ISARIC/WHO Clinical Characterisation Protocol for Severe Emerging Infections

ISARIC CCP Version 3.1

Version history

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1. Background and Objectives

1.1 Purpose of the Study

This is a standardized protocol for the rapid, coordinated clinical investigation of severe or potentially severe acute infections by pathogens of public health interest. Patients with acute illness suspected to be caused by emerging and unknown pathogens 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 H7N9 in 2013, viral haemorrhagic fever (Ebolavirus) in 2014, Monkeypox & MERS-coronavirus in 2018, Tick-borne encephalitis virus (TBEV) in 2019 and nCoV-2019 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.

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 severe acute or potentially severe infections due to the pathogens of interest. 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 by collecting similar data investigators will be willing to share their results and 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 the illness or syndrome
- Describe, where appropriate, the response to treatment, including supportive care and novel therapeutics.
- 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, circulating levels of immune signalling molecules and gene expression profiling 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
- Determine infectivity and appropriate infection control measures of the various pathogens
- Develop clinical guidance documents and offer clinical recommendations to policy makers on the basis of evidence obtained
- Understand the broader epidemiology of an emerging infection through studying potential contacts and asymptomatic individuals

1.7 Structure of this document: 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 a 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.

Tiers included in this protocol are:

- **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 (Single biological sample)** - Clinical samples will be collected on enrolment day (Day 1; ideally at initial presentation to a health care facility). Clinical information will be collected at enrolment and discharge.
- **Tier 2 (Serial biological sampling)** - Clinical samples and data will be collected on enrolment day (Day 1; ideally at initial presentation to a health care facility), and then alternate days for the first 2 weeks, then weekly until resolution of illness or discharge from hospital, and again at 3 and 6 months after enrolment.
- **Tier 3 (Population pharmacokinetics** of antimicrobial/immunomodulatory drugs)

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.

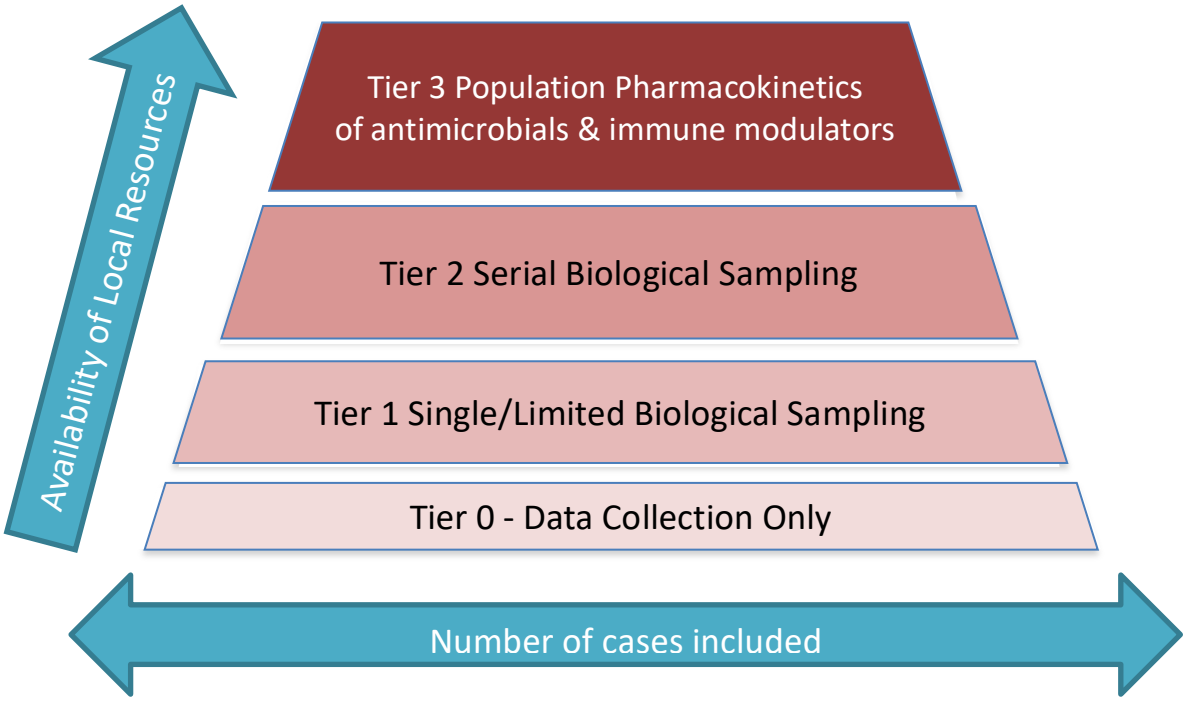

Figure 1. Tiered approach to recruitment in settings with different resources. This information is included to demonstrate the integration of this study with other studies following the same approach in other parts of the world.

1.8 Entry Criteria

This study will enrol eligible patients (children and adults) with confirmed or suspected infection with a pathogen relevant to the study objectives. Recruitment of patients with Day 1 (enrolment) data is the priority. The local study team will dictate whether laboratory confirmation of infection is required prior to enrolment.

Daily follow-up and convalescent visits of patients (Table 1 - Tier 2) should proceed according to local resources.

Inclusion criteria

Suspected or proven novel Coronavirus (nCoV) infection as main reason for admission to hospital.

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 Sample Size

This is a descriptive study of a syndrome, which may be caused by a number of different known or poorly understood pathogens. Therefore, the sample size is not prospectively determined. Recruitment of participants will depend on the emergence and spread of the various pathogens and the resources available to the recruitment centres. The sample size will vary for each location but should be as large as feasible and preferably without limit in order to capture as much clinical data as possible early in the outbreak.

This protocol will be opened at sites with capacity and capability to recruit to any tier of study intensity. The study has no set end date.

3. Methods

3.1 Identification of Potential Patients

In hospital, potential participants will be identified through hospital workers upon presentation at recruiting sites and through public health agencies. When resources limit the number of patients enrolled to less than the number of patients presenting, sites should establish procedures to minimize bias in the selection of participants.

3.2 Approach to Potential Participants

Patients will only be considered for enrolment if appropriate local infection control and prevention measures are in place and can be maintained.

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 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.

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.

For studies that collect or collate only anonymised data that is normally collected, as part of routine care consent may not be required.

Internal pilot study

An internal pilot study will only collate data that is being recorded or generated as part of routine clinical care (e.g. microbiology results). We will seek consent, be it deferred, proxy or assent, in order to test the processes within the overarching Clinical Characterisation Protocol, which include obtaining consent.

All patients will be treated according to clinical requirements regardless of their participation in the study.

3.3 Standard of Care

Provision of care will vary by site 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 benefit the health of the participants.

3.4 Data Collection and Sampling for Patients

Samples and data will be collected according to the protocol tier approach, available resources and the weight of the patient, to prevent excessive volume sampling from children, young people and small adults.

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 1). We recognise that -80°C storage is not available at all sites. In this case please store at coldest available temperature and at least -20°C.

For patients with VHF such as Ebola virus, the biological sampling will at times be limited to extra volumes of blood taken at times to coincide when blood is being taken for clinical purposes and then only at the discretion of the clinical team.

3.5 Sample and Data Collection Schedules - Tables 1, 2 and 3

Table 1. Proposed samples to be obtained.

REQUIREMENTS	Samples	Processing/ storage	Purpose
CONSENT FORM		Site file	
SINGLE SAMPLE SET TAKEN AT RECRUITMENT	Pathogen samples: Urine (up to 10ml) Stool (up to 10ml) or rectal swab; respiratory samples [combined nose and throat swab, AND endotracheal aspirate if intubated, AND, where resources permit, nasopharyngeal aspirate (NPA) OR (if NPA impossible) flocked nose and throat swab]; samples from infected sites/sores. Also store any residual from samples taken for clinical care.	Aliquot stored at -80°C*	Pathogen studies to reveal changes in pathogen during infection and during spread between individuals, detect development of resistance.
	Blood sample in serum (clotted) tube (patients > 40kg only)	Centrifuge 1500g for 10mins. Serum (3 aliquots -80°C*)	Test for mediators and potential biomarkers
			Serology to detect development of antibodies
	Blood sample in EDTA tube	Centrifuge 1500g for 10mins at 4°C. Plasma (3 aliquots -80°C*)	Test for mediators, metabolites and potential biomarkers Test for drug levels.
			Extract RNA/DNA from causative pathogen and other circulating pathogens.
		Cell fraction (1 aliquot -80°C*)	Extract host DNA for genomic studies
			Extract RNA/DNA from causative pathogen and other circulating pathogens; leftover cellular fractions from research or clinical samples can be used for PBMC isolation if feasible.
	Blood sample in blood RNA tube (e.g Tempus™ or PAXgene®)	Freeze at -20°C; transfer to -80°C after 24h where possible	Microarray and CAGE analysis of host immune cell transcriptome
	Cerebrospinal fluid sample (if suspected CNS disease) If after recruitment a lumbar puncture is clinically indicated, an additional sample of up to 5mls will be collected in a	3 aliquots stored at -80°C*	Extract RNA/DNA from causative pathogens and other circulating pathogens for molecular testing, genomic studies and virus

	universal sterile tube, provided it is deemed appropriate by the supervising clinician. Any residual CSF from samples taken as part of routine clinical care will be collected and stored if available.		isolation
			Perform serological testing for pathogen-specific antibodies
			Test for mediators, metabolites and potential biomarkers
CASE REPORT FORM	Complete CORE CRF or WHO NATURAL HISTORY PROTOCOL (depending on local resources) For VHFs collect any amount of clinical data e.g. <50 cases.	Site file	Clinical data

SERIAL SAMPLES THROUGHOUT ACUTE ILLNESS, CONVALESCENT SAMPLES WHERE POSSIBLE	Pathogen samples: Urine (up to 10ml) Stool (up to 10ml) or rectal swab; respiratory samples [combined nose and throat swab, AND endotracheal aspirate if intubated, AND, where resources permit, nasopharyngeal aspirate (NPA) OR (if NPA impossible) flocked nose and throat swab; samples from infected sites/sores. Also store any residual from samples taken for clinical care.	Freeze at -80°C	Pathogen studies to reveal changes in pathogen during infection and during spread between individuals, detect development of resistance.
	Blood sample in serum (clotted) tube (patients > 40kg only)	Centrifuge 1500g for 10mins. Serum (3 aliquots -80°C*)	Test for mediators and potential biomarkers
			Serology to detect development of antibodies
	Blood sample in EDTA	Centrifuge 1500g for 10mins at 4°C. Plasma (3 aliquots -80°C*) Cell fraction (1 aliquot -80°C*)	Test for mediators, metabolites, and potential biomarkers Test for drug levels.
			Serology to detect development of antibodies
			Extract RNA/DNA from causative pathogen and other circulating pathogens.
		Cell fraction (1 aliquot -80°C*)	Extract RNA/DNA from causative pathogen and other circulating pathogens; leftover cellular fractions from research or clinical samples can be used for PBMC isolation if feasible.
	Blood sample in blood RNA tube	Freeze at -20°C; transfer to -80 after 24h where possible	Microarray and CAGE analysis of host immune cell transcriptome
	Cerebrospinal fluid sample (if suspected CNS disease) If after recruitment a lumbar puncture is clinically indicated, an additional sample of up to 5mls will be collected in a universal sterile tube, provided it is deemed appropriate by the supervising clinician.	3 aliquots stored at -80°C*	Extract RNA/DNA from causative pathogens and other circulating pathogens for molecular testing, genomic studies and virus isolation
			Perform serological testing for pathogen-specific antibodies

	Any residual CSF from samples taken as part of routine clinical care will be collected and stored if available.		Test for mediators, metabolites and potential biomarkers
SERIAL CLINICAL DATA	Complete ISARIC DAILY RECORD FORM	Site file	Clinical data
ADDITIONAL SAMPLES FOR POPULATION PHARMACOKINETICS STUDIES	Blood sample in EDTA or fluoride oxalate tubes	Centrifuge 1500g for 10mins at 4°C. Plasma (2 aliquots -80°C*)	Test for drug levels. Store aliquot for other studies.

*freeze at -80°C where possible, or at least at -20°C. #Detailed pathogen analysis will be organised by local authorities, clinicians or reference laboratory.

Table 2. Sampling pattern - In Patient Recruitment

		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		S		S		S		S		S					S	C
20 to 40kg	R		S		S		S		S		S					S	C
10 to 20kg	R		S		S		S		S		S					S	C
4 to 10kg	R		S		S		P		S		P					S	C
<4kg	R		S		S		P		S		P					S	C
Sample priority	1		2		5		7		3		8					6	4

R = recruitment samples. S = serial samples including pathogen samples; P = research pathogen samples only; C = convalescent samples (see Table 3). 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 3. Sample volumes by patient weight

Weight	Samples at recruitment (R)	Serial samples (S)	Convalescent samples	Total Volumes of blood taken
>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 Up to 3 additional 1ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies. Research pathogen samples	3ml EDTA blood 3ml blood in serum(clotted) tube 3ml blood in blood RNA tube Research pathogen samples	Maximum any day: 15ml (0.38ml/kg) Maximum any 4 weeks: 96ml (maximum 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 Up to 3 additional 0.5ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies. 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 (maximum 2.1ml/kg)
10 to 20kg	2ml (2x1ml) EDTA blood 2ml blood in serum(clotted) tube	1ml EDTA blood 1ml blood in blood RNA tube Up to 3 additional 0.2ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for	1ml EDTA blood 1ml blood in serum(clotted) tube 1ml blood in	Maximum any day: 6ml (0.6ml/kg) Maximum any 4 weeks: 23.6ml (maximum 2.36ml/kg)

	2ml blood in blood RNA tube Research pathogen samples	pharmacokinetic/pharmacodynamic studies. Research pathogen samples	blood RNA tube Research pathogen samples	
4 to 10kg	1ml EDTA blood 1ml blood in serum(clotted) tube ml blood in blood RNA tube Research pathogen samples	1ml EDTA blood Up to 3 additional 0.2ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies. 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 (maximum 2.35ml/kg)
< 4kg	0.5ml EDTA blood 0.1ml blood in serum(clotted) tube ml blood in blood RNA tube Research pathogen samples	0.2ml EDTA blood Up to 3 additional 0.1ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies. 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 (maximum 2.4ml/kg)
Research pathogen samples (all patients)	**Pathogen samples taken solely for research purposes:** In all SARI or respiratory infection patients: combined nose and throat swab, otherwise a throat swab or nasopharyngeal swab alone In all intubated patients with SARI or respiratory infection: endotracheal aspirate also where resources permit in a respiratory case: 1. Nasopharyngeal aspirate (NPA) OR (if NPA impossible) flocked nose and throat swab 2. Urine (up to 10ml in sterile universal container, if available) 3. Rectal swab or stool (up to 10ml in sterile universal container or stool specimen container, if available) 4. samples/swabs from infected sites or sores.			No patient will give more than 0.6ml/kg (>1% blood volume) on any one day, or more than 2.4ml/kg (approx 3% blood volume) in any four week period (MCRN recommendations).
Clinician-requested CSF	**Separate aliquot of Cerebrospinal fluid (CSF) collected solely for research purposes:** When a lumbar puncture is clinically-indicated and performed in an infant or older patient, **an additional sample of up to 5mls of CSF** will be collected in a separate universal sterile tube, provided it is deemed appropriate by the supervising clinician. The volume of CSF taken from a child younger than an infant will be at the discretion of the attending clinician. Any residual CSF from samples taken as part of routine clinical care will be collected and stored			See table 4 (below) for guidance on total safe volumes of CSF to take at lumbar puncture
Clinician-requested pathogen samples (all patients)	Where possible, we will obtain an aliquot of any residual and unwanted sample volume from specimens that have been sent by clinicians for pathogen detection, including those obtained before recruitment to the study: urine; stool; respiratory tract samples (NPA, ETA, BAL, sputum, ENT swabs); cerebrospinal fluid			

Table 4. Cerebrospinal Fluid Volume Guidance

Estimates of CSF production rate, total CSF volume and the safe recommended CSF volume taken at lumbar puncture for different age groups. Taken from the British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children (2009).

Age	Mean CSF production rate (ml/h)	Total CSF Volume (mls)	Safe CSF volume to take at LP (mls)
Adult	22	150-170	Maximum: 15-17
Adolescent	18	120-170	Maximum: 12-17
Young child	12	100-150	Maximum: 10-15
Infant	10	60-90	Maximum: 6-9
Term Neonate	1	20-40	Maximum: 2-4

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:

1. Separate and store an aliquot of all routine clinical samples taken at baseline/presentation including (as indicated) blood, cerebrospinal fluid (if CNS disease), infected sites/sores, sputum, respiratory tract specimens, urine and stool or rectal swab. Any research pathogen samples which have not been taken for clinical care will be collected.
2. Take a blood sample (0.8 - 15ml dependent on weight).

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.

During the one week of test activation for the internal pilot study, we will collect only anonymous data from patients that meet the selection criteria defined in Appendix A.

3.7 Case Report Form and Patient Numbers

Case Report Forms (CRFs) completed after site registration at https://redcap.medsci.ox.ac.uk/ .

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 core case report form (Rapid CRF) 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 outlines in Table 1. 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.

Procedures for serial sampling as shown in table 2

Collection of clinical information, blood sample (volume dependent on weight - see Table 3), urine, sputum (if possible), stool or rectal swab, infection site and respiratory samples.

Procedures for pathogen-only serial sampling as shown in table 2

Collection of clinical information, urine, sputum (if possible), stool or rectal swab, infection site and respiratory samples.

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.

Procedure for additional sampling for pharmacokinetic/ pharmacodynamics studies.

[Where a pharmacokinetic study is run concurrently with this protocol] 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 SARI 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.

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 must 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 personal protective equipment (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. Laboratories planning to participate in the study should consider how they would fulfil a requirement to handle research samples in addition to clinical samples.

All samples collected must be labelled according to local hospital policy with appropriate identification (full patient identifiers) and hazard labelling and ideally marked 'ISARIC RESEARCH' with a solvent resistant marker. Samples will be processed as per the table below. 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

4.4 Use of Stored Samples
Access to samples for additional analyses will be governed by a committee comprising the clinical lead investigators and scientific investigators for this study (the Data and Materials Access Committee), in collaboration with the individual recruiting sites. Linked anonymised data generated during the course of these studies may be shared between investigators. Each local site will hold their own data.

Where possible and within the constraints of international law and specific requirements of local ethical 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 local regulations. This database will be held on servers. Access to data for outside investigators will be reviewed by the data and materials access committee.

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 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/or export of specific samples to collaborating institutions for investigations that cannot be performed locally. Any proposed plans to use samples other than for those investigations detailed in this protocol will be submitted to the relevant ethics committees prior to any testing. 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 research 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. Clinical data will be collected locally with the relevant CRF for SARI, VHF, CNS or other emerging infections of public health interest will be completed by a study staff as appropriate. The data will be anonymised at site and a study number issued.

6.2 Data Management
When available, data collected by staff at each site 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. The records kept will not include any information that allows patients to be identified.

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 Site Investigators. All electronic data transfer between study site and database will be username and password protected. Each centre will maintain a trial 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) is maintained locally and is not to be transferred to any other location. The sites 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 the site that contributed it. All analysis of pooled data will be undertaken with the explicit agreement of each site who contributed.

Data and results from central laboratory analysis for individual patients will be available to the clinicians looking after those patients as soon as possible. Often, this may not be in time to affect treatment decisions. Research data will be shared with public health authorities 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;

Data queries may be generated, depending on resource availability. Any information that is not available for the investigator will not be considered as missing. No assumptions will be made for missing data.

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 site visits will not be feasible, given the scope of the study.

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 ethical and regulatory review boards required by the recruiting site and the study sponsor. No patients will be enrolled until all approvals have been obtained for the applicable site.

7.2 Informed Consent

Consent forms will be provided in plain English. Illiterate participants will have the consent form read 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, verified translations will be made when possible. If it is not possible to prepare a translation in a required language, verbal translation of the document and the consent discussion (if required) will be used. In this case, 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.

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.

Parents or guardians of children under the age of 16 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. Should the UK rules on consent by young people for research purposes alter during the period of this study to allow consent by competent minors, then these new rules will be applied to this study without further amendment.

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.

Discomfort of lumbar puncture

Collection of cerebrospinal fluid with lumbar puncture will only be performed if clinically indicated, as decided by the responsible physician. Clinical investigations are the priority, with any remaining sample collected for use in research. Guidance on the safe recommended daily total volume of CSF to take in different age groups is provided (Table 4). Lumbar puncture can be associated with discomfort at the site of needle insertion, headache, and rarely bleeding or infection.

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.

Specific risks for VHF patients

Participants with VHF may be at increased risk of bleeding from venepuncture sites. The decision to perform venepuncture for research purposes will only be performed following discussion with the attending clinician and only if venepuncture is deemed not to pose unacceptable risk to the patient and/or staff. When at risk venepuncture will be minimised by limiting research venepuncture to coincide with clinical venepuncture.

7.5 Benefits to Participants

There will be no direct benefit to research 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 to improving the participant's health. The results of this study will not be available in time to contribute to the participant's care. Where possible, test results with potential relevance to patient care will be informed to the participant and/or treating doctor. The feasibility of this will depend on local resources. Some assays cannot immediately benefit the patient because data will need to be pooled with others, or because the assays take time.

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 great effort has been expended 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 international and local 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 pseudoanonymised 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 must 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.