ALERRT

African coaLition for Epidemic Research, Response and Training

ALERRT AFRICA-READY COVID-19 CCP PROTOCOL

Clinical Characterization Protocol for COVID-19 disease

Table of Contents

LIST OF ABBREVIATIONS

ISARIC	International Severe Acute Respiratory and Emerging Infections Consortium
CCP	Clinical Characterization Protocol
COVID-19	Corona Virus Disease-19
WHO	World Health Organization
ID	Identification
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
TBEV	Tick-Borne Encephalitis Virus
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
CNS	Central Nervous System
RT-PCR	Reverse Transcriptase- Real Time PCR
CSF	Cerebrospinal Fluid
eCRF	electronic Case Report Form
EID	Emerging Infectious Disease
VHF	Viral Haemorrhagic Fevers
EEG	Electroencephalographic
PPE	Personal Protective Equipment
EDTA	Ethylenediaminetetraacetic Acid
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic acid
CRF	Case Report Form
ERC	Ethics Review Committee
IgA	Immunoglobulin A
IgG	Immunoglobulin G
HIV	Human Immunodeficiency Virus
PI	Principal Investigator
SARI	Severe Acute Respiratory Infections
ICH	International Conference on Harmonisation
R	Recruitment Samples Including Pathogen Samples
S	Serial Samples Including Pathogen Samples
P	Research Pathogen Samples Only
C	Convalescent Samples
NPA	Nasopharyngeal Aspirate

1. Background and Objectives

1.1 Purpose of the Study

The purpose of this study is to clinically characterise COVID-19 disease using an adaptation of a standardized protocol for rapid, coordinated, clinical investigation of severe or potentially severe acute infections by pathogens of public health interest while examining the preparedness of health workers for COVID-19 disease. Patients with acute illness suspected to be caused by SARS-CoV-2 will be enrolled. This protocol has been designed to enable data and biological samples to be prospectively collected and shared rapidly in a globally-harmonised sampling schedule. Multiple independent studies can be easily aggregated, tabulated and analysed across many different settings globally. The protocol is the product of many years of discussion among international investigators from a wide range of scientific and medical disciplines (Lancet ID 14(1):8; https://doi.org/10.1016/S1473-3099(13)70327-X).

Recruitment under this protocol has been initiated in response to Middle Eastern Respiratory Syndrome coronavirus (MERS-CoV) in 2012-2013, influenza A H7N9 in 2013, viral haemorrhagic fever (Ebola virus) in 2014, monkeypox & MERS-CoV in 2018, tick-borne encephalitis virus (TBEV) in 2019 and Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) causing COVID-19 in 2020.

1.2 Background Information

Infectious disease is the single biggest cause of death worldwide. New infectious agents, such as the SARS, MERS and other novel coronavirus, novel influenza viruses, viruses causing viral haemorrhagic fever (e.g. Ebola), and viruses that affect the central nervous system (CNS) such as TBEV & Nipah require investigation to understand pathogen biology and pathogenesis in the host. Even for known infections, resistance to antimicrobial therapies is widespread, and treatments to control potentially deleterious host responses are lacking.

In order to develop a mechanistic understanding of disease processes, such that risk factors for severe illness can be identified and treatments can be developed, it is necessary to understand pathogen characteristics associated with virulence, the replication dynamics and in-host evolution of the pathogen, the dynamics of the host response, the pharmacology of antimicrobial or host-directed therapies, the transmission dynamics, and factors underlying individual susceptibility.

Due to the evolution of the pandemic around the world and its relatively late onset in Africa, most publications on COVID-19 are Chinese, European or American in origin. While useful, this information will not always be relevant to the African context because of our differences by way of health determinants. Africa-specific realities (epidemiological, environmental, genetic, ecological etc.) that may be protective, or risk factors for disease need to be better understood. For a more comprehensive understanding of this pandemic, it is imperative that Africa-specific data be collected.

The work proposed here may require sampling that will not immediately benefit the participants. It may also require analysis of the host genome, which may reveal other information about disease susceptibility or other aspects of health status.

1.3 Target Audience of this Document

This document is of primary interest to clinicians (including emergency and critical care providers) and others engaged in identification, triage and treatment of patients with COVID-19. Any individuals or members of research units/networks are invited to use this document to facilitate their own studies. and contribute data to the centralized database. We encourage any and all centres to contribute to this effort. The primary data remain with the individual sites but we hope that by agreeing to use this protocol, researchers commit to provide data for the centralized database. This will allow a much more complete analysis of the data.

1.4 Source of this Protocol

This document is a product of collaboration between the World Health Organization (WHO) and the International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC), and builds on a global consensus on observational research in emerging infections of public health interest.

1.5 Primary Objectives

In potential participants meeting the entry criteria, our primary objectives for each individual pathogen are to:

- Describe the clinical features of COVID-19 in [insert country] and monitor the progress of all hospitalized patients including what is working and what is not
- Describe, where appropriate, the response to treatment, including supportive care and novel therapeutics.
- Identify and adjust treatment protocols early in hospitalized patients who are deteriorating
- Track the size and severity of the pandemic using national level clinical data
- Identify areas for improvement in the current and future epidemics
- Evaluate the diagnostic predictive values of RT-PCR and ELISA at different stages of COVID-19
- Observe, where appropriate and feasible, pathogen replication, excretion and evolution, within the host, and identify determinants of severity and transmission using high-throughput sequencing of pathogen genomes obtained from respiratory tract, blood, urine, stool, CSF and other samples.
- Characterise, where appropriate and feasible, the host responses to infection and therapy over time, including innate and acquired immune responses, levels of immune signalling molecules in relevant body compartments and gene expression profiles in peripheral blood.
- Identify host genetic variants associated with disease progression or severity.
- Understand transmissibility and the probabilities of different clinical outcomes following exposure and infection.

1.6 Secondary Objectives

Secondary objectives are to collect evidence in order to:

- Facilitate effective triage and clinical management of patients with infections relevant to this protocol
- Develop clinical guidance documents and offer clinical recommendations to policy makers on the basis of evidence obtained

1.7 Structure of the study: stratified recruitment according to local resource.

The study will be conducted at multiple sites (to be determined by the spread of disease and availability of resources). It is appreciated that settings will vary in terms of clinical infrastructure, resources and capacity. Distinction is made to allow for resource-appropriate implementation of the protocol, and it is understood that data and/or specimen collection may be limited in certain settings. Observational analyses will be stratified according to available samples and data.

In all cases, a proportionate case report form (paper CRF or web-based electronic "eCRF") will be completed.

Implementation of data and biological sample collection has been stratified into four tiers, each comprising a different intensity of recruitment activities (Figure 1):

- **Tier 0 (Clinical data collection only)** – Clinical data will be collected but no biological samples will be obtained for research purposes. The minimum clinical data set will summarise the illness episode and outcome, with the option to collect additional detailed clinical data at frequent intervals, according to local resources/needs.
- **Tier 1 (1 biological sample set)** - Clinical samples will be collected on recruitment day (Day 1; ideally at initial presentation to a health care facility) but subsequent serial samples will not be obtained. Clinical information will be collected at recruitment and discharge.
- **Tier 2 (Serial biological sampling, schedules 2-11)** - Clinical samples and data will be collected on recruitment day (Day 1; ideally at initial presentation to a health care facility) followed by serial samples obtained at timepoints defined by the schedule (illustrated in Tables 3-6). The schedule ranges from 2 (recruitment and day 3) to 11 (recruitment, every second day for the next 14 days, then weekly [until maximum 100 days], then convalescent samples 3 & 6 months after recruitment). Within Tier 2, we shall aim for at least Schedule 3.
- **Tier 3 Optional modular sub-studies** in addition to Tier 2 recruitment.

Frequency of CRF data collection is also stratified into tiers, shown in Figure 2. Each site will recruit at a given tier. This will be recorded in the site file "Tier Record Form". Changes to the tier active at a given site will be documented by the PI. As an outbreak progresses, and more cases occur, it is anticipated that both the research priorities and the local resource availability will change. It is

therefore likely that, within a given institution, cases recruited later in an outbreak will be sampled at a lower intensity and may be recruited to a lower tier of the study.

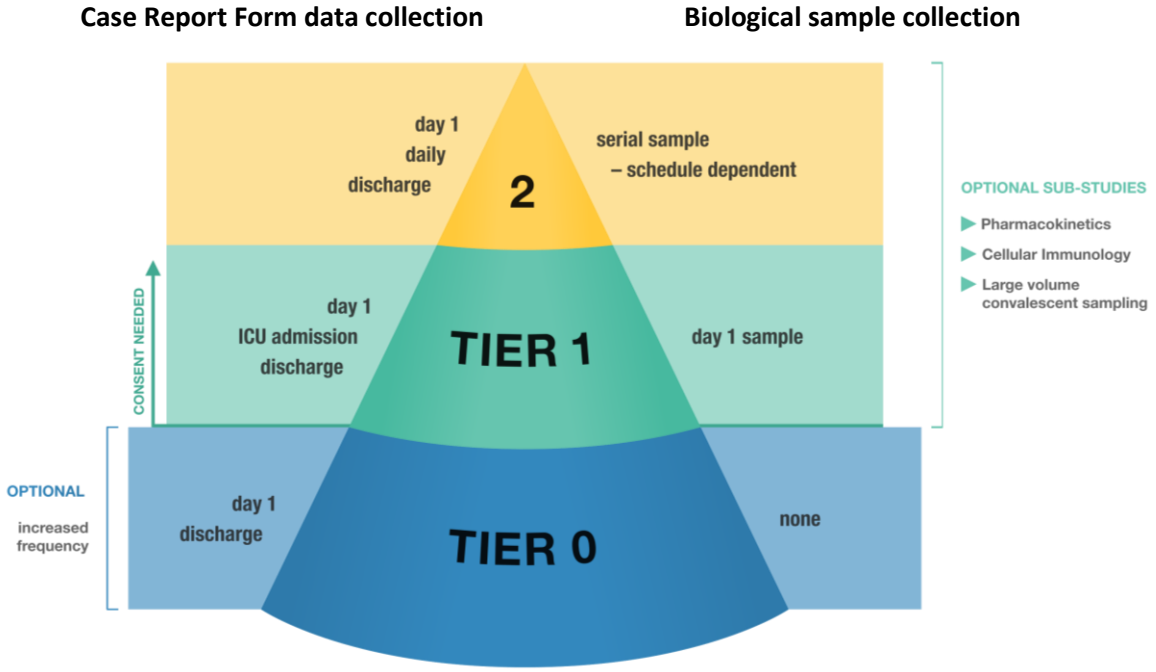

Figure 1. Tiered approach to recruitment in settings with different resources. This information is included to demonstrate the integration of this study with other studies following the same approach in other parts of the world.

1.8 Entry Criteria

This study will enrol eligible patients (children and adults) with confirmed or suspected infection with COVID-19. Recruitment of patients with Day 1 (enrolment) data and biological samples is the priority. Suspected cases with a laboratory confirmation will be a priority and will be recruited. In the unlikely situation where there is a suspected case but delayed lab confirmation, such a case will be recruited for the study pending laboratory confirmation. Once COVID -19 is ruled out, the participant will be dropped and not followed up. However, since this protocol is also designed to allow the health system to detect any unknown severe acute respiratory infection (SARI) which might be hitherto unknown, unless a substantive diagnosis is established, the patient shall be maintained in the study and followed up for a period which will be determined by the implementing group.

Daily follow-up and convalescent visits of patients should proceed according to local resources.

1.8.1 Inclusion criteria

- Suspected or proven infection with SARS-CoV-2, causing COVID-19 as main reason for reporting to the hospital

1.8.2 Exclusion criteria:

- Confirmed diagnosis of a pathogen unrelated to the objectives of this study and no indication or likelihood of co-infection with a relevant pathogen.
- Refusal by participant, parent or appropriate representative.

2. Study Design

This protocol is for a prospective observational cohort study.

2.1 Study Area

The Study will be conducted in [insert country] where COVID-19 cases have been reported. The exact site of patient recruitment will be based on hospitals that have reported cases and are managing patients. Thus, recruitments will be nationwide at approved centres that offer clinical care to COVID-19 patients. In the event that COVID-19 spreads to poorly resourced hospitals anywhere in [insert country], the research team will then restrict recruitment to well-resourced hospitals with adequate preparation and capacity for safe sample collection.

2.2 Sample Size

Since this protocol is more of hypothesis generating and there is very limited or no data currently available to be used for this sample size estimation process, as much information as resources will permit will be collected for this study.

This protocol will be opened at sites with capacity and capability to recruit to any tier of study intensity. The study end date will be based on when the [insert country] Health Service Declares the epidemic as over or that the disease no longer poses a public health threat.

2.3 Timeline for the study

Duration of the Funding: 2 years

- Set up: 3 months
- Recruitment: 12 months
- Follow up: 6 months
- Data cleaning, data analysis article draft: 3 months

3. Methods

3.1 Identification of Potential Patients

In hospital, potential participants will be identified through hospital workers upon presentation at health facilities and through the [insert country] Health Service. When resources limit the number of patients enrolled to less than the number of patients presenting, we shall establish procedures to sample sub population of potential study participants based on the needs of the study at the time. A quota of participants to be recruited per week will be established by the study management team and the recruiting sites will use convenience sampling to recruit participants.

3.2 Approach to Potential Participants

Tier Zero activity, which involves data collection only, requires collection of limited clinical data from the routine health record in a form that does not identify the patient. This does not require consent. This is because the patient is not identifiable and the data is collected by a health care professional who has access to this information by virtue of their clinical role.

Tier One and Two Patients will only be considered for enrolment if appropriate local infection control and prevention measures are in place and can be maintained, and staff trained in use of the required Infection Prevention Consumables (IPC) are available to approach patients and obtain biological samples.

When it has been decided that biological sampling can be performed safely and appropriate consent has been obtained, samples taken early may be most useful for identification or evaluation of risk

factors for disease progression at a clinically-relevant decision point. Therefore, it is desirable to enrol and begin sampling as early as possible during a patient's illness.

Where patients lack capacity to consent to participation, an appropriate representative/ consultee/ parent/guardian will be approached by staff trained in consent procedures that protect the rights of the patient, and adhere to the ethical principles within the Declaration of Helsinki. Staff will explain the details of the study to the participant or parent/guardian/consultee and allow them time to discuss and ask questions. The staff will review the informed consent form with the person giving consent and endeavour to ensure understanding of the contents, including study procedures, risks, benefits, the right to withdraw and alternatives to participation. The consenting party will be asked to sign and date an informed consent form. If the patient is a child, the person with parental responsibility and the child, if competent, should both provide consent/ assent. Specific proxy consent procedures, assent procedures and definition of a competent child will vary based on local legislation which should be consulted and inform specific consent procedures.

In view of the importance of early samples, participants or their parent/guardian/consultee will be permitted to consent and begin to participate in the study immediately if they wish to do so. Those who prefer more time to consider participation will be approached again after an agreed time, normally one day, to discuss further.

An outbreak involving a pathogen of public health interest or pandemic is an emergency. Patients who are incapable of giving consent in emergency situations are an exception to the general rule of informed consent in clinical research. This is clearly acknowledged in the Declaration of Helsinki (2008). The process of consent will comply with the principles of Good Clinical Practice and with the laws regulating clinical research in the recruiting centre.

3.3 Standard of Care

Provision of care will vary by facility and by treating physician. It is not possible to define a single standard of care and therefore to define what samples will be taken as a part of medical management and when. Participants in this study may have samples taken in addition to those required for medical management. The results of tests performed on research samples are unlikely to directly benefit the health of the specific participants from which they are obtained.

3.4 Data Collection and Sampling for Patients

Samples required for clinical management will at all times have priority over samples taken for research tests. Aliquots or samples for research purposes should never compromise the quality or quantity of samples required for medical management. Wherever practical, taking research samples should be timed to coincide with clinical sampling. The research team will be responsible for sharing the sampling protocol with health care workers supporting patient management in order to minimise disruption to routine care and avoid unnecessary procedures. Some samples should be processed and stored at -80°C (Table 2). We recognise that -80°C storage is not available at all facilities. In this case we shall make effort to store at coldest available temperature and at least -20°C and arrange for transportation to a facility in [insert country] that has -80°C storage capabilities. Samples and data will be collected according to the protocol tier approach based on available resources and the weight of the patient (Table 7), to prevent excessive volume sampling from children, young people and small adults.

3.5 Sample and Data Collection Schedules

3.5.1 Overview

The following sub-sections detail specific samples to be collected, with reference to the sample sets in Table 1. We recognize that it will not be possible to collect all samples from all facilities, however as much effort as possible shall be made to adhere to these recommended actions.

Table 1: Recruitment, data and sampling actions.

	ACTIONS
R: Recruitment sample set	Consent form (store in site file) Initiate CCP case report form Obtain core sample set (Table 2) with larger blood volume (Table 9) ± additional samples for sub-studies
S: Serial sample set	Obtain core sample set (Table 2) ± additional samples for sub-studies Update CCP case report form
P: Pathogen-only sample set	Obtain only the *Pathogen samples* from the core sample set (Table 2)
On hospital discharge	Update CCP case report form Plan convalescent visit
C: Convalescent sample set	Obtain core sample set (Table 2) ± additional samples for sub-studies Update CCP case report form

Table 2: Core sample set.

BIOLOGICAL SAMPLES	PROCESSING	RATIONALE
PATHOGEN SAMPLES		
• Respiratory samples: - nasal swaps, - throat swab in virus transport medium, - endotracheal aspirate if intubated, - where resources permit, in infants/children who cannot take SAM strip, nasopharyngeal aspirate OR flocked nose swab in virus transport medium; • Urine (up to 10ml); • Stool (up to 10ml) or rectal swab; • Also store any residual from samples taken for clinical care.	**Do not process at site.** Keep double-bagged. Store at -80°C*	Pathogen studies to reveal changes in SARS-CoV-2 during infection and during spread between individuals, detect development of resistance and detect co-infections.

HOST SAMPLES		
Blood sample in serum (clotted) tube	Serum (3 aliquots) Store at -80°C*	Mediators/biomarkers Serology
Blood sample in EDTA tube (Note – larger volume at recruitment, Table 6)	Plasma (3 aliquots) Store at -80°C* Cell fraction (1 aliquot) Store at -80°C*	Mediators/ metabolites/ biomarkers Detect SARS-CoV-2 RNA. Extract host DNA for genomic studies SARS-CoV-2 RNA, cellular immunology.
Blood sample in blood RNA tube (Tempus™ or PAXgene®)	Freeze at -20°C; transfer to -80°C after 24h where possible	Microarray/RNA sequencing pathogen & host transcriptome

*freeze at -80°C where possible, or at least at -20°C. Further details of processing are provided in Table 10.

3.5.2 Tier zero

Collect data in CRF only. There must be no biological sampling for research purposes. ***Since we are collecting CRF data only and not biological samples, we defer to the ERC as to whether ethical approval or consent is required. If ethical clearance is not required, this protocol is not applicable.***

3.5.3 Tier 1

A single sample set is obtained at, or as soon as practical after, recruitment (‘**recruitment sample set’**). Collect data in CRF.

3.5.4 Tier 2

Schedule-dependent ‘**serial sample sets’** and one ‘**convalescent sample set’** are obtained. Collect data in CRF. The decision as to which Tier 2 sampling schedule shall be followed shall be determined by the human and other physical resources available. As far as possible in [insert country], Tier 2 Sampling Schedule 2 shall be attempted.

Table 3: Tier 1 sampling schedule 1.

		Serial samples.															
	Recruitment	Week 1						Week 2								Further samples	Convalescent samples
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Weekly until max 100 days	3 months and 6 months after recruitment
>40kg	**R**																
20 to 40kg	**R**																
10 to 20kg	**R**																
4 to 10kg	**R**																

<4kg	**R**																
Sample priority*	**1**																

Table 4: Tier 2 sampling schedule 2.

			Serial samples.															
	Recruitment	Week 1						Week 2									Further samples	Convalescent samples
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Weekly until max 100 days	3 months and 6 months after recruitment
>40kg	**R**		**S**						**S**							**S**		**C**
20 to 40kg	**R**		**S**						**S**							**S**		**C**
10 to 20kg	**R**		**S**						**S**							**S**		**C**
4 to 10kg	**R**		**S**						**S**							**S**		**C**
<4kg	**R**		**S**						**S**							**S**		**C**
Sample priority*	**1**		**2**						**3**									**4**

Key (refer to Table 1)**:**
R: recruitment sample set
S: serial sample set
P: pathogen-only sample set
C: convalescent samples

***In the event that local resource limitations require sampling frequency to decrease, samples will be prioritised as shown (1=highest priority). Serial sampling will stop when acute illness resolves, or a patient is discharged from hospital: next samples taken will be the blood sample at 3 months and 6 months post recruitment.**

Table 5 List of Laboratory Parameters-days of sampling and frequency

Test	Sampling days*				Frequency
	Day 1	Day 3	Day 9	Day 16	
URINE PREGNANCY TEST (ßhCG)	X				1
COMPREHENSIVE METABOLIC PANEL (BUE & CREATININE - UREA, CREATININE, ELECTROLYTES (NA, K, CL CO2), TOTAL BILI, DIRECT BILI, ALP, , ALT, AST, TP, ALB), GGT	X		X	X	3
					3
LIPID PROFILE (TOTAL CHOL, TRIG, LDL, HDL)	X				1
HBA1C	X				1
FULL BLOOD COUNT, coagulation tests (PT/PTT/INR), G6PD	X	X	X	X	4
HB ELECTROPHORESIS	X				1

MALARIA TEST (MICROSCOPY per HPF)	X			X	2
URINE R/E	X				1
STOOL R/E	X				1
HEPATITIS B SCREEN	X				1
HEPATITIS C ANTIBODY SCREEN	X				1
HIV 1 & 2 SCREEN ONLY	X				1
C-REACTIVE PROTEIN (hs-CRP)	X		X		2
COVID-19 PCR	X		X	X	3

Table 6: Blood sample volumes stratified by patient weight.

	Samples at recruitment (R)	**Serial samples (S)**	**Convalescent samples (C)**	**Total blood volume**
>40kg	**9ml** (3x3ml) EDTA blood **3ml** blood in serum(clotted) tube **3ml** blood in blood RNA tube Research pathogen samples	**3ml** EDTA blood **3ml** blood in serum(clotted) tube **3ml** blood in blood RNA tube Research pathogen samples	**3ml** EDTA blood **3ml** blood in serum(clotted) tube **3ml** blood in blood RNA tube Research pathogen samples	Maximum any day: 20ml (0.38ml/kg) Maximum any 4 weeks: 96ml (max 2.4ml/kg)
20 to 40kg	**6ml** (3x2ml) EDTA blood **3ml** blood in serum(clotted) tube **3ml** blood in blood RNA tube Research pathogen samples	**1ml** EDTA blood **2ml** blood in blood RNA tube Research pathogen samples	**1ml** EDTA blood **3ml** blood in serum(clotted) tube **2ml** blood in blood RNA tube Research pathogen samples	Maximum any day: 12ml (0.6ml/kg) Maximum any 4 weeks: 42ml (max 2.1ml/kg)
10 to 20kg	**2ml** (2x1ml) EDTA blood **2ml** blood in serum(clotted) tube **2ml** blood in blood RNA tube Research pathogen samples	**1ml** EDTA blood **1ml** blood in blood RNA tube Research pathogen samples	**1ml** EDTA blood **1ml** blood in serum(clotted) tube **1ml** blood in blood RNA tube Research pathogen samples	Maximum any day: 6ml (0.6ml/kg) Maximum any 4 weeks: 23.6ml (max 2.36ml/kg)
4 to 10kg	**1ml** EDTA blood **0.5ml** blood in serum(clotted) tube **0.5 ml** blood in blood RNA tube Research pathogen samples	**1ml** EDTA blood Research pathogen samples	**1ml** EDTA blood **1ml** blood in serum(clotted) tube Research pathogen samples	Maximum any day: 2ml (0.5ml/kg) Maximum any 4 weeks: 9.4ml (max 2.35ml/kg)
< 4kg	**0.5ml** EDTA blood **0.1ml** blood in serum(clotted) tube **0.1ml** blood in blood RNA tube Research pathogen samples	**0.2m**l EDTA blood Research pathogen samples	**0.2ml** EDTA blood **0.2ml** blood in serum(clotted) tube Research pathogen samples	Maximum any day: 0.8ml (~0.27ml/kg) Maximum any 4 weeks: 2.4ml (max 2.4ml/kg)

3.5.3 Optional sub-studies

In addition to the Tier 2 sampling schedule, optional sub-studies from Table 7 may be included. However, prior to this, an amendment shall be submitted to the ERC for consideration.

Table 7: Optional sub-studies.

OPTIONAL SUB-STUDY	SAMPLE SET AND SAMPLE	PROCESSING/STORAGE	RATIONALE
(Each sub-study will only operate in a small minority of sites. Any site participating in a sub-study will alert staff to this fact in the TIER RECORD FORM at the front of the site file)			
PHARMACOKINETICS	ADD TO ALL SAMPLE SETS (R, S, and C) Blood sample in EDTA or fluoride oxalate tubes. Volumes >40kg: 3ml 20 to 40kg: 0.5ml 10 to 20kg: 0.2ml 4 to 10kg: 0.2ml < 4kg: 0.2ml	Separation and storage of plasma. (2 aliquots -80°C)	Test for drug levels. Store aliquot for other studies.
CELLULAR IMMUNOLOGY (if patient not included in pharmacokinetic study)	ADD TO ALL SAMPLE SETS (R, S, and C) Blood sample in EDTA Volumes >40kg: 3ml 20 to 40kg: 0.5ml 10 to 20kg: 0.2ml 4 to 10kg: 0.2ml < 4kg: 0.2ml	Extract and store peripheral blood mononuclear cells	Study host immune response, generate monoclonal antibodies.
ENVIRONMENTAL TRANSMISSION	Specimen collection devices placed in vicinity of patient		
LARGE-VOLUME CONVALESCENT SAMPLING (in a small number of selected patients in specific institutions)	Up to 240mls of blood in fully recovered patients	Separation and storage of plasma. Extraction of peripheral blood mononuclear cells (PBMCs)	Serology tests, development of products including international standards, cellular immunology, generation of monoclonal antibodies for research, diagnostic and therapeutic use
HUMORAL IMMUNE RESPONSE	Inclusion of oral (crevicular) fluid sampling with a cute and convalescent samples	Determination of IgG and IgA	Non-invasive determination of humoral immune response

3.5.3.2 Large volume convalescent sampling

In a small number of patients (likely to be less than 10 patients for COVID-19) there is a need for additional sampling after recovery from acute illness to enable generation of serological tests, setting of reference standards for serology, extraction and culture of peripheral blood mononuclear cells (PBMCs) for cellular immunology studies, and generation of monoclonal antibodies for research, diagnostic and therapeutic use. These studies are often extremely valuable in the global response to a new pathogen.

Immune cells, including monocytes, monocyte-derived macrophages, neutrophils and lymphocytes will be isolated from peripheral blood and studied immediately or following culture. Gene expression, protein synthesis and degradation, cytokine release and other functional studies will be measured in immune cells from cases and age- and sex- matched controls. Cells will be stored for future analysis. This is due to a number of reasons. One being challenges getting the right antibodies during this period for flow cytometry analysis secondary to lockdowns, international travel and courier stoppage, and closure of some manufacturing companies. Another is that we gain more insight into the immune response to SARS-CoV-2 and this may affect the design of laboratory experiments to understand the interplay of cells in the immunopathogenesis of COVID-19. The challenge is that there is a small window to recruit patients and take samples and if it so happens that the pandemic ends at a time we have more insight that requires extra or different analysis, it will be difficult to get new patients or get samples within the timelines to support extrapolation of findings to map immunopathogenesis. As such extra cells are stored to overcome this challenge. This will allow the team to follow the progression of the disease while analysing new cells or markers of interest to gain better understanding.

Patients who participated, with appropriate consent, in this study may be invited to provide additional samples under separate consent for this part of the study. All blood samples will be obtained by an experienced phlebotomist. Participants will be fully recovered, otherwise healthy individuals with no contraindications to blood donation, including:

- Infection with any blood borne diseases (e.g. HIV, Hepatitis B or Hepatitis C)
- Previous or current intravenous drug abuse
- Current anaemia
- Blood clotting disorders
- Current anticoagulant (blood thinning) drug therapy
- History of donations to the blood transfusion service (or any other donation) within the last 12 weeks.

Depending on the participant's weight, the following maximum volumes of blood will be obtained:

- >40kg: 240mls (6.0mls/kg)
- 20-40kg: 80mls (4.0mls/kg)

3.6 Enrolment Procedures for Patients

Patients who meet the inclusion/exclusion criteria and who have given informed consent to participate directly, or have been consented by a parent/guardian or whose wishes have been declared by a consultee, or be it deferred, proxy or assent, will be enrolled to the study.

All patients will have clinical information collected either directly through examination including a review of medical, contact and travel history, or from available medical notes. Information will be recorded in the case report form.

At enrolment, sites with available resources will obtain a core sample set (see above). The day of initial sample collection will be counted as Day 1. All study days will be counted from this point forward. Clinical information will also be collected on discharge.

3.7 Case Report Form and Patient Numbers

Case Report Forms (CRFs) completed after site registration at [insert link to REDCap].

Patient numbers consist of a **3-digit site code** and a **4-digit patient number**. Local investigators should be assigned patient numbers sequentially for each site beginning with 0001. In the case of a single site, recruiting patients on different wards, or where it is otherwise difficult to assign sequential numbers, it is acceptable to assign numbers in blocks. E.g. Outpatient ward will assign numbers from 0001 onwards. In-patient ward will assign numbers from 5001 onwards. The patient identification code is entered at the top of each and every sheet. For settings or circumstances in which resources are constrained, an abbreviated case report form is provided.

3.8 Follow-Up Procedures for Patients

Follow-up procedures (e.g. serial sampling) will be undertaken only when resources allow according to Tier 2 sampling outlined in Section 3.5. Follow-up procedures will only be undertaken if appropriate biological safety measures can be maintained. Sites unable to perform daily follow-up as described below may reduce the frequency of follow-up procedures or exclude follow-up if necessary.

Regular clinical assessment and sampling will follow local guidelines. All patients will have further clinical information recorded in the case report form to record events and treatment experienced during hospitalization and outcome. Some of the samples described below will coincide with clinical management. The number of these will depend on the applicable care guidelines, the treating physician and the health of the patient.

Once acute illness is resolved, or once patients are discharged from hospital, sampling will discontinue until the 3 month and 6-month visits. All patients will be asked to return for a convalescent visit and blood sample at 3 months and 6 months post recruitment.

Resolution of acute illness is defined as: Clearance of pathogen from appropriate samples, return of systemic inflammatory response to considered 'normal' values and one of: 1) recovery from organ failure(s)/need for organ support, 2) resolution of the presenting complaint(s), 3) return to life-style prior to illness OR the national recommendations for resolution.

3.8.1 Procedure for additional sampling for pharmacokinetic/ pharmacodynamics studies.

As previously mentioned, such additional studies shall be carried out only following the approval of an amendment submitted to the COUNTRY ERC. Up to 3 additional samples may be obtained at intervals spread throughout the dosing schedule (ideally including one sample immediately before a dose) of the drug being studied. The spread of the samples can be determined on a case-by-case basis to fit in with clinical care; provided the precise times of administration and the precise time of blood sampling are recorded, samples taken at any time will be of use for analysis using population pharmacokinetic methods.

Samples will be taken in conjunction with those required for clinical care in order to minimize research-specific intervention. Samples taken outside of the scheduled days can be used for study testing and should be recorded with the accurate sampling date.

For respiratory samples for COVID-19 patients, a combined nose and throat swab will be collected from all patients. If a patient is intubated an endotracheal aspirate will also be collected. Also, where resources permit, a Nasopharyngeal aspirate (NPA) OR (if NPA impossible) a flocked nose and throat swab sample will also be collected. A sputum sample will be collected when a productive cough is present, and the patient is able to produce one.

Infection site samples are samples of tissue or fluid or swabs taken from infected sites such as an inflamed oropharynx or inflamed conjunctiva. Residual volumes of all other samples taken for clinical care will be stored for not more than 5 years for research.

3.9 Withdrawal of Patients

Patients enrolled to the study whose illness is subsequently confirmed to be the result of infection with a pathogen which is not relevant to the objectives of this study, and who have no indication or likelihood of co-infection with a relevant pathogen, will be withdrawn. No further follow-up will be conducted. Patient autonomy to withdraw from the study at any time shall be respected.

4. Specimens and Laboratory Analysis

4.1 Specimen Sampling, Storage Procedures and Transport

Appropriate selection and timely collection of high-quality specimens, proper storage procedures and comprehensive diagnostic testing will ensure the quality of data.

Local hospital protocols will be used to collect and handle specimens. Guidance on the collection of specimens from patients with emerging infections can be found on the WHO website.

In dealing with novel pathogens where little is known about transmissibility and/or virulence, great care must be exercised to ensure the safety of hospital staff and other patients. Strict adherence to collection protocols, biosafety and adequate PPE is essential. Biosafety procedures will be as per local policy/guidance, will be in keeping with any national and/or international regulations, and will be applied to the collection, storage, transfer and laboratory handling of research samples.

Emerging or remerging pathogens may be classified as requiring BSL2, BSL3 or BSL4 safety management and guidelines should be consulted as per hospital protocol. In addition, an emergent agent may also be risk assessed as posing a threat to animal health, and may be regulated under the specified animal pathogens order as well.

All samples collected must be labelled according to local hospital policy with appropriate identification (full patient identifiers) and hazard labelling and ideally marked ‘COVID RESEARCH’ with a solvent resistant marker. Samples will be processed as per Table 10 ‘Processing/storage’. Testing that cannot be done in country may be exported. Samples sent to laboratories other than those listed in the Protocol and Material Transfer Agreement will be anonymised with unique coded identifiers to protect the identity of the patient. National guidance must be adhered to for the transport of specimens

Clinical samples will be labelled with standard hospital information, including the date and sent with the standard lab request forms.

Residual volumes available after clinical and research testing is complete will be retained by the lab.

4.2 Additional Data Collection – Pharmacokinetic/Pharmacodynamics Studies

Where local resources allow, additional information and samples will be sought during treatment with antimicrobial or immunomodulatory therapies in order to investigate the relationship between dose and plasma drug concentrations, to determine the variability in pharmacokinetics in patients receiving these drugs, and to identify the key pharmacokinetic drivers of pharmacodynamic outcomes (measured using pathogen load, inflammatory markers, illness severity scores or drug toxicity). This information will be collected on the pharmacokinetics record form, and includes both the precise (to the minute) times of drug administration and the precise time of blood sampling.

Samples obtained will be split as required for pharmacokinetic/pharmacodynamic analysis of each antimicrobial or immunomodulatory therapy prescribed; the volume of blood to be drawn will not increase.

4.3 Sample Processing

Samples will only be processed if authorised biological containment and laboratory facilities appropriate to the relevant pathogen are available.

Table 9: Initial processing of biological samples

SAMPLE	INITIAL PROCESSING	ALIQUOTS	ULTIMATE USE
Blood (serum)	Centrifuge 1500g for 10mins.	Supernatant: freeze at -80°C*	Serology
		Supernatant: freeze at -80°C*	Circulating mediators by multiplex cytokine/chemokine assays and proteomics
		Supernatant: freeze at -80°C*	Mediators/proteomics other assays
Blood (EDTA)	Centrifuge 1500g for 10mins ideally at 4°C.	Supernatant: freeze at -80°C*	Serology
		Supernatant: freeze at -80°C*	Circulating mediators by multiplex cytokine/chemokine assays
		Supernatant: freeze at -80°C*	Other studies (eg pharmacokinetics/ pharmacodynamics)
		Cell pellet: freeze at -80°C*	High-throughput genotyping and/or high coverage genome sequencing
Blood (RNA tube)	Freeze at -20°C	Where possible, freeze at -80°C* after 24hrs	Microarray analysis and/or RNA seq analysis of host and pathogen RNA
CSF (if acquired)	Freeze at -80°C*	Aliquot if safe to do so into 3 aliquots Freeze at -80°C*	Pathogen detection, quantification, viral genome sequencing and isolation
			Serology
			Circulating mediators by multiplex cytokine/chemokine assays and proteomics
Pathogen samples	**Do not process**	Freeze at -80 °C*	Pathogen detection, quantification and viral genome sequencing and isolation.

*freeze at -80°C where possible, or at least at -20°C. If necessary (eg. weekends/public holidays) store in refrigerator until processing.

4.4 Use of Stored Samples

Access to samples for additional analyses will be governed by a local committee which will include the PI, [insert appropriate] and ERC. Linked anonymised data generated during the course of these studies may be shared between co-investigators in-country.

Where possible and within the constraints of international law and specific requirements of the country ERC and institutional management approvals, data will be shared centrally within one master database held in Oxford, which will be fully compliant with standard data management processes and [insert country] data management regulations. This database will be held on servers.

Samples will only be stored in containment facilities that have appropriate biological safety measures in place and have received necessary authorisation to store samples (according to any national regulations for the pathogen being studied).

4.5 Future Use of Samples

Samples collected will be used for the purpose of this study as stated in the protocol and consented for future use. The standard consent form will request consent from subjects for sample storage and

future analysis. In the event that such an analysis cannot be performed locally, samples would be transferred to the lab of an overseas collaborator. Permission will be sought from the country ERC to prepare a material and data transfer agreement prior to such transfer of biological samples. In the event that the advanced analysis doesn't fall within the planned work, a submission will be made to the COUNTRY ERC for a protocol amendment before including any extra work other than what the COUNTRY ERC has approved. Collaborating centres must have appropriate biological safety measures and regulatory approvals in place in order to receive samples.
Any database detailing clinical data will only identify participants by a participant number. Participant names or any other identifying details will NOT be included. Data may be used alone or in combination with data from related studies in secondary analyses. Data is hosted on REDCap, a secure web platform for building and managing online databases and surveys.

5. Medical Management and Safety Reporting

5.1 Medical Management

Medical management will be according to standard of care at the treating site and not a part of this protocol. Research interventions include only collection of clinical information and specimens and therefore adverse event reporting is not applicable as there is no intervention.

6. Data Management

6.1 Data Collection

Clinical and laboratory data will be collected throughout the acute illness period according to local resources. Priority at all times will be given to the collection of clinical information. Research data will be integrated as much as possible with information available from hospital and regulatory files. The data will be anonymised at site and a study number issued.

6.2 Data Management

When available, data collected will be submitted electronically to a protected online database. Anonymised data may be entered by study staff in order to minimize the workload on site clinical staff. Quality checks will be built into the data management system and there will be quality control checks of critical data points entered into the CRFs to ensure standardization and validity of the data collected. Patients' identities will be protected and their information held securely. We will use special alphanumeric identifiers to conceal the identities of the study participant. They will not appear in any analysis, report or database. The use of the special participant identifiers will allow the PI and any other authorized personnel from the facility to unblind and identify any participant when it is necessary. Such a procedure will be done following protocols that will ensure privacy and confidentiality of the participant information. The records kept will not include any information that allows patients to be identified after the pandemic is over.

For the Clinical Characterisation Protocol access to the data entry system will be protected by username and password. Username and password will be assigned during the registration process for individual facility Investigators. All electronic data transfer between collection location and database will be username and password protected. Each facility will maintain a study file including a protocol, ethics approval documentation, and paper CRFs. A participant list will be used in each study site to match identifier codes in the database to individual patients in order to record clinical outcomes and supply any missing data points.

The Participant List (enrolment log) will be maintained in [insert country] and shall NOT be transferred to any other location. We will compile an enrolment log including the patient's name, date of birth, hospital identification number and unique study number. Subsequent data will be identified by the unique patient study number only. The enrolment log and study data will be kept separately.

6.3 Data Access and Data Sharing

This study will adhere to the research policies of ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium, www.isaric.org). A fundamental principle of this work is that clinical investigators contributing to research efforts, often in extremely difficult circumstances, must be given full recognition for their efforts and the opportunity to access data and samples. Ownership of any data transferred to the eCRF and centralized database will be retained by [insert country], and investigators that contributed it. All analysis of pooled data will not be undertaken with the explicit agreement of the investigators.

Data and results from central laboratory analysis for individual patients will be available to the clinicians looking after those patients as soon as possible. Research data will be shared with public health authorities in as timely a manner as needed.

6.4 Data Quality

Several procedures to ensure data quality and protocol standardisation will help to minimise bias. These include:

- A detailed data dictionary will define the data to be collected on the case report form;
- Quality checks will be built into the data management system and there will be quality checks of critical data points entered into the CRFs to ensure standardization and validity of the data collected;

The ability for data queries to generate a response will depend on the quality of data collected. However, since different patients might have different sample collection frequencies, we shall not consider as missing, those which have lower sample collection frequencies. Where data is actually missing for any reason, no assumptions shall be made.

6.4.1 Monitoring

Data monitoring will be conducted on a randomly selected subset (up to 5%) of cases, through discussion with the local site investigator to discuss data collection techniques. Direct facility visits will be conducted where possible.

7. Ethical Considerations

This study is to be conducted during a disease outbreak or presentation of cases of disease of public health interest. This is a challenging research situation because this falls in the area between clinical care, public health and clinical research (WHO Ethical Review in Disease Outbreak Expert Meeting 2009). Normally research activities are defined by anything conducted outside standard clinical care. In these situations, there may be no definitive standard guidelines or treatment protocols and therefore there is often little difference between what can benefit the patients and what is very important for building knowledge on the pathogenesis of the disease to guide future treatment and management.

Medical management of participants in this study must never be compromised by study procedures. At all times, priority will be given to samples required for medical management. Research sampling should never compromise the quantity or quality of samples taken for medical management, nor create a significant diversion for clinical teams from the day-to-day care of the patients.

7.1 Regulations, Guidelines and Ethical Review

This study will be conducted in compliance with the principles set out in the Declaration of Helsinki. Where applicable, the principles of Good Clinical Practice (ICH 1996) and other applicable regulations and guidelines will be used to guide procedures and considerations.

This protocol will be reviewed and approved by the COUNTRY ERC. No patients will be enrolled until all approvals have been obtained.

7.2 Informed Consent

Consent forms will be provided in plain English/French. Illiterate participants will have the consent form read or explained to them in the presence of a witness, who will sign to verify the accurate reading of the form and agreement of the participant. For participants who cannot understand the

language of the available forms, verbal translation of the document and the consent discussion will be used. This can either be in-person with a physical barrier, phone call or recorded messages. In the case of a physical translator, the translator may act as the witness for consent and sign the consent form so that patients who cannot read the language of the forms are not excluded from this research. Note that in many cases the required infection prevention and control measures will mean that once the form has been in contact with the participant to be signed, it will be 'contaminated' and considered a biohazard. In this case, the signed and dated form should be shown through a window to a member of staff outside of room who will complete a copy and sign as witness. All infection prevention protocols need to be followed to protect the witness and the translators.

In the case of adult participants who are unable to give informed consent due to mental or physical status, the wishes of the participant may be declared by an appropriate consultee according to the site policy on obtaining consent for medical procedures. If, during the course of the study, the participant's status changes such that they are able to consider consent independently, informed consent must be discussed and obtained (regained consent).

Parents or guardians of children under the age of 18 years old will give consent for their child. Study staff obtaining consent will consider the ability of the child to understand the principles of the study and will discuss the study with the child in age appropriate language. Where appropriate, children will be invited to give assent, which will be recorded on the informed consent form. The right to withdraw at any time without negative impact will be reinforced with the child and their parent/guardian.

A copy of the informed consent form will be given to the person who gives consent.

7.3 Alternatives to Participation and Withdrawal

Prospective participants are freely able to decline participation in this study or to withdraw from participation at any point without suffering any implied or explicit disadvantage. All patients will be treated according to standard practice regardless of if they participate.

7.4 Risks to Participants

Inconvenience.

Participation in this research study poses a minimal risk of inconvenience through household visits and attendance of follow-up visits. Appropriate compensation for travel costs to attend follow-up visits and for time of attending visits will be given according to the standard policies of the sponsor.

Phlebotomy.

Participants may have blood drawn more often than is required for standard care. Phlebotomy can be associated with pain at the draw site and rarely with infection. Daily blood draw volumes have been restricted according to weight so that combined clinical and research sampling is within recommended limits. Discomfort will be minimized by having expert staff obtain blood samples, and by combining research sampling with routine clinical sampling, where possible, which normally occurs daily in acutely unwell patients in hospital.

Discomfort of respiratory swabs.

Collecting respiratory swabs may be cause transient discomfort. Discomfort and risk will be minimized by using experienced clinical staff at each site, and samples will be taken at the same time as clinical samples in order to minimize these risks.

Incidental findings in genetic testing.

This study includes genetic testing to identify host genetic variants associated with disease progression or severity. There is a very small chance that these tests may result in the incidental discovery of information that is relevant to the participant's health. Since the samples will be analysed anonymously in batches, and generally in non-clinical laboratories with investigational techniques, we will not attempt to identify and inform participants of any results from genetic tests. If we were to do so, there would be a considerable risk of accidental harm in the form of unnecessary anxiety and distress.

7.5 Benefits to Participants

The study may include biological sampling in addition to sampling required for medical management. The results of the tests done on these samples may not contribute directly to improving the specific participant's health. For example, some assays cannot immediately benefit the patient because data will need to be pooled with others, or because the assays take time. As far as possible, test results with potential relevance to patient care will be informed to the participant and/or treating doctor.

7.6 Participation in Other Research Studies / Co-enrolment

Particularly in the case of emerging infections, it is likely that other research projects, including clinical trials, will also recruit participants in this study. In fact, it is important that they do so, and effort has been made to ensure that this observational study is compatible with, and complementary to, other possible research projects.

7.7 Confidentiality

This study will be conducted by clinical staff and those involved in the study will ensure that each study participant's privacy and confidentiality is maintained. Participants will not be identified in any published reports of this study. All records will be kept confidential to the extent provided by [insert country]'s law. All laboratory specimens, evaluation forms, reports, study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party.

Paper and electronic medical records may be accessed during the study to confirm, verify or complete clinical information provided in the case report form.

Site files will at all times be accessible only to clinical and research staff. Consent will be sought for investigators to access patient data. Local research staff will access personal information, but all data will be pseudo-anonymised before transfer by eCRF.

It is important that data generated now is not destroyed unnecessarily, since they will be of considerable potential value to future generations faced with similar outbreaks of infectious disease. Electronic data and electronic copies of paper documents will be stored for at least 5 years.

7.8 Custody of Data and Samples

Custody of site data will remain with the responsible physician at the site. Samples will be shipped (depending upon pathogen of interest) to a reference laboratory for analysis as approved by the appropriate ethics/institutional review committee. Any residual sample will remain in the custody of the site until use can be decided upon.

7.9 Additional Ethical Considerations

Recruitment of critically ill patients who are not able to consent. This is a ubiquitous problem in acute and critical care research and there is a clear legal framework under which these patients may be recruited to research studies. In all cases, efforts will be made to obtain informed consent from patients early in the course of illness, before critical illness interferes with their capacity to make decisions and to confirm consent at the earliest point in recovery. This principle applies equally to adults and children.

Perceived coercion because of individual responsibilities to society, and the implications of this research for public health. We are sensitive to the fact that some patients or their representatives may feel under an unusually strong moral obligation to participate given the nature of this research and the wide, and often inaccurate, publicity surrounding emerging infections. In view of this, we have tried to make both the potential benefits and limitations of this simple observational study clear in the information sheet. In the informed consent form we also stress that participation is entirely voluntary and there is no penalty of any kind for declining to join the study.

Balance between public health and research. Patients with emerging infections are commonly the subject of public health investigations. The work proposed here is research and will be clearly presented as such. There is no primary gain to the patient from participating. In designing and describing this research we are clear that, in accordance with the guiding principles of Good Clinical Practice, the needs and autonomy of the individual are paramount and the potential benefits to wider society do not take precedence.

Risks to clinical and research staff treating the participants. Staff who enrol, examine and take samples from study patients are at risk of infection. Care of study participants will require increased sampling and contact frequency added to normally heavy clinical workloads. All staff will need to be trained in recognised infection control measures and have ready access to appropriate personal protective equipment. In collaboration with the public health authorities, there will be on-going communication with hospital staff to ensure the appropriate training is given, to support the work and to ensure that there is no excess burden on the health system. Where appropriate, dedicated research staff will be available to support the study activities.

7.10 Scientific and Peer Review

The proposed research is the product of several years of discussion within a group of international experts who were brought together following the 2009 influenza pandemic to plan the global research response to future severe and emerging infections: the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC). ISARIC working group 3 (genomics, pathogenesis and pharmacology) comprised senior clinical scientists from 5 continents working together to promote and harmonise observational research during outbreaks of severe infectious disease.

8.1 Study Limitations

We expect some limitations for both the clinical work and the social sciences components. However, we will put adequate measures in place to ensure that these limitations do not significantly affect the quality of the research as follows:

1. The window of sampling could be narrow. This is because the Government and its stakeholders are working to stop the outbreak. To overcome this, we are working with collaborators and stakeholders to hasten preparation and planning to ensure we are able to get samples during the time there are cases.
2. Integration of clinical care of COVID-19 patients with research. The selection of the study participants will be based on facilities managing COVID-19 cases. This will mean that most of these hospitals may not have anyone who already works with the applicants directly. In order to overcome this, a member of the clinical management team for COVID-19n at the hospital will be identified and included as a collaborator. This way, we will not break hospital protocol by using an external person to obtain samples but at the same time allowing the local team to adapt the protocol in order to get the right samples per protocol.
3. None response and bias of a particular health worker grouping could also be a limitation in the social science section. To overcome this, we will sample from hospitals across the nations using a contact person in each hospital. The survey will be administered electronically and will ensure confidentiality and privacy. The focal person at these facilities can follow up on them to ensure that we do get feedback for the surveys.

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