhals, D Steyn, Samantha Potgieter
Gauteng	Charlotte Maxeke Johannesburg Academic Hospital	Sarah Stacey, Trusha Nana, Vindana Chibabhai, Florette Treunicht
Gauteng	Helen Joseph Hospital	Jeremy Nel, Rispah Chomba
Gauteng	Dr George Mukhari Hospital	Maphoshane Nchabaleng, Grace Shikwambane-Ntlemo, Nomandla Madala
Gauteng	Steve Bhiko Academic Hospital	Anwar Hoosen, Mohammed Said, Anton Stoltz
KwaZulu Natal	King Edward VIII Hospital, RK Khan Hospital, Addington Hospital, Mahatma Ghandi Memorial, Durban	Prasha Mahabeer, Praksha Ramjathan, Khine Swe Swe Han, Javid Mulla, Soma Pillay, Lisha Sooka, Nomonde Dlamini, Junaid Bayat
KwaZulu Natal	Greys' Hospital, Edendale Hospital, Northdale Hospital	Halima Dawood, Sumayya Haffejee, Surendra Sirkar
Limpopo	Polokwane/Mankweng Hospital, Seshego Hospital	Ruth Lekalakala, Phetho Mangena, Samuel Cana
Mpumalanga	Rob Ferreira Hospital, Themba Hospital	Sindisile Ntuli, Matamela Madua
North West	Klerksdorp/ Tshepong Complex	Ebrahim Variava
Western Cape	Tygerberg Hospital	Kessendri Reddy, Andrew Whitelaw, Jantjie Taljaard
Western Cape	Mitchells Plain Hospital	Thomas Crede

3. Introduction

On 31st December 2019, the World Health Organization (WHO) was alerted to a cluster of pneumonia of unknown etiology in patients in Wuhan City, Hubei Province of China (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). One week later, on 7th January 2020, Chinese authorities confirmed that they had identified a novel (new) coronavirus as the cause of pneumonia [1]. This novel coronavirus has been named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses due to its genetic similarity (different strain of the same species) to the severe acute respiratory syndrome coronavirus (SARS-CoV) that emerged in 2002 (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1.full.pdf). The disease associated with SARS-CoV-2 has been named by WHO as COVID-19 (for **co**rona**vi**rus **d**isease 20**19**) (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it). Initially person-to-person transmission was not apparent and the majority of the cases were epidemiologically linked to a seafood, poultry and live wildlife market (Huanan Seafood Wholesale Market) in Jianghan District of Hubei Province. Available evidence, and experience from the SARS-CoV and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) suggests that the novel coronavirus has a possible zoonotic origin [2,3]. However, the number of cases continued to increase rapidly, and evidence of person-to-person transmission mounted in travellers diagnosed with SARS-CoV-2 who had visited Wuhan [4]. The WHO International Health Regulations Emergency Committee declared that the outbreak of COVID-19 meets the criteria for a Public Health Emergency of International Concern (PHEIC) on 30th January 2020 (https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(COVID-19)).

As of 3rd March 2020, 90,870 laboratory-confirmed cases had been reported to WHO, of which 80,304 (88.4%) had been detected in China and China special territories, including Hong Kong Special Administrative Region (SAR) (745 cases), Macau SAR (66 cases), and Taipei (2,359 cases). The remaining 10,566 cases have been reported from 72 other countries in the following WHO

Regions: Western Pacific: 9 (Japan, Republic of Korea, Viet Nam, Singapore, Australia, Malaysia, Cambodia, Philippines and New Zealand); South-East Asia: 5 (Thailand, Nepal, Sri Lanka, Indonesia and India); Region of the Americas: 6 (The United States of America, Canada, Brazil, Mexico, Ecuador and Dominican Republic), European Region: 34 (Italy, France, Germany, Spain, The United Kingdom, Switzerland, Norway, Austria, Netherland, Sweden, Israel, Croatia, Iceland, San Marino, Belgium, Finland, Greece, Denmark, Azerbaijan, Czechia, Georgia, Romania, Russian Federation, Portugal, Andorra, Armenia, Belarus, Estonia, Ireland, Latvia, Lithuania, Luxembourg, Monaco and North Macedonia); Eastern Mediterranean: 15 (Iran, Kuwait, Bahrain, United Arab Emirates, Iraq, Oman, Pakistan, Lebanon, Afghanistan, Egypt, Qatar, Jordan, Morocco, Tunisia and Saudi Arabia); and African: 3 (Algeria, Nigeria and Senegal). Outside of China, cases of in-country transmission has been reported in 20 of 58 infected countries and community transmission is presumed to occur in South Korea, Italy, Iran, Japan and Singapore (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200303-sitrep-43-covid-19.pdf?sfvrsn=2c21c09c_2). This is a rapidly evolving situation. The daily WHO situation update can be found at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/. The WHO assessment of the risk of this event is very high in China and at the regional and global level.

The South Africa National Department of Health (NDoH) activated the Emergency Operation Centre (EOC) on 31st January 2020 and is currently actively screening persons under investigation (PUI) for COVID-19 using a standard case definition (see Section 6.1). The EOC task force assessed the risk of introduction of SARS-CoV-2 in South Africa to be high. Laboratory diagnosis of SARS-CoV-2 infection is conducted at the National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS) and at sentinel NHLS laboratories around the country. The first positive case of SARS-CoV-2 infection in South Africa was reported on 5 March 2020 from a patient in KwaZulu-Natal.

3.1. Clinical presentation

There are currently limited clinical data available from confirmed COVID-19 cases. Most of the available information is from the initial and more severe cases identified. A minority, but not negligible proportion of patients have had acute, severe respiratory illness with fever, cough,

shortness of breath, and breathing difficulties. As of 3rd March 2020, of the 90,870 cases confirmed globally, 3,112 (3.4%) were fatal (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200301-sitrep-41-covid-19.pdf?sfvrsn=6768306d_2). Because investigations initially focused on severe cases the severity of the infection may be overestimated among the currently known confirmed cases.

The clinical features of 41 severe cases initially detected in China have been described in detail. These patients were hospitalized in a dedicated hospital in Wuhan city. 73% were men, the median age was 49 years (IQR: 41-58 years) and 66% had been exposed to Huanan seafood market. 32% had underlying medical conditions, including diabetes (20%), hypertension (15%) and cardiovascular disease (15%). Common symptoms at onset included fever (98%), cough (76%) and myalgia or fatigue (44%). Less common symptoms were headache (8%), haemoptysis (5%) and diarrhoea (3%). Dyspnoea developed in 22% of patients. The median time of dyspnoea development was 8 days (IQR: 0-13 days). 63% of patients had lymphopenia and all patients had pneumonia with abnormal findings on chest X-ray. Complications included acute respiratory distress syndrome (29%) and acute cardiac injury (19%). 32% of patients were admitted to an intensive care unit (ICU) and 15% died [5].

Another description of the clinical characteristic of 99 confirmed severe cases from China reported a median age of 55.5 years. 51% of patients had chronic diseases, 83% had fever, 82% had cough, 31% had shortness of breath, 11% had muscle ache, 9% had confusion, 8% had headache, 5% had sore throat, 4% had rhinorrhoea, 2% had chest pain, 2% had diarrhoea and 1% had nausea and vomiting. 75% of patients showed bilateral pneumonia, 14% showed multiple mottling and ground-glass opacity and 1% had pneumothorax. 17% of patients developed acute respiratory distress syndrome and of those 11% worsened in a short period of time and died of multiple organ failure [6].

The first COVID-19 case identified in the United State presented at first with mild illness with progression to pneumonia on day 9 of illness onset [7].

3.2. Duration of viral shedding and antibody response

There is a dearth of information on the duration of shedding (including the fluctuation of the viral load during progression of infection) and the antibody response to infection with COVID-19. The first case of COVID-19 identified in the United States tested positive by real time polymerase chain reaction (PCR) up to 12 days after onset of illness. However, it is unclear if the patients continued to shed the virus thereafter. Patient's stools tested positive on day 7 of illness onset. No stools were collected thereafter. The same patient tested negative on serology until day 7 of illness onset. The seroconversion thereafter is unknown [7]. SARS-CoV-2 has been detected in respiratory, stool and blood (whole blood and serum) specimens [8].

Data from previous novel coronaviruses (MERS-CoV and SARS-CoV) indicated that these viruses were shed for a prolonged period of time (up to 30 days) with peak viral load 12-14 days post symptom onset. This had important implications for quarantine and infection control measures [9,10,11,12].

4. Justification

The detection and spread of an emerging respiratory pathogen are accompanied by uncertainty over the key epidemiological, clinical and virological characteristics of the novel pathogen and particularly its ability to spread in the human population, its virulence (case-severity) and its behavior in HIV-coinfected individuals. This is the case for the SARS-CoV-2. Gaining an early understanding of the transmissibility potential and clinical features of illness associated with SARS-CoV-2 infection are key to guide containment and mitigation measures in local settings and at global level. The WHO recommends the implementation of special studies at the early stage of introduction of SARS-CoV-2 to inform national and global control and mitigation strategies (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance).

Understanding the duration of viral shedding, the clinical features of illness and the association between viral shedding/viral load and the symptomatology of the infection has direct practical implications to: (i) determine the required duration of isolation of infected individuals; (ii) assess

the transmissibility potential of the virus; and (iii) understand the clinical spectrum and progression of illness for improved treatment and mitigation strategies. This protocol forms part of the South Africa preparedness plan for the investigation of SARS-CoV-2 infections .

5. Study aim and objectives

5.1. Aim

To assess the magnitude (viral load) and duration of SARS-CoV-2 shedding and the clinical features associated with SARS-CoV-2 infection among HIV-Infected and HIV-uninfected hospitalized, confirmed cases of COVID-19 in South Africa.

5.2. Objectives

5.2.1. Primary objectives

i. To determine the time period (range and median) in days that individuals continue to shed SARS-CoV-2 from respiratory and stool specimens after symptoms onset using real-time reverse transcription polymerase chain reaction (rRT-PCR) and viral culture.
ii. To determine the temporal variations of SARS-CoV-2 viral-load in respiratory and stool specimens using reverse transcriptase polymerase chain reaction (rRT-PCR).
iii. To determine the viremia of SARS-CoV-2 during infection.
iv. To determine the antibody response to SARS-CoV-2 during and early-post infection and whether there is an association with patient outcome.
v. To determine the clinical features of COVID-19 over time, including signs and symptoms, hematological changes, severity, and progression of illness.
vi. To correlate the duration and magnitude of SARS-CoV-2 shedding detected by rRT-PCR and culture and the antibody response to the clinical features of illness.

5.2.2. Secondary objectives

i. To assess factors that may affect SARS-CoV-2 shedding duration.
ii. To assess factors that may affect SARS-CoV-2 viral load.
iii. To assess factors that may affect SARS-CoV-2 duration and severity of illness.

iv. To determine the genetic characteristics of SARS-CoV-2 of infected patients and assess the impact of prolonged shedding on within-host SARS-CoV-2 evolution through whole genome sequencing.

v. To compare validity of using nasal swab versus nasopharyngeal swab specimens for detection of SARS-CoV-2 by rRT-PCR

6. Methods

6.1. Case definition and identification of cases

A confirmed case of COVID-19 is a person with laboratory confirmation (detection by rRT-PCR) of infection with SARS-CoV-2.

Confirmation of infection will be conducted according to the procedures outlined in the *"COVID-19 Guidelines for Case-Finding, Diagnosis, Management and Public Health Response in South Africa"* (http://www.nicd.ac.za/wp-content/uploads/2020/02/Guidelines-for-case-finding-diagnosis-management-and-public-health-response-in-South-Africa.pdf). Identification of confirmed cases will be implemented through active surveillance and case finding as highlighted in the national guidelines (http://www.nicd.ac.za/wp-content/uploads/2020/02/Guidelines-for-case-finding-diagnosis-management-and-public-health-response-in-South-Africa.pdf). Briefly, active surveillance and case finding will be conducted by clinicians, health facilities and designated staff through implementation of routine screening for the identification of suspected cases at healthcare facilities and identification and follow-up of contacts of confirmed cases.

A suspected case (person under investigation – PUI) is defined as a person with acute respiratory illness with sudden onset of at least one of the following: cough, sore throat, shortness of breath or fever [≥ 38°C (measured) or history of fever (subjective)] irrespective of admission status.
Those at highest risk for COVID-19 include persons who have an acute respiratory illness and who, in the 14 days prior to onset of symptoms, met at least one of the following epidemiological criteria:

- Were in close contact with a confirmed or probable case of SARS-CoV-2 infection;

OR

- Had a history of travel to areas with presumed ongoing community transmission of SARS-CoV-2 (e.g., Mainland China, South Korea, Singapore, Japan, Iran, Hong Kong and Italy).

OR

- Worked in, or attended a health care facility where patients with SARS-CoV-2 infections were being treated.

OR

- Admitted with severe pneumonia of unknown etiology

From all PUI a standard case investigation form (CIF – Appendix I) will be completed and an upper and/or lower respiratory tract sample will be collected as detailed in the national guidelines. Samples with accompanying specimen submission form (Appendix II) will be sent to the national reference laboratory [the Center for Respiratory Diseases and Meningitis (CRDM), National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS)] or to the nearest NHLS virology laboratory offering SARS-CoV-2 rRT-PCR for testing. Figure 1 illustrates the laboratory algorithm followed for testing of specimens collected from a suspected COVID-19 case (http://www.nicd.ac.za/wp-content/uploads/2020/02/Guidelines-for-case-finding-diagnosis-management-and-public-health-response-in-South-Africa.pdf).

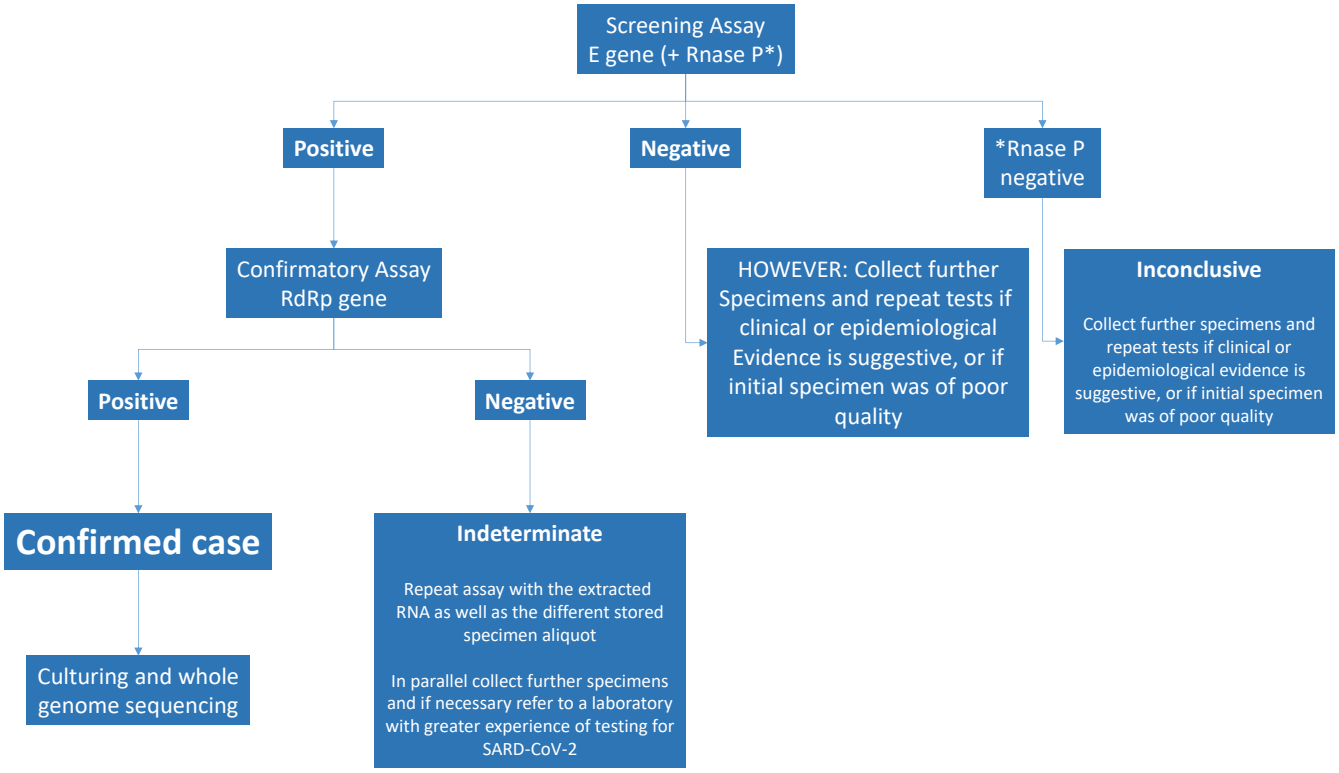

Figure1: Algorithm for testing suspected cases for COVID-19 by rRT-PCR and sequencing

Routine confirmation of cases of SARS-CoV-2 infection is based on amplification and detection of unique SARS-CoV-2 viral nucleic acid sequences by real-time reverse-transcription polymerase chain reaction (rRT-PCR), with confirmation by nucleic acid sequencing when necessary. Currently, the National Institute for Communicable Diseases conducts testing for SARS-CoV-2 using the Charite Institute rRT-PCR assay. The protocol for testing is based on a method described by Corman *et* al, 2020 [13]. A unique identifier will be assigned to all COVID-19 confirmed cases and the laboratory results and the information collected on the CIF (Appendix I) will be entered daily in central databases kept at NICD.

6.2. Isolation of COVID-19 confirmed cases

As per national guidelines, confirmed cases will be isolated in designated public healthcare facilities or where the patient was first admitted if the admitting facility (public or private) has adequate isolation space and they will receive relevant medical care (http://www.nicd.ac.za/wp-content/uploads/2020/02/Guidelines-for-case-finding-diagnosis-management-and-public-

health-response-in-South-Africa.pdf). Patients will be isolated in an airborne infection isolation room if available or in adequately ventilated single rooms.

6.3. Eligibility criteria

6.3.1. Inclusion criteria

i. Patient meets the case definition of COVID-19 confirmed case.
ii. Patient is hospitalized and warrants admission for medical reasons at a participating GERMS-SA syndromic or enhanced laboratory-based surveillance hospital site (see table 2).
iii. Patient is ≥18 years on the day of specimen collection for COVID-19 testing
iv. Patient resides within 50km radius of the admitting hospital
v. Patient is enrolled within 5 days of specimen collection for COVID-19 diagnosis

6.3.2. Exclusion criteria

i. Patient does not meet the case definition of COVID-19 confirmed case.
ii. Confirmatory HIV test result is not available and patient refuses HIV testing.
iii. Patient is admitted and hospitalized for self-isolation purposes only

6.4. Sample size

Two hundred participants meeting the eligibility criteria will be enrolled in the study: 100 HIV-infected and 100 HIV-uninfected adults (≥18 years). Participants will be enrolled from the GERMS-SA syndromic and enhanced laboratory-confirmed surveillance hospitals.

6.5. Enrollment and study procedures

6.5.1. Enrollment of COVID-19 confirmed cases

A list of all confirmed COVID-19 cases will be obtained daily from the centralized national database kept at NICD (see Section 6.1) and from each isolation facility of the participating hospitals. The

focal point/clinical director/infection control practitioner at the isolation facility where the COVID-19 confirmed case is admitted will be contacted by NICD dedicated staff. The initial laboratory report and any available residual respiratory swab specimen will be collected from the initial testing laboratory. Trained NICD study personnel will approach the patient within 24 hours from admission for consenting (Appendix VIII) and enrollment. Following enrollment a study calendar will be completed (Appendix VII) and a detailed Case Record Form (CRF) (Appendix III) will be administered to collect key demographic, exposure and clinical data from the patient.

HIV testing should be conducted as part of clinical management. However, if HIV testing was not done, a finger prick for HIV rapid testing will be conducted with consent, if needed, to define HIV status as described below.

- If the patient has a documented positive HIV result (including viral load or evidence of HAART) available in the hospital records this will be considered the patient's HIV status.
- If the patient is not aware of their status or a previous result (>6 months old) documents a negative result the patient will be offered voluntary counselling and testing (VCT) by rapid enzyme-linked immunosorbent assay (ELISA).

The HIV status of the patient, along with a recent CD4 count and HIV-viral load will be recorded in the CRF.

6.5.2. Follow-up of COVID-19 confirmed cases

If the hospital has adequate capacity, hospital staff will be trained by NICD/DoH personnel on the day of enrollment for sequential sample and clinical data collection during the admission period. If the hospital has insufficient staff the sequential and clinical data collection will be implemented by trained NICD/DoH personnel.

All procedures required after patient's discharge will be conducted by NICD/DoH personnel. This includes: (i) home follow-up visit for sample and clinical data collection if the patient was discharged before cessation of shedding; and (ii) the collection of convalescent serum. For home

visits, a visit calendar will be agreed upon before discharge using a visit calendar form (Appendix VII). For each scheduled procedure (whether at the isolation facility or at home) a window period of 1 day will be allowed after the study visit due date to accommodate emergencies and missed visits. Patients will be considered to be no longer shedding if they test negative for SARS-CoV-2 on rRT-PCR on two consecutive respiratory specimens in concomitance with remission of symptoms.

6.5.2.1. Sequential specimens collection

Respiratory specimens: Oropharyngeal (NP) and nasopharyngeal (OP) swabs (placed in the same vial containing universal transport medium) will be collected on the day of enrollment and every two days from enrollment until cessation of shedding or death of the patient. In addition, a self /HCW administered nasal swab (taken from the middle nasal turbinate) will be collected at each of these time points. If the patient is transferred to a different facility or discharged before cessation of shedding the sequential respiratory specimen's collection will continue at the referred facility or at home with the same frequency until cessation of shedding or death of the patient. Respiratory specimens will be tested by SARS-CoV-2 rRT-PCR and culture assays (see Sections 6.6.1 and 6.6.2). The procedures for the collection, storage and transportation of respiratory samples are provided in Appendices IX, X, XI and XIV.

Stool specimens: Stool specimens, including rectal swabs and nappy liners as appropriate, will be collected following the same schedule of respiratory specimens' collection as described above. If the patient is transferred to a different facility or discharged before cessation of shedding the sequential stool specimen collection will continue at the referred facility or at home with the same frequency until cessation of shedding or death of the patient. Stool specimens will be tested by SARS-CoV-2 rRT-PCR and culture assays (see Sections 6.6.1 and 6.6.2). The procedures for the collection, storage and transportation of stool specimens are provided in Appendices IX, XII and XIV.

Blood specimens: Three blood specimens (±13ml) will be collected at each blood draw (yellow top (5ml), red top (5ml) and purple top (3ml)) on the day of enrollment and on day 7, 14, 21, 28 and 56 post symptoms onset irrespective of time of cessation of shedding or until death of the patient. The following blood specimen(s) collection schedule will be implemented:

- If the enrolment occurs within 0-5 days from symptoms onset, a blood specimen will be collected at enrollment and at days 7, 14, 21, 28 and 56 post symptoms onset.
- If the enrolment occurs within 8-12 days from symptoms onset, a blood specimen will be collected at enrollment and at days 14, 21, 28 and 56 post symptoms onset.
- If the enrolment occurs within 15-19 days from symptoms onset, a blood specimen will be collected at enrollment and at days 21, 28 and 56 post symptoms onset.
- If the enrolment occurs within 22-26 days from symptoms onset, a blood specimen will be collected at enrollment and at days 28 and 56 post symptoms onset.
- If the enrolment occurs 7, 14, 21 or 28 days post symptom onset a blood specimen will be collected at enrollment and at the remaining scheduled follow-up visits (e.g., if the enrolment occurs at 7 days post symptom onset a blood specimen will be collected at enrollment and at days 14, 21, 28 and 56 post symptoms onset).
- If the enrollment occurs 6, 13, 20 or 27 days post symptoms onset (one day before the due sample collection date) a blood specimen will not be collected at enrollment but at the next scheduled visit on the following day (e.g. day 7, 14, 21, or 28 post symptoms onset) and at the remaining scheduled follow-up visits.

If the patient is transferred to a different facility or discharged during the scheduled follow-up period the sequential blood specimen's collection will continue at the referred facility or at home according to schedule. For the visit scheduled at 56 days post symptoms onset, 3 days will be allowed on either side of the scheduled study visit date to accommodate weekends, emergencies and missed visits. Serum samples will be tested for the detection of SARS-CoV-2 using rRT-PCR and culture (to assess viremia – see Sections 6.6.1 and 6.6.2) and the detection and quantification of SARS-CoV-2 immunoglobulin class M (IgM) and class G (IgG) (see Section 6.6.3). In addition, whole blood will be tested for the detection of SARS-CoV-2 using rRT-PCR and culture (see Sections 6.6.1 and 6.6.2) and the results will be compared to those obtained from serum samples. The procedures for the collection, storage and transportation of whole blood and serum specimens are provided in Appendix IX, XIII and IX.

Laboratory slip: A laboratory slip (Appendix VI) will be completed for all samples collected and sent along with the relevant samples to the national reference laboratory at NICD within 24 hours

from specimen collection. The patient's unique identifier provided by NICD will be used to identify the patient and it will be placed on the laboratory slip and the specimen(s). A sequential number reflecting the sequential sample collection day post enrollment will be added to the unique identifier. The first sequential sample will be identified as "Dx" (i.e., where x is the number of days post enrollment). One laboratory slip will be completed for all samples that will be shipped to the laboratory with the same shipment. Samples that are collected on the same day, but will be shipped to the laboratory on a different shipment (either the same day or not) will require a different laboratory slip to be completed. The exact date of sample collection will be reported for each allowed sample collection day within the follow-up window (i.e. the due sample collection date or +1 day of the due sample collection date).

6.5.2.2. Sequential clinical data collection

A clinical follow-up form (Appendix IV) will be completed for every day of follow-up post enrollment until cessation of shedding or death of the patient. The form will be completed through patient interview and, if available, review of clinical records (including hospital laboratory results) by the designated hospital staff or NICD/DoH personnel. If the patient is transferred to a different facility or discharged before cessation of shedding the daily clinical follow-up form will be completed at the referred facility or at home with the same frequency until cessation of shedding or death of the patient. If the form cannot be completed daily (e.g. no available hospital staff and NICD/DoH personnel visits the patient every 2 days either at the isolation facility or at home) one form will be completed retrospectively for each day after the last completed form.

A final outcome form (Appendix V) will be completed at any of the following instances: (i) transfer to a different facility, (ii) discharge (either before or after cessation of shedding), (iii) cessation of shedding (if the patient was not discharged before cessation of shedding), and (iv) death of the patient.

6.6. Laboratory procedures

All collected samples will be tested at the national reference laboratory situated at the CRDM/NICD. The infection control measures and the procedures for collection, storage and transportation of specimens are provided in Appendices IX-XIV.

6.6.1. Molecular detection

Total nucleic acids will be extracted using the MagNA Pure Compact or MagNA Pure 96 instrument, as per manufacturer's instructions. rRT-PCR will be performed according to the method described by Corman et al, 2020 [13]. This assays detects two gene targets of the SARS-CoV-2 virus (E gene and RdRP gene). A specimen will be considered positive for SARS-CoV-2 if both targets are detected (Figure 1). A real-time PCR assay targeting the human RNaseP gene will be performed for all specimens to control for the quality of the specimen and PCR inhibition. For positive specimens, further virus characterization will be performed by virus culture and whole genome sequencing.

6.6.2. Virus isolation

Vero and Vero E6 cell lines, maintained in a monolayer and supplemented with 10% FBS (foetal bovine serum) DMEM (Dulbecco's modified Eagle's medium) with antibiotics (100 units/ml penicillin and 15 µg/ml streptomycin) will be used for virus isolation. PCR-positive samples will be centrifuged at 8.000 g for 15 min, filtered and diluted 1:2 with DMEM supplied with 16 µg/ml trypsin before adding to cells. After incubation at 37°C for 1h, the inoculum will be removed and replaced with fresh culture medium containing antibiotics and 16 µg/ml trypsin. The cells will be incubated at 37°C and observed daily for cytopathic effect (CPE). Viral growth will be confirmed by rRT-PCR.

6.6.3. Antibody detection

A virus neutralization test will be performed in 48-well plates. Patient serum samples will be heat-inactivated by incubation at 56 °C for 30 min before use. Serum samples (5 µL) will be diluted 1:10, 1:20, 1:40 or 1:80, and following addition of an equal volume of virus stock will be incubated at 37°C for 60 min in a 5% CO2 incubator. Diluted serum samples from healthy people will be used as negative control. After incubation, 100 µL of the virus-serum mixtures will be inoculated onto monolayer Vero E6 cells for 1 hour. Each serum will be repeated duplicate. After removal of the

supernatant, the plate will be washed twice with DMEM medium and then incubated with DMEM supplemented with 2% FBS for 24 hours. Finally, cells will be fixed with 4% formaldehyde and virus detected will be performed using the SL-CoV Rp3 NP antibody followed by Cy3-conjugated mouse anti-rabbit IgG. Nuclei will be stained with DAPI. Infected cell numbers will be counted by high-content cytometers. In addition, an under-development in-house ELISA to detect IgG and IgM responses in serum samples to the spike (S) protein of SARS-CoV-2 will be used.

6.7. Study timelines

The study will start after the first cases of COVID-19 are confirmed in South Africa and will continue until the sample size is reached.

6.8. Ethical Considerations

6.8.1. Privacy

All laboratory specimens and questionnaires data will be stored in a password-protected database with limited access. All human biological materials collected as part of this protocol will be used only for the study described in this protocol.

6.8.2. Institutional Review Board

This study is considered operational research and is in line with the NICD's public health mandate to conduct clinical surveillance. The study has been structured in accordance with the **Declaration of Helsinki** (last updated: October 2013), which deals with the recommendations guiding doctors in biomedical research involving human participants. Ethics approval and permissions for the study will be obtained from the relevant ethics committees, Provincial Departments of Health and other authorities prior to study start-up

6.8.3. Informed Consent

All study participants will be required to sign a consent form to participate in the study. A copy of the consent form will be given to the participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. Consent will be

obtained at the bedside and documented in writing. Surveillance officers will approach adult patients directly to obtain consent. For patients who are not able to give consent (confused, unconscious or ventilated patients) consent will be sought from a surrogate decision maker (e.g., a next of kin or doctor managing the patient who is not part of the study); and when the patient is better and is able to make decisions the consent will be sought from the patient. If at this stage the patient refuses participation, the decision made by the patient will override that made by the surrogate decision maker at the time of enrolment and the data and samples already collected for the study will be destroyed and not used for the study. Sample patient information and consent forms are provided in Appendix VIII. Working versions will be held by NICD and sent for approval to the relevant ethics committees if changes are made.

6.9. Risk associated with study participation

6.9.1. HIV testing

Patients who undergo HIV testing will receive pre- and post-test counselling by trained nurses on site at the hospital. The choice of rapid test will be led by the current protocol being used by the National Department of Health. Referrals for continued support, CD4 testing and treatment will be facilitated within the primary health care. One drop of whole blood will be collected from the fingertip for use in the HIV rapid test using a sterile lancet cap, which may cause mild transient discomfort and bruising. The volume of blood to be drawn and the collection method pose minimal risk of harm.

6.9.2. Nasopharyngeal, oropharyngeal and nasal specimen collection

Nasopharyngeal and oropharyngeal specimen collection may cause transient mild discomfort but poses minimal risk of harm. Self-administered nasal swabs cause less discomfort.These procedures are done as part of standard diagnostic procedures for the diagnosis of respiratory viruses and are considered low-risk procedures.

6.9.3. Stool specimen collection

Stool specimen collection poses no risk of harm.

6.9.4. Blood collection

Blood will be collected from all patients at enrollment and a maximum of 5 times during the study period at an interval of 7 days for the first 4 draws and 28 days for the last draw: 3 blood tubes will be collected at each blood draw (5ml in Red top, 5ml in Yellow top and 3ml in Purple top). Blood will be collected by venipuncture, which may cause mild transient discomfort and bruising. The volume of blood to be drawn, the frequency of collection and the collection method pose minimal risk of harm.

6.10. Study Discontinuation

Study participants will be encouraged to continue participating voluntarily in the study. A study participant may choose to discontinue participation in the study at any time without threat of reprisal or risk that his or her future medical care will be compromised. Study participants will be considered "lost to follow-up" if they miss two sequential scheduled study visits and cannot be contacted after at least three attempts by telephone or by home visit if the patient was discharged before cessation of shedding.

6.11. Anticipated Benefits

This study will be of benefit to the individual participant as they will be closely monitored for the development of severe illness until cessation of shedding. In addition, the study will provide key information to guide isolation and clinical management of confirmed COVID-19 cases.

6.12. Compensation

No compensation will be given to participants. It is anticipated that most of the participant follow-up will happen while the patient is still hospitalized. In the event that the patient is discharged before the end of follow-up period, study staff will visit the patient at home or reimburse participants for reasonable transport costs incurred for follow-up at the admission facility.

7. Data management

Data entry will be centralized at the CRDM data centre at NICD and will be performed by trained data clerks. Data clerks will be supervised by the database manager and data will be stored in a password-protected database with limited access. Data will be entered into a Microsoft Access® and REDCap databases and Go.Data (https://www.who.int/godata).

Data quality systems are already in place at the CRDM data center and they will be applied for this study. Data collection will be standardized and questionnaires will be checked by the research manager at the NICD for consistency and errors prior to data capture and queries will be generated. Regular data checks will be performed post-data entry. A data-manager will be responsible for data quality issues.

8. Reporting and data analysis

Key severity indicators will be monitored and reported in real time to the EOC/NDoH as part of national surveillance for COVID-19 guidelines. These indicators will monitor worsening of illness (e.g. development of acute respiratory distress syndrome) and admission to ICU and/or mechanical ventilation. The final outcome of illness (discharge or death) will also be reported in real time. In addition, the duration of shedding (based on rRT-PCR) will be calculated immediately following the cessation of shedding and communicated to the EOC/NDoH to inform isolation guidelines. Viral evolution within an individual and at a population level will be monitored through whole genome sequence analysis with specific focus on the spike protein, which is responsible for receptor engagement and is a current lead candidate for an antibody-mediated vaccine, as well as other genes targets of future drug and treatment regimens. This mutation data, frequency and potential impact will be reported to the EOC/NDoH as they become available.

The duration of shedding will be calculated from the day of symptoms onset to the day of cessation of shedding. At the end of the study the mean, median and interquartile range (IQR) of the duration of shedding will be calculated for respiratory and stool specimens. The correlation of rRT-PCR positivity in NP/OP and stool specimens will be assessed using Chi-square and k-statistics. If the

number of enrolled participants is sufficient the factors associated with the duration of shedding will be assessed using Weibull accelerated time-failure regression. Trends of the viral load (using the rRT-PCR cycle-threshold value as a proxy) will be described over the duration of shedding and summarized with mean, median, min and maximum values. If the number of enrolled participants is sufficient the factors associated with the viral load will be assessed using quantile regression. The correlation between positive virus cultures and viral load (using the rRT-PCR cycle-threshold value as a proxy) will be evaluated using ordinal regression. The antibody response will be described over time during and early-post infection and the correlation with cessation of shedding and viral load (using the rRT-PCR cycle-threshold value as a proxy) will be evaluated using Weibull accelerated time-failure and logistic regression. The clinical characteristics of the patients will be summarized using mean and medians with associated standard deviation or IQR for continuous variables and frequencies for categorical variables. Key symptoms will also be described over the duration of illness and the correlation of the duration and severity of illness with viral shedding, viral load and antibody response will be assessed using Weibull accelerated time-failure and logistic regression. The results of this study will be disseminated through presentation at conferences and publications.

9. References

1 Zhu N, Zhang D, Wang W et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. **2020**;382(8):727-733. doi: 10.1056/NEJMoa2001017.

2 Gong SR, Bao LL. The battle against SARS and MERS coronaviruses: Reservoirs and Animal Models. Animal Model Exp Med. **2018**;1(2):125-133. doi: 10.1002/ame2.12017.

3 Lu R, Zhao X, Li J et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. **2020**. pii: S0140-6736(20)30251-8. doi: 10.1016/S0140-6736(20)30251-8. [Epub ahead of print]

4 Chan JF, Yuan S, Kok KH et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. **2020**;395(10223):514-523. doi: 10.1016/S0140-6736(20)30154-9.

5 Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. **2020**;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.

6 Chen N, Zhou M, Dong X et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. **2020**;395(10223):507-513. doi: 10.1016/S0140-6736(20)30211-7.

7 Holshue ML, DeBolt C, Lindquist S et al. First Case of 2019 Novel Coronavirus in the United States. N Engl J Med. **2020**. doi: 10.1056/NEJMoa2001191. [Epub ahead of print]

8 Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect. **2020**;9(1):386-389. doi: 10.1080/22221751.2020.1729071.

9 Cheng PK, Wong DA, Tong LK et al. Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. Lancet. **2004**;363(9422):1699-700.

10 Park WB, Poon LLM, Choi SJ et al. Replicative virus shedding in the respiratory tract of patients with Middle East respiratory syndrome coronavirus infection. Int J Infect Dis. **2018**;72:8-10. doi: 10.1016/j.ijid.2018.05.003.

11 Bin SY, Heo JY, Song MS, Lee J, Kim EH, Park SJ, Kwon HI, Kim SM, Kim YI, Si YJ, Lee IW, Baek YH, Choi WS, Min J, Jeong HW, Choi YK. Environmental Contamination and Viral Shedding in MERS Patients During MERS-CoV Outbreak in South Korea. Clin Infect Dis. **2016**;62(6):755-60. doi: 10.1093/cid/civ1020.

12 Memish ZA, Assiri AM, Al-Tawfiq JA. Middle East respiratory syndrome coronavirus (MERS-CoV) viral shedding in the respiratory tract: an observational analysis with infection control implications. Int J Infect Dis. **2014**;29:307-8. doi: 10.1016/j.ijid.2014.10.002.

13 Corman VM, Landt O, Kaiser M et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. **2020**;25(3). doi: 10.2807/1560-7917.ES.2020.25.3.2000045.

10. Appendices

10.1. Appendix I: Suspected case – case investigation form

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division in the National Health Laboratory Service

health
Department: Health
REPUBLIC OF SOUTH AFRICA

Version 3, 17 February 2020
CENTRE FOR RESPIRATORY DISEASES AND MENINGITIS
OUTBREAK RESPONSE, DIVISION OF PUBLIC HEALTH SURVEILLANCE AND RESPONSE

Person under investigation (PUI) form for coronavirus disease 2019 (COVID-19): Request for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing

Internal use
CRDM unique no: _____

Tel: (+27) 386 6392/ (+27) 386 6410 | Fax: (+27)11 882 9979 | **Hotline: (+27)82 883 9920 | (+27)66 562 4021**
Forward original forms with the specimen collected.
Email completed specimen submission form and PUI form to ncov@nicd.ac.za

Today's date: DD/MM/YYYY **Form completed by (Name, Surname):** _____ **Contact number(s):** _____

All suspected COVID-19 cases are Category 1 **notifiable medical conditions** under "Respiratory disease caused by a novel respiratory pathogen". Notify as per NMC procedures. If using NMC app provide case ID indicated on alert email.

Case ID : _____

Is this a: New clinical query ☐ Contact of a known case ☐ **If contact of a known case, provide case details:** **Known case first name:** _____ **Known case surname:** _____ **Known case DOB:** DD/MM/YYYY

Detected at point of entry? Y☐ N☐ Unkn☐ **If yes, date:** DD/MM/YYYY **Please specify the point of entry:** _____

PATIENT DETAILS		DOCTOR'S DETAILS	
Patient hospital number (if available):		**First name:**	
First name:	**Surname:**	**Surname:**	
DOB: DD/MM/YYYY	**Sex:** Male ☐ Female ☐	**Facility name:**	
Residency: SA resident ☐ Non-SA resident ☐ Specify:		**Contact number/s:**	
Current residential address[1]:			
Patient's contact number(s): Include alternative number		**Email address:**	
Please indicate occupation (tick all that apply):	Student ☐ Unemployed ☐ Working with animals ☐ Health laboratory worker ☐ Healthcare worker ☐ Facility name: Other ☐ Specify:		

NEXT OF KIN CONTACT DETAILS (alternative contact details)

First name: _____ **Surname:** _____

Relationship to the patient: _____ **Contact number(s):** _____

CLINICAL PRESENTATION AND HISTORY

Date of symptom onset: DD/MM/YYYY **Date of current consultation/admission:** DD/MM/YYYY

Symptoms (tick all that apply):						
Fever (≥38˚C)	Y☐ N☐	Sore throat	Y☐ N☐	Myalgia/body pains	Y☐ N☐	
History of fever	Y☐ N☐	Shortness of breath	Y☐ N☐	General weakness	Y☐ N☐	
Cough	Y☐ N☐	Nausea/vomiting	Y☐ N☐	Irritability/confusion	Y☐ N☐	
Chills	Y☐ N☐	Diarrhoea	Y☐ N☐	Other	Y☐ N☐	Specify_____

DIAGNOSIS

- Did the patient have clinical or radiological evidence of pneumonia Y☐ N☐
- Were chest X-rays (CXR) done: Y☐ N☐ If yes, CXR Findings: _____
- Did the patient have clinical or radiological evidence of acute respiratory distress syndrome (ARDS)? Y☐ N☐

Page 1 of 2 Please refer to www.nicd.ac.za for most recent version of this document before use. Version 3, 17 February 2020

This section is a prerequisite for testing, therefore, please fill out the below section to the best of your ability.
Laboratory testing will be delayed if forms are incomplete or were filled in incorrectly.
In the 14 days before symptom onset did the patient (mark all that apply):

- Have close physical contact[2] with a **known** COVID-19 case? Y☐ N☐ Unkn☐
- If the patient has been in a close physical contact with a known COVID-19 case, please indicate contact setting:

  Healthcare setting ☐ Family setting ☐ Work place ☐ Public transport setting ☐ Other ☐ Specify:_____
- Patient is a healthcare worker (HCW) who was exposed to patients with severe acute respiratory illness, unless another aetiology has been identified to explain the clinical presentation of the HCW? Y☐ N☐ Unkn☐
- Is the patient part of a severe respiratory illness cluster of unknown aetiology that occurred within a 14-day period? Y☐ N☐ Unkn☐
- Patient has visited a health care facility (as a patient or visitor) in a country where hospital-associated COVID-19 cases have been reported? Y☐ N☐ Unkn☐ (If yes, complete travel section)
- Has the patient travelled to/from China or area/s with evidence of sustained SARS-CoV-2 (cause of COVID-19) human-to-human transmission, or a declared outbreak? Y☐ N☐ Unkn☐ (If yes, complete travel section)

TRAVEL HISTORY

If patient traveled outside South Africa in the last 14-days, please complete section below for countries visited

Country and city or cities visited	Date of departure (travel to area)	Date of return (travel from area)
1.	DD/MM/YYYY	DD/MM/YYYY
2.	DD/MM/YYYY	DD/MM/YYYY

UNDERLYING FACTORS/CO-MORBID CONDITIONS

Asthma:	Y☐ N☐ Unkn☐	Cardiac disease:	Y☐ N☐ Unkn☐	Chronic kidney disease:	Y☐ N☐ Unkn☐	Chronic liver disease:	Y☐ N☐ Unkn☐
Chronic neurological/ neuromuscular disease:	Y☐ N☐ Unkn☐	COPD/ Chronic pulmonary disease:	Y☐ N☐ Unkn☐	Diabetes:	Y☐ N☐ Unkn☐	Immuno-deficiency (excluding HIV)	Y☐ N☐ Unkn☐
HIV:	Y☐ N☐ Unkn☐	Is the patient virally suppressed?	Y☐ N☐ Unkn☐	Recent viral load:	_____	On ARVs	Y☐ N☐ Unkn☐
Obesity:	Y☐ N☐ Unkn☐	Pregnancy:	Y☐ N☐ Unkn☐	Trimester:	_____	Tuberculosis:	Y☐ N☐ Unkn☐
Other:	Y☐ N☐ Unkn☐	Specify:	_____				

TREATMENT/MANAGEMENT

Patient hospitalised:	Y☐ N☐ Unkn☐	Admitted to ICU:	Y☐ N☐ Unkn☐	Ventilation:	Y☐ N☐ Unkn☐	On ECMO:	Y☐ N☐ Unkn☐
Antibiotics:	Y☐ N☐ Unkn☐	if Yes, list:	_____	Tamiflu/ other antiviral drugs:	Y☐ N☐ Unkn☐		
White cell count total:	_____	Differential neutrophils/lymphocytes%:	_____				

Has the patient been isolated at: Home ☐ Healthcare facility ☐ Not isolated ☐ Other ☐ Specify: _____

If patient has been isolated at home or at a healthcare facility, please provide date of isolation: DD/MM/YYYY

OUTCOME (at time of specimen submission)

Currently hospitalised:	☐		
Discharged	☐	**Discharge date:**	DD/MM/YYYY
Transferred	☐	**Name of facility:**	
Died	☐	**Date of death:**	DD/MM/YYYY
Other	☐	**Specify:**	

[1]If patient is a not a permanent resident, please provide their current residential address while residing in South Africa. [2]Close contact: A person having had face-to-face contact or was in a closed environment with a COVID-19 case; this includes, amongst others, all persons living in the same household as a COVID-19 case and, people working closely in the same environment as a case. A healthcare worker or other person providing direct care for a COVID-19 case, while not wearing recommended personal protective equipment or PPE). A contact in an aircraft sitting within two seats (in any direction) of the COVID-19 case, travel companions or persons providing care, and crew members serving in the section of the aircraft where the index case was seated. [3]Areas with presumed ongoing community transmission of SARS-CoV-2: http://www.nicd.ac.za/diseases-a-z-index/covid-19/

Please also complete the contact line list provided and submit with specimen submission form and PUI form to ncov@nicd.ac.za

10.2. Appendix II: Suspected case – specimen submission form

CRDM unique no: CRDM lab no: Trak no: Date received:

SARS-CoV-2 (COVID-19) Specimen Submission form

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

Centre for Respiratory Diseases and Meningitis

For SARS-CoV-2 testing only, for any other diagnostic testing please use this form:

http://www.nicd.ac.za/wpcontent/uploads/2020/02/CRDM_specimen_submission_form_v3_14_Feb_2020_Elect.pdf

Patient Information		**Submitter Information (contact person for results)**	
RSA identification number or Passport number **(REQUIRED*)**		Surname	
		First name	
Surname		Hospital/Practice/Facility	
First name		Laboratory	
Date of birth		Country (if SA, Province)	
Age (if no DOB)	☐ Years ☐ Months ☐ Days	Contact number (country code)	+ ( )
Gender	☐ Male ☐ Female	Email address	
Province (Patient)			

Specimen Details

Is this a repeat specimen? ☐ Yes ☐ No

Specimen collection date: dd-mm-yyyy

Specimen type:

☐ Combined NP/OP swab	☐ Nasopharyngeal (NP) aspirate	☐ Pleural fluid
☐ Nasopharyngeal (NP) swab	☐ Bronchoalveolar lavage (BAL)	☐ Sputum
☐ Oropharyngeal (OP) swab	☐ Tracheal aspirate (TA)	☐ Serum
☐ Other, specify: _____		

*Please note that either a RSA identification number or passport number is required to allow linking of repeat specimens. Testing will be delayed for specimens submitted without this information.

Note: Please access results via TrakCare

CRDM SARS-CoV-2 Submission Form V1 March 2020 Date entered: Initials:

10.3. Appendix III: Confirmed case – case record form (on enrollment)

COVID-19 Case Record Form- Admission CNN|___|___|___|___|___|___|___|___|___|___|

To be completed for every patient admitted to a facility with CONFIRMED COVID-19. Use case notification number generated at notification as patient ID. **Surname**_____ **Hospital#**_____

Section A: Inclusion criteria/ epidemiological factors			
1.	interviewdate	**Date of interview (dd/mmm/yyyy)** |___|___|/|___|___|___|/|___|___|___|___|	
2.	facility	**Name of facility:**	
3.	district	**Name of District:**	
4.	province	**Name of Province:**	
5.	country	**Country of Residence**	
Section B : Demographics			
6.	age	**Age (in years and months)** |___|___|y |___|___|m	
7.	dateofbirth	**Date of birth (dd/mmm/yyyy)** |___|___|/|___|___|___|/|___|___|___|___|	
8.	gender	**Sex at birth** **Male ___|** **Female___|**	
9.	ethnicgrp	**Ethnic group** Black African ___| White ___| Colored ___| Asian/Indian ___| Other ___|	
10.		**Have you travelled outside of South Africa in the previous 2 weeks?** Yes___| No ___| UNK___| If yes, which country: _____	
11.	hcw	**Employed as a Healthcare Worker?** Yes___| No ___| UNK___|	
12.	hcf	**Visited a health care facility in previous 14 days** **Yes___| No ___| UNK___|** If yes, which one?_____	
13.	mlw	**Employed in a laboratory?** **Yes___| No ___| UNK___|** If yes, which one?_____	If male go to Q17
14.	pregnant	**Pregnant?** **Yes___| No ___| UNK___|** If yes, gestational age: |___|___|weeks	ifyes go to Q17
15.	pp	**POST PARTUM? (delivered a baby in the past 40 days)** **Yes___| No ___| UNK___|**	if No /UNK go to Q17
16.	po	**Pregnancy Outcome:** Delivery date: |___|___|/|___|___|___|/|___|___|___|___| Gestational age of child: |_|_|weeks Baby tested for SARS-COV2? **Yes___| No ___| UNK___|** If YES: ___| Positive ___| Negative___|	
Section C: Co-morbidities			
17.	comorbids	**Does the patient have past history of the following** - Chronic cardiac disease, including congenital heart disease (not hypertension) **Yes___| No ___| UNK___|** - Hypertension) **Yes___| No ___| UNK___|** -ACE inhibitor or Angiotensin Receptor Blocker use **Yes___| No___| UNK___|** -Chronic pulmonary disease (not asthma) **Yes___| No ___| UNK___|** -Asthma (physician diagnosed) **Yes___| No ___| UNK___|** -Tuberculosis (pulmonary) **Yes___| No ___| UNK___|** -Tuberculosis (extra-pulmonary) **Yes___| No ___| UNK___|** -Chronic kidney disease **Yes___| No __ | UNK___|** -Liver disease **Yes___| No ___| UNK___|** -Chronic neurological disorder **Yes___| No ___| UNK___|** -Malignant neoplasm (cancer) **Yes___| No ___| UNK___|** -Chronic hematologic disease **Yes___| No ___| UNK___|**	

Republic of South Africa, National Institute for Communicable Diseases; 2019-nCov CRF v1.1; 6 April 2020

COVID-19 Case Record Form- Admission CNN|___|___|___|___|___|___|___|___|___|___|__|

To be completed for every patient admitted to a facility with CONFIRMED COVID-19. Use case notification number generated at notification as patient ID. **Surname**_____ **Hospital#**_____

		-Obesity (BMI>30) **Yes___| No ___| UNK___|** -Diabetes **Yes___| No ___| UNK___|** -Rheumatologic disorder **Yes___| No ___| UNK___|** -Dementia **Yes___| No ___| UNK___|** - Immunosuppresivie therapy (cortisone, chemo, radiation therapy, biologicals) **Yes___| No ___| UNK___|** -Smoking **Current smoker___| Former smoker ___| Non smoker___|** -Other relevant risk factor **Yes___| No ___| UNK___|** If other Specify _____	
Section D: Onset and admission			
18.	onsetdate	**Onset date of first/earliest symptom: (dd/mmm/yyyy)** |___|___|/|___|___|___|/|___|___|___|___|	
19.	admdatetime	**Date of admission (dd/mmm/yyyy)** : |___|___|/|___|___|___|/|___|___|___|___|	
20.	transfer	**Transfer from other facility**? **Yes___| No ___| UNK___|** If YES: Name of transfer facility:_____ If YES: Admission date at transfer facility (dd/mmm/yyyy): |___|___|/|___|___|___|/|___|___|___|___|N/A___|	
Section E: Signs/symptoms			
21.	earlysymptoms	**First/earliest symptoms associated with episode** History of fever **Yes___| No ___| UNK___|** Cough **Yes___| No ___| UNK___|** Cough with sputum production **Yes___| No ___| UNK___|** Cough with bloody sputum/haemoptysis **Yes___| No ___| UNK___|** Sore throat **Yes___| No ___| UNK___|** Runny nose (Rhinorrhoea) **Yes___| No ___| UNK___|** Ear pain **Yes___| No ___| UNK___|** Wheezing **Yes___| No ___| UNK___|** Chest pain **Yes___| No ___| UNK___|** Muscle aches (Myalgia) **Yes___| No ___| UNK___|** Joint pain (Arthralgia) **Yes___| No ___| UNK___|** Fatigue / Malaise **Yes___| No ___| UNK___|** Shortness of breath (Dyspnea) **Yes___| No ___| UNK___|** Lower chest wall indrawing **Yes___| No ___| UNK___|** Headache **Yes___| No ___| UNK___|** Altered consciousness/confusion **Yes___| No ___| UNK___|** Seizures **Yes___| No ___| UNK___|** Abdominal pain **Yes___| No ___| UNK___|** Vomiting / Nausea **Yes___| No ___| UNK___|** Diarrhoea **Yes___| No ___| UNK___|** Conjunctivitis **Yes___| No ___| UNK___|** Skin rash **Yes___| No ___| UNK___|** Skin ulcers **Yes___| No ___| UNK___|** Lymphadenopathy **Yes___| No ___|UNK___|** Bleeding (Haemorrhage) **Yes___| No ___| UNK___|** If Bleeding: specify site(s):_____	
22.	symptoms	**Symptoms on admission** Fever **Yes___| No ___| UNK___|** Cough **Yes___| No ___| UNK___|**	

COVID-19 Case Record Form- Admission CNN|___|___|___|___|___|___|___|___|___|___|___|

To be completed for every patient admitted to a facility with CONFIRMED COVID-19. Use case notification number generated at notification as patient ID.

Surname_____ **Hospital#**_____

		Cough with sputum production **Yes**___| **No** ___| **UNK**___| Cough with bloody sputum/haemoptysis **Yes**___| **No** ___| **UNK**___| Sore throat **Yes**___| **No** ___| **UNK**___| Runny nose (Rhinorrhoea) **Yes**___| **No** ___| **UNK**___| Ear pain **Yes**___| **No** ___| **UNK**___| Wheezing **Yes**___| **No** ___| **UNK**___| Chest pain **Yes**___| **No** ___| **UNK**___| Muscle aches (Myalgia) **Yes**___| **No** ___| **UNK**___| Joint pain (Arthralgia) **Yes**___| **No** ___| **UNK**___| Fatigue / Malaise **Yes**___| **No** ___| **UNK**___| Shortness of breath (Dyspnea) **Yes**___| **No** ___| **UNK**___| Lower chest wall indrawing **Yes**___| **No** ___| **UNK**___| Headache **Yes**___| **No** ___| **UNK**___| Altered consciousness/confusion **Yes**___| **No** ___| **UNK**___| Seizures **Yes**___| **No** ___| **UNK**___| Abdominal pain **Yes**___| **No** ___| **UNK**___| Vomiting / Nausea **Yes**___| **No** ___| **UNK**___| Diarrhoea **Yes**___| **No** ___| **UNK**___| Conjunctivitis **Yes**___| **No** ___| **UNK**___| Skin rash **Yes**___| **No** ___| **UNK**___| Skin ulcers **Yes**___| **No** ___| **UNK**___| Lymphadenopathy **Yes**___| **No** ___|**UNK**___| Bleeding (Haemorrhage) **Yes**___| **No** ___| **UNK**___| If Bleeding: specify site(s):_____	
23.	admissionvitals	Temperature: [_][_].[_]°C Heart rate: [_][_][_]beats per minute RR: [_][_]breaths per minute Systolic BP: [_] [_] [_]mmHg Diastolic BP: [_][_][_]mmHg Severe dehydration (requiring IV fluid bolus): **Yes**___| **No** ___| **UNK**___| Sternal capillary refill time >2seconds **Yes**___| **No** ___| **UNK**___| Oxygen saturation: [_][_][_]% On: Room air ___|Oxygen therapy ___| UNK ___| Central cyanosis: **Yes**___| **No** ___| **UNK**___|	
24.	HIVstatus	**HIV status of patient** **Pos** ___| **Neg** ___| **Unk** ___| Source of info Track lab result ___| Rapid test ___| Verbal ___| Date of test |___|___|/|___|___|___|/|___|___|___|___| N/A___| If HIV Pos, please supply latest results from the last 3 months CD4 count (absolute) ____/mm^3 Not done __| Viral load _____ copies per ml Not done __| If HIV Pos, ARV therapy Yes ___| No ___| Unk ___| If yes, please specify antiviral agents: 1._____ 2._____ 3._____	Skip if HIV neg

10.4. Appendix IV: Confirmed case – daily assessment form

COVID19 DAILY ASSESSMENT FORM

To be completed for every patient with confirmed COVID-2019.
Use the Case Notification Number (CNN) generated at notification as patient ID

CNN|___|___|___|___|___|___|___|___|___|___|

Surname_____DOB_____Hospital#_____

Complete for each day patient is admitted in facility (for period 07h00 previous day to 07h00 day of assessment)

Section A: Inclusion criteria/ epidemiological factors		
1.	dateassess	**Date of assessment (dd/mmm/yyyy)** |___|___|/|___|___|___|/|___|___|___|___|
2.	admissionplace	**Admission in past 24 hours (tick all that apply)** Intensive Care Unit **Yes___| No ___| UNK___|** Intensive Treatment Unit/ Critical Care Unit **Yes___| No ___| UNK___|** Intermediate Care Unit/Step-down ward **Yes___| No ___| UNK___|** High dependency Unit **Yes___| No ___| UNK___|** Other _____
3.	symptoms **(UNK if patient unconscious or information not available)**	**Any current signs/symptoms (during the period 07h00 the previous day to 07h00 today)** Fever **Yes___| No ___| UNK___|** Cough **Yes___| No ___| UNK___|** Cough with sputum production **Yes___| No ___| UNK___|** bloody sputum/haemoptysis **Yes___| No ___| UNK___|** Sore throat **Yes___| No ___| UNK___|** Runny nose (Rhinorrhoea) **Yes___| No ___| UNK___|** Ear pain **Yes___| No ___| UNK___|** Wheezing **Yes___| No ___| UNK___|** Chest pain **Yes___| No ___| UNK___|** Muscle aches (Myalgia) **Yes___| No ___| UNK___|** Joint pain (Arthralgia) **Yes___| No ___| UNK___|** Fatigue / Malaise **Yes___| No ___| UNK___|** Shortness of breath (Dyspnea) **Yes___| No ___| UNK___|** Lower chest wall indrawing **Yes___| No ___| UNK___|** Central cyanosis **Yes___| No ___| UNK___|** Headache **Yes___| No ___| UNK___|** Altered consciousness/confusion **Yes___| No ___| UNK___|** Seizures **Yes___| No ___| UNK___|** Abdominal pain **Yes___| No ___| UNK___|** Vomiting / Nausea **Yes___| No ___| UNK___|** Diarrhoea **Yes___| No ___| UNK___|** Conjunctivitis **Yes___| No ___| UNK___|** Skin rash **Yes___| No ___| UNK___|** Skin ulcers **Yes___| No ___| UNK___|** Lymphadenopathy **Yes___| No ___| UNK___|** Bleeding (Haemorrhage) **Yes___| No ___| UNK___|** If Bleeding: specify site(s):_____
4.	readings	**Worst ever measurements during the period 07h00 the previous day to 07h00 today**

Done (Y/N)	**Test**	**Reading**
	AVPU	Alert ___| Verbal ___| Pain ___| Unresponsive ___|
	Glasgow Coma Score (GCS / 15)	
	Systolic Blood Pressure	mmHg
	Diastolic Blood Pressure	mmHg
	Oxygen saturation	%

5.	treatment	**Is the patient currently receiving, or has received?** Non-invasive ventilation (e.g. BIPAP, CPAP)? **Yes___| No ___| UNK___|**

Republic of South Africa, National Institute for Communicable Diseases; COVID 2019, Daily Assessment Form v1.1; 6 April 2020

COVID19 DAILY ASSESSMENT FORM CNN|___|___|___|___|___|___|___|___|___|___|___|

To be completed for every patient with confirmed COVID-2019.
Use the Case Notification Number (CNN) generated at notification as patient ID

Surname_____**DOB**_____**Hospital#**_____

		Invasive ventilation (through ET tube)?	**Yes**___| **No** ___| **UNK**___|
		Extra corporeal life support (ECLS)?	**Yes**___| **No** ___| **UNK**___|
		High-flow nasal canula oxygen therapy	**Yes**___| **No** ___| **UNK**___|
		Dialysis/Hemofiltration?	**Yes**___| **No** ___| **UNK**___|
		Neuromuscular blocking agents?	**Yes**___| **No** ___| **UNK**___|
		Tracheostomy inserted?	**Yes**___| **No** ___| **UNK**___|
		Prone positioning?	**Yes**___| **No** ___| **UNK**___|
		Any vasopressor/inotropic support?	**Yes**___| **No** ___| **UNK**___|
		Other intervention or procedures	**Yes**___| **No** ___| **UNK**___|
		If Yes, Specify	_____
6.	labresults	**Daily laboratory results**	

Done (Y/N)	**Test**	**Reading**
	Haemoglobin	g/dl
	WBC	10^3/µL
	Neutrophil count	cells/ µL
	Lymphocyte	cells/ µL
	Haematocrit	%
	Platelets	$X10^3$/µL
	APTT/APTR	
	PT	seconds
	INR	
	ALT/SGPT	U/L
	Total Bilirubin	µmol/L
	AST/SGOT	U/L
	Glucose	mmol/L
	Blood Urea Nitrogen (urea)	mmol/L
	Creatinine	µmol
	Sodium	mEq/L
	Potassium	mEq/L
	Calcium	mEq/L
	Magnesium	mEq/L
	Procalcitonin	ng/mL
	CRP	mg/L

Republic of South Africa, National Institute for Communicable Diseases; COVID 2019, Daily Assessment Form v1.1; 6 April 2020

10.5. Appendix V: Confirmed case – final outcome form

COVID19 FINAL IN-HOSPITAL OUTCOME FORM

To be completed for every patient with confirmed COVID-19 at discharge, transfer or death. Use the Case Notification Number (CNN) generated at notification as patient ID

CNN|___|___|___|___|___|___|___|___|___|___|

Surname_____DOB_____Hospital#_____

Complete at patient's in-hospital discharge, transfer, cessation of shedding or death

Section A: Complications			
1.	assessdate	**Date of assessment (dd/mmm/yyyy)** |___|___|/|___|___|___|/|___|___|___|___|	
2.	inhospoutcomedt	**Date of in-hospital outcome (dd/mmm/yyyy)** |___|___|/|___|___|___|/|___|___|___|___|	
3.	anycomplic	**At any time during hospitalization did the patient experience any of the following complications?** Pneumonia **Yes___| No ___| UNK___|** Pneumothorax **Yes___| No ___| UNK___|** Pleural effusion **Yes___| No ___| UNK___|** Cryptogenic organizing pneumonia (COP) **Yes___| No ___| UNK___|** Bronchiolitis **Yes___| No ___| UNK___|** Meningitis / Encephalitis **Yes___| No ___| UNK___|** Seizure **Yes___| No ___| UNK___|** Stroke / Cerebrovascular accident **Yes___| No ___| UNK___|** Congestive heart failure **Yes___| No ___| UNK___|** Endocarditis / Myocarditis / Pericarditis **Yes___| No ___| UNK___|** Cardiac ischaemia **Yes___| No ___| UNK___|** Cardiac arrhythmia **Yes___| No ___| UNK___|** Cardiac arrest **Yes___| No ___| UNK___|** Bacteremia **Yes___| No ___| UNK___|** Coagulation disorder / Disseminated Intravascular Coagulation **Yes___| No ___| UNK___|** Anemia **Yes___| No ___| UNK___|** Rhabdomyolysis / Myositis **Yes___| No ___| UNK___|** Acute renal injury/ Acute renal failure **Yes___| No ___| UNK___|** Gastrointestinal haemorrhage **Yes___| No ___| UNK___|** Pancreatitis **Yes___| No ___| UNK___|** Liver dysfunction **Yes___| No ___| UNK___|** Hyperglycemia **Yes___| No ___| UNK___|** Hypoglycemia **Yes___| No ___| UNK___|** Other **Yes___| No ___| UNK___|** **Specify** _____	
4.	labtests	**Were other pathogens tested for during admission?** Yes___| No ___| UNK___| **If yes complete below**: Influenza : YES___| NO ___| UNK___| if Yes, result: POS ___| NEG ____ Bacteria: YES___| NO ___| UNK___| if Yes, Specimen sent: Blood ___| CSF ___| Respiratory secretion ___| Other ___| Culture result: NO Growth ___ | Organism Isolated __ | Organism Name: _____	

Republic of South Africa, National Institute for Communicable Diseases; COVID 2019, Discharge Form v1.1; 6 April 2020

COVID19 FINAL IN-HOSPITAL OUTCOME FORM CNN|___|___|___|___|___|___|___|___|___|___|

To be completed for every patient with confirmed COVID-19 at discharge, transfer or death. Use the Case Notification Number (CNN) generated at notification as patient ID

Surname_____**DOB**_____**Hospital#**_____

		Other Infectious (lab confirmed) Respiratory diagnosis: YES__| NO __|UNK___| If Yes, specify _____	
5.	imaging	**Was a chest xray or CT scan performed?** **Yes___| No ___| UNK___|** If yes, was there: Ground glass/diffuse opacity **Yes___| No ___| UNK___|** Lobar consolidation **Yes___| No ___| UNK___|** Bilateral/multilobar consolidation **Yes___| No ___| UNK___|** Pleural effusion **Yes___| No ___| UNK___|** White out **Yes___| No ___| UNK___|**	
6.	treatment	**At ANY time during hospitalisation, did the patient receive/undergo?** -ICU or High Dependency Unit admission? **Yes___| No ___| N/A___|** If yes, Date of ICU admission: |___|___|/|___|___|___|/|___|___|___|___| N/A___| Date of ICU discharge: |___|___|/|___|___|___|/|___|___|___|___| N/A___| -Oxygen therapy? **Yes___| No ___| UNK___|** Non-invasive ventilation? (e.g. BIPAP, CPAP) **Yes___| No ___| UNK___|** If YES, total duration: _____days Invasive ventilation? (with endotracheal tube inserted) **Yes___| No ___| UNK___|** If YES, total duration: _____ days Prone Ventilation? **Yes___| No ___| UNK___|** Tracheostomy inserted **Yes___| No ___| UNK___|** Extracorporeal support? **Yes___| No ___| UNK___|** Renal replacement therapy (RRT) or dialysis? **Yes___| No ___| UNK___|** Inotropes/vasopressors? **Yes___| No ___| UNK___|** If YES: First/Start date: |___|___|/|___|___|___|/|___|___|___|___| UNK___| Last/End date: |___|___|/|___|___|___|/|___|___|___|___| UNK___| OTHER intervention_____	
7.	medication	**While hospitalised or at discharge, were any of the following administered?** Antiviral agent? **Yes___| No ___| UNK___|**	

COVID19 FINAL IN-HOSPITAL OUTCOME FORM

To be completed for every patient with confirmed COVID-19 at discharge, transfer or death. Use the Case Notification Number (CNN) generated at notification as patient ID

CNN|___|___|___|___|___|___|___|___|___|___|

Surname_____DOB_____Hospital#_____

If YES:

Ribavirin **Yes___| No ___| UNK___|**

Lopinavir/Ritonavir **Yes___| No ___| UNK___|**

Interferon alpha **Yes___| No ___| UNK___|**

Interferon beta **Yes___| No ___| UNK___|**

Neuraminidase inhibitor **(oseltamivir)** **Yes___| No ___| UNK___|**

Other _____

Antibiotics? **Yes___| No ___| UNK___|**

If yes, specify which antibiotic

Agent: _____Route: Oral___| Intravenous___| Inhaled___|

Agent: _____Route: Oral___| Intravenous___| Inhaled___|
Agent: _____Route: Oral___| Intravenous___| Inhaled___|
Agent: _____Route: Oral___| Intravenous___| Inhaled___|
Agent: _____Route: Oral___| Intravenous___| Inhaled___|

Antifungal agent? **Yes___| No ___| UNK___|**

If yes, specify which antifungals

Agent: _____Route: Oral___| Intravenous___| Inhaled___|
Agent: _____Route: Oral___| Intravenous___| Inhaled___|
Agent: _____Route: Oral___| Intravenous___| Inhaled___|

Antimalarial?

Cholorquine **Yes___| No ___| UNK___|**

Corticosteroids? **Yes___| No ___| UNK___|**

If yes, specify which corticosteroids:
Agent: _____Route: Oral___| Intravenous___| Inhaled___|

Nonsteroidal antiinflammatories? **Yes___| No ___| UNK___|**

If yes, specify which NSAID:

Agent: _____Route: Oral___| Intravenous___| Inhaled___|

Immunoglobulin? **Yes___| No ___| UNK___|**

If yes, specify:

Agent: _____Route: Oral___| Intravenous___| Inhaled___|

COVID19 FINAL IN-HOSPITAL OUTCOME FORM

To be completed for every patient with confirmed COVID-19 at discharge, transfer or death. Use the Case Notification Number (CNN) generated at notification as patient ID

CNN|___|___|___|___|___|___|___|___|___|___|

Surname_____DOB_____Hospital#_____

		Was the patient enrolled in any other COVID-19-related trial? **Yes___| No ___| UNK___|** If yes, specify study name: _____ Study identification number: _____ Principle investigator: _____	
Section B: Patient Outcomes			
8.	outcomes	**What is the patient's outcome:** Stopped shedding: ___| Discharged: ___| If discharged, was this a Palliative Discharge: Yes __| No ___| Unk ___| Transfer to step down facility: ___| Died: ___| Unknown: ___| Outcome date: |___|___|/|___|___|___|/|___|___|___|___| UNK___|	

10.6. Appendix VI: Confirmed case – follow-up laboratory slip

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

CENTRE FOR RESPIRATORY DISEASES AND MENINGITIS
OUTBREAK RESPONSE, DIVISION OF PUBLIC HEALTH SURVEILLANCE AND RESPONSE

COVID-19 Shedding and Clinical Characterization Study: Follow-Up Laboratory Slip

Patient Identification No.:_____

This number has been provided by CRDM following confirmation of COVID-19 and it should be used to identify all samples collected from this patient

Complete ONE laboratory slip for EACH collected specimen even if multiple specimens are collected on the same day.

Specimen collection date: DD/MM/YYYY

Facility/Hospital (if applicable): _____

Days since symptom onset: _____days

Form completed by (Name, Surname): _____

Contact number(s): _____

Respiratory / Stool Specimens

☐ Combined Nasopharyngeal (NP)/Oropharyngeal (OP) Swab
☐ Tracheal aspirate
☐ Stool/Rectal swab
☐ Nasal swab

Nasal swab collected by: ☐ HCW ☐ Patient

Follow up visit:

00*☐ 02☐ 04☐ 06☐ 08☐ 10☐ 12☐ 14☐ 16☐ 18☐ 20☐ 22☐ 24☐ 26☐ 28☐ 30☐ 32☐ 34☐ 36☐ 38☐ 40☐

NOTE: Not all listed follow-up visits must be completed. The collection of respiratory/stool specimens will stop after cessation of shedding (i.e., test negative for SARS-CoV-2 on two consecutive respiratory samples on rRT-PCR on respiratory specimens.
* Enrollment

Blood Specimen)

☐ Serum separator tube (yellow top) – 5ml
☐ EDTA tube (purple top) – 3ml
☐ Red top tube – 5ml

Follow up visit (in days post symptoms onset):

00*☐ 07☐ 14☐ 21☐ 28☐ 56☐

NOTE: All follow-up visits must be completed.
* Enrolment, then on the next available scheduled date (e.g., If the patient is enrolled on day 4 post-symptoms onset, then collect a blood specimen on enrollment (day 4) and days 14, 21, 28 and 56 post symptoms onset. If the patient is enrolled on day 9 post-symptoms onset, then collect a blood specimen on enrollment (day 9) and days 14, 21, 28 and 56 post symptoms onset. If the patient is enrolled on day 19 post-symptoms onset, then collect a blood specimen on enrollment (day 19) and days 21, 28 and 56 post symptoms onset).

For laboratory use only:

TCD Number: Note: For lab use only.

CRDM Number Note: For lab use only.

Received by (Name and Surname):_____

Date received: DD/MM/YYYY

Page 1 of 1

10.7. Appendix VII: Confirmed case – follow-up calendars

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division in the National Health Laboratory Service

CENTRE FOR RESPIRATORY DISEASES AND MENINGITIS
OUTBREAK RESPONSE, DIVISION OF PUBLIC HEALTH SURVEILLANCE
AND RESPONSE

COVID-19 Shedding and Clinical Characterization Study:
Follow-Up Calendar – Forms, Respiratory and Stool Specimens

Follow-up procedure due day
Follow-up procedure catch-up day

Patient Identification No.:_____

This number has been provided by CRDM following confirmation of COVID-19 and it should be used to identify all samples collected from this patient

Complete the calendar follow-up dates (DD/MM) at enrolment, then complete the procedures sections (☐Y ☐N) when implemented

Enrolment date: DD/MM/YYYY

Facility/Hospital: _____

Symptoms onset date: DD/MM/YYYY

Completed by (Name and Surname): _____

Follow-up number:	00 (Enrolment)*		02		04		06		08		10	
Follow-up date:	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM
Consent Form:	☐Y ☐N											
Case Record Form:	☐Y ☐N											
Daily Follow-up Form:		☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
NP/OP Swabs:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Nasal Swab:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Stool:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Outcome Form:§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y ☐N	☐Y ☐N
Follow-up number:	**12**		**14**		**16**		**18**		**20**		**22**	
Follow-up date:	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM
Daily Follow-up Form:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
NP/OP Swabs:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Nasal Swab:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Stool:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Outcome Form:§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y ☐N	☐Y ☐N
Continue on next page..												

* The first date is the date of enrolment. Then complete the remaining dates with sequential dates.
§Outcome form to be completed at any of the following instances only: (i) transfer to a different facility, (ii) discharge (either before or after cessation of shedding), (iii) cessation of shedding (if the patient was discharged after cessation of shedding), and (iv) death of the patient.

Follow-up number:	24		26		28		30		32		34	
Follow-up date:	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM
Daily Follow-up Form:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
NP/OP Swabs:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Nasal Swab:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Stool:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Outcome Form:§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y ☐N	☐Y ☐N
Follow-up number:	36		38		40		42		44		46	
Follow-up date:	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM
Daily Follow-up Form:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
NP/OP Swabs:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Nasal Swab:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Stool:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Outcome Form:§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y ☐N	☐Y ☐N
Follow-up number:	48		50		52		54		56			
Follow-up date:	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM
Daily Follow-up Form:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
NP/OP Swabs:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Nasal Swab:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Stool:	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N	☐Y ☐N
Outcome Form:§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y§ ☐N§	☐Y ☐N	☐Y ☐N

Date of first negative NP swab: DD/MM/YYYY

Date of second negative NP swab: DD/MM/YYYY

Reason for study cessation:

___| 2 sequential negative NP swabs noted

___| death

___| lost to follow up

* The first date is the date of enrolment. Then complete the remaining dates with sequential dates.

§Outcome form to be completed at any of the following instances only: (i) transfer to a different facility, (ii) discharge (either before or after cessation of shedding), (iii) cessation of shedding (if the patient was discharged after cessation of shedding), and (iv) death of the patient.

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division in the National Health Laboratory Service

CENTRE FOR RESPIRATORY DISEASES AND MENINGITIS
OUTBREAK RESPONSE, DIVISION OF PUBLIC HEALTH SURVEILLANCE AND RESPONSE

COVID-19 Shedding and Clinical Characterization Study: Follow-Up Calendar – Whole Blood (in serum separator tubes)

Patient Identification No.:_____

This number has been provided by CRDM following confirmation of COVID-19 and it should be used to identify all samples collected from this patient

Follow-up procedure due day
Follow-up procedure catch-up day (due date +1 day)

Complete all information in the yellow boxes at enrolment, then complete the procedure section when implemented

- If the enrolment occurs within 0-5 days from symptoms onset, collect a blood specimen on enrollment (follow-up visit number 00) and at days 7, 14, 21, 28 and 56 post symptoms onset.
- If the enrolment occurs within 8-12 days from symptoms onset, collect a blood specimen on enrollment (follow-up visit number 00) and at days 14, 21, 28 and 56 post symptoms onset.
- If the enrolment occurs within 15-19 days from symptoms onset, collect a blood specimen on enrollment (follow-up visit number 00) and at days 21, 28 and 56 post symptoms onset.
- If the enrolment occurs within 22-26 days from symptoms onset, collect a blood specimen on enrollment (follow-up visit number 00) and at days 28 and 56 post symptoms onset.
- If the enrolment occurs 7, 14, 21 or 28 days post symptom onset complete the enrolment follow-up visit number (00), skip the corresponding follow-up visit and complete the remaining follow-up visits (e.g., if the enrolment occurs at 7 days post symptom onset complete the form for follow-up visit number 00, skip follow up visit number 07 and complete the remaining follow-up visits: 14, 21, 28 and 56).
- If the enrollment occurs 6, 13, 20 or 27 days post symptoms onset (one day before the due sample collection date) do not collect a blood specimen at enrollment (mark follow-up visit number 00 as: ☒No) and collect a blood specimens at the next scheduled visit (e.g. day 7, 14, 21, or 28 post symptoms onset). Complete the remaining scheduled visits thereafter.

Enrolment date: DD/MM/YYYY

Facility/Hospital: _____

Symptoms onset date: DD/MM/YYYY

Completed by (Name and Surname): _____

Number of days post symptoms onset at enrolment: DD

Follow-up number:	00 (Enrolment)*		07		14		21		28		56	
Days post symptoms onset:	DD	DD	07	08	14	15	21	22	28	29	56	57
Follow-up date:	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM	DD/MM
Blood specimens	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top	☐No ☐Red top ☐Yllw top ☐Prpl top

Reasons for cessation of study: all specimens completed ___| death of patient ___| lost to follow up ___|

* The first date is the date of enrolment. Then complete the remaining dates with the relevant dates post symptoms onset.

10.8. Appendix VIII: Participant information leaflet and informed consent

Information leaflet for cases of coronavirus (COVID-19): Adults

STUDY TITLE: Characterization of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Shedding and the Novel Coronavirus Disease (COVID-19) Clinical Features among the First Confirmed Cases in South Africa

---

Persons contemplating participation in this study are asked to read this document or have it read to them, if they are unable to read it for themselves and sign the attached informed consent before any study-related procedure is done.

Institution: National Institute for Communicable Diseases, South Africa; funded by core NICD funds.
Investigator: Professor Cheryl Cohen 011.386.6593 (daytime) and 082.803.8093 (afterhours).

Hello, my name is Professor Cheryl Cohen. I am the Head of the Centre for Respiratory Disease and Meningitis at the National Institute for Communicable Diseases in Johannesburg. I would like to invite you to think about helping us with a research study called the *COVID-19 shedding and disease progression study* (short title)

- Before you decide on whether to agree to take part in this study, we would like you to read this information sheet about the study.
- Before you say whether you would like to take part, please make sure you understand what you will be asked to do.
- You should also make sure you understand the purpose of the study, the study procedures, benefits, risks, discomforts and precautions and your right to withdraw from the study at any time.
- This information leaflet is to help you to decide if you would like to participate.
- If you have any questions, do not hesitate to ask me, or the study staff who will introduce the study to you.
- You should not agree to take part unless you are satisfied with all the procedures involved.
- Please be open with me regarding your health history, since you may otherwise harm yourself by participating in this study.
- If you decide to take part in this study, you will be asked to sign a Consent Sheet to confirm that you understand the study. You will be given a copy of the Information and Consent sheets to keep.

Background/Purpose

Infectious diseases are caused by different germs (virus, bacteria or parasites). By infectious we mean that the illness can be passed (transmitted) from one person to the next. It is important to understand the way these infections are passed from one person to the next so that we can design ways to stop transmission. This information is particularly important for new viruses such as the SARS-CoV-2 (novel coronavirus), the virus that causes a novel coronavirus disease which emerged in 2019 (COVID-19). We do not have all the information about this virus and how the virus passes from one person to the next. One of the important pieces of information is to understand how long people shed the virus (this means how long people who are infected have the virus in the nose/throat or chest after they are infected or become ill. It is also possible that the virus is in the stool). Because this is a new virus we would also like to describe what medical signs and symptoms people who are infected with this virus experience.

The information from the study will help to make guidelines for the use of vaccines and other interventions when these become available, to help prevent these illnesses in people. This information will also help us to understand how we can protect people by isolating them from non-infected people.

We would also like to describe some of the characteristics of people who become ill and those who become very ill, such as your age, if you have any underlying conditions (such as heart disease, diabetes and other lung conditions) and people who have weak immune systems (like people with HIV). We may also ask questions about people that you may have been in contact with before and during your illness.

In order to help us explain all these things we are planning a study that will enroll people who are confirmed to be infected with the coronavirus in South Africa. If you agree to take part (enroll), we will be following you for at least a month (and up to 2 months). This will mean visiting you in hospital or at home, every second day, until we are not able to find the virus in your airways (nose/throat or in their lungs) and also we will take stool and blood samples. Once we cannot find the virus we will visit you at wider intervals (like once a week) until 56 days after your symptoms started. We are doing all these samples because we are still not sure where your body sheds the virus. We are following you up for longer to see how your body reacts after the virus has gone (we do this with blood samples). During this study we will ask you to participate in the following study procedures:

1. Complete a detailed questionnaire about your age, medical history and symptoms.
2. We will ask about your HIV status and if necessary do testing for HIV (as explained in detail below).
3. Every second day we will take samples from your nose and throat with a swab.
4. If you are in hospital we may take samples from your lungs, like sputum (phlegm).
5. Stool samples will be taken at all the visits; a nurse will talk you through the procedure.
6. A small finger prick test to test for your body's reaction to the virus, a small drop of blood will be taken from your finger.
7. Blood samples (from your arm, like routine blood tests) will be taken on the day of enrolment and as detailed below, this will be a 3-10ml sample (less or equal to one teaspoon of blood) taken by a registered nurse using routine sample collection techniques:

a. If the enrolment occurs within 0-5 days from symptoms onset, a blood specimen will be collected at enrollment and at days 7, 14, 21, 28 and 56 post symptoms onset.
b. If the enrolment occurs within 8-12 days from symptoms onset, a blood specimen will be collected at enrollment and at days 14, 21, 28 and 56 post symptoms onset.
c. If the enrolment occurs within 15-19 days from symptoms onset, a blood specimen will be collected at enrollment and at days 21, 28 and 56 post symptoms onset.
d. If the enrolment occurs within 22-26 days from symptoms onset, a blood specimen will be collected at enrollment and at days 28 and 56 post symptoms onset.
e. If the enrolment occurs 7, 14, 21 or 28 days post symptom onset a blood specimen will be collected at enrollment and at the remaining scheduled follow-up visits (e.g., if the enrolment occurs at 7 days post symptom onset a blood specimen will be collected at enrollment and at days 14, 21, 28 and 56 post symptoms onset).
f. If the enrollment occurs 6, 13, 20 or 27 days post symptoms onset (one day before the due sample collection date) a blood specimen will not be collected at enrollment but at the next scheduled visit on the following day (e.g. day 7, 14, 21, or 28 post symptoms onset) and at the remaining scheduled follow-up visits.

As HIV infection weakens the immune system, HIV-infected people react differently to infection, so knowing the HIV status of people in this study is very important. There are a number of ways for us to do this. We can offer you a rapid test and you will get the result after about 15 minutes. We can take a blood sample and test it in the laboratory (you will get the result in about 2 weeks), or if you do not wish to know your results we can test the blood sample, but not give you the results. Because HIV is a treatable infection we recommend that you know your status, so that you can access treatment for the infection and live a healthy life. Our teams are trained in HIV pre- and post-test counselling and will provide this before and after testing. You may change your mind at any stage of the study and we can arrange for you to receive the latest HIV test results.

Length of study and number of participants

- The study will be performed in South Africa only.
- We will enrol 200 hospitalised persons who have the corona virus identified.

Your rights as a participant

It is your right to choose if you want to take part in this study. If you chose not to participate this will not affect your right to health care, other services or your right to participate in future studies.

Expected duration of participation

The first visit will take about 1 hour this will include reading through this form, signing consent, collecting additional information on your signs and symptoms and collecting the initial samples. The follow-up visits will be about 30 minutes. As we will try to arrange these visits when it suits

you, these visits may include weekend days. At each visit the study staff will tell you when the next visit will be and what you can expect at the next visit (just to remind you).

Risks of this study
There is minimal risk to you from participating in this study. You may find some of the questions uncomfortable. There may be minimal discomfort when we take the nasopharyngeal or throat sample. Some people may gag when we take the sample or experience a small nose bleed. However, the procedure only takes a few seconds.

Venipunctures and finger pricks (i.e. drawing blood) are done as part of routine medical care and present a slight risk of discomfort. Drawing blood may result in faintness, inflammation of the vein, pain, bruising or bleeding at the puncture site. There is also a slight possibility of infection. Your protection is that experienced personnel perform the procedures under sterile conditions.

Testing for HIV may be stressful; we will have a trained counsellor to explain to you about the test and what the results will mean to you. We will also refer you directly into the treatment programme if you do test positive for HIV.

Benefits of this study
By taking part in this study, you will help us learn more about how certain infectious diseases affect different people and who may benefit from vaccines. You will have a chance to get the results of your HIV test and if you test positive for this infection, you will get the benefit of early treatment. As infection with coronavirus may be stressful, you will have regular contact with a nurse, who will be able to answer your questions about your illness and concerns.

Confidentiality
Every effort will be made to protect your confidentiality: study forms and blood samples will be marked with a number and not a name. Study staff will keep a log of people enrolled; these will be kept in secure locked offices. No reference to personal detail will be made in any study report or in the results of the study.

Withdrawal from the study
Your participation in this study is entirely voluntary and you can decline to participate, or stop at any time, without stating any reason. Your withdrawal will not affect your access to other medical care.

- The investigators retain the right to withdraw you from the study if it is considered to be in your best interest.
- If you did not give an accurate history or did not follow the guidelines of the study and the regulations of the study facility, you may be withdrawn from the study at any time.

Reimbursement for Participation
You will not be paid to participate in this study, nor will there be any cost to you. The study will not pay for any care that you need for any illness diagnosed during this study. We will share the results of the tests with you so that you get information such as when the virus is no longer detectable in your body. In addition, we are able to refer you for medical help if this is necessary.

Ethical approval

- I do not have any financial or personal interests with this organisation that may bias my actions.
- This study has been approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg ("Committee"). A principal function of this Committee is to safeguard the rights and dignity of all human subjects who agree to participate in a research project and the integrity of the research.
- If you have any concern over the way the study is being conducted, please contact the Chairperson of this Committee who is Dr Clement Penny, who may be contacted on telephone number 011 717 2301, or by e-mail on Clement.Penny@wits.ac.za. The telephone numbers for the Committee secretariat are 011 717 2700/1234 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za and Rhulani.Mukansi@wits.ac.za

Thank you for reading this Study Information Sheet.

April 2020

Informed consent for cases of coronavirus (COVID-19): Adults

STUDY TITLE: Characterization of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Shedding and the Novel Coronavirus Disease (COVID-19) Clinical Features among the First Confirmed Cases in South Africa

---

I hereby confirm that I have been informed by the study team member, _____ _____ *(Insert name of study team member)*, about the nature, conduct, benefits and risks of the COVID-19 shedding and clinical features study.

- I have also received, read and understood the Participant Information Leaflet regarding the clinical study.
- I am aware that the results of the study, excluding personal details regarding my sex, age, date of birth, initials and diagnosis, will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerised system by NICD or on their behalf.
- I understand that anyone wishing to use this data in any future study must first be given clearance by the Human Research Ethics (Medical) Committee of the University of the Witwatersrand, Johannesburg
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- By signing this form I agree to (please tick and initial as appropriate):
  - All the samples mentioned in the Information Leaflet being taken at the intervals described
  - An HIV test
    - ☐ By rapid test
    - ☐ By testing at NICD laboratory
    - ☐ An HIV test and I do not wish to get my results
  - Be contacted by study staff to be invited to enrolled in to other COVID-19-related studies

PARTICIPANT:

Printed Name	Signature / Mark or Thumbprint	Date and Time

I, _____ *(Insert name of study team member)*, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY TEAM MEMBER:

Printed Name	Signature	Date and Time

TRANSLATOR / OTHER PERSON EXPLAINING INFORMED CONSENT (for participants unable to read)

_____ *(Insert designation)*:

Printed Name	Signature	Date and Time

WITNESS (If applicable)**:**

Printed Name	Signature	Date and Time

10.9. Appendix IX: Infection control measures

Infection control measures should be implemented in accordance with the National Guidelines for COVID-19 as follows:

Key contact and droplet precautions include the following:

- isolate the patient (ideally an airborne infection isolation room if available, otherwise adequately ventilated single rooms)
- place patient in a separate room
- healthcare workers to use a medical (surgical/medical) mask and eye (goggles) or facial protection (face shield)
- healthcare workers should wear a clean, non-sterile, long-sleeved gown and use gloves
- healthcare workers should not touch their eyes, nose or mouth with potentially contaminated gloves or bare hands
- limit movement of patient (eg. use designated portable X-ray equipment)
- routinely clean and disinfect surfaces with which the patient is in contact
- limit the number of healthcare workers, family and visitors in contact with suspected or confirmed cases

Additional precautions to be used during aerosol-generating procedures include:

- performing procedures in an adequately ventilated room
- minimising respirator face-seal leakage to fully protect the worker from exposure to aerosolized infectious droplets when using particulate respirators e.g. N95 mask
- eye protection (goggles or face shield) to protect the eyes from respiratory splash or spray
- contact protection (non-sterile, long-sleeved gown and gloves)

10.10. Appendix X: Procedures for the collection of oropharyngeal (throat) and nasopharyngeal swabs

Type of swabs

Only nylon or rayon flocked nasopharyngeal and oropharyngeal swabs with perforated, flexible plastic shaft must be used for collection of specimens. There is evidence to suggest some benefit to using flocked swabs for recovery of pathogens over other types. An appropriate size of the nasopharyngeal swab should be used. Cotton-tipped, calcium alginate swabs or swabs with wooden shafts should not be used as residues present in these materials may inhibit PCR assays. Both swabs should be put in the same vial containing universal transport medium (UTM).

Collecting the nasopharyngeal swab

- Gently insert nasopharyngeal flocked swab into the nostril aiming backwards, along the floor of the nasal cavity, until the nasopharynx is reached. Be careful not to insert swab upwards. If resistance is encountered during insertion of the swab, remove it and try the other nostril. The distance from the nose to the ear gives an estimate of the distance the swab should be inserted
- Gently rotate the swab and hold in place for a few seconds
- Slowly withdraw swab
- Unscrew and remove the cap from the tube with transport medium.
- Insert the swab directly into a vial containing universal transport medium (UTM)
- Break plastic shaft at the break point so that it can fit in the universal transport medium tube
- Close the tube with the lid
- Refrigerate at 2-8 °C

Collecting oropharyngeal swab (OPS)

- Keeping the same pair of gloves on, and holding the UTM with the nasopharyngeal swab in, take a second flocked swab and open it at the plastic shaft
- Ask the patient to tilt their head back and open mouth wide

- Hold the tongue down with a tongue depressor
- Have the patient say “aahh” to elevate the uvula
- Swab each tonsil first, then the posterior pharynx in a “figure 8” movement
- Avoid swabbing the soft palate and do not touch the tongue with the swab tip as this procedure can induce the gag reflex.
- Insert the swab directly into the same UTM vial containing the nasopharyngeal swab
- Break plastic shaft at the break point so that it can fit in the universal transport medium tube
- Close the tube with the lid
- Refrigerate at 2-8 °C

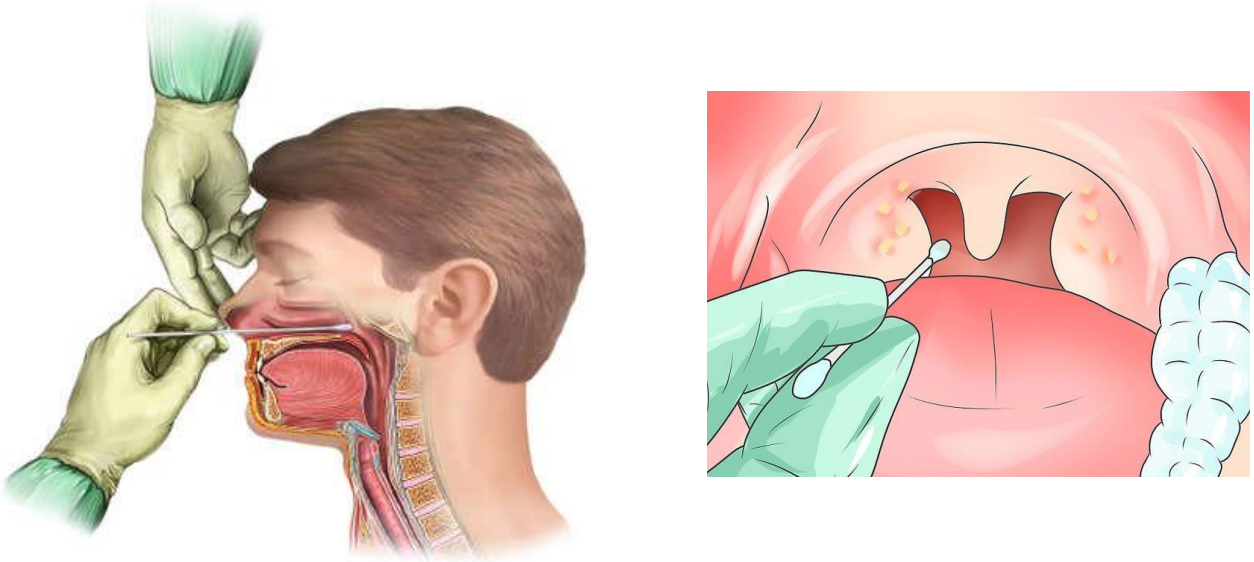

Figure 2: How to collect a nasopharyngeal swab (left) and oropharyngeal swab (right)

10.11. Appendix XI: Procedures for the collection of stool specimens

Type of specimens to be collected

Raw stool specimens will be collected from the nappy, underpants or bedpan of the patient. In cases where the stool is liquid and no solid material can be collected from a nappy, the whole nappy or the nappy liner/section of the nappy liner can be sent to the laboratory for analysis. In adults who are reluctant to provide a raw stool specimen, a damp wet-wipe tissue may be submitted, provided the stool material is visible on the wipe. Where a raw stool specimen cannot be obtained from the patient, a rectal swab with visible stool material (brown swab) may suffice for molecular testing.

Materials and procedures for specimen collection

Wooden tongue depressors may be used to scrape solid stool material out of the nappy and into a specimen container. In cases where the stool is liquid and no solid material can be collected from the nappy, scissors will be cleaned with an ethanol-based cleaner and used to cut out a stool-soiled section of the nappy liner. Forceps, cleaned with an ethanol-based cleaner, will then be used to transfer the nappy liner to a specimen container. Where cloth nappies are used, clean forceps will be used to transfer the nappy liner to a specimen container. Where the patient is collecting their own specimens using wet-wipes or rectal swabs, gloves and specimen collection materials will be provided.

Collection containers

Plastic specimen containers will be used for storage of raw stools or nappy liners. Each container should contain at least a 10 cm x 10 cm (palm sized) nappy liner, a R5 coin sized solid stool or 5ml liquid stool specimen. Ideally, surveillance officers will try to fill the containers with as much raw stool as possible as this permits the laboratory to conduct multiple tests. For specimens collected on damp wet-wipes and rectal swabs, surveillance officers must ensure that these collection devices have visible stool material to ensure sufficient specimen for molecular screening. Wet-wipes will be properly sealed in zip-lock bags and rectal swabs will be stored dry in plastic containers after the collection straw is cut to size. All collection containers will be marked with a unique identifier using stick-on labels supplied by the study. The container itself (not the cap) will always be marked with identifying details.

Labelling of specimens

As soon as the specimens are collected, a laboratory specimen form (Appendix VI) will be completed for each patient. Laboratory specimen forms must be transported with the specimen to the CRDM laboratory, preferably in a dual compartment specimen bag to avoid contamination of documentation should specimen contents leak from the primary plastic container.

Spill clean-up procedure

Surveillance officers will wear appropriate personal protective equipment including coats or plastic aprons and gloves during specimen collection. Any liquid or solid stool spills will be wiped up with paper towels and discarded in biohazard boxes. The area will be cleaned with an appropriate ethanol-based or disinfectant solution (10% bleach), discarding paper towels after use. All collection materials not discarded will be cleaned after each use.

Specimen storage at study site

Specimens will be refrigerated (2°C to 8°C) at the study sites and transported to the CRDM laboratory, NICD, using the NHLS transport system.

Packaging and transport

The samples should be packed properly to avoid leakage during transportation. The specimen container and zip-lock bags should be watertight. The zip-lock bags will be placed in a polystyrene cooler box (or equivalent) filled with frozen ice packs to maintain a cool temperature during transport to NICD.

10.12. Appendix XII: Procedures for the collection of blood specimens

Blood collection for adults

Specimens will be collected using the vacutainer collection system (see Figure 3): in older children and adults, as follows.

- By twisting, remove the bottom white cap (**D**) of the needle.
- Now, firmly screw the needle in the needle holder/barrel (**B**).
- Remove the top cap (**C**) to ready the needle (**A**) for use just before the sample is taken.

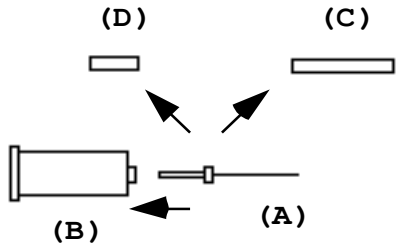

Figure 3: The Vacutainer Needle & Barrel System

- Apply the tourniquet to the patient's arm (Figure 4a) and disinfect the site of venepuncture.
- Hold the needle holder between thumb and index finger of the right hand and insert into the vein (Figure 4b).
- Now change the position of the hand i.e. fixes the needle holder on the patient's arm with the thumb and index finger of the left hand. The left hand now serves to keep the needle Immobilized in the vein during the different actions.
- Position the first tube in the holder (serum separator, yellow top tube) and press it onto the needle to allow the tube to fill (Figure 4c).
- Fill the first tube and remove once full (Figure 4d).
- Position the second tube in the holder (EDTA, purple top tube) and press it onto the needle to allow the tube to fill (Figure 4c).
- Fill the second tube and remove once full (Figure 4d).

- Position the third tube in the holder (red top tube) and press it onto the needle to allow the tube to fill (Figure 4c).
- Fill the third tube and remove once full (Figure 4d).

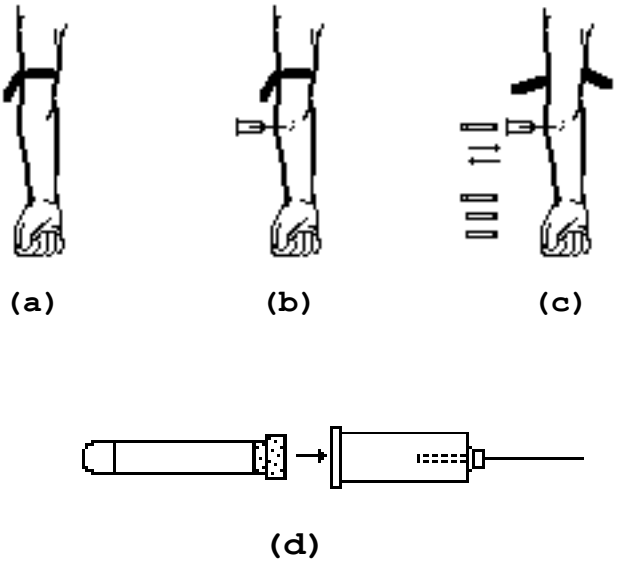

Figure 4: Collecting the Sample

- Release the tourniquet then remove the needle from the patients arm and immediately apply pressure to the venipuncture site. Firm pressure at this time will minimize bruising and therefore discomfort and distress to the patient.
- Ask the patient to take over applying pressure to the site.
- Invert the tube(s) gently 6 to 8 times (See Figure 5).

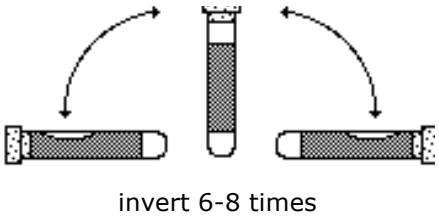

Figure 5: Inverting the tube

10.13. Appendix XIV: Procedures for packaging and submission of specimens

Step 1: Complete one follow-up laboratory slip (see Section 6.5.2.1 and Appendix VI) for all specimen collected and shipped together

Step 2: Package specimens

The principle of triple layer packaging should be followed (Figure 6).

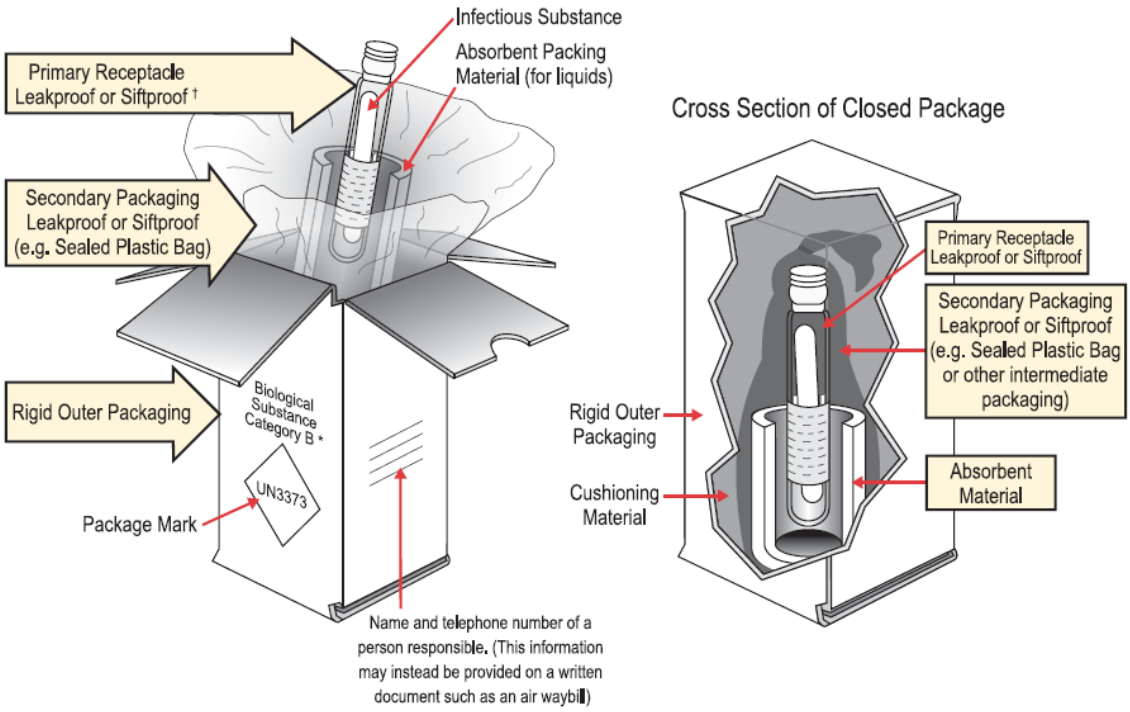

Figure 6. Example of the triple packaging system for the packing and labelling of Category B substances.

It is required that designated staff members per site are trained by approved provider in the packaging and transport of dangerous goods.

Primary specimen containers (properly labelled) should be wrapped in sufficient absorbent material (paper towels or tissues) to absorb the entire contents in the event of leakage.

The wrapped primary containers will be placed in durable, leak-proof secondary containers (i.e. rigid screw-cap plastic containers). The secondary container will be taped closed to prevent leakage.

The secondary containers and data forms, sealed separately in plastic, will be then be placed in a rigid outer (tertiary) container such as a fiber carton or polystyrene cold box with cold packs. The outer wrapping will be addressed to:

NHLS/NICD
Centre for Respiratory Disease and Meningitis (CRDM)
Lower North Wing, SAVP building
1 Modderfontein Rd, Sandringham, Johannesburg, 2131
South Africa

The parcel should bear appropriate outer warning that it contains biohazardous material.

Step 3: Submit specimens

- The specimens should be stored and shipped at temperatures indicated in Table 3 below. Specimens should be packaged in accordance with the guidelines for the transport of dangerous biological goods (triple packaging using absorbent material) and transported directly and urgently.
- Transport specimens to NICD on same day as specimen collection.
- Please notify by email the CRDM staff below of any pending shipments:
    - Linda de Gouveia lindad@nicd.ac.za (011-555-0327) or
    - Amelia Buys ameliab@nicd.ac.za (011-386-6373) or
    - Cardia Fourie cardiaf@nicd.ac.za (011-386-6373)

- Ensure that the (i) complemented follow-up laboratory slip (Appendix VI) and (ii) all daily follow-up clinical forms (Appendix IV) completed from the last shipment accompanies the specimens.
- The case report form completed on enrolment (Appendix III) and the final outcome form(s) completed on transfer, discharge and/or cessation of shedding (Appendix V) should be shipped with the next sample shipment or immediately after completion if no specimens shipment will be done after completion of the form (i.e., form completed after cessation of shedding).

- Avoid repeated freezing and thawing of specimens
- From NHLS laboratories make use of usual overnight regional courier service to ship samples to NICD. From private clinicians / clinics / hospitals transport will be organized by CRDM.

Table 3. Specimens' types and the transport requirements of these specimens

Specimen type	Collection materials	Storage and transportation	Dangerous goods shipping category	Comment
Nasopharyngeal and oropharyngeal swab	Dacron or nylon flocked swab in Universal Transport Medium (UTM) in a sterile leak proof container	Refrigerate and ship at 2-8 °C up to 48 hrs, if >48 hrs freeze at -70°C and ship on dry ice	Biological substance, Category B	Nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load
Stool	Raw stools or nappy liners or rectal swab in a sterile leak proof container	Refrigerate and ship at 2-8 °C up to 48 hrs, if >48 hrs freeze at -70°C and ship on dry ice	Biological substance, Category B	None
Serum	Serum separator tube	Store upright for at least 30 minutes after collection. Refrigerate and ship at 2-8 °C within 48 hrs	Biological substance, Category B	None
Whole blood	EDTA tube	Refrigerate and ship at 2-8 °C within 48 hrs.	Biological substance, Category B	None

* Aerosol-generating procedures may pose an infection risk for health care workers.