Study Protocol

Longitudinal Observational Study of the Natural History of Andes Virus Infection

Short Title: Natural History of Andes Virus Infection (**NAVIS**)

Protocol Number: NAVIS 1.1, Protocol Version: 1.1, Protocol Date: 28 May 2026

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Clinical Trial Registration: Not applicable (observational) unless local policy requires registration of observational studies. Not a clinical trial.

Contents

0. Protocol Summary

This is a longitudinal observational cohort study enrolling individuals with a defined exposure to Andes Virus (ANDV) who are confined or quarantined. Subjects can be included in one of the three tiers and are all followed from one tier to the other and/or to end of quarantine: (a) **Tier 1 (Exposure/Enrolment):** from X0/E0 to P0 (first RT-qPCR positive); (b) **Tier 2 (Pre-symptomatic infection):** from P0 to S0 (first symptom onset); (c) **Tier 3 (Symptomatic disease):** from S0 to clinical outcome (clinical resolution or death). Epidemiological information from their **exposure (X0)** is also collected, and retrospective data prior to enrolment collected where available. The overarching goal is to delineate the natural history and the virologic and immunologic mechanisms and consequences of infection with sampling intensity matched to biological inflection points, i.e., higher frequency around P0 and symptom onset (S0) and lower intensity elsewhere. Clinical care is not directed by the protocol. All medical decisions remain under treating clinicians. This protocol **remains observational and purposely low-intensity** because it **does not direct clinical care** and uses a trigger-based, phase-adaptive tier structure (X0/E0, P0, S0) that limits biospecimen collection to fixed, low-frequency schedules (generally 1–2 collection days/week with step-down to 1 day/week in weeks 5–6 post-trigger; less frequently for children) while daily follow-up is restricted to non-invasive clinical monitoring. Participation in the NAVIS protocol **does not restrict or prevent enrollment in other Hantavirus-related emergency responses or interventional clinical trials**.

1. Introduction

1.1 Background

Hantaviruses are globally distributed, enveloped, negative-sense RNA viruses within the order Bunyavirales that cause two major, severe human syndromes: haemorrhagic fever with renal syndrome (HFRS), predominantly in Europe and Asia, and hantavirus cardiopulmonary syndrome (HCPS, also referred to as hantavirus pulmonary syndrome), predominantly in the Americas[1–4]. Their geographic distribution closely mirrors that of their reservoir hosts, principally rodents, with human infection occurring largely as a consequence of ecological and behavioural interfaces that bring people into contact with virus-contaminated rodent excreta[1,5–11]. Multiple large outbreaks and sporadic clusters over the last decades[10,12–17] have underscored that hantaviruses represent a continuing public health threat whose incidence, detectability, and geographic footprint can fluctuate with climate, land use, and changes in human activity.

Andes virus (ANDV) and its distinctive public health relevance

Within the Americas, Andes Virus (ANDV) is a principal cause of HCPS in parts of South America, particularly Chile and Argentina, and is associated with high clinical severity and substantial case fatality[1,4,7,18–20]. ANDV is maintained in nature in association with specific rodent hosts (notably *Oligoryzomys longicaudatus* in endemic regions)[21]. Human acquisition in many settings reflects rural or peridomestic exposures that aerosolize infectious particles from contaminated urine, faeces, or saliva. The epidemiology of HCPS is strongly shaped by reservoir dynamics, including population surges linked to environmental conditions, and by human behaviours that increase inhalational exposure risk (e.g., activities that generate dust in enclosed spaces).

A uniquely important feature of ANDV, distinguishing it from other hantaviruses, is well-documented person-to-person transmission[13], which has been reported in Argentina and Chile, including household and rare nosocomial transmission events. In a Chilean prospective study of household contacts of confirmed cases, secondary infections occurred with an overall secondary attack rate of 3.4%.[22] Risk was substantially higher among sexual partners than among other household contacts. Outbreak investigations have further implicated close contact around the febrile prodrome (e.g., sleeping in the same room and intimate contact) as key risk contexts, supporting the importance of precisely characterizing the temporal window of infectiousness and early infection kinetics. However, the precise mechanism of ANDV transmission, specifically whether inhalation of infectious respiratory particles, direct mucosal deposition, or contaminated fomite exposure,

remains incompletely characterized. The role of vertical transmission from mother to neonate is unclear.[23] This mechanistic gap constrains outbreak containment strategies.

Clinical course of HCPS and the importance of early-phase characterization

HCPS typically begins after an incubation period that can extend for weeks, followed by a prodrome of non-specific symptoms (often fever, myalgias, headache, and frequently gastrointestinal symptoms). [1,20,24,25] Then, among those who progress, there is an abrupt transition to cardiopulmonary compromise with cough, dyspnoea, hypotension, and rapidly evolving pulmonary oedema and shock. ANDV-associated HCPS can be severe, and clinical deterioration may occur over hours once the cardiopulmonary phase begins, emphasizing the need for high clinical suspicion and rapid diagnostic pathways. Laboratory abnormalities often include early thrombocytopenia, leukocytosis with characteristic peripheral smear features in advanced disease, haemoconcentration, and evidence of capillary leak. Imaging can evolve quickly from normal early studies to bilateral infiltrates and effusions during the cardiopulmonary phase.

Despite decades of research, **major uncertainties remain regarding the earliest virologic and immunologic events that define transition points from exposure to detectable infection, from detectable infection to symptom onset, and from prodrome to severe cardiopulmonary disease.** There are additional uncertainties regarding sub-populations such as pregnancy and paediatrics, where responses and disease trajectories may differ. These uncertainties limit evidence-based risk stratification, constrain optimal timing of potential future therapeutics (e.g., antivirals or passive immunotherapies), and complicate outbreak containment strategies where ANDV person-to-person transmission is possible.

Pathogenesis: endothelial targeting, vascular leak, and immunopathology

Across hantavirus syndromes, increased vascular permeability (capillary leak) is central to pathogenesis.[26–36] Endothelial cells of capillaries and small vessels are principal targets, and clinical disease reflects dysfunction of the endothelial barrier rather than direct viral lysis. Mechanistic work supports a multifactorial model in which viral interactions with endothelial receptors and host inflammatory responses converge to disrupt barrier integrity. Proposed and observed contributors include cytokine-mediated effects (with clinical associations reported for inflammatory cytokines such as IL-6 and TNF-α in severe disease contexts), complement activation (including associations between complement activation markers and severity), and dynamic platelet–endothelial interactions that plausibly contribute to thrombocytopenia and coagulopathy.

Thrombocytopenia is a hallmark of hantavirus disease and may arise from platelet consumption/activation and adhesion phenomena involving infected endothelium and integrin-mediated interactions. The immunopathology paradigm is further supported by observations of strong innate immune activation and vigorous T-cell responses in acute infection, with evidence that immune response patterns and host genetic factors (including HLA-associated susceptibility/severity signals) can modulate clinical outcomes. Collectively, these mechanisms highlight why dense, time-resolved sampling around biological “inflection points” (conversion and early prodrome) is particularly valuable for identifying mechanistic correlates of progression versus non-progression.

Diagnostics and why timing matters for natural history studies

For clinical diagnosis, serology is widely used and IgM is often detectable at the onset of febrile prodrome, with IgG typically present by the end of prodrome. Molecular diagnosis by RT-qPCR is sensitive and specific, and for ANDV, viral RNA can be detectable before symptoms and before antibody detection. Viral loads may be higher in buffy coat than in plasma, which has direct implications for specimen selection and early detection strategy. Importantly, RT-qPCR has been reported to detect ANDV RNA up to approximately two weeks before symptom onset and for weeks after symptom resolution in some contexts, providing an opportunity to characterize viral kinetics across pre-symptomatic, symptomatic, and convalescent phases if sampling is sufficiently frequent and appropriately compartmentalized.

Because incubation can be variable and prolonged, defining a single “best” sampling calendar is challenging. Instead, schedules that adapt to individual-level biological triggers (first PCR positivity; symptom onset) are scientifically efficient and can reduce unnecessary procedures outside the most informative windows. This logic is explicitly embedded in the

NAVIS design, which anchors inclusion of participants in 3 tiers and follow-up from one-tier to the next: from enrolment (E0), first PCR positivity (P0), and symptom onset (S0), and concentrates sampling intensity around P0 and S0 to capture rapid transitions in virologic and immune dynamics.

Treatment landscape and unmet need

At present, there is no broadly accepted, specifically effective antiviral or immunomodulatory treatment for HCPS, and management is primarily supportive. Trials of candidate interventions in established cardiopulmonary phase have not consistently demonstrated benefit (e.g., ribavirin[3,37,38] and high-dose corticosteroids [39,40] in certain contexts), reinforcing that if future therapeutics are to succeed, they may need to be delivered earlier and targeted to the right biological stage. Observational evidence also suggests that early antibody features may correlate with outcome in hantavirus infections, supporting the value of carefully mapped humoral kinetics and functional antibody profiling as part of mechanistic natural history work. In addition, the field is actively exploring neutralising antibodies and other countermeasures, but rational trial design and optimal timing require a more precise description of the exposure-to-viraemia-to-symptom timeline and its variability across individuals and exposure contexts.

The 2026 MV Hondius shipboard Andes virus outbreak

A cluster of severe illness and deaths has been linked to the Dutch-flagged expedition cruise ship MV Hondius during a voyage from South America across the Atlantic[41]. The identification of Andes virus as the cause has triggered an international public-health response. The Andes virus is the only hantavirus known to allow limited person-to-person transmission. However, human-to-human spread generally requires close, prolonged contact, for example among household/intimate contacts or similarly high-intensity exposures. At the time of writing this protocol, both WHO and ECDC have reported eight cases linked to the cluster (with ECDC further categorising them as confirmed/probable/suspected) and three deaths. However, additional cases could still be detected given the prolonged incubation period. A major operational complication of the outbreak containment is that some passengers disembarked at a stopover on Saint Helena before the outbreak was recognised, necessitating multi-country contact tracing and monitoring to manage the possibility of onward spread across borders. In response, WHO is coordinating under the International Health Regulations (IHR), has deployed an expert onboard to support comprehensive medical assessment and risk evaluation, arranged shipment of diagnostic kits to strengthen laboratory capacity, and is developing step-by-step operational guidance for safe and respectful disembarkation and onward travel of passengers and crew.

1.2. Study rationale

The NAVIS protocol ("Natural History of Andes Virus Infection") is designed as a longitudinal observational cohort enrolling adults and children with a defined exposure to ANDV after the MV Hondius shipboard outbreak who are confined/quarantined. This setting provides a rare and scientifically powerful natural experiment: exposure timing and contact structure are more sharply bounded than in typical community outbreaks, follow-up is feasible with high adherence during confinement, and repeated sampling can be aligned to biological transitions rather than fixed calendar visits. Beyond this outbreak, the protocol is designed to be applied to other cases, outbreaks and settings, helping to support a more harmonized approach to disease characterization worldwide. NAVIS follows participants from inclusion, which can be in one of the three tiers, to progression to next tiers and end of quarantine, with an explicit goal to delineate infection kinetics and underlying virologic/immunologic mechanisms and immunopathology, while keeping clinical care independent of study procedures.

This design is particularly relevant to ANDV because (i) incubation is variable and can span multiple weeks, (ii) RT-qPCR can detect viral RNA prior to symptoms and antibodies, with higher yields expected in cellular fractions such as buffy coat, and (iii) the pathogenesis involves dynamic endothelial–immune–platelet interactions that are unlikely to be captured by sparse sampling. By pairing intensive sampling windows with clearly defined triggers (P0 and S0), NAVIS is positioned to generate mechanistic insights that are directly actionable for outbreak containment (including clarifying early infectious windows in settings where person-to-person transmission is possible) and for informing future trials of early-phase

countermeasures. Finally, NAVIS is sponsored/coordinated by WHO and is explicitly framed as an observational protocol (registration optional per local policy). This governance and the outbreak-specific cohort structure support rapid implementation while maintaining an ethically proportionate approach (tiered sampling intensity, trigger-based escalation) appropriate for a confined-exposure context.

2. Objectives

2.1 Primary Objective

Define the natural history timeline of Andes virus infection from exposure (X0) to:

- First detectable viral RNA (P0)
- Peak viral load, viral clearance
- Seroconversion (IgM, IgG) and durable immunity signals
- Symptom onset (S0)
- Clinical outcomes

2.2 Secondary Objectives

A. Virology, shedding, and compartmental dynamics

(i) Quantify viral kinetics across body compartments (buffy coat vs plasma, and non-blood specimens including saliva, urine, feces and semen; for pregnant women vaginal swabs and amniotic fluid if available; for breastfeeding women, breastmilk).

(ii) Compare time-to-detection and viral load magnitude across specimen types (buffy coat, plasma, nasopharyngeal, saliva, urine, feces and semen) to define optimal matrices for early detection and monitoring.

(iii) Quantify viral expansion and decay rates (growth/decline slopes), time to peak, and area-under-the-curve (AUC) in each compartment, aligned to P0 and S0 to capture rapid transitions.

(iv) Define the duration of compartment-specific PCR positivity and shedding relative to symptom onset and symptom resolution, including persistence patterns and discordance between compartments.

(v) Assess within-host compartmental concordance (correlation and lead/lag structure) between systemic viraemia (blood fractions) and mucosal/urinary shedding compartments.

(vi) Evaluate whether early systemic viral burden (e.g., buffy coat viral load at/near P0) predicts subsequent peak viral load, time to clearance, and severity outcomes.

(vii) Using Nanopore / Illumina Andes virus (ANDV) sequencing:

a. Describe within-outbreak viral genetic diversity
b. Explore associations between viral variation and kinetics, shedding, and clinical outcomes (hypothesis-generating).

B. Humoral immunity

(i) Characterize seroconversion kinetics (time to IgM positivity; time to IgG positivity) and their temporal relationship to P0 and S0.

(ii) Quantify IgM and IgG titre trajectories over time and relate titre dynamics to viral load decline and clinical resolution.

(iii) Identify early humoral features associated with clinical outcome (e.g., earlier/later IgM/IgG appearance, steeper rise, higher early titres).

(iv) Characterize functional antibody responses (neutralisation potency, Fc-mediated effector functions) and relate functional profiles to viral kinetics, shedding, and severity (hypothesis-generating, biomarker-enabling).

(v) Determine the frequency and clinical significance of discordant virology/serology patterns (e.g., serology-positive with low/undetectable RNA, prolonged RNA positivity after seroconversion).

(vi) Evaluate durability and waning of humoral immunity signals, including predictors of durability.

C. Innate and adaptive cellular immunity

(i) Characterize innate and adaptive immune dynamics temporally aligned to P0 and S0 (e.g., cytokine patterns, cellular phenotypes, humoral responses).

(ii) Define early innate activation signatures (e.g., interferon-associated patterns, inflammatory cytokine trajectories) and evaluate their association with viral growth rate and symptom onset timing.

(iii) Characterize cellular immune trajectories (PBMC phenotyping where available) to identify patterns associated with (a) abortive/asymptomatic infection, (b) symptomatic disease without progression, and (c) progression to severe cardiopulmonary disease.

(iv) Identify immune "inflection points" preceding S0 (e.g., rapid immune activation immediately after P0) and quantify the lead time of immune changes relative to symptoms.

(v) Evaluate associations between immune dysregulation markers and clinical deterioration consistent with the protocol's mechanistic emphasis on endothelial–immune–platelet interactions and immunopathology.

(vi) (If transcriptomics feasible) Describe blood gene-expression signatures associated with early infection, symptom onset, and severity (hypothesis-generating; supportive of biomarker-driven development).

D. Endothelial, haemostatic, and capillary-leak biology (severity mechanisms)

(i) Quantify longitudinal changes in platelet and coagulation-related measures (as available clinically or via study sampling), and relate them to viral load, immune activation, and severity

(ii) Characterize early biomarkers linked to clinical outcomes (including progression to severe disease, requirement of organ support and death) including, but not necessarily limited to, ferritin, c-Reactive protein, LDH, IL-6, IL-1 and capillary leak/endothelial dysfunction (where feasible).

(iii) Define temporal relationships between thrombocytopenia (if observed), rising viral load, immune activation, and onset of hypoxaemia/hypotension signals captured via daily monitoring

(iv) Describe placental histopathology findings, where available and performed for a clinical reason.

E. Clinical natural history, phenotypes, and outcome prediction

(i) Relate early virologic/immunologic signals to clinical trajectory and severity (including hospitalization, organ support, and mortality where applicable).

(ii) Develop and internally validate risk stratification models for clinically relevant outcomes (e.g., hospitalization, ICU admission, organ support, mortality), using predictors available early (near P0 and early after S0): viral loads, serology timing, immune markers, and baseline clinical covariates.

(iii) Identify predictors of time to symptom onset among RT-qPCR-positive individuals (P0→S0) to improve early warning and clinical monitoring strategies.

(iv) Define and compare clinical phenotypes (e.g., predominantly febrile/GI prodrome vs early respiratory involvement; age group) and evaluate whether phenotypes map to distinct virologic/immune trajectories.

(v) Quantify the prognostic value of simple daily monitoring signals (temperature trends, SpO_2 trajectories, blood pressure trends, diuresis) for predicting deterioration, to support decision-relevant clinical monitoring in confined settings.

(vi) Evaluate the contribution of baseline factors (age, comorbidities, smoking, medications, pregnancy) to risk of infection (X0 →P0), risk of symptoms (P0→S0), and risk of severe outcomes (S0→outcome), consistent with the protocol's planned subgroup framework.

(vii) (While hospitalised) Describe trajectories of standardized inpatient severity measures captured clinically (e.g., WHO ordinal scale, SOFA and p-SOFA where available) and relate these to early biological predictors.

(viii) Relate potential investigational interventions performed by the study sites (not mandated in this protocol) to clinical outcomes and analytical (virological, immunological, biomarker) parameters.

F. Transmission-relevant endpoints and outbreak decision support

(i) Identify correlates of shedding intensity and duration in respiratory/saliva compartments and evaluate whether early clinical or immune features predict higher shedding, to inform infection control and public health strategies.

(ii) Define the timing of detectable shedding relative to symptoms to help refine the plausible window of infectiousness, supporting outbreak containment and contact tracing priorities.

(iii) Evaluate whether early biomarkers can support tiered monitoring intensity (e.g., identifying low-risk individuals for de-escalation and high-risk individuals for intensified clinical observation), aligned with the protocol's proportionality/feasibility principles.

(iv) Describe any differences in pregnancy as well as neonatal outcomes

G. Convalescence and longer-term outcomes

(i) Describe duration and pattern of convalescence and longer-term outcomes.

(ii) Quantify time to return to baseline functional status and persistence of symptoms (fatigue, dyspnoea, exercise limitation) and relate convalescent trajectories to acute-phase viral and immune kinetics.

(iii) In sites with expertise, describe the psycho-social impact of ANDV on mental health and well-being, experience of stigma, social reintegration

(iv) Assess whether acute-phase biological signatures predict prolonged recovery or persistent symptom burden at follow-up (hypothesis-generating; relevant for future intervention trials and modelling).

H. Host genetic determinants of infection susceptibility and disease outcomes (Substudy)

(i) Perform host genotyping (e.g., genome-wide SNP analysis or whole-genome sequencing on baseline Day 1 blood samples) to identify host genetic factors associated with susceptibility or resistance to Andes virus infection, such as differences between individuals who remain uninfected or asymptomatic after exposure and those who develop infection.

(ii) Identify host genetic polymorphisms associated with disease progression and severity (e.g., allelic variants linked to severe cardiopulmonary syndrome, need for intensive care, or mortality) to determine genetic contributors to adverse clinical outcomes in infected participants.

(iii) Evaluate associations between host genotype and key molecular or immunological observations (e.g., viral load kinetics, antibody responses, cytokine profiles) to elucidate how host genetic variation influences infection pathogenesis and immune response dynamics.

(iv) Identify host genetic signals with potential immediate or future therapeutic relevance (e.g., variants implicating drug-targetable pathways), thereby highlighting opportunities for translating genetic findings into improved preventive or therapeutic strategies.

3. Study Design

3.1 Overall Design and Setting

Longitudinal observational cohort study enrolling adults and children (all ages) with a well-defined exposure to Andes virus (ANDV). Whereas NAVIS is primarily designed to collect prospective information, given the nature of the current outbreak and the scarcity of knowledge on key biological, epidemiological and clinical ANDV determinants, subjects who initiated follow-up prior to study commencement are included, provided they fulfill all NAVIS inclusion criteria.

Participants are followed from enrolment (E0) through:

- first detectable ANDV infection by RT-qPCR (P0),
- first symptom onset (S0), and
- clinical outcome (death or clinical resolution)

Sampling intensity is explicitly matched to biologically meaningful inflection points while purposely keeping the study within a low-intensity framework. Environmental surveillance will be integrated as a parallel data stream in a specific annex and

will only be performed in sites where such analyses are available. Clinical care is not directed by the protocol. All clinical decisions remain under the responsibility of treating clinicians and local public health authorities.

3.2 Trigger-based, phase-adaptive framework

The study is operationalised as a trigger-based, phase-adaptive design, structured into three biologically defined tiers, with transitions anchored to objective triggers: RT-qPCR positivity (E0→P0) and symptom onset (P0→S0). This structure is intended to maximise temporal resolution during critical early transitions (exposure→viraemia, viraemia→symptoms), ensure precise temporal alignment of virologic, immunologic, and clinical measures to the same biological "time zero" markers (P0 and S0), and reduce misclassification and recall bias that can occur with symptom- or calendar-only alignment in infections with variable incubation. This structure concentrates sampling around biologically critical transitions (particularly around P0 and S0), while adaptively reducing intensity later to maintain feasibility and proportionality.

The tiered framework is summarised as:

- **Tier 1 (Exposure):** from X0/E0 to P0 (first RT-qPCR positive).
- **Tier 2 (Pre-symptomatic infection):** from P0 to S0 (first symptom onset).
- **Tier 3 (Symptomatic disease):** from S0 to clinical outcome (clinical resolution or death). Enable retrospective clinical characterization of symptomatic patients.

Participants can be included in any tier. Specific participant flow and tier transitions are described in Section 9 (Schedule of events) and Figure 1

4. Study Population

4.1 Population Overview

Consenting persons of any age who have a confirmed exposure to Andes virus and persons with confirmed Andes virus infection who are placed under confinement or quarantine or clinical care for active Andes virus infection. Participation in the NAVIS protocol does not restrict or prevent enrollment in other Hantavirus-related emergency responses or interventional clinical trials.

4.2 Inclusion Criteria

1. **No age restriction**.
2. **Persons diagnosed with or exposed to Andes Virus (ANDV).** Exposure must comply with the national definition of ANDV exposure in each country, or satisfy at least one of the following criteria:
   - **a. Direct physical exposure to a person with ANDV infection (Box 1)**
   - **b. Environmental and proximity exposure to a person with ANDV infection, i.e.:**
     - Prolonged presence in an enclosed or poorly ventilated shared airspace.
       Note: Prolonged exposure is defined as cumulative exposure of 15 minutes or more within a 24-hour period in a confined space, or shared occupancy of an enclosed environment for more than 2 hours (ECDC).
     - Co-habitation in the same household, room, or cabin (e.g., maritime or shared residential settings).
     - Documented proximity during long-haul travel exceeding **4 hours**.
       Note: Proximity is defined as sitting in an adjacent seat, defined as the same row or within two rows in front or behind. *Long haul travel* includes flight, bus, car or train. See Box 2 for justification of the 4 hour time threshold.
   - **c. Occupational or caregiving exposure**
     - Provision of direct healthcare or personal care to a person with ANDV infection without the consistent use of recommended Personal Protective Equipment (PPE).

- Direct handling of potentially contaminated fomites, such as soiled linens, clothing, or bedding used by a confirmed case.
- Direct handling of laboratory samples form a confirmed case with noncompliance with standard biosafety protocols.

3. Ability to **comply with confinement sampling and follow-up procedures**.
4. **Informed consent** by participant, parent/legal guardian, or surrogate where allowed and applicable; assent or consent for children aged <18 years or <16 years as per local regulations.

4.3 Exclusion Criteria

1. Current imprisonment (quarantine does not count).

Box 1: Definition of Exposure to ANDV (CDC, ECDC, UKSHA, and WHO)

Direct Physical Contact

- *Touch and Body Fluids:* Having direct physical contact with an infected person or their body fluids (e.g., saliva, respiratory secretions).
- *Intimate Acts:* Activities like kissing or sexual contact.
- *Sharing Items:* Sharing eating utensils, drinking glasses, or medical equipment.

Prolonged Shared Airspace

- *Enclosed Spaces:* Spending long periods in close proximity within an enclosed or poorly ventilated environment.
- *Living Together:* Household members or those sharing a room/cabin (as seen in the MV Hondius cruise ship outbreak) are at highest risk.
- *Long-haul Travel:* Sitting directly next to an infected person on a long-distance flight or bus journey.

Caregiving and Handling Materials

- *Healthcare/Caregiving:* Providing direct care to a symptomatic patient without appropriate PPE.
- *Contaminated Items:* Handling linens, clothing, or bedding used by an infected person.

Box 2. Justification for Inclusion Criterion: 4-8 Hour Proximity

The inclusion of individuals seated in close proximity to a confirmed case during long-haul travel exceeding 4-8 hours is justified based on the following epidemiological principles:

Established Public Health Standards: This threshold aligns with the World Health Organization (WHO) and European Centre for Disease Prevention and Control (ECDC) protocols for contact tracing in aviation and public transport for severe respiratory pathogens.

Aerosol and Droplet Dynamics: Research indicates that in environments with high occupancy and mechanical ventilation (such as aircraft cabins or long-distance buses), the risk of transmission for viral pathogens increases significantly after 8 hours of shared airspace, particularly when direct physical proximity (adjacent seating) facilitates the inhalation of concentrated respiratory droplets.

Precautionary Principle for ANDV: Given the high case-fatality rate and the documented potential for human-to-human transmission of the Andes Virus (ANDV), a conservative threshold is necessary to ensure the identification of individuals who may have been exposed during the symptomatic peak of the index case.

Ventilation Limitations: While modern transport systems use HEPA filters, they are most effective when the vehicle is in operation. The 8-hour criterion accounts for cumulative risk factors, including periods of boarding, deplaning, or potential ventilation disruptions where proximity to a shedding patient poses the highest risk. **This time threshold can be reduced to 4 hours if the vehicle has deficient ventilation of the contact was close and with a symptomatic case. Based on the aforementioned variability, the NAVIS study team has chosen 4 hours as a conservative time threshold.**

5. Study Outcomes

Because participants may range from asymptomatic to severe HCPS, outcomes include both outpatient and inpatient measures.

5.1 Primary Outcomes

The primary outcomes are designed to define the core natural history timeline from exposure (X0) through first virologic detection (P0) and subsequent infection dynamics.

5.1.1 Time to first virologic detection (X0→P0)

- Outcome: Time from enrolment (X0) to first detectable ANDV RNA (P0).
- Definition: P0 is the date/time of the first ANDV RT-qPCR positive result.
- Data source: Study RT-qPCR testing performed per Schedule of Events.

5.1.2 Blood viral kinetics (trajectory endpoints)

- Outcome: Viral load trajectories in blood, including peak, slope of increase/decrease, and time to clearance.
- Definition: Viral load is quantified by ANDV RT-qPCR in venous blood (EDTA → buffy coat preferred, ± plasma). Summary parameters (peak, slopes, clearance timing) will be derived from longitudinal measurements.
- Data source: Serial blood RT-qPCR measurements collected per Schedule of Events.
- Clearance operationalisation: The specific rule for "viral clearance" (e.g., sustained negativity requirement) will be prespecified in the Statistical Analysis Plan (SAP).

5.1.3 Post-symptom RT-qPCR persistence

- Outcome: Duration of RT-qPCR positivity after symptom resolution (where measured).
- Definition: Time from documented symptom resolution to the last observed RT-qPCR positive result (and/or time to clearance post-resolution), as prespecified in the SAP.
- Data source: RT-qPCR results linked to daily symptom monitoring and follow-up assessments.

5.1.4 Serologic conversion timing

- Outcome: Time to IgM positivity.
- Outcome: Time to IgG positivity.
- Definition: Time from P0 to first IgM positive and first IgG positive, respectively (additional trigger-aligned derivations may be analysed as secondary/exploratory; see below).
- Data source: Serology (IgM/IgG; serum) collected longitudinally per Schedule of Events.

5.2 Secondary Outcomes

5.2.1 Humoral immune kinetics

- Outcome: IgM and IgG titres over time.
- Definition: Longitudinal titre trajectories for IgM and IgG, including time-varying patterns across phases (Tier 1–3).
- Data source: Repeated serology (IgM/IgG; serum) per Schedule of Events.

5.2.2 Longitudinal immune marker trajectories

- Outcome: Longitudinal immune marker trajectories aligned to P0 and S0 (pre-specified panels).

- Definition: Time-series of immune markers (including cellular phenotypes and other immune measures in PBMC-based and related panels) analysed with alignment to P0 and/or S0 to capture early inflection points.
- Data source: Longitudinal immunological panel (PBMCs) collected per Schedule of Events (and other immune assays described in the protocol's biospecimen plan).

5.2.3 Symptom onset and symptom duration

- Outcome: Symptom onset date (S0).
- Outcome: Symptom duration.
- Definition: S0 is the first documented symptom onset during follow-up; symptom duration is the time from S0 to symptom resolution as captured in daily monitoring.
- Data source: Daily clinical monitoring (symptom diary + temperature + SpO_2 + blood pressure + diuresis).

5.2.4 Clinical severity and healthcare utilisation outcomes

- Outcome: Hospitalisation.
- Outcome: ICU/ NICU/ PICU admission.
- Outcome: Organ support (as applicable).
- Outcome: Mortality (as applicable).
- Definition: Occurrence (and timing, where available) of each event during study follow-up, as documented through clinical evaluation pathways and medical record abstraction when care is sought.
- Data source: Medical record abstraction for any hospitalisations, complemented by structured clinical assessments at protocol visits.
- Biomarker association with clinical symptoms in RT-qPCR positive participants

5.2.5 Standardised in-hospital severity metrics (if hospitalised; clinical data only)

- Outcome: WHO Ordinal Scale.
- Outcome: SOFA score (if clinically available) for adults and pSOFA score for children (https://pmc.ncbi.nlm.nih.gov/articles/PMC11162817/).
- Definition: Severity measures captured from routinely available clinical data only (no additional clinical testing mandated by the protocol).
- Data source: Hospital clinical records.

5.2.6. Host genetic correlates of infection and disease outcomes

- Outcome: Genetic associations with infection susceptibility (i.e., infected vs. uninfected among exposed participants), disease severity/progression (e.g., severe vs. mild disease outcomes), key clinical events (e.g., hospitalisation, ICU / PICU/ NICU admission, organ support, mortality) and molecular and immune markers (e.g., differences in viral load kinetics or immune response levels).
- Definition: Identification of associations between baseline host genotype (from Day 1 EDTA blood sample genotyping or whole genome sequencing) and each respective endpoint: (i) infection susceptibility (PCR-confirmed infection vs. no infection), (ii) disease severity or progression (clinical severity categories or progression metrics), (iii) occurrence of the specified clinical events during follow-up, and (iv) variation in relevant molecular or immunological measures (e.g., viral kinetics, serological or immune markers).
- Data source: Baseline DNA samples (Day 1 EDTA blood; host genotyping/WGS results) combined with study clinical data (exposure and infection status, severity outcomes, event occurrences) and laboratory data (virological and immunological results) corresponding to each endpoint.

5.3 Exploratory Outcomes

5.3.1 Multi-compartment detection and shedding dynamics

- Outcome: Time-to-first-detection in each compartment (blood/buffy coat, plasma if collected, nasopharyngeal swab, saliva, urine, feces) and lead/lag structure relative to P0.
- Outcome: Compartment-specific kinetic summaries (peak, time-to-peak, growth/decay rates, and AUC) per compartment.
- Outcome: Compartment-specific time to clearance and discordance patterns (persistence in non-blood compartments after blood clearance, or vice versa).
- Data source: RT-qPCR across blood and non-blood specimens as specified in the Schedule of Events and objectives.

5.3.2 Trigger-aligned serology endpoints (P0/S0 anchored)

- Outcome: P0→IgM+, P0→IgG+, S0→IgM+, and S0→IgG+ intervals (trigger-aligned seroconversion timing).
- Outcome: Early titre kinetic features (e.g., early rise patterns) as predictors of downstream clinical outcomes (hypothesis-generating).
- Data source: Serial IgM/IgG serology collected longitudinally.

5.3.3 Immune "inflection points" and trajectory phenotypes

- Outcome: Earliest detectable immune activation relative to P0 and relative to S0 ("immune inflection point timing"), and peak/resolution kinetics of immune markers.
- Outcome: Data-driven immune trajectory phenotypes (e.g., clustered longitudinal patterns) and their association with clinical outcomes.
- Data source: Longitudinal immune marker panels aligned to P0/S0.

5.3.4 Time-to-event progression endpoints

- Outcome: Time-to-event endpoints such as P0→hospitalisation/ICU and S0→hospitalisation/ICU/organ support/death (as data allow), for early risk modelling and natural history characterization.
- Data source: Clinical event dates from medical record abstraction and follow-up documentation.

5.3.5 Daily monitoring signal summaries

- Outcome: Descriptive trajectories and derived summaries of daily monitoring measures (temperature, SpO_2, blood pressure, diuresis) and their association with subsequent clinical outcomes (hypothesis-generating).
- Data source: Daily clinical monitoring recorded throughout follow-up.

5.3.6 Convalescence and longer-term outcomes

- Outcome: Convalescence pattern descriptors and longer-term outcomes through Month 6 (and beyond if extended), including persistence or resolution patterns as captured in follow-up.
- Data source: Longitudinal follow-up assessments.

6. Approach to Data and Biospecimen Collection

6.1 Overview

Data in NAVIS will be collected using a multi-source, longitudinal approach that integrates: (i) daily participant-reported measures during confinement/quarantine, (ii) direct measurements (e.g., temperature, SpO_2, blood pressure, diuresis), and (iii) medical record abstraction when participants seek or require clinical care. This approach is designed to support the protocol's trigger-based, phase-adaptive framework anchored to exposure/enrolment (X0/E0), first RT-qPCR positivity

(P0), and symptom onset (S0), enabling precise temporal alignment of clinical, virologic, and immunologic trajectories to biologically meaningful time origins. Clinical care is not directed by the protocol. All clinical decisions remain under the responsibility of treating clinicians and local public health authorities. Environmental surveillance will be integrated in a specific substudy in sites where it is feasible, as a systematic, contemporaneous data stream parallel to human biospecimen collection. Environmental specimens (air and surface samples) will be collected from quarantine isolation rooms and shared facility spaces occupied by RT-qPCR-positive participants using standardized protocols and processed alongside human specimens to enable direct temporal alignment and kinetic comparison.

6.2 Conceptual framework: trigger-based, phase-adaptive collection

NAVIS operationalizes biospecimen and data collection using a trigger-based, phase-adaptive design structured into three biologically defined phases ("tiers"), with transitions determined by objective triggers. Sampling intensity is concentrated around P0 and S0 to capture rapid changes in viral kinetics and immune dynamics and is adaptively reduced outside these windows to maintain feasibility and proportionality.

6.3 Frequency and timing

The following procedures will be implemented:

- Daily Clinical Monitoring: Subjects will maintain a daily symptom diary (Annex B) and undergo assessments for body temperature, oxygen saturation (SpO_2), blood pressure, and diuresis.
- Virological Surveillance: Serial RT-qPCR testing will be performed on venous blood (buffy coat preferred), nasopharyngeal swabs, saliva, urine, and feces. The frequency of these collections is determined by the specific study week and assigned tier.
- Immunological Profiling: Longitudinal serological testing (IgM/IgG) and PBMC-based immunological panels will be conducted at intervals specified for each tier and study phase.

To ensure operational feasibility and intra-study standardization, biospecimen collection should preferably adhere to a fixed weekday schedule: twice weekly (Mon/Thu), or once weekly (Mon), as dictated by the SoE.

6.4 Specimen handling, labeling, and linkage to data

Participants will be assigned a unique study ID, and specimens will be labeled with study ID and managed with chain-of-custody and cold-chain procedures, with direct identifiers stored separately from research datasets and access will be restricted to authorized personnel. High-level specimen handling guidance and biosafety statements are described as protocol appendices, including reference to biosafety handling requirements for relevant workstreams.

6.5 Data integrity, completeness, and proportionality

NAVIS is designed to maximize data integrity in a confined setting by combining (i) high adherence feasibility during confinement, (ii) biologically anchored time origins to minimize misclassification and recall bias, and (iii) adaptive reduction of sampling intensity when not scientifically necessary to reduce burden while preserving core inferential value. Data protection and confidentiality controls (pseudonymization, role-based access, encryption where feasible, audit trails, secure storage, and controlled sharing) are described under the protocol's Human Subjects section and apply to all data streams described in Section 6.

7. Data Collection

7.1 Baseline at enrolment

Subjects can be enrolled at any of the mutually exclusive Tiers defined in section 9 (Schedule of Events). Following screening and informed consent/assent, baseline data collection at enrolment will establish the participant's starting status for subsequent trigger-aligned follow-up. At enrollment, the study will capture: (i) an epidemiological assessment

focused on exposure context, and (ii) baseline demographics, comorbidities, and other relevant pre-existing clinical conditions including physical and psycho-social aspects, as specified in the Schedule of Events. Enrollment also initiates the study's daily clinical monitoring stream and aligns baseline measurements with downstream biological triggers used throughout the protocol's trigger-based, phase-adaptive framework.

Passengers from the MV Hondius completed exposure questionnaires administered by RIVM/ ECDC on disembarkation from the ship. These exposure data will be shared by RIVM/ECDC with NAVIS and may be the preferred exposure data to be used for NAVIS analyses as they are less susceptible to recall bias. Equally any data collected by NAVIS which might fill any gaps in the RIVM/ECDC dataset on exposures will be shared by NAVIS with RIVM/ECDC. Passengers will be asked to consent to this data sharing as part of enrollment, and data will only be shared for those consenting to this data reuse.

7.2 Daily data collection during confinement/quarantine

During confinement/quarantine, NAVIS will implement daily clinical monitoring, consisting of a symptom diary (Annex B) coupled with direct measurements of temperature, SpO_2, blood pressure, and diuresis, captured daily as specified in the Schedule of Events. This daily stream is maintained across tiers/phases, including the exposure phase (X0/E0–P0), the pre-symptomatic infection phase (P0–S0), and the symptomatic disease phase (S0–outcome), ensuring consistent temporal coverage and comparability across biologic stages. Daily monitoring outputs are intended to support descriptive characterization of trajectories and derived summaries (e.g., temperature and SpO_2 trajectories) and to enable linkage of clinical signals to virologic and immunologic measures collected under the Schedule of Events. For children below 16 years of age, the symptom diary will be completed by the child together with the parent. Participants aged 16 or above will complete the diary on their own.

7.3 Data collection at protocol-defined visits after trigger events (P0 and/or S0)

NAVIS uses objective triggers to structure follow-up phases and concentrate measurement density around biologically informative transitions. Accordingly, follow-up data collection after P0 and/or S0 will incorporate: (a) continued daily clinical monitoring (symptom diary + temperature + SpO_2 + blood pressure + diuresis), maintained through the protocol-defined follow-up windows, and (b) capture of clinically relevant outcomes (e.g., hospitalization, ICU/ PICU/ NICU admission, organ support, mortality where applicable) through a combination of structured follow-up processes and medical record abstraction when care is sought.

7.4 Medical record abstraction

NAVIS is observational and does not direct clinical care. Therefore, when participants seek or require clinical evaluation/hospitalization, medical record abstraction will be used to capture clinically documented events and routinely collected data relevant to study outcomes. This abstraction is the primary mechanism for documenting severity and healthcare utilization endpoints (including hospitalization and ICU/ PICU/ NICU admission, where applicable) and for capturing timing of clinical outcomes used in analyses.

7.5 Data elements and minimization principle

NAVIS follows a data minimization and purpose limitation approach, collecting data necessary to meet stated objectives. The protocol explicitly anticipates collection of exposure history, demographics/comorbidities, daily symptom monitoring, serial virology/serology/immune assay outputs, and medical record abstraction for hospitalizations (where applicable). These data elements are therefore limited to those needed to define the natural history timeline and support mechanistic and prediction analyses aligned with protocol objectives.

7.6 Identifiers, confidentiality, and linkage to biospecimens

All participants will be assigned a unique study ID. Direct identifiers will be stored separately from research datasets (linkage file), with access restricted to authorized personnel. Specimens are labeled with the study ID, and chain-of-

custody/cold-chain procedures apply as described in protocol appendices. These identifiers allow linkage of daily monitoring and clinical outcomes to laboratory outputs without embedding direct identifiers in analytic datasets. Data protection measures include role-based access control, encryption where feasible, audit trails, secure storage of source documents (if any), and secure specimen transport with coded identifiers only.

7.7 Operational documentation

Operationally, sites should ensure that all data sources (daily monitoring records, protocol visit assessments where applicable, and medical record abstraction outputs) are recorded in a format that supports auditability and traceability to the study ID, consistent with the confidentiality and security controls described in the protocol. Where local implementation requires it, sites may use site-approved case report forms and/or secure study databases, provided that the minimal dataset specified by the protocol is preserved and data handling remains consistent with the protocol's pseudonymization and access-control requirements.

8. Biospecimen Collection

8.1 Scientific justification for specimen types and timing

Biospecimen selection and collection timing are structured to optimise early detection of Andes virus infection and to characterise the biological processes that underpin progression to severe disease. Viral loads are higher in buffy coat than in plasma and RT-qPCR can detect ANDV RNA up to ~2 weeks before symptom onset and before antibody detection, supporting prioritisation of EDTA whole blood processed to cellular fractions for early molecular diagnosis and viral-kinetic profiling. Serum is included to define infection stage and immune kinetics, as IgM is often detectable at the onset of the febrile prodrome and IgG is usually present by the end of the febrile prodrome, enabling precise seroconversion mapping relative to molecular positivity. Serial immune and haemostatic measurements are justified by the same mechanistic framework emphasising endothelial targeting and increased vascular permeability as central features of hantavirus pathogenesis, with downstream platelet/coagulation perturbations in relevant syndromic contexts. Accordingly, NAVIS anchors sampling intensity to biologically meaningful transitions—exposure (X0), first RT-qPCR positivity (P0), and symptom onset (S0)—and concentrates sampling around P0/S0 to capture rapid changes that sparse, calendar-only sampling is likely to miss

8.2 Biospecimen types

Biospecimen types include:

- Blood (venous): EDTA whole blood processed to buffy coat and plasma, Serum and PBMCs; microsampling where available, and
- Non-invasive / minimally invasive specimens: Nasopharyngeal swab and/or saliva for RT-qPCR, Urine for RT-qPCR , Feces for RT-qPCR, Semen for RT-qPCR (optional), milk and vaginal swab for pregnant women where planned

8.3 Blood volume and frequency safeguards The protocol adopts an explicit ethical principle of collecting no more blood than is necessary to achieve the scientific objectives, while accounting for clinically indicated blood draws when participants undergo medical evaluation or hospital care. Consistent with this approach, sampling intensity is structured within a tiered framework to ensure proportionality of risk to anticipated knowledge gain.
Blood volumes taken from children should not exceed maximum allowable weight-based limits (Annex F).

9. Schedule of Events

9.1 Rationale

The NAVIS Schedule of Events (SoE) is a trigger-based, phase-adaptive sampling strategy designed to capture the full virological and immunological trajectory of ANDV infection, from exposure through pre-symptomatic viraemia to clinical disease, while maximizing temporal resolution during biologically critical transitions and maintaining operational feasibility. The design is structured into three biologically defined tiers: **exposure** (X0/E0–P0), **pre-symptomatic infection** (P0–S0), and **symptomatic disease** (S0–outcome), with transitions anchored to objective triggers (RT-qPCR positivity and symptom onset). This ensures precise temporal alignment of virological, immunological, and clinical data, minimising misclassification and recall bias. Sampling intensity is concentrated during critical early phases, where viral replication, immune activation, and progression risk are highest, enabling (a) high-resolution characterisation of viral kinetics and shedding across compartments, (b) precise timing of seroconversion and immune responses, and (c) identification of early biomarkers of severity and transmission potential

A multi-compartment approach (blood, respiratory, saliva, urine, feces, semen) ensures comprehensive assessment of systemic infection and clinically relevant shedding, directly informing diagnostics, infection control, and public health strategies. The adaptive reduction of sampling intensity in later phases and in PCR-negative participants ensures proportionality, feasibility, and minimisation of participant burden, in line with regulatory expectations.

Overall, this design delivers fit-for-purpose, decision-relevant evidence to: (a) optimise clinical management and risk stratification, (b) inform public health interventions (isolation, contact tracing), (c) enable biomarker-driven therapeutic development and (d) support health technology assessment modelling and future intervention trials. The SoE is therefore methodologically robust, operationally feasible, and directly aligned with clinical, regulatory, and health system decision-making needs.

9.2 Participant flow and tier transitions

Study enrollment is open to participants in any of the three mutually exclusive Tiers: Exposure, Presymptomatic Infection, or Symptomatic Disease.

Participants who enter **Tier 1 (Exposure: X0/E0/Q0–P0)** are followed until one of two mutually exclusive outcomes occurs:

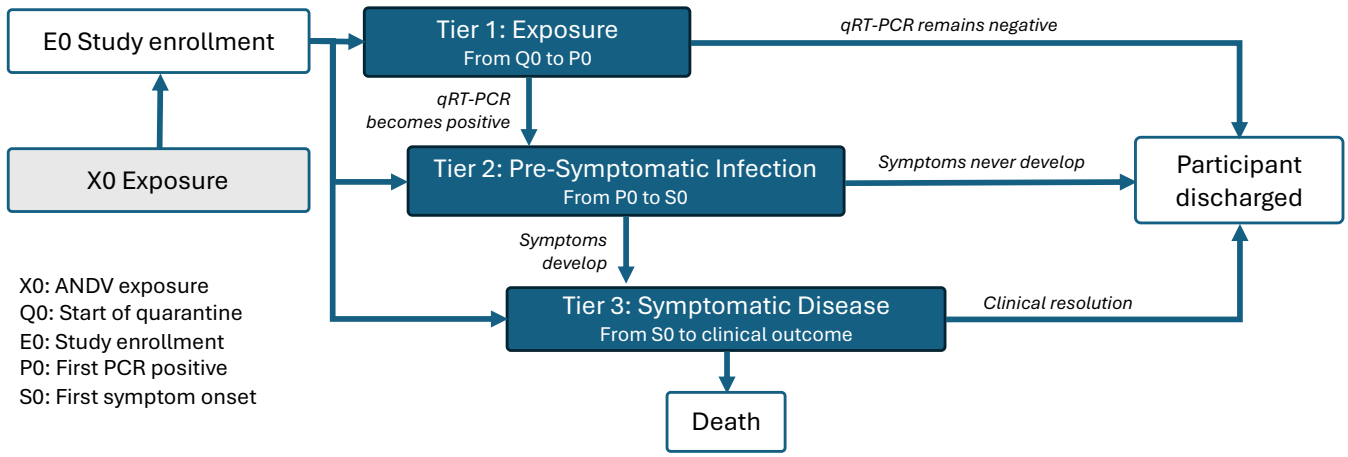
- RT-qPCR becomes positive (P0): the participant transitions to Tier 2 at the date/time of the first positive RT-qPCR result (P0).
- RT-qPCR remains negative through the end of Week 6 from start of quarantine (Q0): the participant exits study follow-up and is discharged (per protocol operational pathway).

Participants who enter **Tier 2 (Pre-symptomatic Infection: P0–S0)** are then followed until one of two outcomes occurs:

- Symptoms develop (S0): the participant transitions to Tier 3 at the first documented symptom onset (S0).
- Symptoms do not develop through the end of Week 6 from P0: the participant exits study follow-up and is discharged.

Participants in **Tier 3 (Symptomatic Disease: S0–outcome)** are followed through clinical resolution or death, with continued phase-adaptive sampling through Week 6 of the symptomatic phase as specified in the Schedule of Events.

Figure 1. Overview of schedule of events



9.3 Schedule of Events Tables

Tier 1. Exposure

From E0 (enrollment) to P0 (first PCR positive)

Test / Assessment	Enrollment (E0)	Time since start of quarantine					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Informed consent /Assent	X						
Verbal consent^		Each visit	Each visit	Each visit	Each visit	Each visit	Each visit
Epidemiological assessment Exposure, contact tracing	X						
Clinical assessments							
Demographics, comorbidities and other relevant pre-existing clinical conditions	X						
Self-reported daily clinical monitoring (symptom diary[$] + temperature + SpO_2 (where available)	X	Daily	Daily	Daily	Daily	Daily	Daily
Mental health (e.g. PHQ2 and GAD2[~])	X		1×/week		1×/week		1×/week
Blood Hematology, Coagulation and Biochemistry	X	1×/week	1×/week	1×/week	1×/week	1×/week	1×/week
Virological assessments							
ANDV RT-qPCR in venous blood (EDTA → buffy coat preferred, ± plasma)	X	1×/week	2×/week	2×/week	2×/week	2×/week	1×/week
ANDV RT-qPCR in nasopharingeal swab	X	1×/week	2×/week	2×/week	2×/week	2×/week	1×/week
ANDV RT-qPCR in saliva*	X	1×/week	2×/week	2×/week	2×/week	2×/week	1×/week
ANDV RT-qPCR in urine	X	1×/week	2×/week	2×/week	2×/week	2×/week	1×/week
ANDV RT-qPCR in feces	X	1×/week	2×/week	2×/week	2×/week	2×/week	1×/week
Optional: RT-qPCR in semen**	X	none	none	X	none	none	X
Immunological assessments							
Serology (IgM/IgG; serum)	X	1×/week	2×/week	2×/week	2×/week	2×/week	1×/week
Longitudinal immunological panel (Peripheral Blood Mononuclear Cells, PBMCs)	X	1×/week	1×/week	1×/week	1×/week	1×/week	1×/week
Host genetics testing (Substudy)							
4mL EDTA tube	X	none	none	none	none	none	none

*Note: For feasibility, sample shipment and follow-up clarity: 2x / week sampling may be performed on Mondays and Thursdays; 1x / week sampling may be performed on Mondays. * Consider obtaining gingival crevicular fluid as well. **Adult biological males only. [$] Symptom diary to be completed by participants themselves if aged ≥16 years, and by participants with their parent if participant <16 years. ^Parent consent for participants aged <16 years or <18 years (depending on local regulations/ IRB approval). ~ PHQ2 - Patient Health Questionnaire-2. GAD2 – Generalized Anxiety Disorder 2-item. Both for age ≥12 years only.*

Two possible outcomes:

- ANDV RT-qPCR becomes positive: The study participant enters Tier 2
- ANDV RT-qPCR remains negative through end of week 6: The study participant is discharged home

Tier 2: Presymptomatic Infection

From P0 (first PCR positive) to S0 (first symptom onset)

Test / Assessment	Enrollment (P0)	Time since first PCR positive (P0)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Informed consent/Assent [a]	X						
Verbal consent^		Each visit	Each visit	Each visit	Each visit	Each visit	Each visit
Epidemiological assessment [a] Exposure, contact tracing	X						
Clinical assessments							
Self-reported daily clinical monitoring (symptom diary[$] + temperature + SpO_2 (where available)	X	Daily	Daily	Daily	Daily	Daily	Daily
Mental health (e.g. PHQ2 and GAD2~	X		1×/week		1×/week		1×/week
Blood Hematology, Coagulation and Biochemistry	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
Virological assessments							
ANDV RT-qPCR in venous blood (EDTA → buffy coat preferred, ± plasma)	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
ANDV RT-qPCR in nasopharingeal swab	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
ANDV RT-qPCR in saliva*	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
ANDV RT-qPCR in urine	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
ANDV RT-qPCR in feces	X	1×/week	2×/week	2×/week	2×/week	2×/week	1×/week
Optional: RT-qPCR in semen**	X	none	none	X	none	none	X
Immunological assessments							
Serology (IgM/IgG; serum)	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
Longitudinal immunological panel (Peripheral Blood Mononuclear Cells, PBMCs)	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
Host genetics testing (Substudy)							
4mL EDTA tube	X	none	none	none	none	none	none

[a] *If not provided before, i.e. the study participant is first enrolled in this phase (Tier 2)*

*Note: For feasibility, sample shipment and follow-up clarity: 2x / week sampling may be performed on Mondays and Thursdays; 1x / week sampling may be performed on Mondays. * Consider obtaining gingival crevicular fluid as well. **Adult biological males only. [$] Symptom diary to be completed by participants themselves if aged ≥16 years, and by participants with their parent if participant <16 years. ^Parent consent for participants aged <16 years or <18 years (depending on local regulations/ IRB approval). ~ PHQ2 - Patient Health Questionnaire-2. GAD2 – Generalized Anxiety Disorder 2-item. Both for age ≥12 years only.*

Two possible outcomes:

- Symptoms (S0) develop: The study participant enters Tier 3
- Symptoms do not through end of week 6 from P0: The study participant is discharged home

Tier 3: Symptomatic Disease

From S0 (initial symptom onset) to clinical outcome

Test / Assessment	Enrollment (S0)	Time since first Symptoms (S0)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Informed consent/Assent [a]	X						
Verbal consent^		Each visit	Each visit	Each visit	Each visit	Each visit	Each visit
Epidemiological assessment [a] Exposure, contact tracing	X						
Clinical assessments							
Self-reported daily clinical monitoring (symptom diary[$] + temperature + SpO_2 (where available)	X	Daily	Daily	Daily	Daily	Daily	Daily
Mental health (e.g. PHQ2 and GAD2~	X		1×/week		1×/week		1×/week
Blood Hematology, Coagulation and Biochemistry	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
Virological assessments							
ANDV RT-qPCR in venous blood (EDTA → buffy coat preferred, ± plasma)	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
ANDV RT-qPCR in nasopharingeal swab	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
ANDV RT-qPCR in saliva*	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
ANDV RT-qPCR in urine	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
ANDV RT-qPCR in feces	X	1×/week	2×/week	2×/week	2×/week	2×/week	1×/week
Optional: RT-qPCR in semen**	X	none	none	X	none	none	X
Immunological assessments							
Serology (IgM/IgG; serum)	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
Longitudinal immunological panel (Peripheral Blood Mononuclear Cells, PBMCs)	X	2×/week	2×/week	2×/week	2×/week	1×/week	1×/week
Host genetics testing (Substudy)							
4mL EDTA tube	X	none	none	none	none	none	none

[a] *If not provided before, i.e. the study participant is first enrolled in this phase (Tier 3)*

*Note: For feasibility, sample shipment and follow-up clarity: 2x / week sampling may be performed on Mondays and Thursdays; 1x / week sampling may be performed on Mondays. * Consider obtaining gingival crevicular fluid as well. **Adult biological males only.* [$] *Symptom diary to be completed by participants themselves if aged ≥16 years, and by participants with their parent if participant <16 years. ^Parent consent for participants aged <16 years or <18 years (depending on local regulations/ IRB approval). ~ PHQ2 - Patient Health Questionnaire-2. GAD2 – Generalized Anxiety Disorder 2-item. Both for age ≥12 years only.*

10. Statistics

10.1 Purpose and analysis objectives

This section gives an overview of the types of statistical analyses planned. A separate Statistical Analysis Plan (SAP) will be developed and finalised prior to the lock of the database. The purpose of the SAP is to predefine analysis populations, estimands, and statistical methods to address the primary and secondary objectives of NAVIS, including (i) the natural-history timeline from exposure (X0)) to virologic detection (P0), symptom onset (S0), and clinical outcomes, and (ii) stage-aligned virologic and immunologic kinetics and their association with downstream severity. The SAP will be finalized prior to the lock of primary endpoints and will provide a technical description of all analyses summarized in the protocol.

10.2 Study overview relevant to analysis

NAVIS is an open-ended observational cohort. Its final sample size depends on the exposure event size and conversion rate (numbers of RT-qPCR positive (P0) and symptomatic (S0) cases may be low; the SAP will describe implications for precision and interpretation, particularly for exploratory analyses). Sampling is tiered and phase-adaptive, with predefined weekly sampling rules for operational feasibility. Participants are followed prospectively from enrolment, with retrospective collection of clinical and biospecimen data recorded prior to enrolment where available.

10.3 Timepoint definitions

The working operational definitions for X0, P0 and S0 are provided below. Exact definitions, and relevant sensitivity analyses will be finalised in the SAP in consultation with relevant stakeholders

- **Exposure (X0)**. Defined as the first known exposure date. Sensitivity analysis will explore alternative definitions, as a single date may not be available but rather a range of dates.
- **Virologic detection (P0).** Defined as date of first RT qPCR positive result. Alternative definitions may include date of true biological onset of viraemia assumed to occur between the last RT qPCR negative and first RT qPCR positive result.
- **Symptom onset (S0).** Defined as date of symptom onset. A list of symptoms and clinical indicators, rules for handling non-specific symptoms or incomplete symptom recording with be described in the SAP.

10.4 Analysis populations (sets)

All analysis populations are subsets of enrolled participants with informed consent and at least baseline assessment at study enrollment (E0).

- **Full Analysis Set (FAS**). All enrolled participants with any (retrospective or prospective) data (clinical monitoring and/or any biospecimen result) (SAP-defined operational rule; aligns with open-ended cohort design).
- **Virology Analysis Set.** All participants with ≥1 valid ANDV RT-qPCR result in any compartment during follow-up(SAP-defined).
- **Immunology Analysis Set.** All participants with ≥1 valid serology (IgM/IgG) result and/or ≥1 valid immune marker result (e.g., PBMC panel). (SAP-defined; serology and PBMC are specified assessments)
- **Symptomatic Disease Set.** Participants who develop symptoms (S0) during follow-up (used for S0-anchored estimands) (SAP-defined; outcomes include S0 and symptom duration.)
- **Hospitalized/Severe Disease Set.** Participants with hospitalization and/or ICU admission and/or organ support and/or mortality outcomes (used for severity estimands).
- **PCR-negative follow-up set (Tier 1 completers).** Participants who remain RT qPCR negative through end of follow-up (per protocol flow) will contribute to censoring for time-to-event endpoints (SAP-defined; censoring rule specified below.)

The SAP will further define how each analysis population contributes to specific estimands, including handling of retrospectively reconstructed data and minimum data requirements for inclusion.

10.5 General statistical principles

- **Descriptive summaries.** Baseline characteristics and follow-up data will be summarized using descriptive statistics (counts/percentages for categorical variables; appropriate summaries for continuous variables), overall and by relevant strata (e.g., PCR status; symptomatic status; hospitalization; age group).
- **Time origins ("anchors").** Analyses will use biologically meaningful time origins consistent with the protocol: X0 (Exposure) for infection acquisition/detection endpoints, P0 (first RT qPCR positive) for within-infection kinetics and pre-symptomatic progression endpoints, and S0 (symptom onset) for clinical progression/severity endpoints.
- **Significance level and inference.** NAVIS is an observational study primarily focused on estimation and prediction. The SAP will predefine which analyses are descriptive / estimation-focused versus inferential and hypothesis-generating.
- **Multiplicity.** Given high-dimensional immune marker panels, multiplicity control (e.g., false discovery rate) will be prespecified for families of related biomarkers.

10.6 Estimands

The tables below define, for each endpoint, the target population, endpoint variable, time origin, intercurrent events/censoring, and the population-level summary measure (estimand). Where operational rules are not yet specified in NAVIS (e.g., "clearance"), the SAP will prespecify them.

10.5.1 Primary estimands (Primary endpoints)

ID	Estimand (endpoint)	Target population	Time origin	Variable / endpoint definition	Intercurrent events / censoring	Summary measure
P1	Time to first virologic detection (X0→P0)	FAS with RT-qPCR follow-up	X0	Time from X0 to first RT qPCR positive result (P0).	Censor at last negative test date for those without P0 by end of follow-up.	Kaplan–Meier median/time quantiles; hazard ratio(s) from Cox model or appropriate alternatives for interval-censored data, as appropriate and prespecified in the SAP
P2	Blood viral load kinetics (buffy coat primary; plasma supportive) including peak, slope, time-to-clearance	Participants with ≥1 blood RT-qPCR value	P0	Longitudinal blood viral load values over time; derived peak, slopes, and clearance time.	Missing measures handled per longitudinal model assumptions; clearance rule prespecified.	Model-based mean trajectories; derived kinetic summaries with CIs.
P3	Time to IgM positivity	Participants with serial serology	P0	Time from XP0to first IgM positive result.	Censor at last serology date if never positive.	Kaplan–Meier summaries; Cox models or appropriate alternatives for interval-censored data, as appropriate and prespecified in the SAP.
P4	Time to IgG positivity	Participants with serial serology	P0	Time from P0 to first IgG positive result.	Censor at last serology date if never positive.	Kaplan–Meier summaries; Cox models as appropriate or appropriate alternatives for interval-censored data, as appropriate and prespecified in the SAP.
P5	Duration of RT-qPCR positivity after symptom resolution (where measured)	Symptomatic Disease Set with post-resolution testing	Symptom resolution date (derived)	Time from symptom resolution to clearance (rule prespecified).	Competing risk of death handled if applicable.	Time-to-event summaries; cumulative incidence if competing risks apply.

10.6.2 Secondary estimands (key secondary endpoints)

ID	Estimand (endpoint)	Target population	Time origin	Variable / endpoint definition	Intercurrent events / censoring	Summary measure
S1	Time to symptom onset (X0/S0)	FAS	X0and P0	Time from X0/ to first documented symptom onset (S0).	Censor at last symptom diary date if never symptomatic. *(SAP-defined.)*	Kaplan–Meier; Cox models.
S2	Symptom duration	Symptomatic Disease Set	S0	Time from S0 to symptom resolution (definition prespecified).	Competing risk of death if applicable.	Time-to-event; cumulative incidence if competing risks apply.
S3	IgM/IgG titre trajectories over time	Immunology Analysis Set	P0	Longitudinal titres across scheduled draws.	Missing handled in longitudinal models.	Mixed-effects model summaries (means/medians by time).
S4	Immune marker trajectories aligned to P0 and S0	Immunology Analysis Set	P0 and S0	Longitudinal immune markers (pre-specified panels) aligned to P0/S0.	Missing handled in longitudinal models.	Mixed effects / GEE summaries of trajectories.
S5	Hospitalization (and timing)	FAS / Symptomatic Disease Set	S0 (primary), P0 supportive	Time to hospitalization and/or binary occurrence.	Censor at end of follow-up.	Cox/log-rank; risk models as prespecified.
S6	ICU admission (and timing)	FAS / Symptomatic Disease Set	S0 (primary), P0 supportive	Time to ICU admission and/or binary occurrence.	Censor at end of follow-up.	Cox/log-rank; risk models as prespecified.
S7	Organ support (and timing)	FAS / Symptomatic Disease Set	S0 (primary)	Time to first organ support and/or binary occurrence.	Censor at end of follow-up.	Cox/log-rank; risk models as prespecified.
S8	Mortality (and timing)	FAS / Symptomatic Disease Set	X0, P0, or S0 (endpoint-specific)	Time to death.	N/A (event).	Kaplan–Meier/Cox.
S9	WHO Ordinal Scale (if hospitalized; clinical data only)	Hospitalized/Severe Disease Set	Hospital admission or S0	Ordinal severity score where available.	N/A	Descriptive and model-based ordinal methods as prespecified (e.g., proportional odds), if data allow.
S10	SOFA score or pSOFA (if hospitalized; clinical data only)	Hospitalized/Severe Disease Set	Hospital admission or S0	Continuous severity score where available.	N/A	Descriptive; longitudinal/peak summaries as prespecified.

10.6.3 Exploratory estimands (mechanistic, transmission-relevant, prediction, and convalescence)

ID	Estimand (endpoint)	Target population	Time origin	Variable / endpoint definition	Summary measure / model
E1	Compartment-specific time to first detection (blood/buffy coat, plasma, NP, saliva, urine, feces)	Virology Analysis Set	X0	Time from X0/E0 to first positive per compartment	Kaplan–Meier/Cox; within-person comparisons
E2	Compartment-specific viral kinetics (peak, time-to-peak, slopes, AUC)	Virology Analysis Set	P0 (preferred)	Derived kinetic parameters per compartment	Mixed-effects trajectory models; derived summaries
E3	Compartment-specific clearance and discordance	Virology Analysis Set	P0 a	Time to clearance per compartment + discordance metrics	Time-to-event; concordance statistics (

E4	Trigger-aligned seroconversion intervals (P0→IgM+, P0→IgG+, S0→IgM+, S0→IgG+)	Immunology Analysis Set	P0 and S0	Time intervals from triggers to seropositivity	Kaplan–Meier/Cox (interval-censoring handled if needed)
E5	Early viral load / immune markers → severity association	Participants with early measures	P0 and/or S0	Association of early measures with hospitalization/ICU/organ support/death	Multivariable Cox/logistic models; prespecified covariates
E6	Convalescence and longer-term outcomes (to Month 6, extendable)	Follow-up set	Clinical resolution or S0	Persistence/resolution patterns through Month 6	Descriptive + longitudinal models as data allow

10.7. Statistical methods (by outcome class)

- **Time-to-event outcomes.** Time-to-event outcomes in NAVIS include X0→P0, P0→S0, and S0→hospitalization/ICU (if applicable). Where the outcome is assumed to occur on an exact date (e.g. date of first RT-qPCR+ or S0) cumulative incidence will be estimated with Kaplan–Meier methods and group comparisons performed with log-rank tests; hazard ratios will be estimated using Cox proportional hazards models adjusted/stratified by relevant characteristics. Alternative approaches, such as flexible parametric survival models or time-varying effects models, will be considered where there is evidence of violation of proportional hazards assumptions. Where the outcome is assumed to occur in an interval (e.g. between a previous RT-qPCR- and first RT-qPCR+ result) interval censoring approaches will be used. Cumulative incidence will be estimated using the Turnbull estimator, and regression models may include parametric or semi-parametric interval-censored survival models, as appropriate.The SAP will predefine a parsimonious set of baseline covariates and risk factors (e.g., age, comorbidities, smoking) for adjusted analyses, consistent with the protocol's plan for subgroup analyses where data allow.
- **Competing risk methods (when applicable).** For time-to-event outcomes where death is a competing risk (e.g., time to sustained recovery or time to symptom resolution if death occurs), competing-risk methods will be used, including Gray's test, Fine–Gray models, and Aalen–Johansen estimators, as appropriate.
- **Longitudinal outcomes (viral load, serology titres, immune markers).** Longitudinal trajectories will be analyzed using mixed-effects models for viral load and immune marker kinetics over time, baseline-adjusted. More generally, longitudinal mixed-effects models or generalized estimating equations may be used to summarize differences in disease trajectories across groups, with baseline levels as covariates.
- **Scale/transformations:** The SAP will prespecify transformations for quantitative assays (e.g., log scale) and handling of values below detection/quantification limits.
- **Ordinal outcomes (hospital severity).** If WHO ordinal scale is available, ordinal outcome methods may be applied (e.g., proportional odds models) with adjustment for relevant confounders, consistent with the STRIVE statistical framework for ordinal endpoints. SOFA (if available) or pSOFA (if available) will be summarized descriptively and may be modeled longitudinally depending on data density.
- **Association and prediction analyses (exploratory but prespecified).** The SAP will prespecify association analyses between early viral load (buffy coat) and downstream severity, as stated in the protocol. For extended objectives related to prediction, the SAP will define: (a) candidate predictors (early viral kinetics parameters; early immune-marker summaries; baseline risk factors), (b) prediction targets (hospitalization, ICU/PICU/NICU, organ support, death; or composite severity), (c) internal validation strategy (e.g., bootstrap or cross-validation), and (d) a minimum confounder set which will be adjusted for where data allow.
- **Subgroup analyses.** Subgroup analyses (e.g., age, comorbidities, smoking, pregnancy) will be conducted where data allow. The SAP will prespecify subgroup definitions, minimum data requirements, and interpretation constraints (exploratory unless otherwise stated).

10.8. Handling missing data and data conventions

Missing data will be described and categorised by cause (e.g. deterioration, death, logistical constraints, invalid samples, refusal). The SAP will specify endpoint specific missing data handling rules including for time-to-event endpoints (censoring

rules, interval censoring where relevant), longitudinal endpoints (assumptions under mixed-effects/GEE; sensitivity analyses), and derived endpoints (e.g., clearance requiring consecutive negatives). The SAP will also specify approaches to handling, and any assumptions made about missing data due to delayed entry into the study.

10.9 Sensitivity analyses

Sensitivity analyses will be prespecified to assess robustness to: (a) alternative clearance definitions (e.g., 1 vs 2 consecutive negatives), (b) alternative time origins (X0 vs P0 vs S0) for selected endpoints, (c) alternative time origin definitions (e.g. last known exposure date, or midpoint of exposure window for X0; biological onset of viraemia for P0), (d) alternative model specifications (e.g., non-linear trajectories vs linear), and (e) missing data mechanisms.

10.10 Quality control and programming plans

QC and programming plans will be documented, including reproducible generation of tables/figures and validation steps, consistent with standard SAP structure.

10.11 Risk of bias

Given the observational design, potential biases include selection bias due to tiered enrolment and delayed entry, leading to incomplete observation of early infection. Differences between prospectively collected and retrospectively reconstructed data may introduce bias, particularly in symptom reporting and data completeness. Informative missingness may occur if participation or sampling varies by disease progression or study tier. Measurement error and misclassification may occur in exposure timing and key event definitions (e.g. X0, P0, S0). The inclusion of multiple exploratory outcomes increases the likelihood of false positive findings. In exploratory analyses, confounding may arise; while adjustment will be made where feasible, this may be limited by sample size and unmeasured confounders.

Analytical approaches described above, and specified in detail in the SAP, are intended to mitigate these risks where possible. Remaining sources of bias will be described and considered when interpreting study findings and exploratory nature of results emphasised.

Box 3. Summary of the statistical testing strategy

Natural history timeline: time-to-event (Kaplan–Meier/Cox).

Viral kinetics and immune kinetics: longitudinal mixed-effects models (baseline-adjusted).

Early predictors of severity: association models; early viral load–severity explicitly planned.

Compartment kinetics and discordance: compartment-specific time-to-event + longitudinal models.

11. Human Subjects

11.1. Risks and Benefits

11.1.1 Overview of anticipated risks

Participation in NAVIS entails procedural and informational risks typical of observational studies with serial biospecimen collection and daily monitoring, alongside context-specific considerations related to recruitment in a confinement/quarantine environment. Risks are mitigated through: (i) a tiered system that escalates sampling intensity only when scientifically justified, (ii) explicit blood volume and frequency safeguards, and (iii) clear separation of clinical/public health decision-making from research participation.

11.1.2 Procedural risks

- **Venipuncture and blood sampling.** NAVIS includes serial blood collection for EDTA whole blood (processed to buffy coat and plasma), serum, and PBMCs. Blood collection for children will not exceed the maximum allowable limits (Annex F).

- **Non-invasive sampling (respiratory/saliva/urine/feces).** NAVIS includes non-invasive or minimally invasive specimens such as nasopharyngeal swabs, saliva, urine and feces for RT-qPCR (and, where planned, other assays). Nasopharyngeal sampling risks may include transient discomfort, gagging/coughing, watery eyes, and (rarely) minor epistaxis. Saliva collection is generally low risk (minor discomfort; potential embarrassment). Urine and feces collection are low risk but may involve privacy concerns and inconvenience.
- **Daily monitoring and clinical measurements.** NAVIS includes daily symptom diary plus direct measurement/recording of temperature, SpO_2, blood pressure, and diuresis during confinement, with trigger flags for clinical worsening (e.g., new dyspnea, SpO_2 decline, hypotension symptoms) leading to immediate clinical evaluation per local policy (clinical care, not mandated by the study). Risks are limited but include inconvenience, burden, and potential anxiety related to symptom tracking.

11.1.3 Psychological and situational risks due to confinement/quarantine context

Because participants are recruited and followed during confinement/quarantine after a shipboard outbreak, additional risks may include: (a) **Stress/anxiety** related to possible infection, repeated testing, and uncertainty regarding incubation and outcomes. (b) **Perceived pressure to participate** due to the confined environment or perceived authority of response teams (risk of undue influence). (c) **Stigma** if infection status is inadvertently disclosed. NAVIS addresses these risks by emphasizing that participation is voluntary, that refusal/withdrawal will not affect clinical care or public health management, and by implementing robust privacy protections (see 11.3) and consent safeguards (see 11.2). The protocol also explicitly separates clinical care decisions from protocol-driven activities. However, each clinical site will be encouraged to have clear referral pathways for people who would need additional psychological support.

11.1.4 Informational risks (privacy, confidentiality, re-identification)

Data collected include exposure history, demographics/comorbidities, daily symptom monitoring, serial virology/serology, immune assays, and medical record abstraction for hospitalizations (where applicable). These may carry risks of confidentiality breach, potential social harms (e.g., stigma), and potential implications for employability/insurance in some jurisdictions if improperly disclosed. Mitigations are detailed in Section 11.3.

11.1.5 Risk minimization and safety oversight (procedural + biosafety)

NAVIS incorporates multiple design features that reduce risk while preserving scientific value, including trigger-based, phase-adaptive sampling concentrates procedures around P0 and S0, while adaptively reducing intensity in later phases and in participants who remain RT-qPCR negative through Week 6, supporting proportionality and reduced burden.

Biosafety: the protocol appendices include a biosafety statement referencing BSL-3 handling requirements for relevant workstreams, and specimen handling guidance including labeling with study ID and chain-of-custody/cold chain processes.

11.1.6 Potential benefits

Direct clinical benefit to participants is not guaranteed, as NAVIS is observational and clinical management is not directed by the protocol. However, potential participant-level benefits may include:

- Earlier identification of infection through serial RT-qPCR testing and structured monitoring, which may support timely clinical evaluation according to local policy (while remaining outside protocol-directed care).
- Societal/public health benefits are expected to be substantial: NAVIS aims to define the natural history timeline from exposure to RT-qPCR positivity, symptom onset, seroconversion, and clinical outcomes, and to quantify compartment-specific kinetics (buffy coat vs plasma, and non-blood specimens). Outputs are intended to inform outbreak containment, diagnostics, infection control, and future countermeasure trial design (especially for early-phase interventions).

11.2. Informed Consent

11.2.1 General principles

Informed consent will be obtained from each participant/parent/legal guardian prior to performance of any study-specific procedures, and verbal consent will be obtained at each subsequent visit or procedure, to ensure that the participant has the ability to withdraw consent at any time. The protocol includes informed consent at enrolment (E0) and permits surrogate consent where allowed and applicable. Consent will be conducted in a manner that ensures comprehension, voluntariness, and protection from undue influence, with additional safeguards appropriate to the confinement/quarantine context. In addition, children and youth with the maturity to understand what is required to participate will also be asked to assent to the study. Children under 6 years of age may be provided with the adapted ISARIC cartoon information sheet (Annex A) if the clinician deems this appropriate. If a child reaches the legal age of consent during the study, the appropriate consent form should be completed.

11.2.2 Consent process in a confinement/quarantine setting

Because participants may be confined or quarantined, consent may need to be operationalized with flexible modalities, such as: (a) In-person consent with infection prevention measures consistent with local policy (when permissible), or (b) Remote consent (e.g., telephone/video) with documented identity verification and provision of the consent form in advance, when in-person contact is restricted. Regardless of modality, the consent discussion will:

- Explain the observational nature of NAVIS and that clinical care is independent of research participation.
- Describe all expected procedures: daily symptom/vitals monitoring and the schedule of biospecimen collection including tier escalation logic around P0/S0.
- Explain foreseeable risks (Section 11.1) and privacy protections (Section 11.3).
- Clarify voluntariness and right to withdraw without penalty.

It is expected that in most cases, eligible children aged <18 years (or <16 years) will be confined or quarantined with a parent. Where this is not the case, the parent should be informed of the study and give consent.

11.2.3 Avoidance of undue influence and separation from public health decisions

NAVIS is conducted in a setting where public health authorities and WHO coordination are active in outbreak response. To prevent undue influence:

- Consent will explicitly state that participation (or refusal) will not affect quarantine management, disembarkation / travel decisions, access to medical care, or any entitlements/services provided during outbreak response.
- Recruitment staff will be trained to present the study neutrally and to avoid any implication that participation is expected.
- Where feasible, consent will be obtained by personnel not directly responsible for enforcement of confinement / quarantine measures.

11.2.4 Participant comprehension and language access

Consent/Assent materials will be provided in a language understandable to the participant. When needed, qualified interpreters will be used. The discussion will include time for questions, with documentation that key elements (procedures, risks, voluntariness, confidentiality) were understood.

11.2.5 Capacity, surrogate consent, and re-consent

For participants who are acutely ill, capacity to consent will be assessed according to local regulations. If a participant lacks capacity and local regulations permit, consent may be obtained from a legally authorized representative. If the participant later regains capacity, re-consent will be sought at the earliest reasonable opportunity.

11.2.6 Withdrawal and discontinuation

Participants may withdraw at any time without penalty. On withdrawal, the protocol should allow participants to specify whether: (a) No further data/specimens will be collected, and (b) Previously collected data/specimens may still be used in de-identified / pseudonymized form, as permitted by local regulation and as described in the consent form. Operationally, given NAVIS's trigger-based design, participants who remain RT-qPCR negative through Week 6 exit follow-up per protocol flow, and participants who do not develop symptoms through Week 6 from P0 may also exit follow-up and be discharged home.

11.3. Confidentiality and Data Protection

11.3.1 Data minimization and purpose limitation

NAVIS collects data necessary to meet stated objectives, including baseline demographics / exposure history / comorbidities, daily monitoring measures, serial virology/serology/immune markers, and medical record abstraction for hospitalizations where applicable. Data elements are therefore limited to those needed for (i) defining the natural history timeline and (ii) supporting mechanistic and prediction analyses consistent with protocol objectives.

11.3.2 Pseudonymization and identifiers management

All participants will be assigned a unique study ID. Specimens will be labeled with study ID and handled with chain-of-custody and cold chain procedures as described in the protocol appendices. Direct identifiers will be stored separately from research datasets (linkage file), with access restricted to authorized personnel only.

11.3.3 Data security controls (technical and organizational)

Data protection measures will include, at minimum:

- Role-based access control to study databases and shared repositories.
- Encryption for data at rest and in transit where feasible.
- Audit trails for data access and modification.
- Secure storage of paper source documents (if any) in locked facilities with controlled access.
- Secure specimen transport with labeling limited to coded identifiers (study ID) and documented chain-of-custody.

11.3.4 Cross-border data sharing and sponsor/coordination context

Given WHO coordination and the multi-country nature of outbreak containment, NAVIS may require external data hosting or transfer of coded data/specimens across sites and potentially across national borders. Any transfers will be governed by appropriate data sharing agreements and compliant with applicable local data protection laws. Only the minimum necessary information will be shared, and wherever possible, shared datasets will be coded/pseudonymized.

11.3.5 Protection of sensitive information (exposure/contact tracing context)

Exposure history and contact tracing-related epidemiological assessments are included at enrolment. These data may be particularly sensitive in a setting where ANDV person-to-person transmission is possible and public health measures are active. Accordingly: (a) Research use of exposure/contact data will be limited to protocol objectives, and (b) Any reporting will be aggregated and de-identified to prevent re-identification of index cases or contact networks.

11.3.6 Confidentiality in publications and reporting

Results will be presented in aggregate form. Any case-level vignettes (if ever used) will be sufficiently masked to prevent identification, particularly given the uniqueness of a shipboard outbreak setting.

11.3.7 Data retention and specimen storage/use

Retention duration, secondary use, and data and specimen destruction policies will be specified in site-specific documentation and aligned with consent language, local requirements, and sponsor policies.

11.4. IRB/EC Review

11.4.1 Ethics review requirements and approvals

Prior to initiation at any site, NAVIS will obtain approval from the responsible Institutional Review Board/Ethics Committee (IRB/EC) and, where required, additional institutional permissions. NAVIS is observational, but includes serial biospecimen collection and daily monitoring, requiring formal ethics review.

11.4.2 Amendments and protocol versioning

Any changes affecting participant risk, sampling frequency/volume, specimen types, data sharing, or consent procedures will be submitted as amendments for IRB/EC approval prior to implementation, except where immediate changes are necessary to eliminate apparent immediate hazards to participants (handled per local regulations).

11.4.3 Local regulatory alignment and registration

Site submissions should document the applicable local requirements for observational study registration and ensure compliance accordingly.

11.5. Ethical Conduct

11.5.1 Ethical principles governing the study

NAVIS will be conducted in accordance with recognized ethical principles for research involving human participants, including respect for persons, beneficence, and justice. These principles are operationalized through the study's proportionality-driven design, tiered safeguards, and emphasis on voluntariness in a constrained environment.

11.5.2 Respect for persons (autonomy, voluntariness, dignity)

Voluntary participation will be emphasized, with clear communication that refusal or withdrawal will not affect clinical care or public health management. Consent processes will be adapted to confinement/quarantine logistics while preserving comprehension and freedom from undue influence (Section 11.2). Participants' dignity will be respected during specimen collection and daily monitoring, with privacy-preserving procedures.

11.5.3 Beneficence (risk–benefit proportionality)

Beneficence is supported by: (a) Minimizing unnecessary procedures via trigger-based sampling anchored to E0, P0, and S0, with adaptive reduction in intensity when not scientifically necessary. (b) Ensuring clinical care remains independent of study procedures, avoiding therapeutic misconception.

11.5.4 Justice (fair selection and equitable treatment)

Eligibility is based on defined ANDV exposure and ability to comply with follow-up procedures, with inclusion of adults and children (all ages) and exclusion of those for whom additional sampling would be unsafe by investigator judgment.

Recruitment will be conducted fairly within the confined cohort without discrimination, and study procedures will be applied consistently.

11.5.5 Safety monitoring, reporting, and clinical escalation pathways

NAVIS is observational and does not direct clinical management. As in other observational trials, adverse events are not systematically collected, however, procedure-related events are to be reported per local IRB requirements. Daily monitoring includes trigger flags for clinical worsening that prompt immediate clinical evaluation per local policy.

12. Adverse Events and Safety Monitoring

12.1 Overview and guiding principles

NAVIS is an observational cohort study in which clinical management remains independent of the protocol. Consistent with the protocol's observational intent, adverse events (AEs) are not systematically collected as trial safety outcomes. However, procedure-related events associated with serial specimen collection and study measurements will be documented and reported per local IRB/EC requirements. Safety oversight is therefore focused on: (i) minimizing procedural risk through tiered, trigger-based sampling and blood volume/frequency safeguards, (ii) rapid clinical escalation when daily monitoring indicates potential deterioration, (iii) timely documentation and reporting of procedure-related AEs/serious events per applicable regulations and site policy, and (iv) identifying any acute or concerning mental health needs of participants (either through normal interactions or the mental health questions in NAVIS) and escalating them as appropriate.

12.2 Scope of safety data: what is and is not captured

12.2.1 Events captured as clinical outcomes (not "study AEs")

Because NAVIS does not assign investigational treatment, the principal disease-related events (e.g., hospitalization, ICU admission, organ support, mortality) are captured as clinical outcomes and health-care utilization endpoints through structured follow-up and medical record abstraction, not as treatment-emergent AEs.

12.2.2 Events captured as procedure-related AEs (safety monitoring focus)

The following will be captured as procedure-related AEs when temporally associated with study procedures and plausibly attributable to them:

- Venipuncture/blood draw events: pain, bruising, hematoma, bleeding, vasovagal reaction/syncope, local infection/phlebitis, nerve irritation (rare).
- Nasopharyngeal sampling events: transient discomfort, cough/gagging, watery eyes, minor epistaxis (rare).
- Other specimen collection/measurements: minor discomfort or anxiety related to repeated sampling or daily monitoring activities.

12.2.3 Events not routinely collected

General intercurrent medical events that are not procedure-related (e.g., unrelated infections, injuries) are not systematically collected as AEs under this observational protocol, unless required by local IRB/EC conditions or the event occurs in the context of a study procedure (e.g., syncope during blood draw).

12.3 Definitions for harmonized documentation

Where sites are required (by local policy/IRB/EC) to document AEs, the following standard definitions apply:

- **Adverse Event (AE):** Any untoward medical occurrence in a participant temporally associated with a study procedure, whether or not considered related to that procedure.
- **Procedure-related AE:** An AE for which a reasonable possibility exists that the event was caused by a study-mandated procedure (e.g., venipuncture, nasopharyngeal swab).
- **Serious Adverse Event (SAE):** An event that results in death, is life-threatening, requires inpatient hospitalization or prolongation of hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.
- **Adverse Event of Special Interest (AESI) – procedure-related:** A procedure-related event that merits expedited review due to clinical importance (e.g., significant bleeding requiring medical intervention after venipuncture, severe vasovagal syncope with injury, clinically significant epistaxis requiring intervention, confirmed local infection requiring antibiotics.
- **Relatedness:** The likelihood that the event is attributable to study procedures (e.g., unrelated, unlikely, possible, probable, definite), per site standard.
- **Severity (intensity):** Mild / moderate / severe intensity (clinical intensity), distinct from seriousness (SAE criteria).

Note: Disease progression events (e.g., hospitalization for suspected or confirmed HCPS) are recorded as clinical outcomes per protocol endpoints and are not presumed "procedure-related."

12.4 Safety monitoring processes and responsibilities

12.4.1 Oversight structure

Safety oversight will be implemented in a proportionate manner appropriate for an observational study, under the leadership of the site Principal Investigator and Sponsor/Coordinating Institution processes. At minimum, each site will designate a responsible clinician (or delegate) to: (a) review procedure-related AEs and ensure appropriate clinical follow-up, (b) ensure reporting to the local IRB/EC per requirements, and (c) confirm that protocol procedures remain appropriate for each participant over time (including temporary holds when clinically indicated).

12.4.2 Training and standardization

Study staff performing procedures will be trained and competency-assessed for: (a) venipuncture and specimen handling consistent with site SOPs, (b) nasopharyngeal swab collection consistent with site SOPs, (c) infection prevention measures appropriate to the outbreak context, and (d) documentation of procedure-related AEs and escalation steps.

12.5 Participant-level safety safeguards: risk minimization

12.5.1 Tiered, trigger-based sampling to reduce unnecessary procedures

The protocol's tiered and trigger-based design concentrates higher-intensity procedures around biologically meaningful inflection points (e.g., P0 and S0), while reducing intensity in later phases and in participants who remain RT-qPCR negative through follow-up, thereby minimizing participant burden and procedural exposure.

12.5.2 Blood volume and frequency safeguards

The protocol adopts an ethical principle to collect no more blood than necessary and incorporates tier-specific safeguards regarding venipuncture frequency and proportionality, including use of microsampling approaches (e.g., DBS) where appropriate to limit venous draws. Operational safeguards (implemented per site feasibility and IRB/EC stipulations) should include: (a) Cumulative research blood volume tracking for each participant across the follow-up period, (b) Pre-procedure clinical check for symptoms or signs that increase venipuncture risk (e.g., dizziness, symptomatic anemia, bleeding tendency by history), (c) Temporary hold criteria for additional research blood draws when clinically indicated

(e.g., clinician concern, symptomatic anemia, repeated vasovagal syncope), (d) Adaptions to the sampling frequency, type and volumes for children, including consideration of microsampling approaches.

12.5.3 Procedure technique safeguards

- **Venipuncture:** standard aseptic technique, minimize attempts, employ age-appropriate pain reduction strategies in infants/children, apply adequate pressure post-draw, document complications.
- **Nasopharyngeal swab:** trained collector, avoid in participants with clear contraindications per clinical judgment (e.g., recent nasal trauma/surgery), document epistaxis and manage per local care pathway.

12.6 Clinical monitoring and escalation pathways (safety-by-design)

NAVIS includes daily clinical monitoring during confinement (symptom diary plus temperature, SpO_2, blood pressure, and diuresis) with trigger flags for clinical worsening leading to immediate clinical evaluation per local policy. To preserve the separation of research from care, escalation operates as follows: Trigger identified (by participant report or measured parameter) → Immediate notification to designated clinical team per site pathway → Clinical evaluation (outside protocol-directed care) → Continuation, modification, or temporary hold of study procedures as determined by clinician judgment and participant preference. Examples of escalation triggers may include new or worsening dyspnea, declining oxygen saturation, symptoms suggestive of hypotension, or other signs that warrant urgent assessment. Sites should operationalize threshold values and escalation workflows in a site-specific appendix/SOP aligned with local clinical practice and outbreak response requirements.

12.7 Documentation, attribution, and reporting

12.7.1 Documentation requirements

For sites required by local policies to track procedure-related AEs, documentation should include:

- event term/description, onset date/time and resolution date/time
- severity (mild/moderate/severe), seriousness (SAE criteria met or not)
- assessment of relatedness to a study procedure
- action taken (e.g., none, procedure hold, medical evaluation, treatment)
- outcome (resolved, resolving, ongoing, sequelae), and
- whether the event was reported to IRB/EC and/or Sponsor per coordinating institution requirements.

12.7.2 Reporting timelines (site- and jurisdiction-dependent)

Because NAVIS is multi-country and implemented under local IRB/EC authority, reporting must follow local regulatory and IRB/EC requirements. A harmonized approach (where acceptable) is recommended:

- Procedure-related SAEs (including death temporally associated with a study procedure, life-threatening reactions, hospitalization resulting from a procedure complication): report to the IRB/EC and Sponsor/Coordinating Institution as expedited reports per local timelines.
- Procedure-related non-serious AEs: summarize and report per IRB/EC continuing review requirements (or as otherwise stipulated).
- Protocol deviations impacting safety (e.g., exceeding approved blood volumes/frequency): report per IRB/EC deviation policy and implement corrective actions.

12.7.3 Pregnancy

This observational study does not assign investigational products. If pregnancy is identified at recruitment or during follow-up, the event should be handled clinically per local standard of care. Continuation in study procedures (particularly blood draws) should be guided by clinician judgment, participant preference, and local IRB/EC guidance.

12.8 Safety review, trends, and corrective actions

12.8.1 Periodic safety review

Site teams should perform periodic review of procedure-related AEs to identify patterns (e.g., repeated vasovagal episodes, higher-than-expected epistaxis rates) and implement risk mitigation, including retraining, procedure modifications, or tightening hold criteria.

12.8.2 Corrective and preventive actions (CAPA)

If safety issues are detected (e.g., cluster of procedure-related complications), the site PI will implement CAPA steps such as: (a) retraining and competency reassessment, (b) modification of sampling approach (e.g., greater use of DBS/microsampling, fewer venous draws where scientifically acceptable), (c) enhanced pre-procedure screening (e.g., hydration, posture, anxiety mitigation), (d) temporary suspension of specific procedures pending IRB/EC review if warranted.

13. Data sharing and publications

After the study has ended and its results have been reported, anonymized, deidentified data sharing will occur as per the Policy Statement on Data Sharing by the World Health Organization*. The final data sets will be available to the protocol group and principal investigators (as listed in the protocol) on the dedicated site (ISARIC) during and after the study results are published. There will be group authorship recognizing the contribution of all national and local investigators and guided by the International Committee of Medical Journal Editors (ICMJE) criteria and recommendations. A writing committee will consist of the protocol group as listed in the protocol – publication will include all international and national collaborators whose team, in the view of the national principal investigator, contributed substantially towards the trial, in line with ICMJE policy. The results of the study will be presented at conferences held in each country and each of the partner countries, as well as at international conferences. All principal investigators and teams will be kept informed about timelines to report and publish data – there will be no publication or dissemination without permission from the protocol team. With the exception of sharing with public health authorities, agreement not to make public or otherwise disseminate any results of the study until these have been formally published, with group authorship recognizing the contribution of all national and local investigators, unless permission is received from the protocol group ahead of time.

**World Health Organization. New WHO policy requires sharing of all research data. 16 September 2022. https://www.who.int/news/item/16-09-2022-new-who-policy-requires-sharing-of-all-research-data.*
* http://www.icmje.org/#author

13. Annexes

Annex A: Participant Information and Consent Forms

*The forms below are only provided as **examples** to the NAVIS Network. Each country must adapt them to their own regulations*

Simplified Consent Form
(Adult/Parents/Legal guardians)
Study: Natural History of Andes Virus Infection (NAVIS)

Multicounty Collaborative Outbreak Observational Cohort Study Facilitated by WHO

What is this study?

We are studying how Andes virus behaves in people who were exposed to the virus. This will help us understand the virus and improve care for future patients.

Why am I being asked?

You may have been exposed to the virus, or you have already tested positive for Andes virus infection. We want to follow your health during your quarantine/treatment period.

What will happen?

- Daily self-reported symptom monitoring and health checks during the 6-week study period
- Nose/throat swabs or saliva samples to test for virus: once or twice per week
- Blood samples: twice per week for 6 weeks (about 1-2 teaspoons each time), or longer if virus is still detectable in your blood
- Optional semen donation (adult men only) to learn if the virus is present in semen
- Follow-up to see how you recover over the 6-week study period

What are the risks?

- Blood draws may cause brief pain, bruising, or lightheadedness
- Nose swabs may cause brief discomfort
- No other significant risks expected

What are the benefits?

- No direct benefit to you
- Information may help future outbreak response and patient care

Is this voluntary?

- Yes, completely voluntary
- You can stop participating anytime
- Your medical care and quarantine requirements won't change whether you participate or not

Privacy?

Your samples and information will be coded (no names used) and shared only with public health agencies and collaborating researchers for this outbreak investigation.

Questions?

Contact: [Study doctor name and phone number]

Your agreement:

☐ I understand this is a research study about Andes virus

☐ I understand the procedures, risks, and that participation is voluntary

☐ I agree to participate

Signature: _____ **Date:** _____

Print name: _____
(Give one signed copy of this form to the participant (or their representative) and keep one for study records.)

Participant Information and Informed Consent Form

(Adults)

Study Title: *Natural History of Andes Virus Infection (NAVIS)*
Sponsor/Coordinator: World Health Organization (WHO) – Outbreak Response under International Health Regulations (IHR)
Protocol Number: NAVIS 1.0. (Version 1.0, Date 18 May 2026)

Introduction and Invitation:
You are being invited to participate in a research study about **Andes virus (ANDV)** infection following potential exposure. This form explains the study and what your participation involves. **Please read it carefully and ask any questions** you may have. Taking part is **voluntary**. If you decide not to participate or to withdraw later, there will be **no penalty or loss of medical care or other benefits** you are entitled to.

Why is this study being done?
The purpose of the **NAVIS** study is to **better understand the infection and illness caused by Andes virus**. Andes virus can cause a serious disease called **hantavirus cardiopulmonary syndrome (HCPS)**. Unlike most hantaviruses, Andes virus may **spread person-to-person** through close contact. This study aims to **track the natural course of infection** from the moment of exposure to when the virus is detected and when symptoms begin, and through recovery. By collecting medical data and biological samples over time, researchers hope to learn **how the virus behaves in the body**, how the immune system responds, **when a person becomes infectious**, **how your genetics (DNA) affect disease**, and what factors might predict severe illness. This knowledge can help improve **outbreak control, patient care, and future treatments or vaccines**. The study is observational, meaning **no experimental treatment is given**; your usual medical care will **not** be altered by the study. WHO is coordinating this study globally as part of the outbreak response under the International Health Regulations.

Why am I being asked to participate?
You are **eligible for this study because** you have had a defined **exposure to Andes virus** (for example, as a passenger or crew member during a shipboard outbreak). This means you might have been in contact with someone who was infected or with the environment where the virus was present. Because of this exposure, you are currently under **public health confinement/quarantine** to monitor your health. The study invites individuals like you to join so we can closely observe what happens after exposure to the virus. **Your participation can help answer key questions** about early infection and immunity in this outbreak setting.

Do I have to take part?
No. Participation is entirely voluntary. If you choose not to take part, it will **not affect your routine medical care, quarantine conditions, or any public health measures** you are subject to. You can also **change your mind and withdraw** from the study at any time, even after signing this form, without giving a reason. There will be **no negative consequences** for you if you decide not to participate or to withdraw. If you withdraw, we will stop collecting any new information or samples from you. You can also request that your unused samples be destroyed. (However, data or samples already collected up to the point of withdrawal may still be used to maintain the integrity of the study results.)

What will happen if I participate?
If you agree to participate, you will be asked to **sign this consent form**. You will then undergo the following **study procedures**:

- **Initial Assessment (Enrollment):** A study doctor or nurse will ask about your **medical history** and any current symptoms. They will perform a brief physical check (for example, measuring your temperature, blood pressure, oxygen level, etc.). We will collect a **baseline set of samples** from you at this time, including a blood sample and possibly a nose/throat swab, saliva, urine, and feces. We will also record some information from your **medical records** if relevant (for example, any test results or treatments you have received as part of routine care). If you were a passenger on the ship, we will also ask RIVM (a public health agency) to share the data they collected from you when you disembarked, about your exposure to Andes on the ship.
- **Daily Monitoring During Quarantine:** While you are in isolation/quarantine (the period after exposure during which you are being observed by public health authorities), the study team will **monitor your health daily**. This will include asking you about any symptoms you might have and measuring basic signs like **body temperature and oxygen saturation** (using a finger-clip device) each day. In many cases, you may be asked to **self-record** these daily measurements with guidance. We will also collect a small specimen once or twice a week to check for the virus – typically a **nasal swab or saliva sample** – to detect the virus as early as possible. Daily blood collection is **not** planned; blood draws will be less frequent and tied to specific triggers as explained below.
- **Trigger-Based Additional Sampling:** This study uses a **special design** where extra sample collections are done around key "triggers":
    - If/when your **virus test first becomes positive** (this is called **P0**, indicating the first detection of virus by PCR), we will initiate a series of additional sample collections around that time. This means, upon the first positive test, you will have **more frequent blood draws and possibly other specimens (like saliva (and gingival crevicular fluid sometimes), urine, feces, and another swab)** in the days immediately following, to capture the early changes in the virus levels and immune response. We expect to take small blood samples perhaps **daily or every other day for a few days** around this event.
    - Similarly, if/when you **develop your first symptoms** of Andes virus infection (e.g., fever, aches, etc., termed **S0**), we will again perform **intensified sampling** around the onset of illness. This could include daily blood samples and other specimens for a short period during early illness.
    - Outside of those trigger periods (before any virus is detected and after acute symptoms have passed), **sampling will be less frequent** – likely a routine schedule such as **once every few days or weekly**, as per the study plan. The schedule is designed to **focus on critical periods** (when most information can be gained) while avoiding unnecessary procedures when they are less informative.
- **Biospecimen Types:** The types of **samples** we will collect from you include:
    - **Blood samples:** Taken by needle from a vein in your arm. We will be careful to collect only the needed volume. *At enrollment*, we may draw around X ml (approximately Y teaspoons) of blood. During trigger periods, we might draw about X ml per day for a few days. In total, the amount will be within safe limits and comparable to a few standard blood tests or less.

  - **Respiratory samples:** Such as **nasal or throat swabs** (using a swab similar to a cotton bud inserted in the nose/throat for a few seconds) or **saliva samples** (you may be asked to spit into a sterile container). These help detect the virus and are generally done daily or as needed.
  - **Urine and Feces samples:** You may occasionally be asked to provide a urine and a feces sample, which will be collected in sterile cups, to check for any virus shedding.
  - **Breastmilk and vaginal swab:** pregnant women and breastfeeding women may be asked to provide a vaginal swab and breastmilk to check for any virus shedding
  - (No surgical procedures or invasive biopsies are involved in this study.)
- **Clinical Data Collection:** The researchers will **record relevant information** about your health status and clinical care. This includes data like test results (e.g., routine blood counts, chest X-ray findings) and medical treatments if you receive any (for example, if you are hospitalized or receive oxygen). They will obtain this from your **hospital or clinic records** and through daily check-ins. The study team will **not interfere with your medical care**; all treatment decisions remain with your doctors. The study just records what happens as part of observing the illness.
- **Duration of Participation:** You will be in the study from the time you sign consent until a defined end point, which is likely **up to a few weeks or months** after exposure, depending on your health. Specifically, we plan to follow you through the acute phase and into **recovery** (convalescence). After you have recovered or after a certain period (for example, a follow-up at a few weeks or months post-illness to see longer-term outcomes), your active participation will end. **No additional procedures** beyond standard medical care will occur after that point.

What will happen to my samples?

Your blood and other samples will be **tested for Andes virus and your body's immune responses** (like antibodies and other immune cells or proteins). We may perform advanced laboratory analyses, such as **genetic sequencing of the virus** (to see if the virus has any mutations) and **laboratory studies on your immune cells or blood components**. In some cases, researchers might attempt to **isolate (grow) the virus** from your samples in a high-security laboratory to study it—this will not affect you, but it is done to learn more about the virus's characteristics. Any isolated virus or genetic data will be handled with strict safety and confidentiality standards.

In addition to studying the virus, if you specifically agree to it in the consent form, then **this research will also look at your genetic material (DNA)**. We will perform genetic analyses (such as genetic marker testing or whole genome sequencing) to see if certain genetic differences might reveal how some people's immune systems respond differently to the virus. The purpose of these genetic studies is purely scientific – to better understand Andes virus disease and possibly help discover new treatments in the future. Previously, genetic research like this led directly to a new treatment for Covid-19.

Use and storage of data and samples: Samples will be processed and possibly **shared with specialized laboratories** collaborating with the WHO for testing. **Any sample or data shared outside the local study site will be identified only by a unique code, and will *not* include your name or personal identifiers.** The results from testing your samples will be used for **research purposes** as outlined by this study's goals. After completing the planned analyses for this study, any leftover samples may be **securely stored for a longer period** (for example, in a biorepository) for **future research related to Andes virus or similar emerging infections**. Any such future use of your samples or data will be subject to appropriate **ethical review and approvals** to ensure it aligns with the original purpose of this study and protects your rights. If you **do not**

want your leftover samples to be stored for future related research, you can inform the study staff and that request will be respected (no further use of your samples beyond this study).

What are the risks or discomforts?
Participating in this study may involve some **risks or inconveniences**:

- **Risks of blood draws:** Drawing blood can cause mild pain, bruising, bleeding, lightheadedness, or, rarely, infection at the needle site. Trained professionals will minimize these risks by using sterile techniques and taking only the necessary amount of blood. If you feel faint or uncomfortable during a blood draw, you should inform the staff immediately and they will take appropriate measures (such as having you lie down).
- **Risks of nose/throat swabs:** Having a nasal or throat swab can cause brief discomfort or a gagging sensation, and occasionally a small nosebleed or irritation. This should be temporary.
- **Daily monitoring and procedures:** Being asked about symptoms and providing daily samples (like saliva or swabs) might become tiresome or inconvenient over time. We will try to make this as easy as possible, working with your schedule and minimizing disruptions.
- **Psychological discomfort:** Some people might feel anxious or stressed about their health status, frequent monitoring, or receiving test results (for example, learning that they have become infected with the virus). The study team will be available to discuss any concerns, and remember that **participating in the study does not change the medical care you receive** if you fall ill.
- **Privacy and confidentiality risks:** We will collect personal and health information about you. There is a very small risk of **loss of confidentiality** (i.e., your personal data or health information being seen by someone not authorized). We have strict safeguards in place to prevent this (see the section on confidentiality below).
- **Incidental findings:** This study is focused on Andes virus infection. It is not designed to diagnose or treat other conditions. It is unlikely, but possible, that tests on your samples might reveal **unexpected medical information** (for example, a previously unknown health issue). If you choose to participate in the genetic part of this study, this would include any findings from you DNA that we think will directly affect your medical care in future. If any **actionable incidental finding** relevant to your health is discovered, the study team will discuss it with you and arrange appropriate medical follow-up. You may choose **not to be informed** of certain types of research findings, unless they are important for your immediate health – in which case we would inform you so you can seek care. Also, it could be possible that tests run by the local hospital may reveal pregnancy.

We do not anticipate other significant risks. Not participating in the study or withdrawing will not remove any **public health obligations** (for example, if you are required to remain in quarantine by public health authorities, you must still do so even if you are not in the study). But joining this study will **not extend your quarantine** or impose extra restrictions beyond standard public health requirements; it only adds research procedures as described.

If any **new information about risks** or anything learned during the study might affect your willingness to continue, we will promptly inform you.

What are the benefits of participating?
You may not receive any direct personal benefit from participating in this research. The study will not provide any new treatment to you; it is for observational and data-collection purposes. **Your health will still**

be monitored and managed by medical professionals as per standard care, regardless of study participation.

The **potential benefit is to society and public health.** Knowledge gained from this study may **help improve understanding of Andes virus infection**, such as how early the virus can be detected, how it affects the body, and how to better predict and prevent severe illness or further transmission. This could benefit **future patients and help public health authorities** to manage similar outbreaks better. You will receive no guaranteed personal health benefit, but **your contribution is valuable** to help others and inform future medical advances.

Pregnancy
We know little about Andes virus infection during pregnancy. We would like to learn more about whether the virus can be passed from a mother to her baby (called vertical transmission). This information could help other pregnant women in the future.
If you know that you are pregnant, please tell the study team. You can decide whether or not to take part. In addition to the tests already described, and only if your doctor confirms that it is safe and you agree, extra samples (such as a vaginal swab, breast milk or amniotic fluid) may be collected. The sample collection frequency will be discussed with the study doctor and based on your health status.

How will my privacy be protected? Who will know my data?
We take **confidentiality and data protection** very seriously. Efforts will be made to **limit the personal data collected to the minimum necessary** for the research (this is called **data minimization**). Here is how your privacy will be safeguarded:

- **Coding of your data:** When you enroll, you will be assigned a **unique study code**. Your samples and all data (like lab results and forms) will be **labeled with this code instead of your name** or directly identifying information. The **key** linking the code to your identity (name, contact info) will be kept secure at the local site and **accessible only to authorized study staff**.
- **Secure data storage:** Study data will be stored on **secure, password-protected computers or locked file cabinets**. We follow **European Union General Data Protection Regulation (GDPR)** and applicable national data protection laws. Under these laws, we must **protect your personal information** and **cannot misuse it**. The data we collect will be **used only for the purposes of this research** (or future research consistent with these purposes, as described above, and with necessary approvals).
- **Who will see my data:** Your coded study data (which does not name you) may be shared with public health agencies and collaborating researchers that are helping with the outbreak investigation. **No one outside the study team will know your identity from the research data.** The results of the study may be published or presented, but **your identity will never be revealed** in any report or publication. Individual data might be combined to generate statistics or scientific findings, but it will not be identifiable as yours.
- **Cross-border data transfers:** Because this outbreak involves multiple countries, your coded data and samples may be **sent to authorized partners in other countries** (for example, a specialized lab in another country may test the samples). Any cross-border transfer of your data will follow **international data protection standards** and will only occur for the purposes of this study under WHO coordination. All parties involved are required to maintain **confidentiality and security** of your data to GDPR standards or equivalent.

- **GDPR Privacy Rights:** As a participant, **you have rights regarding your personal data**. These include:
    - The **right to access** your data (you can ask what information the study has about you and to receive a copy of it).
    - The **right to correct** any inaccurate information.
    - The **right to delete** your data or withdraw consent for its use (**if feasible** – note that some data may need to be retained to comply with laws or for ensuring research integrity, but we will fulfill deletion requests to the extent possible).
    - The **right to restrict or object** to certain data processing (for example, you may choose to withdraw from parts of the study if possible).
    - The **right to data portability** (to receive your personal data in a common format, if applicable).
    - The **right to lodge a complaint** with a data protection authority or ethics committee if you feel your data is being mishandled.

If you have questions or want to exercise your data rights, you can contact the study team (see contact information below). They will assist you or direct you to the appropriate person (such as a Data Protection Officer or Ethics Committee contact).

Will I be paid or incur any costs by participating?

No, **you will not be paid** for participating in this study. There are also **no costs to you** for any of the study procedures. All tests and procedures done for research purposes will be provided **free of charge**. If you require medical care due to illness, those medical services will be provided as per usual standards by the health system or outbreak response teams, not by the research study (though there is also no charge to you under the public health response). The study does not cover any additional expenses beyond research-related procedures, but since you are in a quarantine setting, issues like travel do not apply. If you were to need to travel for any follow-up visits after quarantine, the study would cover reasonable travel expenses or make necessary arrangements (if applicable).

What if I am injured or harmed by the study?

This is an observational study with minimal physical intervention, so the risk of injury is low. However, if you are **harmed or injured as a direct result of taking samples or other study procedures**, the study team will ensure you **receive prompt medical attention**. The **sponsor (WHO) or the local health authorities** will cover the costs of any necessary treatment for such research-related injury, in accordance with WHO policy and local regulations. You will **not be asked to pay** for care required to treat an injury resulting from participation in this study. There is **no special compensation for other harms** beyond medical care; by signing this form, you are not waiving any of your legal rights nor releasing the investigators or sponsor from liability for negligence.

What if new information becomes available?

During the course of the study, you will be promptly informed if any **significant new findings** emerge (for example, new information about risks or benefits) that might influence your decision to continue participation. You can then decide whether to continue or withdraw from the study.

What will happen at the end of the study?

When the study is completed, the researchers will analyze the samples and data. **Individual results (like specific lab tests)** will generally not be provided to you, because their meaning for your health may be uncertain and they are used for research purposes. However, if any of your health-related tests from the

study require **clinical follow-up or action**, the study doctor will discuss these with you and assist in arranging appropriate care. The overall **findings from the study** will be made available (for example, through scientific publications or summary reports). You may ask the study staff if you are interested in receiving a summary of the results when available.

Who has reviewed and approved this study?
This study protocol has been reviewed and approved by **[Name of Ethics Committee/Institutional Review Board]**, an independent committee responsible for ensuring that research is ethically and safely conducted. The study is also conducted under **WHO ethical guidelines for outbreak research** and complies with all **applicable local laws and regulations**. If you have any concerns about your rights as a participant, you can contact **[name of local Ethics Committee office or representative]**.

Who can I contact for questions or in case of any issues?

- **Study Doctor/Investigator:** If you have questions, concerns, or experience any study-related injury or distress, you can contact **[Dr. [Name], Principal Investigator]** at **[phone number/email]**. This is the physician or researcher in charge of the study at this site.
- **Ethics Committee:** If you have questions about your rights as a participant or wish to talk to someone not directly involved with the research, you may contact **[Name of Institutional Review Board or Ethics Committee]** at **[contact information]**. They have approved this study and can answer any questions about your rights.

(Use the above contacts or insert local contact details as appropriate for your location. Since this is a multi-country study, each site will provide local contact information for both the study team and the local ethics board.)

Confidentiality and Data Protection Contact: If you have specific questions about privacy or data protection, you may contact **[Data Protection Officer or appropriate authority, with contact info]**.

Confirmation of Understanding and Signature:
Please read the statements below and sign to indicate your agreement:

- I have been **given a copy of this Patient Information and Informed Consent Form**, which I have read (or had read to me in a language I understand). I have had the opportunity to **ask questions** about the study, and all my questions have been answered to my satisfaction.
- I understand the **purpose and procedures** of the study, including what is expected of me and the duration of my participation.
- I understand the **risks and discomforts** as well as the **potential benefits** (though no guaranteed personal benefit) of participating.
- I understand that participation is **voluntary**, and I can **withdraw at any time** without any effect on my medical care or any other rights.
- I understand how my **data and samples will be used**, and I agree to their use for the purposes described in this form. I understand and agree that my data and samples may be transferred to and used in other countries by the study team, collaborating researchers and public health agencies, with my confidentiality protected.
- For pregnancy only: I understand that if I am pregnant, additional samples may be collected.

- For ship passengers only: I understand that public health authorities (RIVM, Netherlands) will share the data I already provided on my potential exposure to Hantavirus onboard the ship, with this study's investigators
- I have been informed that my **personal information will be kept confidential** and that measures are in place to protect my privacy.
- I am aware of my **data protection rights** under GDPR or local laws.
- I know whom to **contact** if I have questions about the study or my rights or if I have a study-related injury.
- By signing this form, **I freely agree to participate** in this study.

I further agree that my genome (DNA) may be studied for the purpose of better understanding Andes virus infection and its effects on human health.	Yes / No

Participant's Name: ..... (please print)
Participant's Signature: ..... **Date:** .....

If the participant is unable to sign but gives oral consent, a witness may sign instead, confirming that the participant understood the information and consented voluntarily.

Witness's Name (if applicable): ..... (print)
Witness's Signature: ..... **Date:** .....
Relationship to Participant: ..... (only if needed, e.g., impartial witness for oral consent)

Person Obtaining Consent (Investigator or Designee):
I confirm that I have explained the study to the participant and answered all questions. I believe that the participant understands the information described in this form and freely consents to participate.

Name of Investigator/Consent Taker: ..... (print)
Signature: ..... **Date:** .....

(Give one signed copy of this form to the participant (or their representative) and keep one for study records.)

Participant Information and Informed Consent Form (Parents/Legal guardians of children <18 years or <16 years (depending on local regulations))

Study Title: *Natural History of Andes Virus Infection (NAVIS)*
Sponsor/Coordinator: World Health Organization (WHO) – Outbreak Response under International Health Regulations (IHR)
Protocol Number: NAVIS 1.1. (Version 1.1, Date 28 May 2026)

Introduction and Invitation:
You are being approached as we would like to invite your child to participate in a research study about **Andes virus (ANDV)** infection following potential exposure. This form explains the study and what your child's participation involves. **Please read it carefully and ask any questions** you may have. Taking part is **voluntary**. If you decide not to participate or to withdraw later, there will be **no penalty or loss of medical care or other benefits** you or your child are entitled to.

Why is this study being done?
The purpose of the **NAVIS** study is to **better understand the infection and illness caused by Andes virus**. Andes virus can cause a serious disease called **hantavirus cardiopulmonary syndrome (HCPS)**. Unlike most hantaviruses, Andes virus may **spread person-to-person** through close contact. This study aims to **track the natural course of infection** from the moment of exposure to when the virus is detected and when symptoms begin, and through recovery. By collecting medical data and biological samples over time, researchers hope to learn **how the virus behaves in the body**, how the immune system responds, **when a person becomes infectious**, **how your genetics (DNA) affect disease**, and what factors might predict severe illness. This knowledge can help improve **outbreak control, patient care, and future treatments or vaccines**. The study is observational, meaning **no experimental treatment is given**; your child's usual medical care will **not** be altered by the study. WHO is coordinating this study globally as part of the outbreak response under International Health Regulations.

Why is my child being asked to participate?
Your child is **eligible for this study because** they have had a defined **exposure to Andes virus** (for example, as a passenger or crew member during a shipboard outbreak). This means your child might have been in contact with someone who was infected or with the environment where the virus was present. Because of this exposure, your child is currently under **public health confinement/quarantine** to monitor their health. The study invites individuals exposed to the virus to join so we can closely observe what happens after exposure to the virus. **Your child's participation can help answer key questions** about early infection and immunity in this outbreak setting.

Does my child have to take part?
No. Participation is entirely voluntary. If you choose not to take part, it will **not affect your child's routine medical care, quarantine conditions, or any public health measures** they are subject to. You can also **change your mind and withdraw** your child from the study at any time, even after signing this form, without giving a reason. There will be **no negative consequences** for your child if you decide not to participate or to withdraw. If you withdraw, we will stop collecting any new information or samples from your child. You can also request that your child's unused samples be destroyed. (However, data or samples already collected up to the point of withdrawal may still be used to maintain the integrity of the study results.)

What will happen if my child participates?
If you agree for your child to participate, you will be asked to **sign this consent form**. Your child will then undergo the following **study procedures**:

- **Initial Assessment (Enrollment):** A study doctor or nurse will ask you about your child's **medical history** and any current symptoms. They will perform a brief physical check (for example, measuring your child's temperature, blood pressure, oxygen level, etc.). We will collect a **baseline set of samples** from your child at this time, including a blood sample and possibly a

nose/throat swab, saliva, urine, and feces. We will also record some information from your child's **medical records** if relevant (for example, any test results or treatments your child has received as part of routine care). If your child was a passenger on the ship, we will also ask RIVM (a public health agency) to share the data they collected from your child when they disembarked, about their exposure to Andes on the ship.

- **Daily Monitoring During Quarantine:** While your child is in isolation/quarantine (the period after exposure during which you are being observed by public health authorities), the study team will **monitor your child's health daily**. This will include asking you about any symptoms your child might have and measuring basic signs like **body temperature and oxygen saturation** (using a finger-clip device) each day. In many cases, you may be asked to **self-record** these daily measurements with guidance. We will also collect a small specimen once or twice a week to check for the virus – typically a **nasal swab or saliva sample** – to detect the virus as early as possible. Daily blood collection is **not** planned; blood draws will be less frequent (weekly) and tied to specific triggers as explained below.
- **Trigger-Based Additional Sampling:** This study uses a **special design** where extra sample collections are done around key "triggers":
    - If/when your child's **virus test first becomes positive** (this is called **P0**, indicating the first detection of virus by PCR), we will initiate a series of additional sample collections around that time. This means, upon the first positive test, your child will have **more frequent blood draws and possibly other specimens (like saliva (and gingival crevicular fluid sometimes), urine, feces, and another swab)** in the days immediately following, to capture the early changes in the virus levels and immune response. We expect to take small blood samples perhaps twice per week around this event.
    - Similarly, if/when your child **develops their first symptoms** of Andes virus infection (e.g., fever, aches, etc., termed **S0**), we will again perform **intensified sampling** around the onset of illness. This could include daily blood samples and other specimens for a short period during early illness.
    - Outside of those trigger periods (before any virus is detected and after acute symptoms have passed), **sampling will be less frequent** – likely a routine schedule such as **once every few days or weekly**, as per the study plan. The schedule is designed to **focus on critical periods** (when most information can be gained) while avoiding unnecessary procedures when they are less informative.
- **Biospecimen Types:** The types of **samples** we will collect from your child include:
    - **Blood samples:** Taken by needle from a vein in your child's arm. We will be careful to collect only the needed volume. *At enrollment*, we may draw around X ml (approximately Y teaspoons) of blood. During trigger periods, we might draw about X ml per day for a few days. In total, the amount will be within safe limits and comparable to a few standard blood tests or less.
    - **Respiratory samples:** Such as **nasal or throat swabs** (using a swab similar to a cotton bud inserted in the nose/throat for a few seconds) or **saliva samples** (your child may be asked to spit into a sterile container). These help detect the virus and are generally done daily or as needed.
    - **Urine and Feces samples:** Your child may occasionally be asked to provide a urine and a feces sample, which will be collected in sterile cups, to check for any virus shedding.
    - (No surgical procedures or invasive biopsies are involved in this study.)

- **Clinical Data Collection:** The researchers will **record relevant information** about your child's health status and clinical care. This includes data like test results (e.g., routine blood counts, chest X-ray findings) and medical treatments if your child receives any (for example, if your child is hospitalized or receives oxygen). They will obtain this from your child's **hospital or clinic records** and through daily check-ins. The study team will **not interfere with your child's medical care**; all treatment decisions remain with your child's doctors. The study just records what happens as part of observing the illness.
- **Duration of Participation:** Your child will be in the study from the time you sign consent until a defined end point, which is likely **up to a few weeks or months** after exposure, depending on your child's health. Specifically, we plan to follow you through the acute phase and into **recovery** (convalescence). After your child has recovered or after a certain period (for example, a follow-up at a few weeks or months post-illness to see longer-term outcomes), your child's active participation will end. **No additional procedures** beyond standard medical care will occur after that point.

What will happen to my child's samples?

Your child's blood and other samples will be **tested for Andes virus and your child body's immune responses** (like antibodies and other immune cells or proteins). We may perform advanced laboratory analyses, such as **genetic sequencing of the virus** (to see if the virus has any mutations) and **laboratory studies on your child's immune cells or blood components**. In some cases, researchers might attempt to **isolate (grow) the virus** from your child's samples in a high-security laboratory to study it—this will not affect you, but it is done to learn more about the virus's characteristics. Any isolated virus or genetic data will be handled with strict safety and confidentiality standards.

In addition to studying the virus, if you specifically agree in the consent form, then **this research will also look at your child's genetic material (DNA)**. We will perform genetic analyses (such as genetic marker testing or whole genome sequencing) to see if certain genetic differences might reveal how some people's immune systems respond differently to the virus. The purpose of these genetic studies is purely scientific – to better understand Andes virus disease and possibly help discover new treatments in the future. Previously, genetic research like this led directly to a new treatment for Covid-19.

Use and storage of data and samples: Samples will be processed and possibly **shared with specialized laboratories** collaborating with the WHO for testing, including within and outside the EU. **Any sample or data shared outside the local study site will be identified only by a unique code, and will *not* include your child's name or personal identifiers.** The results from testing your child's samples will be used for **research purposes** as outlined by this study's goals. After completing the planned analyses for this study, any leftover samples may be **securely stored for a longer period** (for example, in a biorepository) for **future research related to Andes virus or similar emerging infections**. Any such future use of your child's samples or data will be subject to appropriate **ethical review and approvals** to ensure it aligns with the original purpose of this study and protects your child's rights. If you **do not want your child's leftover samples to be stored** for future related research, you can inform the study staff and that request will be respected (no further use of your samples beyond this study).

What are the risks or discomforts?

Participating in this study may involve some **risks or inconveniences**:

- **Risks of blood draws:** Drawing blood can cause mild pain, bruising, bleeding, lightheadedness, or, rarely, infection at the needle site. Trained professionals will minimize these risks by using sterile techniques and taking only the necessary amount of blood. If your child feels faint or uncomfortable during a blood draw, you should inform the staff immediately and they will take appropriate measures (such as having your child lie down). Anaesthetic creams may be used to relieve discomfort and pain.

- **Risks of nose/throat swabs:** Having a nasal or throat swab can cause brief discomfort or a gagging sensation, and occasionally a small nosebleed or irritation. This should be temporary.
- **Daily monitoring and procedures:** Being asked about daily symptoms and providing regular samples (like saliva or swabs) might become tiresome or inconvenient over time. We will try to make this as easy as possible, working with you and your child's schedule and minimizing disruptions.
- **Psychological discomfort:** Some people might feel anxious or stressed about their health status, frequent monitoring, or receiving test results (for example, learning that they have become infected with the virus). The study team will be available to discuss any concerns, and remember that **participating in the study does not change the medical care your child receives** if your child falls ill.
- **Privacy and confidentiality risks:** We will collect personal and health information about your child. There is a very small risk of **loss of confidentiality** (i.e., your child's personal data or health information being seen by someone not authorized). We have strict safeguards in place to prevent this (see the section on confidentiality below).
- **Incidental findings:** This study is focused on Andes virus infection. It is not designed to diagnose or treat other conditions. It is unlikely, but possible, that tests on your child's samples might reveal **unexpected medical information** (for example, a previously unknown health issue). If you choose for your child to participate in the genetic part of this study, this would include any findings from their DNA that we think will directly affect your child's medical care in future. If any **actionable incidental finding** relevant to your child's health is discovered, the study team will discuss it with you and arrange appropriate medical follow-up. You may choose **not to be informed** of certain types of research findings, unless they are important for your child's immediate health – in which case we would inform you so you can help them seek care. Also, it may happen that during the tests run by the local hospital, the doctor may find out that your child (if female) is pregnant.

We do not anticipate other significant risks. Not participating in the study or withdrawing will not remove any **public health obligations** (for example, if you are required to remain in quarantine by public health authorities, you must still do so even if you are not in the study). But joining this study will **not extend your quarantine** or impose extra restrictions beyond standard public health requirements; it only adds research procedures as described.
If any **new information about risks** or anything learned during the study might affect your willingness to continue, we will promptly inform you.

What are the benefits of participating?
You or your child may not receive any direct personal benefit from participating in this research. The study will not provide any new treatment to your child; it is for observational and data-collection purposes. **Your child's health will still be monitored and managed by medical professionals as per standard care**, regardless of study participation.
The **potential benefit is to society and public health.** Knowledge gained from this study may **help improve understanding of Andes virus infection**, such as how early the virus can be detected, how it affects the body, and how to better predict and prevent severe illness or further transmission. This could benefit **future patients and help public health authorities** to manage similar outbreaks better. Your child will receive no guaranteed personal health benefit, but **the contribution to the study is valuable** to help others and inform future medical advances.
Pregnancy

We have little information on Andes virus infection during pregnancy. We would like to learn more about whether the virus can be passed from a mother to her baby (called vertical transmission). This information could help other pregnant women in the future.
If you know that your child is pregnant, please tell the study team. You can decide whether or not they take part. In addition to the tests already described, and only if your doctor confirms that it is safe and you agree, extra samples (such as a vaginal swab, breast milk or amniotic fluid) may be collected. The sample collection frequency will be discussed with the study doctor based on your child's health.

How will my child's privacy be protected? Who will know my child's data?
We take **confidentiality and data protection** very seriously. Efforts will be made to **limit the personal data collected to the minimum necessary** for the research (this is called **data minimization**). Here is how your child's privacy will be safeguarded:

- **Coding of your child's data:** When your child enrolls, they will be assigned a **unique study code**. Your child's samples and all data (like lab results and forms) will be **labeled with this code instead of your name** or directly identifying information. The **key** linking the code to your child's identity (name, contact info) will be kept secure at the local site and **accessible only to authorized study staff**.
- **Secure data storage:** Study data will be stored on **secure, password-protected computers or locked file cabinets**. We follow **European Union General Data Protection Regulation (GDPR)** and applicable national data protection laws. Under these laws, we must **protect your child's personal information** and **cannot misuse it**. The data we collect will be **used only for the purposes of this research** (or future research consistent with these purposes, as described above, and with necessary approvals).
- **Who will see my child's data:** Your child's coded study data (which does not name your child) may be shared with public health agencies and collaborating researchers that are helping with the outbreak investigation. **No one outside the local study team will know your child's identity from the research data.** The results of the study may be published or presented, but **your child's identity will never be revealed** in any report or publication. Individual data might be combined to generate statistics or scientific findings, but it will not be identifiable as your child.
- **Cross-border data transfers:** Because this outbreak involves multiple countries, your child's coded data and samples may be **sent to authorized partners in other countries** (for example, a specialized lab in another country may test the samples). Any cross-border transfer of your child's data will follow **international data protection standards** and will only occur for the purposes of this study under WHO coordination. All parties involved are required to maintain **confidentiality and security** of your child's data to GDPR standards or equivalent.
- **GDPR Privacy Rights:** Your child, as a participant, **has rights regarding their personal data**. These include:
    - The **right to access** their data (you can ask what information the study has about your child and to receive a copy of it).
    - The **right to correct** any inaccurate information.
    - The **right to delete** your child's data or withdraw consent for its use (**if feasible** – note that some data may need to be retained to comply with laws or for ensuring research integrity, but we will fulfill deletion requests to the extent possible).
    - The **right to restrict or object** to certain data processing (for example, you may choose to withdraw from parts of the study if possible).

- The **right to data portability** (to receive your child's personal data in a common format, if applicable).
- The **right to lodge a complaint** with a data protection authority or ethics committee if you feel your child's data is being mishandled.

If you have questions or want to exercise your child's data rights, you can contact the study team (see contact information below). They will assist you or direct you to the appropriate person (such as a Data Protection Officer or Ethics Committee contact).

Will I be paid or incur any costs by participating?

No, **you and your child will not be paid** for participating in this study. There are also **no costs to you** for any of the study procedures. All tests and procedures done for research purposes will be provided **free of charge**. If your child requires medical care due to illness, those medical services will be provided as per usual standards by the health system or outbreak response teams, not by the research study (though there is also no charge to you under the public health response). The study does not cover any additional expenses beyond research-related procedures, but since your child is in a quarantine setting, issues like travel do not apply. If your child were to need to travel for any follow-up visits after quarantine, the study would cover reasonable travel expenses or make necessary arrangements (if applicable).

What if my child is injured or harmed by the study?

This is an observational study with minimal physical intervention, so the risk of injury is low. However, if your child is **harmed or injured as a direct result of taking samples or other study procedures**, the study team will ensure your child **receives prompt medical attention**. The **sponsor (WHO) or the local health authorities** will cover the costs of any necessary treatment for such research-related injury, in accordance with WHO policy and local regulations. You will **not be asked to pay** for care required to treat an injury resulting from participation in this study. There is **no special compensation for other harms** beyond medical care; by signing this form, you are not waiving any of your child's legal rights nor releasing the investigators or sponsor from liability for negligence.

What if new information becomes available?

During the course of the study, you will be promptly informed if any **significant new findings** emerge (for example, new information about risks or benefits) that might influence your decision to continue participation. You can then decide whether to continue or withdraw your child from the study.

What will happen at the end of the study?

When the study is completed, the researchers will analyze the samples and data. **Individual results (like specific lab tests)** will generally not be provided to you, because their meaning for your health may be uncertain and they are used for research purposes. However, if any of your child's health-related tests from the study require **clinical follow-up or action**, the study doctor will discuss these with you and assist in arranging appropriate care. The overall **findings from the study** will be made available (for example, through scientific publications or summary reports). You may ask the study staff if you are interested in receiving a summary of the results when available.

Who has reviewed and approved this study?

This study protocol has been reviewed and approved by **[Name of Ethics Committee/Institutional Review Board]**, an independent committee responsible for ensuring that research is ethically and safely conducted. The study is also conducted under **WHO ethical guidelines for outbreak research** and complies with all **applicable local laws and regulations**. If you have any concerns about your child's rights as a participant, you can contact **[name of local Ethics Committee office or representative]**.

Who can I contact for questions or in case of any issues?

- **Study Doctor/Investigator:** If you have questions, concerns, or experience any study-related injury or distress, you can contact **[Dr. [Name], Principal Investigator]** at **[phone number/email]**. This is the physician or researcher in charge of the study at this site.
- **Ethics Committee:** If you have questions about your child's rights as a participant or wish to talk to someone not directly involved with the research, you may contact **[Name of Institutional**

Review Board or Ethics Committee] at **[contact information]**. They have approved this study and can answer any questions about your child's rights.

(Use the above contacts or insert local contact details as appropriate for your location. Since this is a multi-country study, each site will provide local contact information for both the study team and the local ethics board.)

Confidentiality and Data Protection Contact: If you have specific questions about privacy or data protection, you may contact **[Data Protection Officer or appropriate authority, with contact info]**.

Confirmation of Understanding and Signature:

Please read the statements below and sign to indicate your agreement:

- I have been **given a copy of this Patient Information and Informed Consent Form**, which I have read (or had read to me in a language I understand). I have had the opportunity to **ask questions** about the study, and all my questions have been answered to my satisfaction.
- I understand the **purpose and procedures** of the study, including what is expected of me and the duration of my child's participation.
- I understand the **risks and discomforts** as well as the **potential benefits** (though no guaranteed personal benefit) of participating.
- I understand that participation is **voluntary**, and I can **withdraw my child from the study at any time** without any effect on their medical care or any other rights.
- I understand how my child's **data and samples will be used**, and I agree to their use for the purposes described in this form. I understand and agree that my child's data and samples may be transferred to and used in other countries by the study team, collaborating researchers and public health agencies, with my confidentiality protected.
- For pregnancy only: I understand that if my child is pregnant, additional samples may be collected.
- For ship passengers only: I understand that public health authorities (RIVM, Netherlands) will share the data my child already provided on their potential exposure to Hantavirus onboard the ship, with this study's investigators
- I have been informed that my child's **personal information will be kept confidential** and that measures are in place to protect my child's privacy.
- I am aware of my child's **data protection rights** under GDPR or local laws.
- I know whom to **contact** if I have questions about the study or my child's rights or if they have a study-related injury.
- By signing this form, **I freely agree for my child to participate** in this study.

I further agree that my child's genome (DNA) may be studied for the purpose of better understanding Andes virus infection and its effects on human health.	Yes / No
I further agree to receive information on potential Incidental Findings emerging from the study	Yes / No

Parent/legal guardian's Name: ..... (please print)

Parent/legal guardian's Signature: ..... **Date:** .....

If the parent/legal guardian is unable to sign but gives verbal consent, a witness may sign instead, confirming that the parent/legal guardian understood the information and consented voluntarily.

Witness's Name (if applicable): ..... (print)
Witness's Signature: ..... **Date:** .....
Relationship to Parent/Legal guardian: ..... (only if needed, e.g., impartial witness for oral consent)
Person Obtaining Consent (Investigator or Designee):
I confirm that I have explained the study to the participant and answered all questions. I believe that the participant understands the information described in this form and freely consents to participate.
Name of Investigator/Consent Taker: ..... (print)
Signature: ..... **Date:** .....
(Give one signed copy of this form to the participant (or their representative) and keep one for study records.)

Participant Information and Informed Assent Form (Indicative age 15yrs-17yrs; competence to be assessed by the responsible investigator)

Study Title: *Natural History of Andes Virus Infection (NAVIS)*
Sponsor/Coordinator: World Health Organization (WHO) – Outbreak Response under International Health Regulations (IHR)
Protocol Number: NAVIS 1.1. (Version 1.1, Date 28 May 2026)

Introduction and Invitation:
You are being invited to take part in a research study about **Andes virus (ANDV)** infection as you may have been exposed to the virus, or you have already tested positive for Andes virus infection. We want to follow your health during your quarantine/treatment period.

Please read this information sheet carefully and ask any questions you may have.

Why is this study being done?
The **NAVIS** study aims to **better understand** how **Andes virus** behaves in people who were exposed to it. By collecting health information and samples over time, researchers hope to learn more about the virus and use this knowledge to improve care for future patients.
The study is observational, which means you will not receive any new or **experimental treatment, and your** usual medical care will **stay the same**. The study is being coordinated by the World Health Organization (WHO) to help respond to the outbreak.

Why am I being asked to take part?
You are **being invited because** you have been **exposed to the Andes virus** (for example, as a passenger or crew member during an outbreak on a ship). This means you might have been in contact with someone who had the virus, or with an environment where the virus was present.
Because of this exposure, you are currently being **quarantined** to keep an eye on your health. By taking part in the study, we can understand what happens after someone is exposed to the virus including how the body responds early on. **Taking part can help answer important questions** and help improve care and responses to future outbreaks.

Do I have to take part?
No. Taking part is entirely voluntary. If you choose not to take part, it will **not change your normal medical care or quarantine conditions**. You can also **change your mind and withdraw** from the study at any time, even after signing this form, without giving a reason. If you withdraw, we will stop collecting any new information or samples from you.

What will happen if I take part?
If you agree to take part, you will be asked to **sign the assent form**. You will then be asked to do the following:

- Have daily checks of your symptoms and general health for about 6 weeks
- Give nose/throat swabs or saliva samples once or twice a week to test for virus
- Provide small blood samples (about 1-2 teaspoons each time), twice a week or longer if virus is still found in your blood
- Take part in follow-up to see how you recover over the 6-week period

If you were a ship passenger, we will also ask RIVM (a public health agency) to share the data they collected from you when you disembarked, about your exposure to Andes on the ship.

What will happen to my samples?
Your blood and other samples will be **tested for Andes virus to understand how your body responds to it**. Sometimes, more detailed laboratory tests may also be carried out to study **the virus more closely**.
If you agree in the assent form, researchers may **also look at your genetic material (DNA). This is** to see if people respond differently to the virus. These genetic tests are only for research purposes, to help better understand Andes virus disease and possibly help develop new treatments in the future.
Any sample or information outside the local study site will *not* include your name or anything that could identify you. Once the main tests are completed, any leftover samples may be **securely stored for future research on Andes virus or similar infections**. If you **do not want your leftover samples to be stored**, you can inform the study staff, and your wishes will be respected.
What are the risks or discomforts?
Taking part in this study may cause some **minor discomforts**:

- Blood samples may cause brief pain, bruising, or feeling light headed
- Nose or throat swabs may feel uncomfortable for a short time
- No other significant risks are expected

What are the benefits of taking part?
You may not receive any direct personal benefit from taking part. **Your normal health care will not change and your will continue to be cared for by healthcare professionals as usual**.
However, the **study may benefit others in the future.** What we learn may **help improve understanding of Andes virus -** such as how early it can be detected, how it affects the body, and how to better prevent severe illness or spread to others.
Pregnancy
We do not have many information on cases of Andes virus infection during pregnancy, therefore, we would like to understand how the virus passes from the mother to the child and if the virus passes to the child through the milk. This may help other pregnant women in the future.
If you are pregnant, please inform the study team. You can freely decide whether to take part in the study and further to the testing described, if your doctor thinks that it is ok and you provide consent, a vaginal swab, breast milk or amniotic fluid will be collected. The sample collection will be discussed with your doctor based on your health status.
How will my privacy be protected? Who will see my data?
Your samples and information will be given a code so your name is not used. This means you cannot be personally identified from the information shared.
Your data will only be shared with public health agencies and collaborating researchers that are helping with this outbreak investigation. Sometimes your sample and information may be sent outside your country for special analysis. All organizations involved must follow strict rules to protect your privacy and keep your data secure in line with UK GDPR or similar standards.

Will I be paid or incur any costs by participating?
No, **you will not be paid** for taking part in this study. You will not have to pay anything either. All tests and procedures done for the study will be **free of charge**.
What if I am injured or harmed by the study?
This study involves very little risk. However, if you are **harmed or injured as a result of the study procedures (such as taking blood samples)** you **receive prompt medical care**. The **sponsor (WHO) or the local health authorities** will cover the costs of treatment for injury related to the study.
What if new information becomes available?
If we learn anything **new during the study** that might affect your decision to continue in the study, we will let you know as soon as possible and you can then decide whether to continue or leave the study.

What will happen at the end of the study?
When the study ends, researchers will analyze the samples and information collected. The overall **results** will be shared (for example, through scientific publications or summary reports). You may ask the study staff if you are interested in receiving a summary of the results when available.

Who has reviewed and approved this study?
This study has been reviewed and approved by **[Name of Ethics Committee/Institutional Review Board]**. The study is also conducted under **WHO ethical guidelines for outbreak research** and complies with all **applicable local laws and regulations**. If you have any concerns about your rights as a participant, you can contact **[name of local Ethics Committee office or representative]**.

Who can I contact for questions or in case of any issues?

- **Study Doctor/Investigator:** If you have questions, concerns, or experience any study-related injury or distress, you can contact **[Dr. [Name], Principal Investigator]** at **[phone number/email]**. This is the doctor or researcher in charge of the study at this site.
- **Ethics Committee:** If you have questions about your rights as a participant or wish to talk to someone not directly involved with the research, you may contact **[Name of Institutional Review Board or Ethics Committee]** at **[contact information]**. They have approved this study and can answer any questions about your rights.

(Use the above contacts or insert local contact details as appropriate for your location. Since this is a multi-country study, each site will provide local contact information for both the study team and the local ethics board.)

Please read the statements below and sign to show you agree:

- I understand what the study is about, what I need to do and how long I will take part. I have had the chance to ask questions and have them answered.
- I understand the possible **risks and discomforts** as well as the **potential benefits** (although I may not benefit personally).
- I understand that taking part is **my choice**, and I can **leave the study at any time** without it affecting my medical care or my rights.
- I understand how my **information and samples will be used**, and I agree to their use as described. I understand that my information and samples may be used in other countries by the study team, collaborating researchers and public health agencies, and that my identity will be protected.
- I understand that if I am pregnant additional samples may be collected.
- For ship passengers only: I understand that public health authorities (RIVM, Netherlands) will share the data I already provided on my potential exposure to Hantavirus onboard the ship, with this study's investigators
- I understand that my **personal information will be kept confidential** and that steps are in place to protect my privacy.
- I know whom to **contact** if I have questions, concerns or if I am harmed as part of the study.
- By signing this form, **I agree to take part** in this study.

I further agree that my genome (DNA) may be studied for the purpose of better understanding Andes virus infection and its effects on human health.	Yes / No

Participant's Name: ..... (please print)
Participant's Signature: ..... **Date:** .....
If the participant is unable to sign but gives oral consent, a witness may sign instead, confirming that the participant understood the information and consented voluntarily.
Witness's Name (if applicable): ..... (print)
Witness's Signature: ..... **Date:** .....
Relationship to Participant: ..... (only if needed, e.g., impartial witness for oral consent)

Person Obtaining Consent (Investigator or Designee):

I confirm that I have explained the study to the participant and answered all questions. I believe that the participant understands the information described in this form and freely consents to participate.

Name of Investigator/Consent Taker: ..... (print)

Signature: ..... **Date:** .....

(Give one signed copy of this form to the participant (or their representative) and keep one for study records.)

Participant Information and Informed Assent Form (Indicative age 11yrs-14yrs; competence to be assessed by the responsible investigator)

Study Title: *Natural History of Andes Virus Infection (NAVIS)*
Sponsor/Coordinator: World Health Organization (WHO) – Outbreak Response under International Health Regulations (IHR)
Protocol Number: NAVIS 1.1. (Version 1.1, Date 28 May 2026)

We are inviting you to take part in a research study

We would like you to take part in a research study. Research helps us learn more about illness, such as the Andes Virus, so that we can improve care in the future.
If you choose to take part, you will be asked to give your assent. This means you understand what the study is about and agree to take part. You do not have to take part if you do not want to, and no one will make you. We have also told your parents or guardians about this study. They have said that you may take part if you want to.

Why is this study being done?
The **NAVIS** study **helps us learn** how the **Andes virus** affects people. We do this by collecting health information and small samples over time. This will help us understand the virus better and improve care for others in the future.

Why are you contacting me?
You have been contacted because you may have been around someone who the **Andes virus** or in a place where the virus was present (for example, on a ship during an outbreak, or through your family). Because of this, your health is being checked for a short time (for example staying at home or in quarantine to keep others safe).

Do I have to take part?
No. It is your choice. You do not have to take part if you don't want to. If you decide to take part you can stop at any time. Just tell your parents or the study doctor and they will make sure that your decision is respected.
What do I have to do?
If you agree to take part, you will be asked to **sign the assent form**. You will be asked to:

- Tell us how you feel every day for about 6 weeks.
- Give a nose or throat swabs or saliva samples once or twice a week
- Give a small blood sample (about 1-2 teaspoons) twice a week for 6 weeks. Have follow-up checks to check your health.

What will happen to my samples?
Your blood and other samples will be **tested for the Andes virus and to see how your body responds**. **Your name will not be on your samples or information. This means no one outside the study will know the information belongs to you. Your information will be kept private and confidential.**

Will anything bad happen to me?
Taking part in this study may involve some **small discomforts**:

- Blood draws may hurt a little, cause bruising, or make you feel a bit dizzy
- Nose or throat swabs may feel a bit uncomfortable for a short time

- No other important risks are expected.

What are the benefits of taking part?
You may not get any direct benefit from taking part. **You will still receive normal care from doctors whether you join the study or not**.
However, the study may help other **people in future**, by helping us understand the virus better – such as how early it can be found, how it affects the body, and how to prevent serious illness or further transmission.

How will my privacy be protected? Who will know my data?
Your samples and information will not include your names. Instead. they will be given a code. Only the study team and researchers and health workers involved in this study will be able to see your file with your information. Your name will not be in any reports that are printed about the study.

Will my parents have to pay the doctor or the hospital?
No, **your parents will not have to pay anything for you to take part** in this study.

What if I am hurt during the study?
This study has very low risk. But if you are hurt or feel unwell, tell your parents or the study doctor. They will make sure you are looked after.

What will happen at the end of the study?
When the study finishes, researchers will look at all the information and samples collected. The results will be shared in reports or science papers.
You can ask the study team if you would like to find out the results.

Please read and sign if you agree:

- I understand what the study is about, what I need to do, and how long it will last. I have had the chance to ask questions.
- I understand the possible risks and that I may not **benefit personally**.
- I **know that taking part is my choice and I can stop at any time** without affecting my health care.
- I understand how my **information and samples will be used**, and agree to this. I understand they may be shared safely with other researchers and public health organisations.
- I understand my **personal information will be kept** private
- I know who to **contact** if I have questions or problems.
- By signing this form, **I agree to take part** in this study.

Participant's Name: ..... (please print)
Participant's Signature: ..... **Date:** .....
If the participant is unable to sign but gives oral consent, a witness may sign instead, confirming that the participant understood the information and consented voluntarily.
Witness's Name (if applicable): ..... (print)
Witness's Signature: ..... **Date:** .....
Relationship to Participant: ..... (only if needed, e.g., impartial witness for oral consent)
Person Obtaining Consent (Investigator or Designee):
I confirm that I have explained the study to the participant and answered all questions. I believe that the participant understands the information described in this form and freely consents to participate.
Name of Investigator/Consent Taker: ..... (print)
Signature: ..... **Date:** .....
(Give one signed copy of this form to the participant (or their representative) and keep one for study records.)

Children Guide
(Indicative age 6yrs-11yrs; competence to be assessed by the responsible investigator)

Study Title: *Natural History of Andes Virus Infection (NAVIS)*
Sponsor/Coordinator: World Health Organization (WHO) – Outbreak Response under International Health Regulations (IHR)
Protocol Number: NAVIS 1.1. (Version 1.1, Date 28 May 2026)

Do you want to help with our study?
We would like to invite you to be part of a research study.
A research study is how we learn more about illness (when people are sick) so that we can help others feel better in the future.
We have already talked to your parents, and they think it is okay for you to take part if you want to.

Why are we doing this study?

We want to learn more about a virus called the Andes virus

We are trying to understand:
- How the virus acts inside the body.
- How people feel when they have it

This will help doctors take better care of people in the future.

Why are you asking me?
You may have been near someone who had the virus or near a place where the virus is found.
Because of this, doctors are already checking how you are feeling to keep you safe.
Do I have to take part?
No, you do not have to.
It is your choice.
- You can say yes or no
- If you say yes now you can change your mind later.
- Just tell your parents or the doctor.

What will I need to do?
If you join the study, we will ask you to:
- Tell us how you feel every day for 6 weeks
- Give a small sample from your nose, throat swabs or spit (saliva) once or twice a week
- Give a small blood sample two times a week. The blood is a small amount - about 1-2 teaspoons each time.

What will happen to my samples?
- We will test your samples **for the virus**
- If you have the virus, we will see how your body is fighting it

Your name will be kept private and no one outside the study will know it.

Will anything uncomfortable happen

Some things might feel a little uncomfortable, like:

- Giving blood might hurt a little or leave a small bruise
- Nose swabs might feel a bit funny or uncomfortable

If you feel worried or uncomfortable, you can tell your parents or the doctor and they will help you.

Children Cartoon
(Indicative age <6yrs; competence to be assessed by the responsible investigator)

Cartoon information sheet for discussing NAVIS study with young children



We want to understand more about how you are feeling so that we can help other children like you. First we will collect information from your medical records, including medicines and test results. Secondly, we will also take some extra samples of blood and other samples while you are in hospital or at home. All information will be studied anonymously and nobody will know it is from you.



Blood samples will be taken by finger prick or existing lines. The sample will be small. Numbing cream can be used.


Throats will be wiped (swabbed) using cotton buds This does not hurt, but can tickle a bit.

Samples of poo (stool), wee (urine), snot (nasal aspirate) and cough spit (phlegm) will also be collected when you make them.

Annex B: Symptom diary

ISARIC

PARTICIPANT IDENTIFICATION #: [___][___][___][___][___]--- [___][___][___][___]

DAILY

Enter the participant number assigned by the study staff It should have 5 characters, a hyphen, and four digits (e.g. Y0401-0001) _____	Date of birth [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]

DAILY ASSESSMENT			
DATE OF ASSESSMENT	[_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]		
Did you measure your temperature today?	○ Yes ○ No	If Yes: What was the highest temperature you measured today	_____ ○°C ○°F
Did you measure your oxygen saturation today?	○ Yes ○ No	If Yes: What was the lowest oxygen saturation value you measured today (%)	_____

SYMPTOMS: Indicate if experienced between 00:00 to 24:00 on day of assessment.					
In the previous 24 hours, did you experience any of the symptoms below?					
Fever	○ Yes ○ No	Chills or rigors	○ Yes ○ No	Fatigue / malaise / lethargy	○ Yes ○ No
Weakness	○ Yes ○ No	Muscle aches (myalgia)	○ Yes ○ No	Skin rash	○ Yes ○ No
Back Pain	○ Yes ○ No			Neck Pain	○ Yes ○ No
Cough	○ Yes ○ No			If Yes: Cough type	○ Non Productive (dry cough) ○ Productive (wet cough) ○ Haemoptysis (coughing up blood) ○ Unknown
Shortness of breath	○ Yes ○ No	Chest pain	○ Yes ○ No	Abdominal pain (stomach pain)	○ Yes ○ No
Diarrhoea	○ Yes ○ No	Vomiting	○ Yes ○ No	Nausea	○ Yes ○ No
Anorexia (lack of appetite)	○ Yes ○ No	Urinary retention	○ Yes ○ No	Increased urination (polyuria)	○ Yes ○ No
Bleeding (haemorrhage)	○ Yes ○ No			If Yes: Severe bleeding (requiring the intervention of a health care provider)	○ Yes ○ No
Headache	○ Yes ○ No	Photophobia (aversion to light)	○ Yes ○ No	Hazy / Blurred vision	○ Yes ○ No
Seizures / convulsions	○ Yes ○ No			Altered consciousness / confusion	○ Yes ○ No
Other symptom(s)	○ Yes ○ No			If Yes:	_____

ISARIC

PARTICIPANT IDENTIFICATION #: [___][___][___][___][___]--- [___][___][___][___]

WELLBEING QUESTIONS					
Please read each statement and indicate how you feel right now, in this moment.					
I feel calm	○ Not at all ○ Somewhat ○ Moderately ○ Very much ○ Prefer not to answer	I am tense	○ Not at all ○ Somewhat ○ Moderately ○ Very much ○ Prefer not to answer	I feel upset	○ Not at all ○ Somewhat ○ Moderately ○ Very much ○ Prefer not to answer
I am relaxed	○ Not at all ○ Somewhat ○ Moderately ○ Very much ○ Prefer not to answer	I feel content	○ Not at all ○ Somewhat ○ Moderately ○ Very much ○ Prefer not to answer	I am worried	○ Not at all ○ Somewhat ○ Moderately ○ Very much ○ Prefer not to answer
How would you rate your health today?	○ Excellent ○ Very good ○ Good ○ Fair ○ Poor ○ Prefer not to answer	How likely do you think it is that you will develop symptoms of hantavirus?	○ Very likely ○ Fairly likely ○ Not very likely ○ Not at all likely ○ Prefer not to answer	How serious do you think it would be for you if you were to develop symptoms of hantavirus?	○ Very serious ○ Fairly serious ○ Slightly serious ○ Not very serious ○ Not at all serious ○ Prefer not to answer
Please indicate how much you agree or disagree with this statement: There are people I can depend on to help me if I really need it.		○ Strongly agree ○ Agree ○ Disagree ○ Strongly disagree ○ Prefer not to answer			

Annex C: Clinical Laboratory Analyses

1. Biochemistry, Haematology and Coagulation[12,42,43]

- Performed by: Local Hospital / Isolation Facility
- Purpose: Clinical monitoring and management
- Timing: Prospective, Real-time feedback to clinicians
- Minimum / Optional items to be included:

Domain	Parameter / Test	Minimum vs Optional	Rationale	Interpretation / thresholds
Haematology	Platelet count (CBC)	Minimum	Thrombocytopenia is a typical clinical laboratory finding in HPS/HCPS.	Platelets <50,000 are an alarm sign of severity.
Haematology	Haematocrit (CBC)	Minimum	Hemoconcentration/elevated haematocrit is a typical laboratory finding in HPS/HCPS.	Haematocrit >45% is an alarm sign of severity.
Haematology	Total WBC (CBC)	Minimum	Neutrophilic leukocytosis/left shift are described among typical clinical laboratory findings used in surveillance/early orientation.	Leukocytosis >20,000/mL is an alarm sign of severity.
Haematology	Differential / left shift (CBC differential)	Minimum	"Left shift in the white blood cell count" is explicitly included among typical clinical laboratory findings in the CDC HPS case definition.	No numeric threshold stated in official definitions, interpret as presence/absence per laboratory reporting.
Haematology	Circulating immunoblasts (manual differential / smear review if available)	Optional	Circulating immunoblasts are a typical laboratory feature. Chile MoH includes immunoblast percentage in alarm signs.	Immunoblasts >45% are an alarm sign of severity.
Coagulation	aPTT (TTPa)	Minimum	aPTT (TTPa) is an admission lab predictor for HCPS diagnosis.	No specific cut off, interpret relative prolongation per local reference ranges.
Biochemistry	AST (GOT)	Minimum	AST (GOT) is an admission lab predictor, particularly in combination with haematocrit/platelets.	No specific cut-off, interpret elevation per local reference ranges.
Biochemistry / Metabolic	Blood lactate	Optional (high-value if clinically unwell)	Lactate is as a poor prognostic indicator in HPS management.	Lactate >2 mmol/L is an alarm sign.
Biochemistry / Metabolic	Blood pH (acid–base status, ABG/VBG as clinically indicated)	Optional (severity assessment)	Low pH is and alarm sign used for urgent escalation decisions.	pH <7.25 is an alarm sign.
Biochemistry / Inflammatory markers	Ferritin, C-reactive Protein, LDH, D-Dimer	Minimum	Indicate systemic hyperinflammation	Predictive cut-offs have not been defined.
Biochemistry / Inflammatory markers	IL-6 (IL-1)	Optional (if available)	Indicates systemic hyperinflammation	Predictive cut-offs have not been defined.

2. ANDV RT-PCR testing in blood, nasopharyngeal swabs, saliva. Semen, urine and feces

- Performed by: Local Hospital / Isolation Facility or by Central Reference Microbiology Laboratory, as per local policy
- Purpose:

    - Primary: Clinical monitoring and management. Detect transition from Tier 1 (Exposure) to Tier 2 (Pre-symptomatic infection)
    - Secondary: Monitor rt-PCR dynamics in a Research context
- Timing:
    - Primary purpose: Prospective, Real-time feedback to clinicians
    - Secondary: Can be done retrospectively, to minimize acute burden, as per local policy
- Methods:

Specimen Collection and Pre-Analytical Handling

- **Blood Samples:** Blood is collected by venipuncture into sterile EDTA tubes (for whole blood, PBMC or plasma) or serum separator tubes (for serum). Whole blood or separated plasma/serum can be tested. However, leukocyte-rich fractions (buffy coat) often contain the highest viral loads and are preferred to maximize sensitivity. Blood samples are kept refrigerated (2–8 °C) and transported on cold packs to maintain RNA stability, ideally delivered within 24–72 hours to avoid RNA degradation. For prolonged storage or batch testing, specimens are frozen at –70 °C or below, avoiding repeat freeze–thaw cycles.
- **Nasopharyngeal (NP) Swabs:** NP swabs (e.g., flocked synthetic swabs) are obtained from the posterior nasopharynx using appropriate personal protective equipment (PPE, including N95 respirator and eye protection) due to the potential for respiratory aerosols. Swabs are immediately placed in viral transport medium (VTM) to preserve viral RNA during transport and storage. NP swab specimens are handled like other respiratory samples: kept refrigerated (2–8 °C) and processed promptly (within 24–48 hours) or frozen at –70 °C for longer-term storage if delays are anticipated.
- **Saliva and Oral Fluids:** Saliva or oral fluid specimens (e.g., sputum or oral swabs from gingival crevices) are collected in sterile containers or swabs, ideally first-morning samples to maximize viral yield. Because saliva contains nucleases and potential PCR inhibitors such as mucins and food debris, pre-analytical measures include using specialized saliva collection kits or adding RNA stabilization buffer, when available, to inhibit nucleases and preserve viral RNA. Saliva samples are kept cold (refrigerated) and processed rapidly or mixed with lysis buffer immediately upon arrival to ensure viral inactivation and RNA stabilization.
- **Urine:** Approximately 5–10 mL of mid-stream urine is collected into a sterile container. Urine samples may contain lower viral titers, If a high sensitivity is required, larger volumes (up to 10–30 mL) can be concentrated (e.g., by centrifugation or filtration) before RNA extraction. Urine is prone to rapid pH changes and potential nuclease activity, so samples are transported on cold packs and stored refrigerated short-term. For extended storage, freezing at –70 °C is recommended to preserve viral RNA integrity.
- **Feces:** Stool specimens are self-collected by participants into a sterile, leak-proof, screw-cap container (target ~2–5 g or an equivalent "pea-sized" aliquot if only a small volume is feasible), taking care to avoid contamination with urine or toilet water. Because stool is heterogeneous and often contains PCR inhibitors (e.g., bile salts, complex polysaccharides, heme compounds), pre-analytical handling should prioritize standardized aliquoting and prompt stabilization: where available, transfer a small aliquot into a pre-labeled tube containing RNA stabilization solution or chaotropic lysis buffer per local SOPs to preserve viral RNA and reduce degradation; otherwise keep the primary container tightly sealed and cold. Handling and transport should use appropriate PPE (gloves; eye protection; consider an N95/respirator if any aerosol-generating manipulation is anticipated) and secondary containment to prevent leakage. Stool samples are transported refrigerated (2–8 °C) and processed as soon as possible (ideally within 24–48 hours). For longer-term storage or anticipated delays, aliquot to minimize future freeze–thaw and freeze at –70 °C or below to maintain RNA integrity.
- **Semen:** Optional and limited to consenting adult male participants where culturally acceptable. Collection: Self-collected by masturbation into a sterile container (aim for ≥1–2 mL). Seminal fluid is viscous and contains proteases and other PCR inhibitors, allow it to liquefy at room temperature (up to ~30 minutes) and use appropriate RNA stabilization or lysis buffer per local SOPs to preserve viral RNA. Handling: Keep semen specimens refrigerated (2–8 °C)

and transport on cold packs for prompt processing (ideally within 24 hours). For delays or longer storage, freeze at –70 °C or below as soon as possible, and avoid repeated freeze–thaw cycles to maintain RNA integrity.

Biosafety Precautions: All clinical specimens from suspected Andes virus cases are considered high-risk infectious materials and must be handled with stringent biosafety practices. Initial processing (e.g., sample aliquoting and lysis) must be conducted in a Class II biosafety cabinet within a BSL-3 laboratory, given that Andes virus can be transmitted through contact with blood or respiratory droplets. Immediately upon receipt in the lab, each sample is chemically lysed in a chaotropic buffer (e.g., guanidinium thiocyanate-based lysis solution) to inactivate any live virus (ensuring a safe transition to BSL-2 processing). This step also preserves viral RNA by denaturing RNases. However, **to isolate viruses from biological samples, they should be freezed and preserved without lysis.** Standard universal precautions (gloves, lab coat, eye protection) and BSL-3 practices must be maintained throughout specimen handling to prevent laboratory-acquired infections.

Viral RNA Extraction

- **Extraction Methods:** Total viral RNA is extracted from each specimen using validated RNA extraction kits or platforms. Both manual silica column-based kits (e.g., spin-column viral RNA extraction kits) and automated magnetic bead-based platforms (for high-throughput processing) are suitable, provided that they yield high-quality, inhibitor-free RNA from diverse specimen matrices.
- **Procedure:** After sample inactivation, lysates are clarified by brief centrifugation and then applied to the chosen extraction system following the manufacturer's protocol. In the manual approach, ethanol is added to the lysate to enable nucleic acid binding to the silica membrane, followed by sequential wash steps and elution of RNA in RNase-free buffer. In automated extraction, lysis buffer-compatible samples are loaded onto a robotic extractor with pre-set protocols (for example, involving magnetic silica particles). Both approaches reliably remove PCR inhibitors (e.g., hemoglobin from whole blood and possible inhibitory substances in saliva, urine or feces) and ensure high-purity RNA suitable for RT-PCR amplification. Co-extraction of an internal control (e.g., a known concentration of non-target RNA viral control added to each sample prior to extraction) is strongly advised to monitor extraction efficiency and the presence of inhibitors in each specimen.
- **Storage of RNA:** Purified RNA is kept at –70 °C or used immediately for RT-PCR. If a two-step RT-PCR protocol is employed, the complementary DNA (cDNA) product is generated promptly and can be stored at –20 °C before PCR amplification.

RT-PCR Assay Design and Amplification

- **Genomic Target Selection:** The RT-PCR assay is designed to amplify a conserved region of the ANDV genome, typically within the S (small) segment encoding the nucleocapsid (N) protein, which is known to be a stable and sensitive target for New World hantaviruses. Targeting the S segment provides broad detection of Andes virus strains while maintaining specificity, as this region is conserved across ANDV variants. The N gene also yields abundant viral RNA copies during infection, improving analytical sensitivity. In some protocols, multiplex designs include dual minor groove binder (MGB) hydrolysis probes to cover sequence variability across different ANDV lineages, but a single well-optimized probe can suffice for species-specific detection.
- **Primer–Probe Strategy:** Oligonucleotide primers and a probe are designed according to published sequences for the chosen target region. For example, a widely used assay (originally described by Kramski et al.[44]) employs a forward primer, reverse primer, and a 5'-fluorophore-labeled TaqMan probe specific to the ANDV S segment. A second probe or degenerate bases can be used if needed to account for minor genetic variations among ANDV strains. Primers are used at an optimized concentration (commonly ~0.4–0.9 µM each) and the probe is labeled with a reporter dye (e.g., FAM) and a quencher (e.g., NFQ-MGB or TAMRA), allowing real-time fluorescence detection of amplification products.

- **One-Step vs. Two-Step RT-PCR:** The assay can be performed in either a one-step RT-PCR format or a two-step format, with each approach offering advantages. In one-step RT-qPCR, reverse transcription and PCR occur sequentially in the same tube using a combined enzyme mix, minimizing hands-on steps and lowering the risk of contamination, which is beneficial for time-sensitive diagnostics and high-throughput testing. In contrast, a two-step RT-PCR (separate cDNA synthesis followed by PCR) may offer slight performance gains in some cases. Both approaches are acceptable: one-step RT-qPCR is commonly used in clinical virology and, when properly optimized, provides rapid and specific detection.
- **Amplification Conditions:** Reverse transcription is generally performed at ~50 °C for ~10–30 minutes, followed by an initial polymerase activation (e.g., 95 °C for ~10–15 minutes) to inactivate reverse transcriptase and activate DNA polymerase. Thermal cycling is typically 40–45 cycles of denaturation (~95 °C for 5–15 seconds) and combined annealing-extension (~55–60 °C for 30–60 seconds). These conditions are tuned to the enzyme chemistry. Amplification is carried out on a validated real-time PCR thermocycler (e.g., ABI 7500/QuantStudio or Roche LightCycler, or equivalent) with fluorescence data acquisition on appropriate channels (e.g., FAM for the ANDV probe, and a different channel like VIC for the internal control probe in a multiplex assay).

Controls and Quality Assurance

- **Internal Extraction Control (IEC):** Each specimen must be processed with an exogenous control to ensure the entire process (extraction through amplification) is functioning properly. A non-target RNA (e.g., canine distemper virus RNA, bacteriophage MS2 RNA, or armored RNA) is spiked into the lysis buffer before extraction. This internal control is co-extracted and co-amplified in a separate detection channel, confirming that RNA extraction was successful and PCR inhibitors (e.g., heme, complex polysaccharides) are not present at levels that significantly impede amplification. A failure to detect the IEC in a sample while controls perform normally indicates possible extraction failure or PCR inhibition. Such samples must be re-extracted or diluted and re-tested.
- **Positive Control:** Each RT-PCR run must include a positive control containing ANDV-specific RNA to verify assay performance. This may be a plasmid construct carrying the target sequence, in vitro transcribed RNA, or a previously characterized positive clinical sample. The positive control is subjected to the same extraction (if using a whole process control) or included in the RT-PCR reaction as a purified RNA/cDNA template. It must yield a positive amplification with a cycle threshold (Ct) within the expected range (e.g., typically mid-20s for ~10^4 copies/reaction) for the run to be considered valid.
- **Negative Controls:** To monitor contamination, a no-template (water) control is included in each PCR plate, which should show no detectable amplification by the end of 40–45 cycles. Additionally, an extraction blank (e.g., an empty extraction with only lysis buffer and no sample) must be processed alongside clinical specimens through RNA extraction and subsequent PCR to ensure no cross-contamination has occurred. Any amplification in negative controls invalidates the run and triggers an investigation. Laboratory personnel must adhere to a unidirectional workflow (separating pre-PCR and post-PCR areas) and decontaminate surfaces with appropriate disinfectants (e.g., hypochlorite or ethanol) to prevent amplicon carryover.
- **Quality Assurance & Acceptance Criteria:** For each batch of tests, results must be accepted only if all control criteria are met: the positive control yields the expected positive result (with Ct within the predefined range), the internal control is detected in all negative and low-titer samples (ensuring no significant inhibition), and all negative controls show no amplification. If any control fails, the affected samples and runs are invalidated and repeated from extraction. Laboratories must follow Good Clinical Laboratory Practices and perform periodic proficiency testing and assay re-validation to ensure consistent performance.

Analytical Considerations Across Different Specimen Types

- **Viral Load and Sensitivity:** Andes virus is typically detected at highest concentration in blood, reflecting systemic viremia during early HPS. Consequently, blood (or its white-cell fraction) offers superior sensitivity for RT-PCR, as confirmed by clinical studies showing near-100% RT-PCR positivity in acute-phase blood samples. Respiratory

samples (NP swabs), saliva, urine and feces generally contain lower viral RNA levels, so their sensitivity is lower. A recent prospective study found ANDV RNA in only ~12–30% of acute-phase saliva/oral fluid samples, compared to almost all blood samples[18]. However, detection in these specimens can still be valuable for patient management and epidemiological investigations of person-to-person transmission, as infectious virus has been recovered from some NP, saliva, and urine samples during acute infection. To maximize yield, larger sample volumes or more sensitive methods (e.g., concentrating urine or combining swab eluates from both nares) may be used when testing non-blood fluids.

- **PCR Inhibition:** Different matrices present distinct challenges. Whole blood contains potential PCR inhibitors (e.g., hemoglobin and heme). These are largely removed by standard extraction, or by processing plasma/serum or buffy coat rather than unprocessed whole blood. Respiratory and saliva specimens may contain mucus, enzymes, or food particles that inhibit PCR. Appropriate sample preparation (e.g., use of mucolytic agents or dilution of viscous samples) and inclusion of an internal control in every reaction help in monitoring and mitigating these effects. Urine generally has fewer PCR inhibitors, but low cellularity can result in low nucleic acid yield, necessitating concentration or larger input volume as noted above.

Result Interpretation, Reporting, and Validation

- **Positive and Negative Result Criteria:** A sample is interpreted as RT-PCR positive for Andes virus if it produces an amplification curve that crosses the threshold (Ct value) within the validated cutoff (typically around Ct ≤35–38, depending on assay calibration). Extremely late signals approaching the limit of detection are considered equivocal and warrant repeat testing or confirmation by an independent target. A negative result (no Ct or Ct > cutoff) indicates that ANDV RNA was not detected in the sample. However, due to assay limits of detection, a negative RT-PCR does not completely exclude infection if clinical suspicion remains high. In such cases, it is recommended to confirm with serological testing (IgM/IgG for hantavirus) and/or obtain a repeat sample for RT-PCR on a subsequent day, as patients in the very early or late disease stages might have undetectable or declining viral RNA levels.
- **Reporting and Diagnostic Use:** In line with public health guidelines (e.g., WHO/PAHO HPS case definitions), a positive RT-PCR for Andes virus is considered confirmatory evidence of infection and qualifies a case as laboratory-confirmed HPS. Cycle threshold values are generally documented but not directly reported as viral load. Instead, results are typically reported qualitatively (Positive/Negative) with a comment if needed (e.g., high Ct indicating low-level viremia). Laboratories may interpret lower Ct values as reflecting higher viral RNA concentrations, which could correlate with disease severity in research settings.
- **Analytical Performance:** The assay has been validated for sensitivity, specificity, and precision. In one clinical validation, the RT-qPCR method demonstrated a limit of detection of approximately 10–12 copies of ANDV RNA per reaction (95% detection probability)[45]. Analytical sensitivity was ~95% in acute blood samples, with 100% specificity (no false-positives among negative controls) and high positive/negative predictive values. Reproducibility has been confirmed across different real-time PCR instrument platforms and operators, yielding consistent threshold cycle results. These performance characteristics underscore that real-time RT-PCR targeting ANDV's S segment is a robust and reliable tool for early diagnosis and investigation of Andes hantavirus infections.
- **Limitations**: False-negative results may occur in cases of very low viral load, improper specimen handling, or RNA degradation. Therefore, negative RT-PCR results should be interpreted with caution. If clinical suspicion for HPS remains, follow-up testing, including repeat RT-PCR or serology, is advised. Furthermore, while RT-PCR detects viral RNA, it does not directly indicate viral infectivity. Hence, results must be correlated with clinical context and other findings. Finally, cross-contamination is a critical concern in PCR-based diagnostics. Strict adherence to biosafety and molecular contamination control practices is essential to maintain the assay's high specificity and to prevent any false-positive results.

Specific Hantavirus RT-PCR protocols. ***WHO does not formally endorse any specific qRT-PCR protocols.***

Only a handful of commercial RT-PCR kits for hantavirus diagnosis are currently available. Most are geographically specialized (e.g., Old World vs New World strains) and differ in their coverage of Andes virus (ANDV). The table below summarizes all known commercially marketed RT-PCR kits for hantavirus with in vitro diagnostic (IVD) regulatory approval or certification in at least one major region (EU, China, etc.). Each kit's ANDV validation status is categorized as:

A – Explicitly validated for ANDV (ANDV is named in the intended use or product documentation)

B – Implicit coverage (intended for broad "HPS" or "hantavirus" detection, implying potential ANDV detection but without explicit mention)

C – Not validated/suitable for ANDV (targets other hantavirus types only, e.g., Old World species)

Table C1. Hantavirus RT-PCR kits with IVD regulatory approval or certification in at least one WHO region (not exhaustive)

Manufacturer	Kit Name	Target(s) / Intended Use	ANDV Validation	Regulatory Status	Specimen Types	Platform Compatibility	Availability
Genekam Biotechnology AG (Germany)	Andes virus (ANDV) Real-Time PCR Kit *Cat. FR546*	Specific detection of Andes orthohantavirus RNA (one-step RT-PCR)	A (Yes – explicit ANDV)	CE-IVD (Europe)	Validated on blood (e.g., buffy coat), plasma/serum (Manufacturer documentation, flexible to other tissues)	Open real-time PCR platforms (standard 96-well cyclers; e.g., ABI, Bio-Rad, Roche, etc.)	Worldwide (directly from Genekam & distributors)
Genetic PCR Solutions (GPS) (Spain)	Andes Hantavirus qPCR Kit	Specific detection of Andes hantaviruslinked to 2026 outbreak (cruise ship MV *Hondius* cluster)	A (Yes – explicit ANDV)	Pre-CE (IVD pending)	Blood serum/plasma (primary), other HPS-relevant clinical samples under validation	Open real-time PCR instruments (common 96/384-well qPCR platforms)	Initial release in *May 2026* to specialized diagnostic labs, broader distribution planned post-validation
Genekam Biotechnology AG (Germany)	Hantavirus Real-Time PCR Kit *(Broad hantavirus detection)*	Generic HantavirusRT-PCR, broad detection system (exact strains not disclosed)	B (Implicit – broad "Hantavirus")	CE-IVD (Europe)	Likely validated on blood and derivates (not explicitly limited, typical usage for suspected hantaviral disease)	Open platform (adaptable to standard qPCR cyclers)	Available globally (via Genekam's international distribution)
Shanghai ZJ Bio-Tech / Liferiver (China)	Hantavirus Pulmonary Syndrome RT-PCR Kit *(HPS broad detection, 2012)*	Qualitative RT-PCR for Hantavirus causing HPS (New World hantaviruses; specific strains not listed in IFU)	B (Implicit – broad "HPS")	CE-IVD (Europe, via EU rep Obelis S.A.)	Human serum (validated)	Open real-time PCRinstruments (validated on ABI, Bio-Rad, Qiagen, Roche, etc.)	Worldwide (widely distributed; older kit, still available via distributors)
GCC Biotech (India)	DiAGSure® Hantavirus Detection Kit *(Pan-HPS / HFRS detection)*	Real-time PCR assay covering HPS & HFRS (targets conserved hantavirus sequence, broad detection)	B (Implicit – broad "HPS/HFRS")	"Certified" (Indian domestic, details not publicly specified)	Whole blood, plasma (EDTA)	Open real-time PCR (validated across major platforms: ABI 7500, Bio-Rad CFX, Rotor-Gene, etc.)	Primarily India & nearby regions (local distribution, limited global visibility)
Jiangsu Bioperfectus Tech. (China)	Hantavirus Real Time PCR Kit *(HFRS–Old World Hantaviruses)*	Qualitative RT-PCR for HFRS-causing hantaviruses (Old World: Hantaan, Seoul, Puumala viruses)	C (No – old-world only)	CE-IVD (Europe, legacy IVDD), China NMPA	Human serum (validated)	Open real-time PCR platforms (multiple mainstream qPCR systems)	Global distribution via Bioperfectus partners (CE marking ensures EU availability)
Jiangsu Bioperfectus Tech. (China)	Sin Nombre Virus Real Time PCR Kit *(HPS–North American strain)*	Qualitative RT-PCR for Sin Nombre virus (SNV), a major HPS hantavirus in North America	C (No – SNV only)	CE-IVD (Europe, legacy IVDD)	Human serum (validated)	Open real-time PCR instruments (multi-platform compatible)	Marketed globally via Bioperfectus (targeted to N. America & int'l labs)

Tianlong (Xi'an) Biotech (China)	Hantaan/Seoul Virus PCR Detection Kit *(HFRS – Hantaan & Seoul viruses)*	Qualitative RT-PCR for HFRS viruses (Hantaan & Seoul)	C (No – Hantaan/Seoul only)	CE-IVD (Europe), NMPA (China)	Human serum/plasma; Urine & Saliva (validated as per kit specs)	Open real-time PCR platforms (e.g., ABI 7500; Tianlong Gentier; Roche LC, etc.)	Primarily in Asia & EU (Tianlong international distributors)

Research Use-Only Hantavirus RT-PCR Kits

The following RT-PCR kits are marketed as Research Use Only (RUO) and lack IVD certification, They are not approved for routine clinical diagnostics but may be used in research or during public health emergencies under special authorization. They are included here for completeness, as they demonstrate additional testing options. RUO kits are not subject to the rigorous validation and external regulatory review that IVD kits undergo. Their performance claims may rely on *in silico* design and limited internal testing. Use in actual diagnostics usually requires further validation or special authorization. Public health laboratories and research institutions often develop their own PCR protocols for hantavirus when commercial kits are lacking or unvalidated for local strains; this has been the case with ANDV historically, where national reference labs in Chile and Argentina employed in-house RT-PCR assays targeting the S gene (nucleocapsid) of ANDV.

Table C2. Reseach Use Only (RUO) Hantavirus RT-PCR kits (not exhaustive)

Kit Name (Manufacturer)	Intended Coverage / Special Notes	ANDV Coverage	Reg. Status
RealStar®Hantavirus-HPS RT-PCR Kit 1.0 *(altona Diagnostics, Germany)*	Qualitative RT-PCR for HPS hantaviruses (New World; no specific strains named in IFU)	B (Implicit – broad HPS incl. likely ANDV)	RUO (Not CE-marked)
RealStar®Hantavirus-HFRS RT-PCR Kit 1.0 *(altona Diagnostics, Germany)*	Qualitative RT-PCR (two-assay format) for Old World HFRS viruses (differentiates Hantaan, Seoul, Dobrava, Puumala)	C (No – Old World only)	RUO (Not CE-marked)
Hantavirus Real-Time PCR Kit *(Creative Diagnostics, USA)*	Qualitative RT-PCR for HFRS hantaviruses (Old World; Hantaan, Seoul, Puumala; implies Dobrava)	C (No – Old World only)	RUO (US – not FDA cleared)
Andes Virus Detection Kit *(Creative Biogene, USA)*	Designed for Andes virus RNA detection (broadly sensitive to ANDV strains)	A (Yes – explicit ANDV target)	RUO (Research-stage assay)
Hanta Virus One-Step RT-qPCR Kit *(Bioingentech, Chile)*	General Hantavirus screening assay (one-step RT-qPCR; broad detection)	B (Implicit – broad "Hantavirus")	RUO (ISO 13485-certified manufacturer; not CE-IVD)

3. Hantavirus Clinical Serology testing

- Performed by: Local Hospital / Isolation Facility or by Central Reference Microbiology Laboratory, as per local policy.
- Purpose:
    - Primary: Clinical monitoring and management. Detect seroconversion
    - Secondary: Monitor IgM/IgG dynamics in a Research context
- Timing:
    - Primary: Prospective, Real-time feedback to clinicians
    - Secondary: Can be done retrospectively, to minimize acute burden, as per local policy
- Methods:

Serum (or plasma) samples from participants will be tested for Andes virus-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) using validated serological assays (e.g., enzyme-linked immunosorbent assays, ELISA), performed at a central laboratory. These assays detect acute-phase IgM and later IgG antibodies against ANDV antigens to confirm infection and determine infection stage, in line with HPS case definitions, where detection of anti-hantavirus IgM or

seroconversion to IgG constitutes laboratory confirmation. Longitudinal IgM/IgG testing of serial samples (obtained per the Schedule of Events) is designed to capture the timing of seroconversion relative to first detectable viral RNA (P0) and symptom onset (S0). The appearance of IgM typically marks a recent or acute infection, often emerging around or shortly after S0, whereas the development of IgG indicates immune maturation and longer-term immunity (convalescence). By aligning serology positivity with trigger events (enrollment/exposure [X0/E0], P0, S0) and follow-up visits, we will map the kinetics of antibody responses, including time from P0 to IgM detection and time from P0 or S0 to IgG seroconversion. Monitoring the persistence of IgG titers over subsequent months further allows assessment of the durability of humoral immunity in survivors. Together, these serological analyses complement molecular diagnostics by providing insights into when and how quickly the adaptive immune response arises after exposure, and by enabling correlation of antibody dynamics with viral clearance and clinical outcomes (e.g., whether timely, robust IgM/IgG responses are associated with less severe disease or rapid viral control).

A more detailed evaluation of immune response functionality will be conducted at a central research laboratory (see Annex D, Research Laboratory Investigations) by measuring the neutralizing activity of antibodies using a complementary set of pseudovirus-based and live infectious virus neutralization assays.

Annex D: Advanced Research Laboratory Investigations

To be performed in proficient research labs within the NAVIS Network.
For NAVIS sites without access to such investigations locally, they are also available at the irsiCaixa Research Institute, Badalona, Spain, upon request. Contact persons: Dr. Julià Blanco, jblanco@irsicaixa.es, and Dr Núria Izquierdo-Useros: nizquierdo@irsicaixa.es

1. Andes Virus Neutralization Assays

***Purpose**: To measure the development and strength of neutralizing antibody responses against Andes virus in participants over time, providing a functional readout of humoral immunity. To Identify when protective antibodies arise relative to first viral detection (P0) and symptom onset (S0), and to exploring whether early neutralization activity correlates with milder disease or reduced viral shedding*

- **Reporter pseudoviruses.** HIV reporter pseudoviruses expressing ANDV Gc/Gn protein and Luciferase will be generated by cotransfection of the plasmid pNL4-3.Luc.R-E- (obtained from the National Institute of Health (NIH) AIDS Reagent Program) and the plasmid expressing encoding the ANDV glycoprotein (isolate Chile-9717869) inserted into pcDNA3.1(+). Expi293F cells will be co-transfected using ExpiFectamine 293 Reagent (Thermo Fisher Scientific). Control pseudoviruses will be obtained by replacing the Gc/Gn protein expression plasmid with a VSV-G protein (Vesicular Stomatitis Virus G protein) expression plasmid as described[46]. Supernatants will be harvested 72 hours after transfection, filtered at 0.45 µm, frozen, and titrated on VeroE6 cells. Serial dilutions of the pseudoviral stock will be prepared in quadruplicate in a 96-well plate using DMEM medium supplemented with 10% fetal bovine serum (D10). Then, 10,000 cells per well will be infected and incubated at 37ºC for 2 days. After incubation, 100 µL of medium will be removed, and 50 µL of Britelite Plus (Revvity), a luciferase substrate, will be added to quantify the activity of the luciferase from infected cells will be measured with a Microplate Reader Ensight (Perkin) and expressed in relative light units (RLUs).
- **Pseudovirus-based neutralization assay (PBNA).** Plasma samples will be heat-inactivated (56ºC 30 min) before analysis. Samples will be serially diluted in D10 medium, at least six different dilutions will be tested (ranging from 1/20 to 1/14580) and incubated in duplicate wells with 50 µL of luciferase reporter pseudoviruses (targeting 20,000 RLUs based on prior titration). After incubation at 37ºC for 45 minutes, mixtures will be used to infect a total of 10,000 VEroE6 cells/in D10 medium in a final volume of 250 uL. After 2 days of incubation at 37ºC, 150 µL of medium will be removed from each well and luminiscance will be measured as described above. The inhibition values will be normalized, and the IC_{50} (concentration inhibiting 50% of the infection) will be calculated by plotting and fitting all duplicate neutralization values against the reciprocal plasma dilution to a 4-parameter equation in Prism v10 (GraphPad Software, USA).
- **Viral neutralization assay (VNA) against ANDV.** ANDV from EVAg repository will be titrated to induce a 40%–80% viral-induced cytopathic effect on Vero E6. One hundred microliters of heat inactivated plasmas were added in duplicate wells, followed by serial 1/3 dilutions. The mix was added to 6 x104 Vero E6 cells per well seeded in triplicate in 96-well plates. To detect any plasma-associated cytotoxic effect, Vero E6 cells were equally cultured in the presence of the same antibody dilutions. Viral-induced and plasma induced cytopathic effects were measured 72 h postinfection using the CellTiter-Glo luminescent cell viability assay (Promega). Luminescence was measured in a Fluoroskan Ascent FL Luminometer (Thermo Fisher Scientific). For IC50 calculation, RLUs were normalized to the no virus condition set as 100% viability, and dose–response curves of Log10 were adjusted to a nonlinear fit regression model calculated with a four-parameter equation in Prism v10 (GraphPad Software, USA).

2. T-cell Immunity against Andes Virus

***Purpose**: To measure and characterize the development of Andes virus-specific T cell responses in participants, capturing how CD4⁺ and CD8⁺ T cells become activated over time, with special focus on immune inflection points around initial exposure (X0), first detectable viral RNA (P0), and symptom onset (S0). This comprehensive T-cell assessment aims to elucidate adaptive immune dynamics and explore whether the magnitude or timing of the T cell response correlate with infection control, disease severity, and other clinical outcomes.*

- **Elispot.** Briefly, PBMCs will be stimulated overnight with recombinant Hantavirus proteins (5ug/ml for each protein) or PHA (15 µg/ml) as the positive control. 100,000 cells per well will be stimulated with the different antigens in triplicates for 24h to reliably capture CD4 and CD8 T cell responses. Spot Forming Cells (SFC) will be determined by Fluorospot, using in house capacities at Irsicaixa. The validation of these assays and positive responses will be based on cell samples viability (>80%), assay background (media only) (<50 SFC/106 PBMC), positive responses against PHA-P (>500 SFC/106 PBMC), and positive ELISpot responses (>50 SFC/106 PBMC and at least ≥3-fold over media control).
- **Flow cytometry:** PBMCs will be stimulated with single viral proteins in duplicate wells using 0.5m PBMC per stimulation/well and incubated for 16 hours. In the immune cell sorter (ICS), we will combine T-cell lineage markers (CD3, CD4, CD8, CD45, CCR7, CD27), activation inducible markers (AIMs: OX40, CD25 and CD137) and cytokines (IL2, IFNg, TNF). Samples will be acquired on an LSR Fortessa cytometer (BD) at the IGTP/IDIBAPS Cytometry Platforms, data analysed using FlowJo software v10 and polyfunctional cytokine profiling represented using SPICE v6 (NIAID, NIH). The analysis will determine the frequency and the effector function profile of each response based on the possible combinations of cytokine expression or combinations of differentiation marker expression. In addition, cluster analysis and principal components analysis at the single-cell level will be performed using Flowjo software (V10) and Prado´s Lab developed R pipeline. Background responses detected in negative-control tubes without antigen stimulation will be subtracted from those detected in stimulated samples for every specific functional combination.
- **ELISPOT epitope mapping.** Synthetic overlapping peptides (15mers with 11 amino acids overlap, covering the individual viral protein sequences), will be generated and tested in IFNg ELISpot assays, initially in matrix pool designs well established at Irsicaixa and then by single peptide deconvolution Elispot to define the single reactive OLP. The number of targeted peptides will then be related to disease course and effector functions defined by flow cytometry, as well as linked to subsequent single cell analyses (including transcriptomics and TCR repertoire breadth) and other immune parameters of humoral immunity.
- **Single cell analyses (to be considered in future exploratory analyses):** For single cell transcriptomics, Hantavirus protein- and OLP-specific T cells will be isolated by employing an activation-induced marker assay that detects activation markers (including CD25, CD69, CD137) on antigen reactive T cells which then allows for single cell sorting. Analyses will be performed using Massively Parallel RNA Single-Cell Sequencing (MARSseq) and SMARTseq2 on single PBMC derived T cells obtained at the different sampling time point by 10X single cell platform. Principal component analysis will be performed with the regularized log transformation of the counts for the topmost 500 variable genes using the 'prcomp' function and the ggplot2 library from R. Differential expression analysis will be performed with DESeq2 with default parameters. Functional enrichment of the differentially expressed genes will be performed with g:Profiler. The data will be compared longitudinally to immune and disease markers in infected and exposed individuals and correlated to the presence, titers, isotype usage and avidity of the vaccine-induced humoral response.

3. Andes virus isolation from clinical samples

***Purpose**: To attempt live-virus culture from participants' clinical samples that test positive (or suspected) for Andes virus. This procedure confirms the presence of infectious virus in different specimens, providing direct evidence of viral shedding and transmission risk in various compartments, and yields viral stocks for detailed virologic analysis (e.g., sequencing) relevant to the outbreak strain.*

We will inoculate pharyngeal or other swab samples that have either a positive detection of ANDV DNA by PCR or negative results into T25 culture flasks with Vero E6 cells. 1.5 x 106 cells will be inoculated with 1 mL of the liquid sample for 1 h at 37 °C and 5% CO2. Then we will add 4 mL of 2% FCS-supplemented DMEM containing 100 U/mL penicillin, 100 µg/mL streptomycin and 2.5 µg/mL amphotericin B (all from Thermo-Fisher Scientific). We will maintain cells in incubation and assess them daily for cytopathic effect (CPE) in order to be able to harvest the supernatant, which will be centrifuged at 410 g for 5 min to remove cell debris and stored at −80 °C. We will propagate the virus for two passages and collect the supernatant. We will titrate the viral stock and confirm the infection by the presence of viral antigens using PCR. As a positive control, we will employ the ANDV stock from the EVAg repository. As a negative control, we will include mock-treated cells. We will assess viral cultures daily and keep them for 14 days or until 50% CPE is observed. In cases where we detect CPE, we will harvest the supernatants, centrifuge them at 410 g for 5 min to remove cell debris, and store them at −80 °C. If we detect no CPE, the cells will remain in culture until reaching a confluent state, when we will pass half of the cells with supernatant from the previous culture to new flasks and add antibiotics and amphotericin B. We will discard any cultures with undesired microorganism growth and will not consider them for the analysis. We will follow the cultures for up to 14 days.

4. Antiviral activity against Andes Virus

***Purpose**: To evaluate candidate antiviral compounds for their ability to inhibit Andes virus replication in vitro, using cell-based cytopathic effect assays. This informs NAVIS's translational aims by identifying potential early interventions that could limit viral growth, supporting the treatment preparedness in an outbreak with no established specific therapies.*

Increasing concentrations of antivirals will be added to 60~10^4 Vero E6 cells per well seeded in 96-well plates and, immediately after, the cells will be infected with ANDV titrated to induce a 50% or above 70% CPE. Untreated non-infected cells and untreated virus-infected cells will be used as negative and positive controls of infection, respectively. To detect any drug-associated cytotoxic effect, Vero E6 cells will be equally cultured in the presence of increasing drug concentrations, but in the absence of virus. Cytopathic or cytotoxic effects of the virus or drugs will be measured 3 days post-infection, using the CellTiter-Glo luminescent cell viability assay (Promega). Luminescence will be measured in a Fluoroskan Ascent FL luminometer (ThermoFisher Scientific).

5. Andes Virus Whole Genome Sequencing

***Purpose**: To sequence the full Andes virus (ANDV) genomes from participant samples, confirming the outbreak viral strain's identity and determining genetic relatedness among cases for transmission chain analysis. This will reveal within-host and between-host viral diversity across key time points (X0/E0/P0/S0), helping to map transmission links and explore whether any viral mutations arise that relate to differences in disease severity or immune escape.*

- **Scope.** This protocol describes two sequencing approaches for the generation of complete ANDV genomes directly from inactivated RNA extracts derived from clinical samples: targeted amplicon-based sequencing and untargeted cDNA-based sequencing, both compatible with Oxford Nanopore Technologies (ONT) and Illumina sequencing platforms. The experimental and bioinformatic analyses described in this protocol are subject to modification and optimization based on ongoing evaluation and accumulated experience.
  The workflows are prioritized in this protocol according to their suitability for routine genomic surveillance, considering turnaround time, technical performance, and scalability. ONT-based workflows are prioritized due to the rapid response application and the real-time sequencing capabilities. Also, due to the low viral loads and the tri-segmented ANDV genome (S, M, and L), untargeted approaches often yield suboptimal genome completeness and uneven segment representation. Therefore, amplicon-based sequencing is recommended as the primary approach for

complete genome recovery, whereas cDNA-based methods may serve as complementary approaches in specific applications.

ANDV belongs to the Orthohantavirus andesense species within the Orthohantavirus genus, a group of negative-sense single-stranded RNA (ssRNA–) viruses in the Hantaviridae family that infect both rodents and humans (https://ictv.global/report/chapter/hantaviridae/hantaviridae). ANDV is an enveloped virus with a segmented ssRNA– genome composed of three segments (https://viralzone.expasy.org/213): (i) S, Small (encodes the nucleocapsid protein N); M, Medium (encodes the envelope glycoproteins Gn and Gc), and L, Large (encodes the RNA-dependent RNA polymerase RdRp).

- **Responsibilities.** Laboratory technicians are responsible for following this SOP to ensure consistent, high-quality genomic data for surveillance.

- **Safety considerations, contamination control, and sample pre-processing requirements**. The procedure is indicated to work with inactivated RNA extracts of clinical samples positive for ANDV, avoiding the need to work on biosafety conditions. Physical separation of pre-PCR (pre-amplification) and post-PCR (post-amplification) areas is mandatory to avoid cross-contamination, and a negative control with only reagents should be included to monitor the whole process. Ideally, to minimize the presence of human DNA and RNA in clinical samples prior to sequencing, a sample filtration step should be performed to remove human cells before viral nucleic acid extraction, followed by a DNase treatment step after extraction to eliminate residual DNA.

Nanopore amplicon sequencing protocol

References: [21,47]

- **Procedure**

- ***Reverse transcription of viral RNA:*** A two-step protocol is followed in order to amplify only viral RNA. For so, viral RNA (vRNA)-specific primers are pooled for cDNA synthesis using SuperScript IV First-Strand Synthesis System (Invitrogen, ref. 18091050). First, genomic and host DNA are digested with the ezDNase™ Enzyme kit (Invitrogen, ref. 11766051) mixing 1 µL 10x ezDNase buffer, 1 µL ezDNase enzyme up to 8 µL of template RNA and filling up to 10 µL of final volume with nuclease-free water. gDNA is digested with an incubation at 37ºC for 2 min, and the reaction is inactivated with an incubation at 55ºC for 5 min in presence of 10 mM DTT (Invitrogen, ref. R0861). The reaction is placed on ice until the next step is prepared. The reaction for the first-strand synthesis consists on the following mix: 1 µL of 2 µM pooled vRNA specific primers, 1 µL 10 mM dNTP mix (10mM each), 10 µL prior reaction RNA, 1 µL DEPC-treated water. Incubate at 65ºC for 5 min, and leave in ice at least 1 min. While on ice, the following mix will be prepared and added to the prior reaction: 4 µL 5x SSIV buffer, 1 µL 100 mM DTT, 1 µL ribonuclease inhibitor and 1 µL SuperScript IV Reverse Transcriptase 200 U/µl. This mix will be incubated at 55ºC for 10 min and inactivated at 80ºC for 10 min. Whole genome amplification will follow immediately.
- ***Whole genome amplification:*** A combination of specific primers previously published by Taylor et al. (2020) and Ulloa-Zepeta et al. (preprint 2026, Supplementary Table 4) are used with the Phusion™ High-Fidelity PCR Master Mix with HF Buffer (NEB, ref. M0531) in three different reactions for the three different pools of primers. Each mix is as follows: 1,25 µL of 10 µM pooled forward primers, 1,25 µL of 10 µM pooled reverse primers, 12,5 µL of 2x Phusion Master Mix, 7 µL of the prior reaction of cDNA and fill-up to 25 µL of nuclease-free water. PCR conditions for the three reactions are 98ºC for 30 s, 30x (98ºC 10s, 60ºC 30s, 72ºC 30s), 72ºC 10 min and hold at 10ºC. Each resulting pool of amplicons is purified using 0.8× Agencourt AMPure XP beads (Beckman Coulter, ref. A63881), quantified using a Qubit™ 2.0 Fluorometer (Invitrogen), and pooled at equimolar concentrations for downstream library preparation.
- ***Library preparation and sequencing:*** Libraries are prepared using the Native Barcoding 201 Kit 24 V14 (ONT, ref. SQK-NBD114.24), according to the manufacturer's instructions. Sequencing libraries are loaded onto an MK1D device using a R10.4.1 flow cell.

- ***Bioinformatic analyses:*** Raw signal data is basecalled using Dorado software in super-accurate mode (dna_r10.4.1_e8.2_400bps_sup@v4.3.0 model). Sequencing metrics were assessed using NanoStats (v1.4.0)21. Adapter trimming is performed with Porechop (v0.2.4)22. High-quality reads with a (Q-score > 10) length over 500 bp, filtered with NanoFilt (v2.8.0)21, are mapped to the ANDV reference sequence using Minimap2 (v 2.24-r1122). Primer sequences are trimmed using iVar (v1.4.2)24 and then the BAM files are used to generate consensus sequences using iVar with a Q>20, 20 and 0.6 as minimum read depth and minimum frequency threshold respectively.

Nanopore cDNA sequencing protocol

Reference: [48]

To date, Nanopore sequencing of ANDV has been reported exclusively using amplicon-based approaches, and PCR free cDNA or direct RNA sequencing has not yet been demonstrated for this virus. However, the following information will be considered as an alternative approach if the amplicon-based method does not perform adequately.

- **Procedure**

- ***cDNA Synthesis:*** Reverse transcription is performed in a 20 µL reaction by combining 16 µL of extracted RNA with 4 µL of LunaScript™ RT SuperMix (NEB, ref. E3010) in a 0.2 mL thin-walled PCR tube. The reaction is gently mixed, briefly centrifuged, and incubated in a thermal cycler under the following conditions: primer annealing at 25 °C for 2 minutes, cDNA synthesis at 55 °C for 10 minutes, heat inactivation at 95 °C for 1 minute, followed by a hold at 4 °C.
- ***Second-Strand Synthesis:*** Second strand synthesis is carried out using Sequenase Version 2.0 DNA Polymerase (ThermoFisher, ref. 15809896). A first Sequenase master mix is prepared consisting of 5× Sequenase Reaction Buffer, nuclease free water, and Sequenase polymerase, and 10 µL of this mix is added to each cDNA reaction. Samples are mixed, briefly centrifuged, and incubated at 37 °C for 8 minutes. A second Sequenase master mix, containing 0.9 µL Sequenase Dilution Buffer and 0.3 µL Sequenase polymerase, is then prepared and added to the reaction. After mixing and centrifugation, the reaction is incubated again at 37 °C for 8 minutes. Upon completion, samples are transferred to clean 1.5 mL Eppendorf DNA LoBind tubes for purification using 45 µL Agencourt AMPure XP beads (Beckman Coulter, ref. A63881), quantified using a Qubit™ 2.0 Fluorometer (Invitrogen), and pooled at equimolar concentrations for downstream library preparation.
- ***Library preparation and sequencing:*** The Rapid PCR barcoding kit is used (ONT, ref. SQK-RPB004). Briefly, 3 µL of template DNA (1–5 ng) is combined with 1 µL of Fragmentation Mix, and tagmentation is performed in a thermal cycler at 30 °C for 1 minute, followed by 80 °C for 1 minute. PCR reactions are prepared by combining nuclease free water, 4 µL of tagmented DNA, 1 µL of Rapid Barcode Primer at 10 µM, and 25 µL of LongAmp™ Taq 2× Master Mix (NEB, ref. M0287), for a final volume of 50 µL. Reactions are incubated under the following conditions: 95 °C for 3 min, 30 cycles of 95 °C for 15 s, 56 °C for 15 s, and 65 °C for 4 min, Final extension at 65 °C for 4 min, Hold at 4 °C. After amplification, samples are transferred to DNA LoBind tubes and purified using 0.6× AMPure XP beads. Libraries are quantified using a Qubit™ fluorometer. Barcoded libraries are pooled at the desired ratios to obtain a total of 50–100 fmol in 10 µL, and 1 µL of Rapid Adapter is added to the pooled library. The reaction is mixed gently, centrifuged briefly, and incubated for 5 minutes at room temperature. Sequencing libraries are loaded onto an MK1D device using a R10.4.1 flow cell.
- ***Bioinformatic analyses*** (to be developed): See https://nanoporetech.com/document/viral-metagenomics-RNA-DNA

Illumina Amplicon sequencing protocol

References: [21,47]

- **Procedure**

- ***Reverse transcription of viral RNA:*** A two-step protocol is followed in order to amplify only viral RNA (vRNA). For so, vRNA-specific primers are pooled for cDNA synthesis using SuperScript IV First-Strand Synthesis System (Invitrogen,

ref. 18091050). First, genomic and host DNA are digested with the ezDNase™ Enzyme kit (Invitrogen, ref. 11766051) mixing 1 µL 10x ezDNase buffer, 1 µL ezDNase enzyme, up to 8 µL of template RNA and filling up to 10 µL of final volume with nuclease-free water. gDNA is digested with an incubation at 37ºC for 2 min, and the reaction is inactivated with an incubation at 55ºC for 5 min in presence of 10 mM DTT (Invitrogen, ref. R0861). The reaction is placed on ice until the next step is prepared. The reaction for the first-strand synthesis consists on the following mix: 1 µL of 2 µM pooled vRNA specific primers, 1 µL 10 mM dNTP mix (10mM each), 10 µL prior reaction RNA, 1 µL DEPC-treated water. Incubate at 65ºC for 5 min, and leave in ice at least 1 min. While on ice, the following mix will be prepared and added to the prior reaction: 4 µL 5x SSIV buffer, 1 µL 100 mM DTT, 1 µL ribonuclease inhibitor and 1 µL SuperScript IV Reverse Transcriptase 200 U/µl. This mix will be incubated at 55ºC for 10 min and inactivated at 80ºC for 10 min. Whole genome amplification will follow immediately.

- ***Whole genome amplification:*** A combination of specific primers previously published by Taylor et al. (2020) are used with the Platinum SuperFi PCR Master Mix (Invitrogen, ref. 12358010) in three different reactions for the three different pools of primers. Each mix is as follows: 1,25 µL of 10 µM pooled forward primers, 1,25 µL of 10 µM pooled reverse primers, 12,5 µL of 2x Platinum SuperFi PCR Master Mix, 7 µL of the prior reaction of cDNA and fill-up to 25 µL of nuclease-free water. PCR conditions for the three reactions are 98ºC for 30 s, 20x (98ºC 10s, 64,6ºC 10s, 72ºC 30s), 72ºC 5 min and hold at 10ºC. Each resulting pool of amplicons is purified using 0.8× Agencourt AMPure XP beads (Beckman Coulter), quantified using a Qubit™ 2.0 Fluorometer (Invitrogen), and pooled at equimolar concentrations for downstream library preparation.
- ***Library preparation and sequencing:*** Library preparation is performed using Nextera XT DNA Library Preparation Kit (Illumina, ref. FC-131-1096) following the manufacturer's protocol with the exception that tagmentation time is extended to 10 min and the final resuspension volume is in 15 µl resuspension buffer. Library concentrations are measured on the Qubit 4 Fluorometer (Invitrogen) and equimolar normalized to 4 nM, and sequencing is performed using a MiSeq platform.
- ***Bioinformatic analyses:*** Raw FASTQ are downloaded from Illumina BaseSpace. Quality is assessed with FastQC and MultiQC tools. Adapters and low-quality bases are trimmed using Trimmomatic, applying the IlluminaClip and SlidingWindow methods. Processed reads are aligned using BWA-MEM, using a reference genome. Finally, consensus sequences are generated using ivar consensus (Q > 30, depth >400x) and aligned with Clustal Omega. Phylogenetic trees are constructed using IQ-TREE, with ModelFinder for model selection. Bootstrap support is based on 1000 maximum-likelihood replicates.

Illumina cDNA sequencing protocol

Reference: [49]

- **Procedure:**

- ***cDNA generation:*** First strand cDNA synthesis is performed using the SuperScript™ IV First Strand Synthesis System (Invitrogen, ref. 18091050). For each reaction, 8 µL of RNA is combined with 1 µL of 10 mM dNTP mix, 3 µL of nuclease free water, and 1 µL of random hexamers. The mixture is incubated at 65 °C for 5 minutes and then cooled on ice for at least 1 minute. A master mix containing 4 µL of 5× SuperScript™ IV Buffer, 1 µL of DTT, 1 µL of RNase inhibitor, and 1 µL of SuperScript™ IV Reverse Transcriptase is prepared, and 7 µL is added to each sample. Reactions are incubated sequentially at room temperature for 10 minutes, 50 °C for 20 minutes, and 80 °C for 10 minutes. Following reverse transcription, 1 µL of RNase H (Thermofisher Scientific, ref. 18021071) is added to each reaction and incubated at 37 °C for 20 minutes. Second strand cDNA synthesis is performed using the NEBNext® mRNA Second Strand Synthesis Module (NEB, ref. E6111L) according to the manufacturer's instructions. Double stranded cDNA is subsequently purified using the QIAquick PCR Purification Kit (Qiagen, ref. 28104) following the manufacturer's protocol.
- ***Library preparation and sequencing:*** Sequencing libraries are generated using the Nextera XT library prep kit (Illumina, ref. FC-131-1024) according to the manufacturer's protocol Library concentrations are measured on the Qubit 4 Fluorometer (Invitrogen) and equimolar normalized to 4 nM, and sequencing is performed using a MiSeq platform.

- ***Bioinformatic analyses:*** PhiX control reads are removed using BBDuk, followed by adapter removal and quality trimming using fastp. Taxonomic classification is performed using Kraken2 in combination with Centrifuge. Reads classified as viral, as well as unclassified reads, are retained for downstream analysis, while non-viral reads are excluded. De novo genome assembly is performed using Shovill, with Unicycler applied to improve assembly continuity. In parallel, reference-based mapping is conducted using Snippy, with reads aligned against reference Andes virus genomes. Consensus sequences generated from de novo assembly and reference-based mapping are compared using MAFFT multiple sequence alignment.

Annex E: Specimen Handling Guidance and Biosafety Requirements for Andes Virus

Scope and Principles: This annex is provided as general orientation for investigators and site personnel to support safe and consistent implementation across NAVIS sites. It does not replace, supersede, or reduce any site's legal, institutional, or competent-authority requirements. In every country, all applicable national and international regulations, standards, and guidance must be strictly followed. This annex provides comprehensive biosafety guidance for handling Andes hantavirus (ANDV) specimens across all phases of the NAVIS study. It applies to all participating sites and personnel, covering specimen collection, bedside handling, labeling, intra-facility transport, laboratory processing (diagnostic and research), storage/biobanking, shipping, and waste disposal. Key principles include biosafety-by-design (minimizing risk through inherently safer workflows), rigorous containment, validated inactivation before de-escalating precautions, strict chain-of-custody tracking, and adherence to international and national biosafety standards (aligned with WHO, ECDC, and comparable frameworks). Responsibilities are shared by all staff: each individual must follow these guidelines, and site investigators and biosafety officers must ensure compliance through training, supervision, and periodic review. "Detailed guidance is provided in the WHO Laboratory Biosafety Manual, 4th edition: https://www.who.int/publications/i/item/9789240011311

Biosafety Containment Requirements: Andes virus is a WHO Risk Group 3 pathogen requiring high-level containment. Biosafety Level 3 (BSL-3) containment is the default requirement for all work involving potentially infectious materials, i.e., any clinical or environmental specimen that may contain viable ANDV, as well as all cultures, manipulations, or assays using live virus. Only after an appropriate, validated inactivation procedure has been completed and documented, may materials be handled at BSL-2. This strict approach is consistent with international best practices and reflects the hazardous nature of ANDV (capable of causing life-threatening disease in humans) and its rare but documented person-to-person transmissibility. By default, no procedures with ANDV or ANDV-containing specimens are to be conducted at BSL-2, even with "enhanced" practices, unless and until the agent is confirmed inactivated. National or local regulations that differ (for example, some diagnostic guidelines allowing BSL-2 with special precautions) must be addressed via documented risk assessment and explicit approval, ensuring they do not compromise the overarching safety standard. In all cases, the more stringent requirement (protocol vs. local regulation) must be followed.

Minimum Biosafety Measures by Workstream: Table F1 summarizes biosafety and specimen-handling requirements for each major workstream in the study, from clinical sampling to laboratory analysis and storage. All personnel must be trained and competent in these precautions before handling any study specimens.

Table F1. Biosafety requirements by workstream.
Each row describes specific specimen categories and procedural contexts, with the minimum containment level and personal protective equipment (PPE) to be used, any permissible conditions to step down precautions (e.g., after inactivation), and key governance controls (documentation, training, etc.). BSL-3 containment is required for all operations with live Andes virus or potentially infectious material, step-down to BSL-2 is allowed only after completing a validated inactivation process and under controlled conditions as outlined.

Workstream / Context	Specimen Types & Infectious Risk	Containment Level (Min.)	PPE Requirements (summary)	Allowable Step-Down Conditions	Key Controls & Documentation
A. Clinical sampling & bedside handling	Patient's blood, respiratory swabs, urine, feces, saliva, etc. collected from suspected or confirmed ANDV cases, fresh and	Isolation precautions equivalent to BSL-3: samples collected in a controlled environment (e.g. airborne isolation room if	At minimum: fit-tested respirator (N95 or higher) for aerosol risk, eye/face protection, impermeable gown or coverall, double gloves, shoe covers. Use puncture-	None during collection. All samples must be sealed in leak-proof containers and surface-decontaminated before leaving patient area.	Clinical staff must follow infection control SOPs. Use buddy system for donning/doffing PPE. Label specimens with biohazard symbol and study ID at collection. Record chain-of-custody from collection to lab.

	thus potentially infectious	available) with full barrier precautions	resistant sharps handling. All PPE donned before patient contact and doffed safely after.		
B. Diagnostic laboratory (e.g. RT-qPCR, general assays)	Human specimens (blood, respiratory fluid, tissues) containing live virus unless inactivated by lysis buffer	BSL-3 until verified inactivation. All pre-inactivation steps (aliquoting, nucleic acid extraction setup, etc.) in Class II BSC inside BSL-3 laboratory	BSL-3 PPE: solid-front gown or suit, double gloves, eye protection, appropriate respiratory protection (powered air-purifying respirator or N95 at minimum). All work inside BSC, with centrifuge safety cups for any centrifugation.	After adding a validated chemical lysis buffer or other proven inactivation step (per SOP), material may be transferred to BSL-2 laboratory for downstream analysis. Transition requires written verification that inactivation conditions (e.g., chaotropic agent contact time) are met.	Document each inactivation (reagent, method, time) in a log. Lab manager must authorize any transfer to BSL-2 post-inactivation. Maintain access control — only trained, approved staff handle specimens. Routine decontamination of surfaces and instruments after use.
C. Serology & immunoassay laboratory	Serum, plasma, or other fluids from participants (potentially infectious prior to processing)	BSL-3 for all live specimen handling (pipetting, aliquoting, preparing dilutions). If heat-inactivation (e.g. 56°C for 30 min) or chemical treatment is used to render serum non-infectious, subsequent assays can be done at BSL-2.	Similar to BSL-3 PPE as above: double gloves, gown, eye protection, respiratory protection if splash or aerosol potential. Work in BSC for open tube manipulations.	Heat-inactivated or chemically inactivated sera may be handled at BSL-2 only after completion of the inactivation protocol and cooling, with a documented procedure and controls confirming effective viral kill.	Inactivation procedure must be validated (e.g. by literature or testing) for ANDV. Records of each batch inactivation are retained. Use universal precautions for all human-derived materials: even at BSL-2 post-inactivation, handle with gloves and lab coat.
D. Research virology (virus culture, neutralization assays, etc.)	Live ANDV propagation in cell culture; neutralization tests (mixing live virus with serum); any work intentionally handling live virus (high titer)	BSL-3 (strict). No step-down to BSL-2 at any stage since live virus is present throughout. Large-scale culture volumes may require additional High-containment measures (BSL-4-level precautions) by risk assessment if exceeding routine amounts	BSL-3 PPE plus enhanced respiratory protection (e.g., PAPR recommended for high-titer culture work). Full BSL-3 practices: BSC for all open handling; sealed rotors/cups for centrifugation; no sharps unless absolutely necessary.	None. All activities remain in BSL-3 containment until virus is completely inactivated or stored in a secured BSL-3 freezer. Neutralized samples (after mixing with antibody) are still handled as infectious until proven otherwise.	Only staff with specialized training in BSL-3 viral culture techniques may perform this work. Required authorized protocol approved by institutional biosafety committee. Daily disinfection of incubators, centrifuges, and work surfaces; all liquid waste disinfected (e.g., with bleach) before disposal
E. Genomics & sequencing	Clinical or culture samples processed for viral RNA sequencing; typically involves nucleic acid extraction (potentially	BSL-3 for pre-extraction handling identical to Workstream B. If standard extraction with chaotropic lysis	BSL-3 PPE for any work pre-inactivation. Once extracts are confirmed non-infectious, standard molecular biology PPE at BSL-2 (lab coat, gloves, eye	Same step-down condition as diagnostic PCR: allowed only post-extraction/inactivation. No live viral cultures are handled in this workstream, so	Rigorous sample labeling to distinguish "live" vs "inactivated" material. Use separate work areas for pre- and post-inactivation. Document movement of samples from BSL-3 to BSL-2 zones. Dispose all

	inactivating) and library prep	is used, that step must occur in BSL-3. Prepared nucleic acids (no live virus) can then be handled at BSL-2.	protection) is acceptable. Sequence instruments (e.g., sequencers) can be located in BSL-2 only if only inactivated RNA libraries are loaded.	BSL-2 stage is permissible only after complete lysis of all infectious material.	extraction waste and consumables as infectious until decontaminated.
F. Environmental sampling (air, surface swabs)	Air filter units, surface swabs, rodent droppings or other environmental samples from outbreak settings – potentially containing live virus but often in low quantities	BSL-3 for laboratory handling of any field-collected environmental sample. Field collection itself must follow enhanced precautions (PPE similar to patient care if in high-risk setting).	Field collection PPE: respirator (P2/N95 or higher), eye protection, gloves, coveralls. Laboratory PPE: treat like infectious clinical samples (BSL-3 PPE and BSC). Avoid dry actions that could aerosolize dust from samples; moisten swabs if needed to minimize dust.	If environmental samples are processed by directly adding them into lysis buffer in the field or upon receipt (thus inactivated), subsequent molecular analysis can occur at BSL-2. Otherwise, maintain BSL-3 until any agent is inactivated or disposed.	Field teams must be trained in safe sampling and sample packaging. Samples transported double-contained (e.g. swab in tube, in sealed secondary bag with disinfectant). Labs log each incoming environmental sample and disinfect external surfaces upon receipt. Waste from environmental samples (used swabs, filters) autoclaved or incinerated after testing.
G. Transport and biobanking	All specimen types (clinical & environmental) in transit between sites or in long-term storage; may include cultured isolates; frozen stocks	Transport **off-site:** Package per dangerous goods regulations. Category B (UN3373) for routine diagnostic specimens; Category A (UN2814) for cultures or materials likely containing high-titer live virus (Biobank storage: Infectious materials must remain in BSL-3-equivalent storage (e.g., locked –80°C in BSL-3) unless inactivated.	Packing staff must wear gloves and lab coat at minimum; face protection if risk of spill during packing. Use approved shipping containers (triple packaging). For biobank handling at site: BSL-3 PPE for retrieving or aliquoting infectious stocks from freezers; mechanical cryogloves for ultra-cold temps in addition.	Specimens leaving containment to ship must be inactivated or sealed per shipping regulations. Biobanked samples that are inactivated (e.g. gamma-irradiated or chemically fixed) may be stored at BSL-2 if clearly labeled as "non-infectious" after validation.	Maintain chain-of-custody forms for all shipments. Only certified dangerous goods shippers to prepare Category A shipments. Include biohazard labeling and shipping manifests with identification of contents (Category A labeled as "Infectious Substance, UN2814 – Suspected Hantavirus" per IATA if appropriate. Inventory logs for biobank samples must indicate containment status (live vs. inactivated) and any removal/use events.

Definitions:

- "Potentially infectious" refers to any material that may contain viable ANDV (e.g., untreated patient specimens, viral cultures, or rodent/environmental samples).
- "Validated inactivation" means a treatment (chemical inactivation, fixation, heat, etc.) proven to effectively destroy or neutralize ANDV, documented by reference to scientific literature or empirical testing.
- "BSL-3" corresponds to biosafety level 3 facilities and practices: a high-containment lab with directional airflow and specialized safety equipment, where work is done in biosafety cabinets with PPE as described.

- "BSL-2" refers to a lower-containment lab suitable for moderate hazards, here permitted only for materials confirmed non-infectious.
- "Containment level" encompasses facility design, engineering controls (like biosafety cabinets and sealed centrifuges), and work practices. All sites should have a biosafety manual or SOP outlining these level definitions and must adhere to national definitions if stricter.

Biosafety by Design: The NAVIS study emphasizes biosafety-by-design, meaning procedures are structured to minimize exposure risk at every step. This includes choosing specimen collection methods and lab assays that reduce aerosol generation, using inactivation steps early in protocols whenever feasible, and segregating "clean" vs. "dirty" workflow stages (e.g., pre- vs. post-inactivation areas). All procedures are to be evaluated via risk assessment prior to implementation, and safer alternatives adopted where possible. For example, if a novel analytic method can use chemically inactivated samples instead of live virus, it should be adopted to eliminate risk at source.

Inactivation and Step-Down Procedures: Each site must have a standard operating procedure (SOP) for specimen inactivation (for instance, use of guanidinium-based lysis buffers for nucleic acid extraction or heat inactivation for serology samples). These SOPs should reference evidence that the method reliably inactivates ANDV (or similar hantaviruses), and include internal validation steps if possible (e.g., attempting virus culture from treated vs. untreated control samples). Inactivation procedures must be documented in a log including date, method, and person responsible. Only once inactivation is confirmed (by procedure completion, not by viral testing) can materials be removed from BSL-3 conditions to BSL-2. The transition from BSL-3 to BSL-2 must be physically controlled (e.g., sealed containers moved via pass-through or double-bagging) and clearly labeled as "Inactivated – safe for BSL-2". If any doubt exists about completeness of inactivation, the material must remain under BSL-3 containment. This conservative approach ensures that even if local regulations might have permitted certain diagnostic work at lower containment, the protocol's default remains protective.

Transport, Shipping, and Receiving: All shipments of NAVIS specimens must comply with IATA/UN transport regulations for infectious substances. By default, diagnostic or clinical research samples are packed and shipped as UN 3373, Biological Substance Category B (triple packaging, marked "BIOLOGICAL SUBSTANCE, CATEGORY B"). If, however, a sample is known or strongly suspected to contain live ANDV at levels posing a severe hazard (for example, a cultivated virus isolate or a high-titer positive material), it must be shipped as UN 2814, Category A Infectious Substance (affecting humans) with all required documentation and labeling. Shippers must be trained and certified according to dangerous goods regulations. Each site should have a shipping SOP addressing classification, packaging (including use of absorbent material and secondary leak-proof containers), labeling, and documentation (e.g., "Shipper's Declaration for Dangerous Goods" when required). On-site transport (e.g., moving specimens from ward to lab, or between BSL-3 and BSL-2 zones) must use robust, leak-proof secondary containment (sealed bags or hard-sided containers labeled with biohazard symbols). Upon receiving shipments, the receiving lab must have a designated unpacking area in BSL-3 (or in a BSC), and all external surfaces of packages should be disinfected prior to opening.

Waste Management and Decontamination: All solid and liquid waste that has contacted potentially infectious material or derived from ANDV processing must be decontaminated prior to disposal. Acceptable methods include autoclaving (steam sterilization), validated chemical disinfection (e.g., bleach solution at appropriate concentration and contact time), or incineration by a licensed facility. Each lab should segregate biohazard waste (e.g., pipette tips, PPE, culture plates) and autoclave it in leak-proof bags before it leaves the high-containment area. Liquid waste (e.g., aspirated supernatants) should be treated with a suitable disinfectant (such as 1:10 bleach) for a minimum recommended contact time (e.g., 30 minutes) before sink disposal or autoclaving. Reusable equipment and materials must be decontaminated after each use: for example, BSC interiors wiped with 1% sodium hypochlorite (or equivalent) followed by 70% ethanol, and centrifuge rotors disinfected after use. Spills involving potentially infectious ANDV material must be managed immediately: evacuate area if aerosolized, wait for aerosols to settle, then cover spill with absorbent and disinfectant working from edges inward; staff must wear full PPE during cleanup, and any materials used in cleanup are treated as infectious waste. [canada.ca]

Incident Reporting and Exposure Management: Any incident (spill, accidental exposure, needle stick, PPE breach, equipment failure, etc.) involving ANDV specimens must be reported immediately to the site's biosafety officer and the study coordinating center. Each site needs a Biosafety Incident Response Plan describing first aid (e.g., wound washing, mucous membrane flushing), medical evaluation for exposed staff, and post-exposure actions. Although no specific post-exposure prophylaxis is established for ANDV, prompt observation and medical care is crucial given ANDV's potential severity; thus, exposed personnel should be monitored for symptoms and may be subject to occupational health protocols (e.g., fever watch, possible quarantine) per local regulations. Near-misses and minor spills should also be logged to identify and mitigate any process weaknesses. Regular safety meetings should review incidents to prevent recurrences.

Training and Competency: All personnel handling NAVIS specimens or working in associated labs must be trained in appropriate biosafety procedures. This training must cover theoretical knowledge (ANDV transmission, health risks, principles of containment) and practical skills (correct PPE use, BSC techniques, spill cleanup, waste disposal, shipping regulations). Staff should demonstrate competency (through assessments or drills) before working independently with ANDV specimens. Refresher training should occur at least annually or if procedures change. Training records must be maintained and available for audit. Additionally, personnel must be enrolled in any required occupational health programs (including routine health monitoring or immunizations if any become available).

Oversight and Compliance: The implementation of this annex at each site is subject to oversight by the study Sponsor (WHO coordinating center) in collaboration with local Institutional Biosafety Committees (IBCs) or equivalents. Prior to initiation of the study, each site will undergo a biosafety compliance assessment to ensure BSL-3 facilities and practices are in place as described. Audits may be conducted during the study (either remote document reviews or on-site inspections) to verify continued compliance with containment measures, sample tracking, and training status. Any deviations from these requirements must be promptly corrected and documented, with potential suspension of specimen handling at that site until resolved if serious gaps are identified.

Alignment with International Frameworks: This annex aligns with guidance from WHO's Laboratory Biosafety Manual (risk-based approach for Risk Group 3 agents) and ECDC outbreak recommendations for containing high-consequence pathogens. It avoids reliance on any single country's biosafety rules, instead, it sets a conservative common standard for all NAVIS sites while permitting additional site-specific measures to meet stricter national requirements where applicable. This ensures harmonization across a multi-country study, supporting safe operations without undercutting any local regulations. In particular, the requirement for BSL-3 containment of hantaviruses reflects global consensus. Sites within jurisdictions that allow certain procedures at BSL-2 must still adhere to the protocol's higher standard unless a formal exemption is granted after risk assessment and sponsor approval.

Version Control: This annex is a controlled document. The current version (v1.0) is dated 18 May 2026. Future revisions will be issued if new evidence or guidance emerges (for example, updated WHO/CDC biosafety advisories or improved inactivation methods) or if required by amendments to the protocol. All participating sites will be promptly notified of any changes, and older versions will be archived. The version number and date should be referenced in any site-specific biosafety documentation to ensure alignment with the latest approved guidance.

Annex F: Maximum blood volumes for children

The blood volumes taken from children for clinical and research purposes should not exceed maximum allowable limits.

Allowable blood draw limits for children (1,2):

For healthy children:

3% total blood volume in a 24 hour period

10% total blood volume in a 30 day period

For sick/unwell children:

2.5% total blood volume in a 24 hour period

5% total blood volume in a 30 day period

Estimated total blood volume for term infants (Wt >2kg) and children is 80 ml/kg.

Wt, kg	TBV, ml	Blood draw volume in 24 hours, ml		Total volume in 30 days, ml	
		Unwell (2.5% TBV)	**Healthy (3% TBV)**	Unwell (5% TBV)	**Healthy (10% TBV)**
3	240	6	**7**	12	**24**
4	320	8	**10**	16	**32**
5	400	10	**12**	20	**40**
6	480	12	**14**	24	**48**
7	560	14	**17**	28	**56**
8	640	16	**19**	32	**64**
9	720	18	**22**	36	**72**
10	800	20	**24**	40	**80**
11-15	880-1200	22-30	**27-36**	44-60	**88-120**
16-20	1280-1600	32-40	**38-48**	64-80	**128-160**
21-25	1680-2000	42-50	**50-60**	64-100	**168-200**
26-30	2080-2400	52-60	**62-72**	104-120	**208-240**
31-35	2480-2800	62-70	**74-84**	124-140	**248-280**
36-40	2880-3200	72-80	**86-96**	144-160	**288-320**
41-45	3280-3600	82-90	**98-108**	164-180	**328-360**
46-50	3680-4000	92-100	**110-120**	184-200	**368-400**

*Adapted from Jack 2001 (2).

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