 

**ISARIC/WHO Severe Acute Respiratory Infection Biological Sampling Study**

**13th May 2013. Version 2.5.0**

Sponsor: [\*\*\*Insert name of sponsor\*\*\*]

Chief Investigator: [\*\*\*Insert name of chief investigator\*\*\*]

Co-Investigators: [\*\*\*Insert names\*\*\*]

Funder: [\*\*\*Insert details\*\*\*]

**License.**

This document was created by members of ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium) in collaboration with the World Health Organisation and is distributed under the Creative Commons Attribution Non-commercial ShareAlike Licence version 3.0 (http://creativecommons.org/licenses/by-nc-sa/3.0/). It is freely available for you to copy, adapt, distribute and transmit under the conditions that: (a.) the original source is attributed; (b.) the work is not used for commercial purposes, and (c.) any altered forms of this document are distributed freely under the same conditions.

**1. Background and Objectives**

**1.1 Purpose of the Document**

This document is a standardized protocol for the rapid, coordinated clinical investigation of emerging infections causing severe acute respiratory illness. Patients with a spectrum of emerging and unknown pathogens will be enrolled. This protocol has been designed to maximize the likelihood that data and biological samples are prospectively and systematically collected and shared rapidly in a format that can be easily aggregated, tabulated and analysed across many different settings globally. The protocol is designed to have some level of flexibility in order to ensure the broadest acceptance and has been initiated in response to the recent cases of novel coronavirus (nCoV) in 2012-2013 and Influenza H7N9 in 2013.

**1.2 Background Information**

Infectious disease is the single biggest cause of death worldwide. New infectious agents, such as the SARS coronavirus and new strains of influenza continually emerge and require new investigations 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, and factors underlying individual susceptibility.

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

This protocol is designed to enroll patients with suspicion, diagnosis or high risk of infection by the following:

**Influenza A H5N1.**

Since 1997, strain A/H5N1 of highly pathogenic avian influenza (HPAI) has emerged as a global zoonosis, and has caused severe sporadic respiratory illness in humans that is associated with an extremely high mortality rate. As of March 2013, the total number of H5N1 cases reported to WHO worldwide is 622; of these 371 have died resulting in a case fatality rate of just under 60%. http://www.who.int/influenza/human\_animal\_interface/H5N1\_cumulative\_table\_archives/en/index.html (accessed April 2013).

**Novel Coronavirus.**

In September 2012 a novel coronavirus was identified in a patient who died of severe acute respiratory syndrome in June 2012. As of 3rd April 2013, the World Health Organization has been informed of a total of 17 confirmed cases, including 11 deaths (www.who.int).

**Influenza A H7N9.**

As of 11th April 2013, thirty-eight laboratory confirmed cases of human infection with influenza A(H7N9) have been reported by the World Health Organization. Ten of the cases have died and fourteen are in a critical condition. The majority of cases presented with respiratory tract infection with progression to severe pneumonia and breathing difficulties.

**Emerging Pathogens causing Severe Acute Respiratory Illness.**

Novel pathogens, new strains of existing pathogens, and re-emergence of known pathogens are a frequent threat to global health. A coordinated clinical research response is critical to identify and describe pathogen and host characteristics to inform a clinical and public health response.

**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 respiratory illness. 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.

This document may also be of interest to other researchers and health care workers conducting basic research into the pathogenesis and treatment of other respiratory pathogens or other emerging infections.

**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.

**1.5 Primary Objectives**

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

* Describe the clinical features and response to treatment of SARI
* Observe 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 and other samples.
* Characterise the host responses to infection and therapy over time, including innate and acquired immune responses, circulating levels of immune signaling molecules and gene expression profiling in peripheral blood.
* Identify host genetic variants associated with disease progression or severity

**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

**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. Participating centres, in many cases working in extremely challenging circumstances, will follow this protocol as much as possible within their means. Observational analyses will be stratified according to available samples and data.

In all cases, a case report form will be completed. In many cases, outwith this research study, case report forms will be completed for audit and public health purposes. This can be regarded as Tier 0 of this protocol, and the data collected will be the foundation for subsequent analyses described here.

Tiers included in this protocol are:

* 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 enrollment. Intensity of sampling will be varied according to local conditions.
* Tier 3C (Population pharmacokinetics of antimicrobial/immunomodulatory drugs)


Figure 1. Tiered approach to recruitment in settings with different resources. 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.

**1.8 Entry Criteria**

This study will enroll eligible patients with confirmed or suspected infection with a pathogen relevant to the study objectives. Recruitment of patients with Day 1 (enrolment) data is the highest priority.

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

Inclusion criteria for all patients:

* Acute respiratory illness patients of all ages with a history of fever or measured fever of >38°C and at least one respiratory symptom
* AND suspected or confirmed infection with a pathogen relevant to the objectives of this protocol
* AND admitted to hospital.

Exclusion criteria for patients:

* Confirmed diagnosis of a pathogen unrelated to the objectives of this study and no indication 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 and unknown 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 open for recruitment for three years in the first instance.

**3. Methods**

**3.1 Identification of Potential Patients**

Approval of the responsible ethics committee will be obtained before patients are recruited at any site. 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 will establish procedures to minimize bias in the selection of participants.

**3.2 Approach to Potential Participants**

Samples taken early may be most useful for identification or evaluation of risk factors for disease progression at a clinically-relevant decision point. It is therefore very desirable to begin sampling as early as possible in a patient's illness.

Participants or an appropriate parent/guardian/consultee 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. Participants who agree to participate (or their parent/guardian or consultee who declares their wishes to do so) will be asked to sign and date an informed consent form.

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.

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

**3.3 Standard of Care for Patients**

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 will have samples taken in addition to what is 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 available resources and the weight of the patient, to prevent excessive volume sampling from children, young people and small adults.

**Samples required for medical 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.

**3.5 Sample and Data Collection Schedules**

Table 1. Sample schedule.

|  |  |  |  |
| --- | --- | --- | --- |
| **REQUIREMENTS** | **Samples** | **Processing/ storage** | **Purpose** |
| CONSENT FORM |  | Site file |  |
| SINGLE SAMPLE SET TAKEN AT RECRUITMENT | Pathogen samples: Urine (up to 10mls) Stool (up to 10mls) or rectal swab Nasopharyngeal aspirate/nasal+throat swab, endotracheal aspirate if intubated Samples from infected sites/sores Also store other 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 | Serum (3 aliquots -80°C\*) | Test for mediators and potential biomarkers |
| Serology to detect development of antibodies |
| Blood sample in EDTA tube | Plasma (3 aliquots -80°C\*) | Test for mediators, metabolites and potential biomarkers |
| 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. |
| Blood sample in blood RNA tube | Freeze at -20°C; transfer to -80°C after 24h where possible | Microarray and CAGE analysis of host immune cell transcriptome |
| CASE REPORT FORM | Complete CORE CRF or WHO NATURAL HISTORY PROTOCOL (depending on local resources) | Site file | Clinical data |
| SERIAL SAMPLES THROUGHOUT ACUTE ILLNESS, CONVALESCENT SAMPLES WHERE POSSIBLE | Pathogen samples: Urine (up to 10mls) Stool (up to 10mls) or rectal swab Nasopharyngeal aspirate/flocked swab OR endotracheal aspirate if intubated Samples from infected sites/sores Also store other 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) | Serum (3 aliquots -80°C\*) | Test for mediators and potential biomarkers |
| Serology to detect development of antibodies |
| Blood sample in EDTA | Plasma (3 aliquots -80°C\*) | Test for mediators, metabolites, and potential biomarkersTest 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. |
| 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 |
| SERIAL CLINICAL DATA | Complete ISARIC DAILY RECORD FORM | Site file | Clinical data |
| ADDITIONAL SAMPLES FOR POPULATION PHARMACOKINETICS STUDIES | Blood sample in EDTA | Plasma (2 aliquots -80°C\*) | Test for drug levels. Store aliquot for other studies. |

\*freeze at -80°C where possible, -20°C otherwise. #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).

Table 3. Samples

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Weight | Samples at recruitment (R) | Serial samples (S) | Convalescent samples | Total Volumes of blood taken |
| >40kg | 9mls EDTA blood3mls blood in serum(clotted) tube3mls blood in blood RNA tubeResearch pathogen samples | 3mls EDTA blood3mls blood in serum(clotted) tube3mls blood in blood RNA tubeUp to 3 additional 1ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies.Research pathogen samples | 3mls EDTA blood3mls blood in serum(clotted) tube3mls blood in blood RNA tubeResearch pathogen samples | Maximum any day: 15mls (0.38mls/kg)Maximum any 4 weeks: 96mls (maximum 2.4mls/kg) |
| 20 to 40kg | 6mls EDTA blood3mls blood in serum(clotted) tube3mls blood in blood RNA tubeResearch pathogen samples | 1mls EDTA blood2mls blood in blood RNA tubeUp to 3 additional 0.5ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies.Research pathogen samples | 1mls EDTA blood3mls blood in serum(clotted) tube2mls blood in blood RNA tubeResearch pathogen samples | Maximum any day: 12mls (0.6mls/kg)Maximum any 4 weeks: 42mls (maximum 2.1mls/kg) |
| 10 to 20kg | 2mls EDTA blood2mls blood in serum(clotted) tube2mls blood in blood RNA tubeResearch pathogen samples | 1mls EDTA blood1mls blood in blood RNA tubeUp to 3 additional 0.2ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies.Research pathogen samples | 1mls EDTA blood1mls blood in serum(clotted) tube1mls blood in blood RNA tubeResearch pathogen samples | Maximum any day: 6mls (0.6mls/kg)Maximum any 4 weeks: 23.6mls (maximum 2.36mls/kg) |
| <4 to 10kg | 1mls EDTA blood1mls blood in serum(clotted) tubemls blood in blood RNA tubeResearch pathogen samples | 1mls EDTA bloodUp to 3 additional 0.2ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies.Research pathogen samples | 1mls EDTA blood1mls blood in serum(clotted) tubeResearch pathogen samples | Maximum any day: 2mls (0.5mls/kg)Maximum any 4 weeks: 9.4mls (maximum 2.35mls/kg) |
| < 4kg | 0.5mls EDTA blood0.1mls blood in serum(clotted) tubemls blood in blood RNA tubeResearch pathogen samples | 0.2mls EDTA bloodUp to 3 additional 0.1ml samples in EDTA or fluoride oxalate tubes spread throughout dosing schedule for pharmacokinetic/pharmacodynamic studies.Research pathogen samples | 0.2mls EDTA blood0.2mls blood in serum(clotted) tubeResearch pathogen samples | Maximum any day: 0.8mls (~0.27mls/kg)Maximum any 4 weeks: 2.4mls (maximum 2.4mls/kg) |
| Research pathogen samples (all patients) | Pathogen samples taken solely for research purposes:1. In all patients: combined nose and throat swab
2. In all intubated patients: endotracheal aspirate

also where resources permit:1. Nasopharyngeal aspirate (NPA) OR (if NPA impossible) flocked nose and throat swab
2. Urine (up to 10mls in sterile universal container, if available)
3. Rectal swab or stool (up to 10mls in sterile universal container or stool specimen container, if available)
4. samples/swabs from infected sites or sores.
 | No patient will give more than 0.6mls/kg (>1% blood volume) on any one day, or more than 2.4mls/kg (approx 3% blood volume) in any four week period (MCRN recommendations). |
| 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. |  |

**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, 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 enrollment, sites with available resources will:

1. Separate and store an aliquot of all routine clinical samples taken at baseline/presentation including (as indicated) blood, 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 - 15 mls 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.

**3.7 Case report form**

In public health emergencies the WHO Natural History Protocol Case Report Form will be used to collect data at, or before, enrolment to this study. For settings or circumstances in which resources are constrained, an abbreviated core case report form is provided.

**3.8 Follow-Up Procedures for Patients**

Follow-up procedures will be undertaken only when resources allow according to Tier 2 sampling outlines in Table 1. 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.

Patients discharged from hospital will discontinue follow-up visits until the convalescent 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 pharmacokinetics studies. Up to 2 additional samples will 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 to on a case-by-case basis to fit in with clinical care; provided the precise times of administration 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, a paired nasopharyngeal aspirate AND endotracheal aspirate if intubated is recommended. In settings where the necessary equipment is not available a combined flocked nasal and throat swab may be taken. A sputum sample will be collected when a productive cough is present and the patient is able to produce one. Paired upper and lower respiratory track samples should be collected when possible.

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 will be withdrawn. No further follow-up will be conducted.

**4. Specimen Sampling, Storage Procedures, Transport and Laboratory Analysis**

**4.1 Specimen Sampling and 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.

Well-established hospital protocols may be used to collect specimens however guidance on the collection of specimens from SARI patients is also found in the WHO draft document "Collecting, preserving and shipping specimens for the diagnosis of influenza virus infection" (2011). In dealing with respiratory 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) are essential. Biosafety procedures will be as per local policy/guidance and will be applied to the collection, storage and laboratory handling of research samples.

All samples collected in hospital will be labeled as per hospital procedure with appropriate identification and hazard labeling according to local policy. Samples will be processed as per the table below. Testing that cannot be done in country will be exported with the permission of the patient/parent/guardian/consultee. Any samples sent to external laboratories will be anonymized with unique coded identifiers to protect the identity of the patient at the site level at the point of enrolment. When required, national guidance will be adhered to for the transport of specimens.

Standard laboratory systems will be used for all clinical and research samples. Clinical samples will be labeled with standard hospital information, including the date and sent with the standard lab request forms.

A unique alphanumeric code will be given to each patient, and the only link between the patient's identifying data and this code will be held securely.

Residual volumes available after clinical and research testing is complete will be stored centrally. All samples will be anonymized before storage. Only residual volumes which remain after the processing of the test for which the sample was taken will be stored.

**4.2 Additional Data Collection - Pharmacokinetics Studies**

Where local resources allow, additional information and samples will be sought daily 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 daily record form, and includes both the precise (to the minute) times of drug administration and the precise time of blood sampling.

**4.3 Sample Processing**

Table 5.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **Initial processing** | **Aliquots** | **Analysis** |
| Blood samples (serum) | Centrifuge 1500g for 10mins. | Supernatant: freeze at -80°C | Serology |
| Supernatant: freeze at -80°C | Circulating mediators by multiplex cytokine/chemokine assays |
| Supernatant: freeze at -80°C |
| Blood samples (EDTA) | Centrifuge 1500g for 10mins 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) |
| Cell pellet: freeze at -80°C | 1mcg: high-throughput genotyping1mcg: targeted resequencing |
| Blood samples (blood RNA tube) | Freeze at -20°C | Where possible, freeze at -80°C after 24hrs | 1mcg: microarray analysis1mcg: high-throughput sequencing (CAGE) |
| Pathogen samples | Aliquots where appropriate | Freeze at -20 or -80 °C | Pathogen detection, quantification and viral genome sequencing |

For sites/regions lacking specific laboratory facilities, an updated list of laboratories offering specific analyses of samples obtained through this protocol is available at www.isaric.org/wg3. Sites that do not have access to specific laboratory facilities are encouraged to contact investigators through the ISARIC network in order to arrange analysis.

**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 database which will be fully compliant with standard data management processes and local regulations. Access to data for outside investigators will be reviewed by the data and materials access committee.

**4.5 Future Use of Samples**

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

Any database 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.

**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 and a CRF completed by a study staff. 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. Anonymized 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. Data protection regulations will be adhered to. Patients' identities will be protected and their information held securely. Centrally maintained electronic records will not include any information that allows patients to be identified.

**6.3 Data Access**

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 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.

**7. Ethical Considerations**

This study is to be conducted during a disease outbreak. 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 should 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 current revision of the Declaration of Helsinki (Somerset West, 1996). 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**

All consent forms will be available in local language. 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 should be discussed and obtained, or else the participant should be withdrawn.

Parents or guardians of children under the age of 16 will give consent for their child. Study staff obtaining consent will consider the ability of the child to understand the basic 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 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, which normally occurs daily in acutely unwell patients in hospital.

**Discomfort of respiratory swabs.**

Respiratory swabs may be uncomfortable to obtain. 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**

There will be no direct benefit to research participants. The study includes serial biological sampling in addition to that 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**

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.

Minimal personal data will be entered into the database for analysis. The patient's identifying personal information will be logged separately and stored securely. The patient might be asked to take part in future research, and therefore their identifiers need to be retained for contact at a future date, subsequent to the appropriate ethical approvals. The stored research data is also likely to be of significant value in the future for other studies and therefore permission is sought for this storing of the research data that does contain minimal patient identifiers such as age, sex and ethnicity.

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 anonymised before transfer.

All samples will be labeled with a unique, non-identifiable subject number. The patient's name and subject number will be recorded on the consent form. This will preserve the link between anonymous and identifiable data. Data and samples obtained from routine clinical care will be anonymised. The only link to identifiable data will be the consent form. Further research questions, subject to appropriate ethical approval, may be answered in retrospect in the future. Since the samples and data generated by this work may be irreplaceable after an outbreak of infectious disease has passed, it is essential that future work is not impeded by unnecessary data loss.

Data will be encrypted before transfer on portable devices. Multiple backups will be maintained on institutional servers. Critical data will be stored in a stable storage format.

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 indefinitely.

**7.8 Custody of Data and Samples**

Custody of site data will remain with the responsible physician at the site. Samples will be shipped to the named reference laboratories for analysis as approved by the appropriate ethics/institutional review committee. Any residual samples will remain in the custody of the site until use can be decided upon according to ISARIC policies/procedures and the appropriate ethical approvals.

**7.9 Additional Ethical Considerations**

**Recruitment of critically ill patients who are not able to consent.**

This is a ubiquitous problem in critical care research. Personal or nominated consultees will be asked to provide an opinion on whether they believe the patient would object to recruitment to the study; recruitment will only take place if a favourable opinion is provided. 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.

**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 staff treating the participants.**

Staff who enroll, 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 loads. All staff will be trained in infection control measures and have ready access to the appropriate personal protective equipment. In collaboration with the public health authorities, there will be ongoing 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 a year-long 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) comprises senior clinical scientists from 5 continents, and aims to promote and harmonise observational research during outbreaks of severe infectious disease.