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Establishment of a multisite umbrella cohort study protocol to describe the epidemiology and aetiologies of acute undifferentiated febrile illness in Latin America

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ABSTRACT

Introduction Acute undifferentiated febrile illnesses (AUFIs) impose a large burden in the tropics. Understanding of AUFI's epidemiology is limited. Insufficient diagnostic capacity hinders the detection of outbreaks. The lack of interconnection in healthcare systems hinders timely response. We describe a protocol to study the epidemiology and aetiologies of AUFI and pathogen discovery in strategic areas of Latin America (LA).

Methods and analysis Global Infectious Diseases Network investigators comprising institutions in Colombia, Dominican Republic, México, Perú and the USA, developed a common cohort study protocol. The primary objective is to determine the aetiologies of AUFI at healthcare facilities in high-risk areas. Data collection and laboratory testing for viral, bacterial and parasitic agents are performed in rural and urban healthcare facilities and partner laboratories. Centralised laboratory and data management cores deploy diagnostic tests and data management tools. Subjects >6 years with fever for <8 days without localised infection are included in the cohort. They are evaluated during the acute and convalescent phases of illness. Study personnel collect clinical and epidemiological information. Blood, urine, nasal or pharyngeal swabs and saliva are collected in the acute phase and blood in convalescent phase. Specimens are banked at −80°C. Malaria, dengue and COVID-19 are tested onsite in the acute phase. The acute-phase serum is PCR tested for dengue, chikungunya, Venezuelan equine encephalitis, Mayaro, Oropouche, Zika, and yellow fever viruses. Paired convalescent and acute serum antibody titters are tested for arbovirus, *Leptospira* spp, and *Rickettsia* spp. Serum is used for viral cultures and next-generation sequencing for pathogen discovery. Analysis includes variable distributions, risk factors and regression models. Laboratory results are shared with health authorities and network members.

Ethics and dissemination The protocol was approved by local ethics committees and health authorities. The results will be published in peer-reviewed journals. All study results are shared with local and regional health authorities.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The protocol combines conventional approaches to pathogen testing and unbiased pathogen detection.
⇒ The protocol provides a framework to define common causes of acute undifferentiated febrile illnesses through comprehensive data collection and laboratory testing.
⇒ The pathogen discovery process is centralised, limiting timeliness of identification and communication of risk.
⇒ A common data capture and management system poses implementation challenges in dissimilar epidemiological settings.
⇒ The surveillance methods proposed may be affected by concurrent events that impose a large burden on the healthcare system.

INTRODUCTION

Non-malarial acute undifferentiated febrile illnesses (AUFIs) are defined as systemic illnesses with fever (>38°C) of less than 8 (or occasionally <14) days duration without evidence of infection localised to a specific organ or system (eg, pneumonia, gastroenteritis and pyelonephritis).[1] Surveillance of AUFI in high-risk groups provides an opportunity to identify clinically relevant, newly emergent pathogens. This is particularly important at the human–animal interface, including unplanned urbanisation, where proximity may promote cross-species transmission and disease emergence/re-emergence.[2–6]

The lack of comprehensive studies of AUFIs limits our understanding of the importance and spread of different pathogens in specific geographical regions. Many studies have focused on malaria and/or a few pathogens using a narrow diagnostic scope.[7] Studies reporting on syndrome-based surveillance have significant limitations because of overlapping clinical presentations. Short-duration studies may not reflect the true prevalence or distribution of seasonal illnesses.[8] Limited geographical coverage and population diversity also decrease many studies' generalisability. A systematic review mapping the aetiological agents of non-malaria febrile illness in Southeast Asia revealed large areas with no information on the causes of AUFI.[9] Similarly, data from LA and the Caribbean are scarce, with significant gaps in AUFI aetiology.[7] A systematic review of the aetiology of severe febrile illness in low-income and middle-income countries (LMICs) noted a lack of rigorous laboratory-based case definitions and did not include LA studies.[10] Most reports on AUFI in South America have a limited geographic representation.[11–13]

Diagnostic testing to determine the aetiology of AUFI in the tropics is challenging. Agent-specific diagnostic tests used to detect known causes of AUFIs in LMICs cannot identify new or unexpected pathogens.[14] The use of diagnostic tests with uncertain performance characteristics or the suboptimal implementation of established tests hinders data interpretation. For example, serological tests without paired acute and convalescent sera or cross-reactive serological tests without confirmatory testing yield difficult-to-interpret data. This leads to a large proportion (27%–60%) of AUFI cases in studies from geographically diverse regions without definitive aetiological diagnoses.[11 12 15–17] A study of AUFIs in Thai children detected only 53% of dengue and 41% of leptospirosis cases testing acute serum.[18] A Tanzanian study found overdiagnosis of malaria and underdiagnosis of arboviral aetiologies.[19]

Arboviruses are a major cause of AUFI in tropical LA, where dengue virus (DENV) is the main cause. However, the majority of AUFIs are attributed to 'dengue infection', leaving coendemic and emerging arboviral diseases hidden under the 'dengue umbrella'.[20–22] Only one-third of AUFI cases clinically diagnosed as dengue are truly caused by DENV.[12] West Nile and chikungunya viruses (CHIKV) spread rapidly in LA, causing significant morbidity and mortality and becoming endemic. Recently, Zika virus (ZIKV) spread to >60 LA countries and territories and exposed suboptimal surveillance in the region. The first cases were recognised in Brazil in 2015.[23] Nevertheless, the virus was circulating in Brazil for at least 12 months and had probably spread to nearby countries before the first case was officially reported.[24] To improve health systems' preparedness for future outbreaks, it is imperative to establish improved surveillance and robust diagnostics in tropical regions. Generating laboratory capacity for real-time surveillance with interconnection between high-risk areas may help identify threats and prevent the spread of emerging infections.

Table 1 List of investigators and participating academic institutions

Investigator	Institution	Country
Francisco J Diaz Juan D Rodas	Universidad de Antioquia, Medellín	Colombia
Marylin Hidalgo	Pontificia Universidad Javeriana, Bogotá	Colombia
Margarita Arboleda	Instituto Colombiano de Medicina Tropical–Universidad CES, Medellín	Colombia
Eugenia S González-Diaz	Universidad Central de Este, San Pedro de Macorís	Dominican Republic
Matilde Jimenez-Coello Antonio Ortega-Pacheco	Universidad Autónoma de Yucatán, Merída	México
Karen Mozo	Universidad Peruana Cayetano Heredia, Lima	Perú
Patricia V Aguilar Miguel M Cabada Mathew M Dacso Peter C Melby David H Walker Scott C Weaver	University of Texas Medical Branch, Galveston, Texas	USA

We present a protocol for the surveillance of AUFI aetiologies considering high-risk arboviral and bacterial infections using conventional testing and next-generation sequencing for pathogen discovery. This protocol is implemented within the Global Infectious Diseases Research Network (GIDRN) sponsored by the University of Texas Medical Branch (UTMB) and includes academic institutions in LA and the Caribbean.

METHODS AND ANALYSIS

The GIDRN was founded in 2017 through the Division of Infectious Diseases and Center for Tropical Diseases at UTMB to foster multilateral research collaborations between academic institutions in low-income and middle-income LA and Caribbean countries. GIDRN's goal is to promote clinical, translational and field research in vectorborne and zoonotic infectious diseases through mutually beneficial, sustainable and synergistic partnerships. Seven academic institutions in Colombia, Dominican Republic, México, Perú and the USA are included (table 1). Participants are diverse and multidisciplinary—physician–scientists, virologists, veterinarians and epidemiologists. An elected steering committee leads the network, guided by member-written and approved bylaws on governance, collaboration, intellectual property, sharing of research data and specimens, joint publication and authorship, and professional development. Annual meetings provide training on research skills (grant writing, scientific writing, good clinical and good data management practices). A common research protocol,

including required activities and procedures, was created to guide a competitive pilot-grant application funded by the network as a corollary to the training. Four applications were funded to perform AUFI research in high-risk areas. The common protocol and diagnostic algorithm in use by the network are described.

Patient involvement

Patients and communities at the sites where this umbrella protocol is implemented did not participate in the study design or endpoint definition. The protocol's multisite character precluded the direct involvement of patients in strategy design.

Primary programme objective

The overall objective is to develop the capacity to study the aetiology and epidemiology of AUFIs in tropical areas of Colombia, the Dominican Republic, México and Perú by establishing a network of collaborating field sites and laboratories using the same protocol and diagnostic pipeline.

Primary research objective

To determine the aetiologies of AUFI among subjects attending healthcare facilities in high-risk areas of countries participating in the GIDRN.

Secondary research objectives

1. To determine the epidemiology and clinical presentations of specific pathogens that cause AUFIs in subjects attending healthcare facilities in GIDRN countries.
2. To implement and support capacity to perform aetiological diagnoses for AUFIs in local laboratories of participating partners.
3. To provide a framework for standardised data collection on AUFIs that will allow the characterisation of local and regional aetiological agents.
4. To provide a framework for early detection and response to aetiological agents of AUFIs causing outbreaks in GIDRN countries.
5. To establish common procedures to create high-quality specimen repositories at GIDRN sites.
6. To provide a platform for 'south-south' collaborations between GIDRN members.

Study organisation

This multisite protocol is organised within the framework of the GIDRN and conducted at clinical facilities and laboratories. Sites use a common research protocol, data and clinical specimen collection methods, specimen testing algorithm, specimen biorepository and a web-based data management platform (figure 1). A central diagnostic testing core at UTMB develops and standardises diagnostic tests for implementation at the study sites. It will receive clinical specimens from the study sites for pathogen identification through viral culture under biosafety level 3 conditions and next-generation sequencing. A central data management core created the data collection tools and web-based data entry platform to be used by the field sites. The administrative coordination of the GIDRN and multisite study is provided through the Center for Tropical Diseases at UTMB.

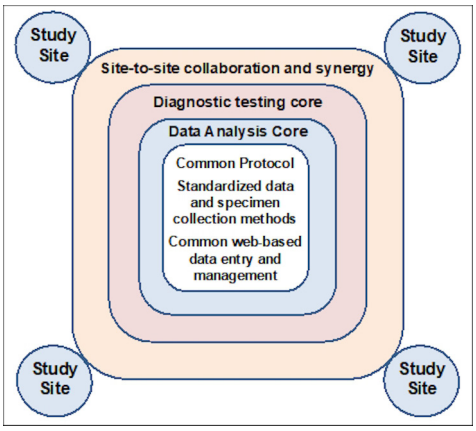

Figure 1 Organisation of the Global Infectious Diseases Research Network Umbrella Protocol.

Study design

The study is a prospective cohort of subjects presenting with AUFI to healthcare facilities located in tropical areas of Colombia, Dominican Republic, México and Perú. Subjects are evaluated during the acute and convalescent periods (≥14 days from first encounter) for aetiology, epidemiology, clinical characteristics and early complications of their illnesses. The GIDRN Steering Committee, Data Management Core and Diagnostic Testing Core provide oversight and coordination for the field and laboratory operations. Each of the study sites has a principal investigator and research team that includes physicians, nurses, community health workers, laboratory technicians and data management personnel. The overall study design and procedures are summarised in figure 2.

Enrolment timeline

Research began asynchronously in September 2021 at the different sites depending on the country's pandemic status and regulations. At the time of the original submission of this manuscript in December 2023, all sites were enrolling subjects to the common study. Study procedures will continue for 1 year in Colombia and Peru where subject enrolment has been completed and the diagnostic algorithm is at 50% completion. In the Dominican Republic and Mexico, subject enrolment will be completed in the next 6 months and completion of the diagnostic algorithm is expected within the next year. The number of subjects enrolled every week has significant seasonal variations ranging from as little as 1–2 subjects during the dry season to as many as 15–20 during the rainy season. Additional subject recruitment was approved in Peru during 2023 (n=600) for a significant increase in AUFI cases associated with DENV outbreaks occurring in Quillabamba.

Inclusion criteria

1. Fever (oral, tympanic or rectal temperature of ≥38°C or axillary temperature of ≥37.5°C) for <8 days without

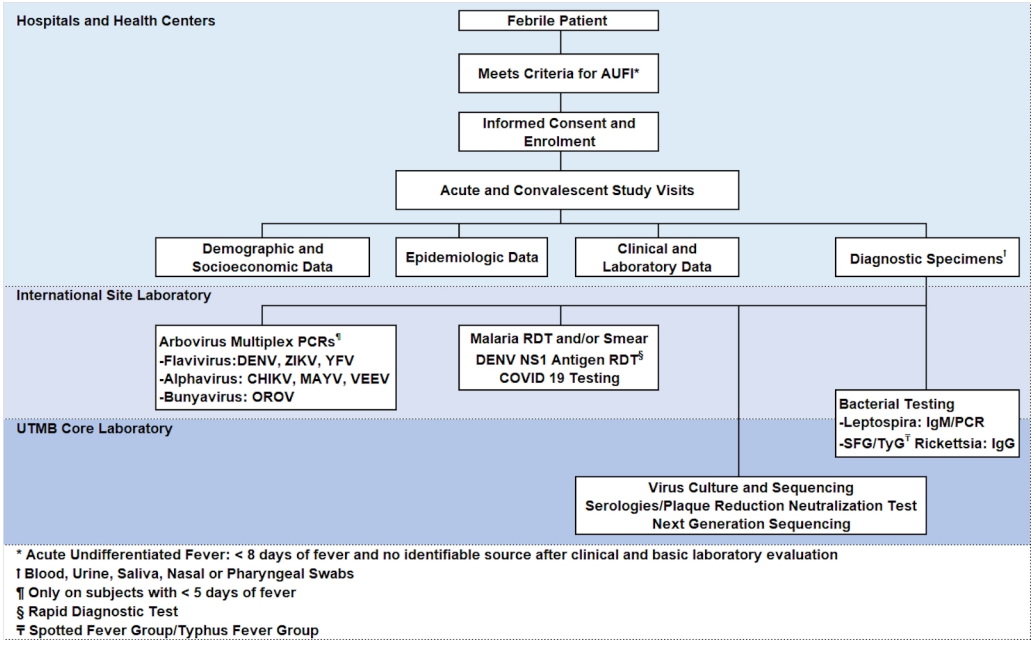

Figure 2 Overall study design and procedures. *Acute undifferentiated fever: <8 days of fever and n identifed source after clinical and basic laboratory evalution. †Blood, urine, saliva, nasal or pharyngeal swabs. ¶Only on subjects with <5 days of fever. §Rapid diagnostic Test. ‡Spotted fever group/typhus group. AUFI, acute undifferentiated febrile illnesses; DENV, dengue virus; ZIKV, zika virus; YFV, yellow fever virus; CHIKV, Chikungunya virus; MAYV, Mayaro virus; VEEV, Venezuelan Equine Encephalitis virus; OROV, Oropouche virus; UTMB, University of Texas Medical Branch.

evidence of a localising infection, documented by the patient or healthcare personnel at the facility within 24 hours of inclusion. At physician discretion, subjects without documented fever may be included in the study if the clinical presentation included other systemic symptoms (eg, chills, rash, arthralgias, myalgias) and laboratory abnormalities (eg, thrombocytopenia, elevated liver enzymes) suggestive of an arbovirus infection.
2. Female and male subjects 6 years or older.
3. Voluntary consent to participate. In the case of minors, persons without the capacity to make decisions, and critically ill patients, consent should be provided by their parent, guardian or legal representative. Minors must provide assent to participate.

Exclusion criteria

1. History of fever for >8 days.
2. Clinical or laboratory evidence of a differentiated bacterial, fungal or parasitic infection capable of causing an acute febrile illness. Patients with an identifiable focus of infection including, but not limited to, pneumonia with focal consolidation, otitis media, sinusitis, purulent pharyngitis, cellulitis, urinary tract infection, dental abscess, septic monarthritis, pelvic inflammatory disease or peritonitis. Subjects with a diagnosis of malaria are not excluded.
3. Subjects unwilling or unable to comply with study procedures and follow-up visits.
4. Any condition which in the opinion of the investigator might interfere with study objectives.
5. Any reason which, in the opinion of the investigator, creates additional risk to the patient.

Sample size

To pilot protocol procedures, identify errors, improve workflows and gain preliminary data on the aetiological causes of AUFI, a convenience sample of at least 200 subjects per site is enrolled. The pilot will also evaluate the feasibility of performing high-quality research in LA as a solid network of academic sites.

Subject selection process

Subjects are selected through active surveillance of patients attending healthcare facilities at each study site. After initial assessment using a standardised screening form (online supplemental materials Screening Documentation Form), subjects fulfilling all inclusion criteria and none of the exclusion criteria are invited to participate. Candidates interested in participating or letting their children or next of kin participate undergo the consent process. Children >6 provide informed assent to participate. Children 2 months to 5 years with AUFI are not included in our pilot protocol because the testing in this age group would require a more complex algorithm, obtaining repeated blood samples may raise concerns for worsening iron deficiency at this particularly vulnerable age, and the experience to obtain the multiple sample types in young children is not available in the settings where these pilots take place.

Acute illness visit

A full medical history and physical examination are performed. Demographic, socioeconomic, epidemiological and routine laboratory data are collected. Subjects admitted to the hospital are followed through their hospitalisation to document their clinical course. Autopsy records are collected when available. All information is recorded on the acute illness data collection form (online supplemental materials Acute Illness Visit Data Collection Form).

Convalescent visit

Subjects are evaluated 3 weeks after the acute illness visit. A full physical examination is performed and information on any new laboratory results, diagnostic procedures, illness course and hospital admissions since enrolment are recorded in the convalescent data collection form (online supplemental materials Convalescent Visit Data Collection Form). Subjects missing the convalescent visit receive home visits.

Study sites

Apartadó, Colombia

Hospital 'Antonio Roldán Betancur,' Apartadó municipality, Antioquia, in northern Colombia. A 120-bed regional referral hospital that serves ~200 000 inhabitants of greater Apartadó. It is affiliated with the Colombian Institute of Tropical Medicine located on the hospital grounds. Specimen testing/storage: Universidad de Antioquia.

Villeta, Colombia

Hospital Salazar de Villeta, Cundinamarca Region, in central Colombia. It serves a rural population of ~25 000. Specimen testing/storage: Pontificia Universidad Javeriana.

La Romana, Dominican Republic

Hospital General Buen Samaritano, La Romana province, southeastern Dominican Republic. It serves migrant Haitian-Dominicans from rural sugar cane plantations ('bateyes'). Specimen testing/storage: Universidad Central del Este in San Pedro de Macorís.

Mérida, México

Unidad Universitaria de Inserción Social San José Tecoh of Universidad Autónoma de Yucatán in Merida city. It serves an urban and periurban population of ~15 438. Specimen testing/storage: Universidad Autónoma de Yucatán.

Molas, México

Módulo Médico Molas, Merida Municipality, Yucatan state. It serves 2400 people in Molas and other rural communities of the Yucatán Peninsula. Specimen testing/storage: Universidad Autónoma de Yucatán.

Quillabamba, Perú

Hospital de Quillabamba, La Convención Province, Cusco Region in southeastern Peru. It serves ~20 000 residents of Quillabamba City and approximately ~180 000 provincial residents. Specimen testing/storage: Sede Cusco–Tropical Medicine Institute, Universidad Peruana Cayetano Heredia in Cusco.

UTMB, Galveston, Texas

UTMB investigators oversee the Data Management Core and the Diagnostic Testing Core. Aliquots of acute and convalescent serum specimens obtained at the international sites are dry-ice shipped to UTMB for viral isolation, serology and next-generation sequencing.

Specimen collection, processing, storage

General procedures

Blood, urine, saliva, and nasal and pharyngeal swabs are collected at the acute study visits and blood at the convalescent study visits. All specimens are immediately transported to designated sample-processing areas for handling, temporary storage at −80°C, and transportation to the testing laboratories. Specimens collected at the subject's residence are transported in cooler boxes with ice packs.

1. Blood: Samples are collected by venipuncture and centrifuged to separate the serum from the clot. The maximum blood volume drawn each time is 10 mL for adults and 5 mL for children. Aliquots of both products are deposited in the biorepository.
2. Urine: Samples are collected in sterile containers, passed through sterile syringe filters (0.22 µm pores), and aliquots are stored in the biorepository using RNAse-free cryovials.
3. Saliva: Samples are collected in sterile wide-mouth containers, passed through sterile syringe filters (0.22 µm pores), and stored in the biorepository using RNAse-free cryovials.
4. Oral and pharyngeal swabs: Swabs are immediately mixed with viral transport media (UTM Universal Transport Media, Copan, Murrieta, California, USA). The supernatants are aliquoted and stored in the biorepository.

Diagnostic testing

Onsite malaria, DENV, SARS-CoV-2

During the acute study visit, a malaria rapid diagnostic test such as the OnSite Malaria Pf/Pv Ag Rapid Test (CTK Biotech, Poway, California, USA) or thin smear is performed on whole blood. A dengue NS1 antigen rapid test such as the OnSite Duo Dengue Ag-IgG/IgM Rapid Test CE (CTK Biotech) is performed on blood samples. A SARS-CoV-2 molecular test is performed on pharyngeal swabs if a test result is not available at the time of the visit. WHO prequalified malaria and dengue NS1 antigen rapid diagnostic tests are recommended according to local market availability.

Arbovirus

Acute study visit serum samples from subjects with ≤5 days of fever are tested at the study site using two in-house trioplex real-time RT-PCR assays to detect RNA from Dengue

virus (DENV), yellow fever virus (YFV), and Chikungunya virus (CHIKV), and for Mayaro virus (MAYV), Oropouche virus (OROV), and Venezuelan equine encephalitis virus (VEEV). A single probe-based PCR assay is used to detect RNA from Zika virus (ZIKV). Reaction, negative and positive controls are included in every run.

Viral isolation is attempted on selected acute-phase serum samples of subjects with ≤5 days of fever using standard laboratory cell lines (ie, Vero and C6/36 cells) in a Biosafety level-3 laboratory at UTMB. Viruses recovered by culture are identified by targeted PCR and sequencing. Methods for pathogen identification will include indirect immunofluorescence assay using polyclonal and/or monoclonal antibodies.

The presence of IgM antibodies is tested on acute and convalescent samples of all subjects by ELISA for DENV, ZIKV and CHIKV. Other serological tests such as plaque reduction neutralisation tests, haemagglutination inhibition assay and complement fixation may be used to expand the serological testing. If these methods fail to identify the aetiological agent, electron microscopy and next-generation sequencing may be performed.

Leptospirosis

Leptospira IgM antibodies are tested by ELISA on convalescent serum samples first and, if positive, the ELISA is performed on the acute samples. A fourfold increase in IgM antibodies between acute and convalescent samples is considered confirmatory of Leptospira infection. On subjects without convalescent samples, an ELISA on the acute samples with an IgM titre >160 is considered suggestive of Leptospira infection. PCR to detect Leptospira DNA in acute serum samples is performed on subjects with ≤5 days of fever and if positive a Leptospira infection is diagnosed. Microagglutination tests with a limited number of Leptospira serovars are used if available.

Rickettsia

Indirect immunofluorescence antibody assays for spotted fever and typhus group rickettsioses are performed. Convalescent serum samples are tested first and, if positive, the paired acute serum sample is tested. A Rickettsia infection is diagnosed if a fourfold increase in antibody titers is documented. In subjects without a convalescent serum sample, an IgG titre >160 in the acute serum sample is considered highly suspicious for Rickettsia infection.

Reporting of results

Laboratory testing results are reported according to the type of test used and the certainty of the diagnosis as described above. When possible, potential cross-reactions in serological tests are confirmed with additional testing including plaque reduction neutralisation or PCR. When serology of unpaired samples is positive, the result will be reported as a possible infection. Dual infections will be reported as such accounting for the diagnosis certainty and/or availability of confirmatory testing.

Data management

Study sites trained their personnel and implemented a data management plan to collect, process, maintain, store, query, clean and report study data. This plan and site-specific standard operating procedures (SOPs) ensure harmonisation of procedures and maintenance of good clinical practices. The data management plan includes (1) training of personnel and harmonisation activities, (2) data sources and types to be collected, (3) data collection tools, (4) data capture software, (5) subject privacy and data confidentiality, (6) data entry and validation, (7) quality assurance and quality control, (8) data and specimen storage and backup and (9) reports, intellectual property and dissemination of findings.

Training

All personnel involved in data collection and management completed training on good documentation and clinical practices. Individual site training includes the study protocol, SOPs and Research Electronic Data capture (REDCap) data entry.

Data sources and types

Data sources include subjects, family members, community leaders and members, hospital and health records, blood, serum, saliva, nasal or pharyngeal swabs, and urine.

Data collection tools

Paper case report forms mitigate the potential for inconsistent internet access in the field. Standardised data collection forms include screening, enrolment/acute visit, convalescent, additional visit and laboratory results.

Data capture software

The data are managed using REDCap hosted by UTMB.[25 26] Two-device authentication for access and UTMB's firewall increase data security. The REDCap database is validated, and the competency of the data-entry personnel confirmed pre-enrolment using dummy datasets.

Data entry/validation

After quality control for completeness and consistency, and all queries have been resolved, forms are entered into REDCap. While a single global dataset is generated, site personnel are designated to specific data-access groups approved by their local investigators and UTMB Data Management Core.

Quality assurance/quality control

Clearly defined written SOPs govern the management of data at each site. These SOPs provide information on specific role-related activities and competencies. Access and modification of the dataset are monitored with an audit trail. Logs for case report forms, specimens, laboratory results, personnel training, protocol revisions and deviations, and audits are maintained. Laboratory procedures at each site include internal and external quality controls. Laboratories have positive control specimens and/or viral RNA for each viral pathogen. Specimens with

positive and negative test results are shipped to UTMB for further testing and confirmation.

Data/specimen storage and backup

Consent and assent forms, study paper forms, specimens, and quality control logbooks are treated as source documents and stored securely at the data management units at each site. Backup copies are maintained securely at the generation sites. Laboratory results are stored in 'raw format' electronically in the equipment used to run the tests. Periodic backup of electronic information, including local datasets and results, is performed in encrypted and password-protected hard drives.

A repository at each site stores RNA, serum, blood clots, saliva, nasal or pharyngeal swabs, and urine samples for which the subjects consented in writing for future use. Only samples processed, preserved and transported according to the SOPs and passing quality controls are stored.

Access and intellectual property

Each site has unrestricted access and publication rights to its own data but must acknowledge GIDRN participation. Any download, presentation, communication or publication requires written approval by the involved sites and investigators. Credit to the investigators from each site is discussed before any data analysis or publication preparation. Novel viruses isolated during the study are deposited in UTMB's World Reference Center for Arbovirus and Emerging Viruses (WRCEVA, NIH grant R24 AI120942).

Statistical analysis

Subject demographics and baseline characteristics will be summarised using descriptive statistics. Mean, SD, median, quartiles, minimum and maximum will be used for continuous variables and numbers and percentages for categorical variables. The percentages of specific aetiological diagnoses and specific clinical features will be compared within and across sites. χ^2 will be used for comparison of categorical data. For continuous data, comparisons between two groups will be evaluated with two-tailed Mann-Whitney U test for non-parametric data or two-tailed unpaired t test for normally distributed data. Comparisons between more than two groups will be performed with Kruskall-Wallis for nonparametric data or analysis of variance for normally distributed data with post hoc correction for multiple comparisons (Bonferroni or Tukey).

Strengths and limitations

A major strength of this protocol is its implementation at multiple sites in multiple countries with diverse geographic, environmental, sociodemographic and AFUI endemicity. The engagement of all network partners in the development of the protocol created a scientific environment rich in diversity of expertise and experience. Participation of network partners from the outset has led to a strong and shared commitment to the success of the study by investigators and their home institutions. Recognition of disparities in resources and human subject research experience enabled focusing efforts on sites that require more support. The broad range of sites and diagnostic testing will provide a comprehensive dataset that will enhance the field and inform future larger-scale studies. A limitation is the diagnostics targeting a selected group of pathogens when we know that many untargeted pathogens (known and unknown) cause AUFIs in the tropics. The protocol attempts to mitigate this limitation by including viral culture on acute-phase samples and unbiased deep sequencing of a subset of diagnostic specimens, but some pathogens will still be missed. The pilot nature of the study and its limited funding will limit its broad applicability and impact. With the complexities of a single global database, errors may surface. The emergence of the COVID-19 pandemic just before subject enrolment delayed activities, wasted limited resources and affected the network's capacity to hold in-person meetings and provide training and professional development. Asynchronous enrolment at the different sites may decrease the validity of seasonality comparisons.

ETHICS AND DISSEMINATION

The site-specific study protocols were approved by the local research ethics committees. These included the Bioethics Committee of Universidad de Antioquia (#F-017-00) in Colombia, the National Counsel in Health Bioethics (#030-2020) in Dominican Republic, the Research Ethics Committee (#CEI-11-2022) at Universidad Autónoma de Yucatán in Mexico, the Institutional Research Ethics Committee (#103608) of Universidad Peruana Cayetano Heredia in Peru and Institutional Review Board (IRB) of The UTMB (#19-0047 and #21-0120). All the investigators and study personnel involved in the study completed human subject protection and good clinical practice training before the start of the activities at their sites. Local IRB and health regulations govern all study activities and supersede the common study protocol and GIDRN bylaws.

The results of this study will be published individually by each site and as a multicentric study in peer-reviewed journals. The results will be disseminated among local health authorities and ministries of health in each country. All viral sequences are submitted to GenBank.

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Contributors Conceptualisation: MMC, PVA and PCM. First draft: MMC and PCM. Field work design: MMC, JDR, DHW, SW and PCM. Laboratory work design: MMC, PVA, DHW, SW and PCM. Overall design: MMC, PVA, JDR, MH, KM, ESG-D, MJ-C, FJD, MMD, AO-P, MA, DHW, SW and PCM. Approval of the final version: MMC, PVA, JDR, MH, KM, ESG-D, MJ-C, FJD, MMD, AO-P, MA, DHW, SW and PCM.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

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