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Bacterial versus non-bacterial infections: a methodology to support use-case-driven product development of diagnostics

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ABSTRACT

Acute febrile illness (AFI) is one of the most common reasons for seeking medical care in low-income and middle-income countries. Bacterial infections account for a relatively small proportion of AFIs; however, in the absence of a simple diagnostic test to guide clinical decisions, healthcare professionals often presume that a non-malarial febrile illness is bacterial in origin, potentially resulting in inappropriate antibiotic use. An accurate differential diagnostic tool for AFIs is thus essential, to both limit antibiotic use to bacterial infections and address the antimicrobial resistance crisis that is emerging globally, without resorting to multiple or complex pathogen-specific assays. The Biomarker for Fever-Diagnostic (BFF-Dx) study is one of the largest fever biomarker studies ever undertaken. We collected samples and classified disease aetiology in more than 1900 individuals, distributed among enrolment centres in three countries on two continents. Identical protocols were followed at each study site, and the same analyses were conducted in each setting, enabling like-with-like comparisons to be made among the large sample set generated. The BFF-Dx methodology can act as a model for other researchers, facilitating wider utility of the work in the future. The established sample collection is now accessible to researchers and companies and will facilitate the development of future fever-related diagnostic tests. Here, we outline the methodology used to determine the sample populations and to differentiate bacterial versus non-bacterial AFIs. Future publications will set out in more detail the study's demographics, the causes of fever identified and the performance of selected biomarkers.

Summary box

- Acute febrile illness (AFI) is one of the most common reasons for seeking medical care in low-income and middle-income countries (LMICs).
- The adoption of malaria rapid diagnostic tests to guide antimalarial treatment has led to reduced use of antimalarials; however, in many malaria-endemic regions there has been an increase in antibiotics given to those who test negative for malaria.
- Although bacterial infections account for a relatively small proportion of AFIs in LMICs, in the absence of a simple diagnostic test clinicians often presume that an AFI is bacterial in origin, which can potentially lead to the inappropriate use of antibiotics.
- Here, we outline the methodology of the Biomarker for Fever-Diagnostic (BFF-Dx) study, one of the largest fever biomarker studies ever undertaken, which enables like-with-like comparisons to be made among epidemiologically different settings and has generated a well-characterised sample set that can be used for future research and development of biomarkers and diagnostic tools.
- The BFF-Dx methodology facilitates the evaluation of the usefulness of biomarkers in differentiating AFIs of bacterial versus non-bacterial origin, the results of which will contribute to efforts to provide appropriate care, reduce the overuse of antibiotics and help curb the threat posed by antimicrobial resistance.

INTRODUCTION

Acute febrile illness (AFI) is one of the most common reasons for seeking medical care in low-income and middle-income countries (LMICs). The diseases underlying AFIs, including malaria, typhoid, leptospirosis, rickettsial illnesses and many illnesses caused by viruses, such as arboviruses, are a major cause of morbidity and mortality, especially among children.[1] The global roll-out of simple, rapid diagnostic tests (RDTs) for malaria has improved our understanding of the role malaria plays in AFIs and led to an awareness that malaria is responsible for a much smaller fraction of fever cases than was once thought.[2 3] In Africa alone, it is estimated that more than 90 million children present to health facilities annually with non-malarial

fevers.[4 5] However, information about the causes of fever in LMICs is scarce.[6 7] Recent studies conducted in Latin America have shown that viruses, including arboviruses and respiratory viruses, are the most frequently reported causative agents of febrile illness.[8 9] A study in Tanzania showed up to 70% of all paediatric patients who present with gastroenteritis, respiratory symptoms or bloodstream infections are infected by viral agents and suggested bacterial agents are implicated in fewer than 25% of AFI cases.[10] Another study of adult and paediatric patients with fever conducted in northern Tanzania identified malaria as the cause of fever in just 1.6% of patients.[11] These studies, and another conducted in South-east Asia,[12] also show great heterogeneity in the causes of febrile illness across regions and even within a country. In such a complex and poorly characterised epidemiological context and in the absence of a simple diagnostic test to guide clinical treatment, especially for cases malaria-negative by RDT, many healthcare professionals prescribe antibiotics as a precaution, since they fear undertreating life-threatening bacterial infections such as pneumonia.[2 13] Therefore, an accurate differential diagnostic tool for AFIs is essential to improve the targeted use of antibiotics and help address the emerging global crisis of antimicrobial resistance,[14] in a context where the primary causes of fever remain unknown, and costly, pathogen-specific detection tools are not available.

Host biomarkers have been suggested as an appropriate means of meeting the challenge of differentiating bacterial from non-bacterial infections.[15] C reactive protein (CRP) and procalcitonin are long-established biomarkers used to guide clinical decisions in hospitals in high-income countries (HICs).[15 16] However, the use of such biomarkers was until recently mostly restricted to hospital-based care and therefore not easily transferable to a decentralised testing approach in LMICs. To define more clearly the needs of LMICs, a consortium of experts in global health and diagnostics developed a target product profile (TPP), which identified the need for an assay to distinguish bacterial from non-bacterial infections in low-resource settings (eg, corresponding to community-based healthcare settings as well as primary care centres) to support evidence-based treatment guidance.[17] From this consensus effort, the ideal characteristics for such a test were defined and the target population was identified as the general febrile population and included all age groups. To determine how effectively any potential solution meets these TPP priorities, it is essential that potential biomarkers are investigated within the intended target population. To date, most biomarker studies have been conducted in HICs and have focused on severe and/or hospitalised patients[18] (also Fernandez *et al*, in preparation). Data that address the challenges of the TPP (eg, target setting, target population) are therefore urgently needed, not least because the health priorities and operational challenges faced in less well-resourced settings differ considerably from those faced in HICs, and the performance of biomarkers may also differ considerably in these settings.[19]

To address this data gap, which until now has impeded targeted diagnostic development to address the emerging needs in LMICs, we conducted the Biomarker for Fever-Diagnostic (BFF-Dx) study; one of the largest fever biomarker studies ever undertaken and one that involved extensive laboratory testing. The primary objective of BFF-Dx was to evaluate the performance in differentiating bacterial versus non-bacterial infections of various host biomarkers across multiple settings in Africa and South America, the intended-use settings of any potential fever biomarker tests. Here, we outline the overall BFF-Dx methodology adopted: the protocols used to determine the BFF-Dx sample populations, how bacterial versus non-bacterial AFIs were differentiated and how the various analytical tools used were employed.

BIOMARKER FOR FEVER-DIAGNOSTIC STUDY: OVERALL APPROACH

Study sites

Several potential study locations were identified based on the following factors: geographical location, type of health facility, endemic pathogen profile, logistical and operational characteristics, laboratory and recruitment capacity and expected study population. An initial assessment led to eight sites being identified for a subsequent site visit. Based on the findings of these on-site assessments, four sites were shortlisted, with three sites finally selected to participate in recruitment for BFF-Dx (table 1).

table 1 The study was conducted in full compliance with the principles of both the Declaration of Helsinki and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines. All participants or their parent/guardian gave written informed consent prior to their participation in the study.

Sample size

The sample size was determined according to previously published formulae,[20] taking into account available performance data for selected fever biomarkers and making the following assumptions:

- Estimate a sensitivity and specificity of 80% and 80%, respectively, based on published reports of the performance of the human neutrophil lipocalin ELISA,[21] the FebriDx RDT[22] and the TRAIL/IP-10/CRP combination[23] in HICs.
- Significance level alpha=0.05 (used for the derivation of CIs).
- Expected width of the 95% CI of the point estimates of sensitivity and specificity, M =±10%.
- An estimated prevalence of 10% bacterial infections in patients presenting with AFI at an outpatient department (based on estimates from the literature

Table 1 Participating study site settings and corresponding ethical boards that approved BFF-Dx

Country	Brazil	Gabon	Malawi
Institute	Instituto Nacional de Infectologia Evandro Chagas (INI), FIOCRUZ, Rio de Janeiro	Center of Medical Research Lambaréné (CERMEL)	Malawi Epidemiology and Intervention Research Unit (MEIRU)
Enrolment site	UPA Rocha Miranda, UPA Manguinhos and Family Health Clinics Armando Palhares	Clinical trials unit, CERMEL	MEIRU, Chilumba campus
Enrolment setting	Primary healthcare facility in an urban area (*favela*)	Hospital in a semirural setting	Primary healthcare facility in a rural setting
Enrolment period	October 2018 to July 2019	May 2019 to November 2019	April 2017 to April 2018
Main causes of fever (expected)	Circulation of arboviruses, including dengue, Zika and chikungunya viruses	Endemic *Plasmodium falciparum*, dengue virus and chikungunya virus	Endemic *Plasmodium falciparum* and possibly chikungunya virus

BFF-Dx, Biomarker for Fever-Diagnostic.

and consultation with on-site infectious disease clinicians).[10]

- Power to detect estimates of sensitivity and specificity with a CI of width M: 80%; power of sampling the necessary number of patients with bacterial infections based on the reported prevalence: 90%.

Based on the above assumptions, the minimum sample size required for the primary discovery cohort was calculated to be 1380 participants; this was rounded up to 1500 participants, that is, 500 participants per study site.

Study design

This was a cross-sectional, observational study that used a convenience sample of children and adults who had clinical signs of AFI and no signs of severe illness. All patients enrolled in BFF-Dx continued to be clinically managed according to local standards of care. Inclusion and exclusion criteria for the study were based on the target population previously identified in the TPP[17]; patients diagnosed with chronic disease were enrolled only when their fever was a new and separate symptom (table 2). Investigators used case report forms (CRFs) for data capture, tailored to local needs. Data items captured included enrolment information, clinical signs and symptoms, laboratory results and patient follow-up details. Templates of CRFs are provided in online supplemental appendix 1. Participant follow-up visits were conducted 14–28 days after their initial healthcare-seeking appointment to allow convalescent samples to be taken for

Table 2 Inclusion and exclusion criteria at the enrolment sites

Study site	Rio de Janeiro (Brazil)		Lambaréné (Gabon)		Karonga (Malawi)	
Criteria	**Inclusion**	**Exclusion**	**Inclusion**	**Exclusion**	**Inclusion**	**Exclusion**
Acute fever	History of fever, last 7 days	History of fever, more than 7 days previously*	History of fever, last 7 days	History of fever, more than 7 days previously*	On presentation†	More than 7 days*
Age (years)	2–65		2–17‡		2–65	
Patient condition	Outpatient only	Critical condition	Outpatient only	Critical condition	Outpatient only	Critical condition
Informed consent/ assent	Yes		Yes		Yes	
Prepared to have follow-up at 2 weeks	Yes		Yes		Yes	
Pregnant		No exclusion		No exclusion		Yes §

*Exclusion of patients with a history of fever of more than 7 days excludes the majority of presumptive tuberculosis cases, who usually present with a fever that has lasted for over 2 weeks.
†In Malawi, patients were unlikely to self-medicate with antipyretics prior to their clinic visit, as was the case in Brazil and Gabon, and therefore history of fever was not added to the inclusion criteria.
‡Children only, due to the setting and to counter the lower rates of child enrolment experienced in Brazil.
§National Health Science Research Committee (NHSRC) requirement. Women of childbearing age were asked about the possibility of pregnancy and offered a urine-based pregnancy test for confirmation.

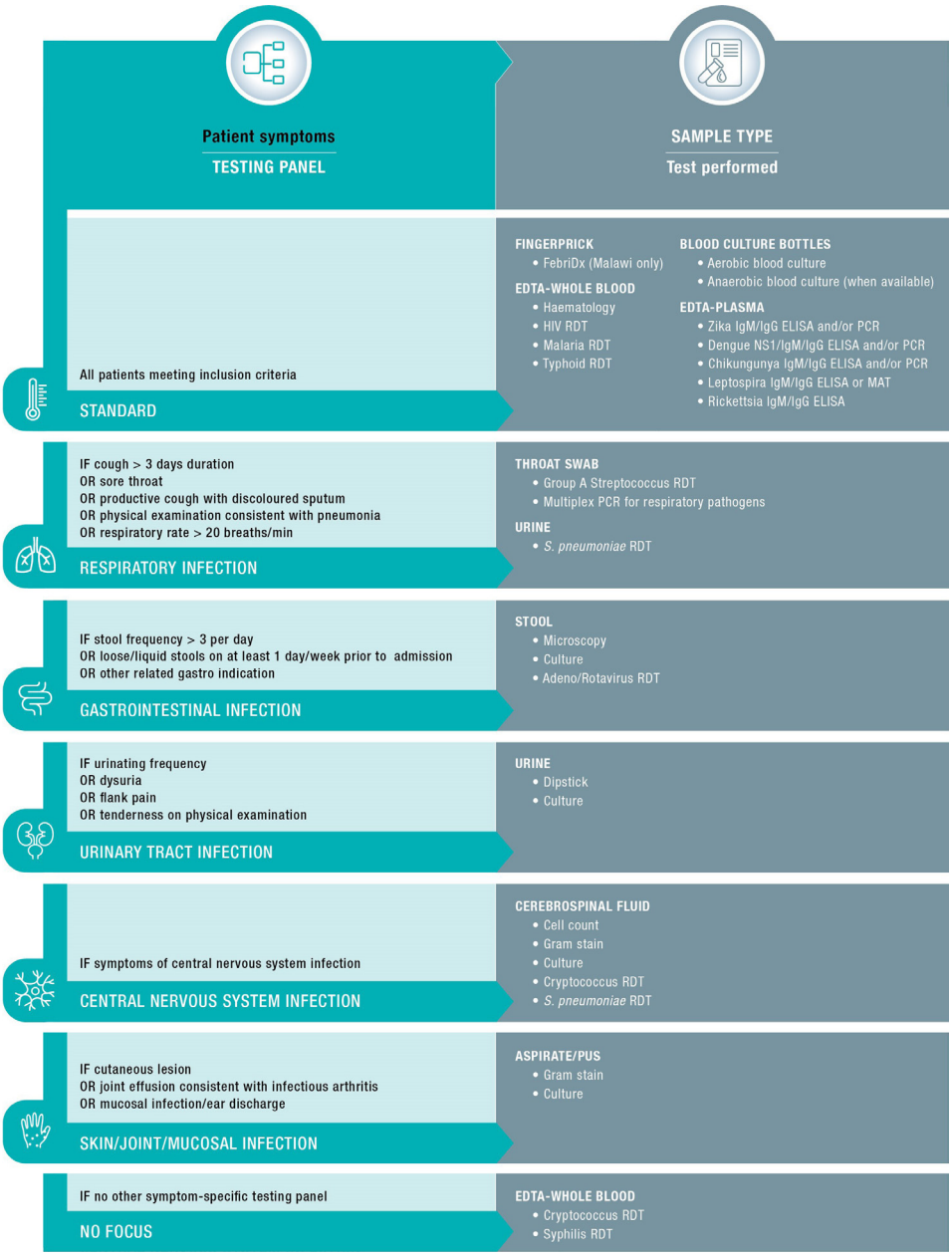


Figure 1 Symptom-based panel of tests. MAT, microscopic agglutination test; NS1, non-structural protein 1; RDT, rapid diagnostic test.

selected confirmatory tests (IgM/IgG testing for dengue, Zika, chikungunya, *Rickettsia* spp and *Leptospira* spp). Based on a participant's clinical presentation, their samples were sent for symptom-based panels of laboratory tests. A standard panel of tests was performed for all participants; other tests were performed only if specific signs or symptoms were present (figure 1). A table listing all tests and sample types used for each panel is provided in online supplemental appendix 2.

Most laboratory tests were performed daily onsite, with further characterisation performed on batched samples. For batched samples from Malawi and Gabon, this characterisation was conducted in a specialised clinical laboratory (Limbach Gruppe SE, Heidelberg, Germany); for samples from Brazil, it was performed by reference laboratories at FIOCRUZ, Rio de Janeiro, Brazil.

Sample transport and storage

Blood and urine samples were collected from all participants on enrolment. Stool, oropharyngeal swab, aspirate and pleural fluid, cerebrospinal fluid and skin swab samples were collected according to the criteria shown in figure 1. Standardised guidance for sample transport and storage prior to laboratory evaluation was provided to all sites (online supplemental appendix 3). All samples for biomarker testing or reference testing were stored at −20°C until being tested at a reference laboratory. Samples collected for the sample collection were stored at

–80°C. All shipments were undertaken via World Courier and followed international shipping requirements.

Data collation and quality control

Data captured using CRFs were added to a secure database (Brazil/Gabon: OpenClinica Enterprise 34, managed by the Foundation for Innovative New Diagnostics (FIND); Malawi: local Microsoft Access project database). PCR and ELISA reference testing yielded qualitative results that were generated as electronic files and directly transferred to the FIND data management team, who reviewed them to ensure consistency with the standard format prior to importing them into the database.

Good clinical practice and good clinical laboratory practice standards were observed at all stages of BFF-Dx. Detailed site initiation, monitoring and close-out visits were undertaken. All paper forms, logbooks and sample containers were labelled with a unique identification number and barcode. Data cleaning was conducted both during the enrolment period and at the end of it; this cleaning comprised five components: (1) during data entry, in response to detailed electronic data capture system logic and range checks; (2) by adopting a double data entry procedure; (3) by site supervisor monitoring of local data managers; (4) by preprogrammed cross-form or other complex checks performed by the FIND data management team and (5) by checking the data for inconsistencies, which was performed by statisticians before they conducted statistical analyses.

Classification of patients with bacterial and non-bacterial causes of fever

We opted for a two-step approach, described in a previous publication[19] as the best method for differentiating patients as having either bacterial-caused or non-bacterial-caused fever. First, an electronic classification was applied; second, there was an expert clinical review of unclassified patient files (figure 2).

The electronic classification was based on predefined and widely accepted laboratory parameters, including direct pathogen detection, a fourfold increase in antibody titre, or a positive PCR or antigen RDT result. The case definitions are listed in figure 3. The classification system prioritised bacterial infections such that in cases of AFIs where both bacterial and non-bacterial criteria were met, the output category would be 'bacterial'. The rationale was that the clinical practice adopted for dealing with bacterial and non-bacterial coinfections would necessarily involve treatment with antibiotics.

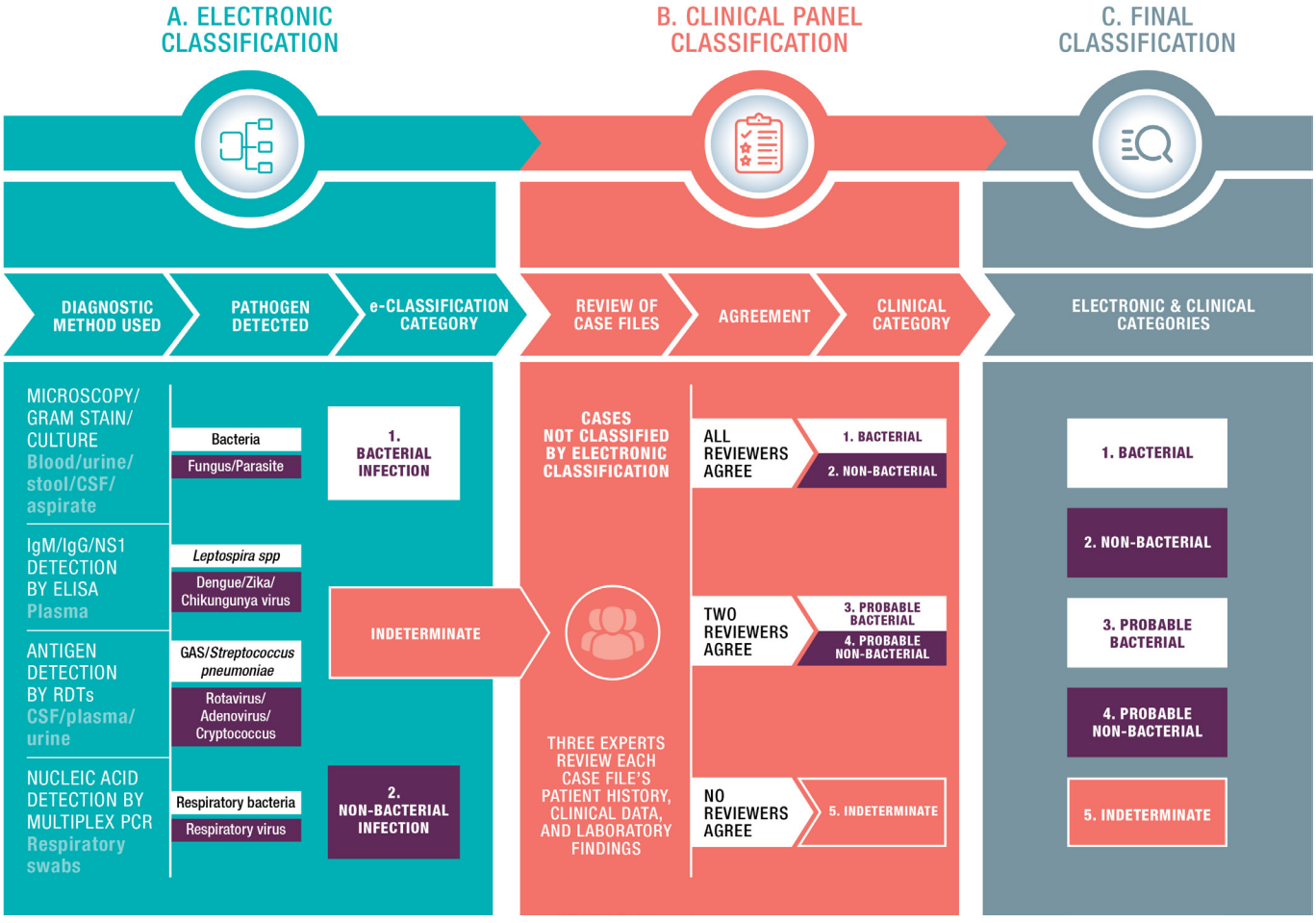
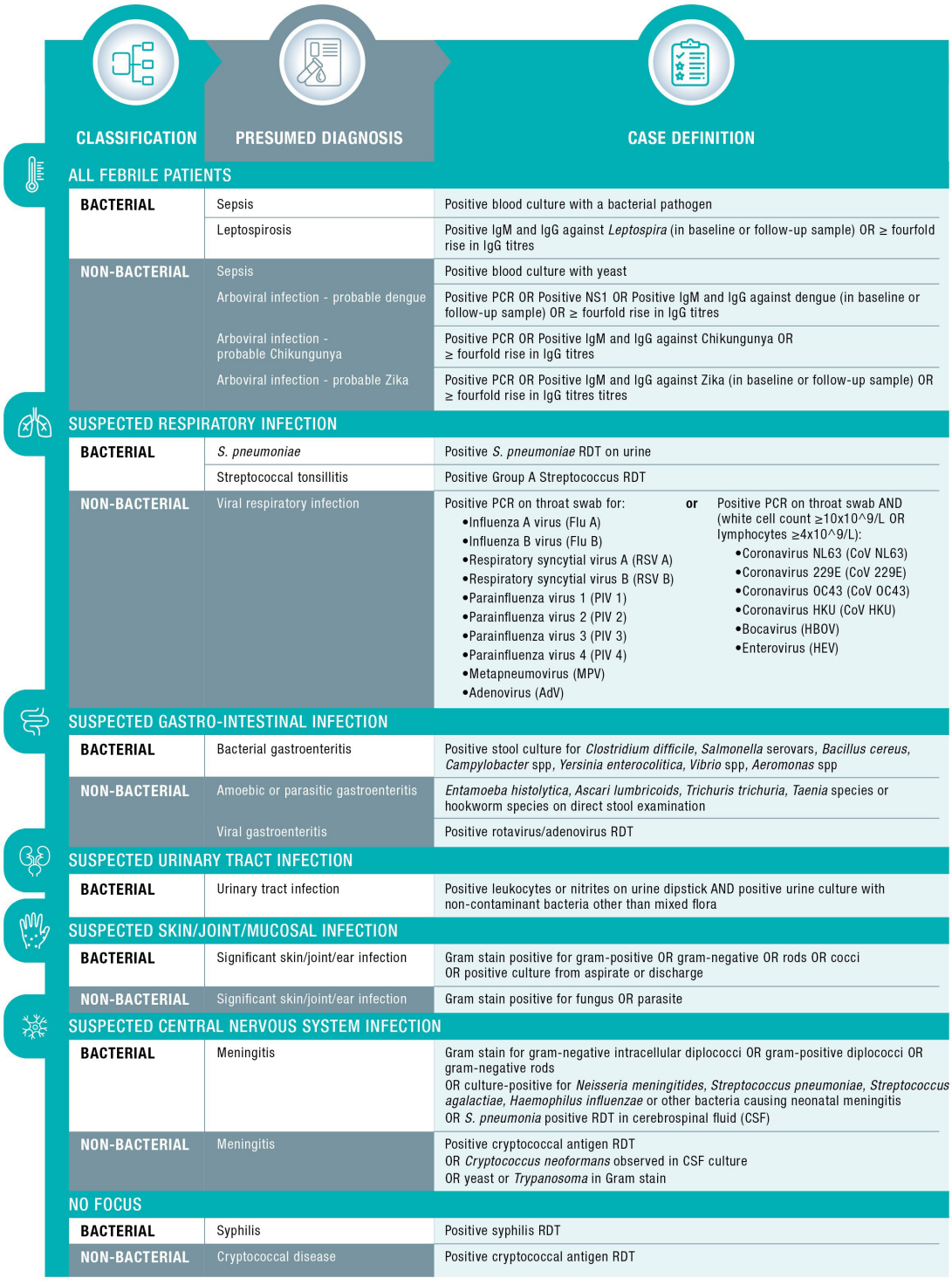


Figure 2 The two-step approach used to differentiate causes of fever: (A) electronic classification, (B) expert clinical panel classification and (C) the final classification categories.



CLASSIFICATION	PRESUMED DIAGNOSIS	CASE DEFINITION
ALL FEBRILE PATIENTS		
BACTERIAL	Sepsis	Positive blood culture with a bacterial pathogen
	Leptospirosis	Positive IgM and IgG against *Leptospira* (in baseline or follow-up sample) OR ≥ fourfold rise in IgG titres
NON-BACTERIAL	Sepsis	Positive blood culture with yeast
	Arboviral infection - probable dengue	Positive PCR OR Positive NS1 OR Positive IgM and IgG against dengue (in baseline or follow-up sample) OR ≥ fourfold rise in IgG titres
	Arboviral infection - probable Chikungunya	Positive PCR OR Positive IgM and IgG against Chikungunya OR ≥ fourfold rise in IgG titres
	Arboviral infection - probable Zika	Positive PCR OR Positive IgM and IgG against Zika (in baseline or follow-up sample) OR ≥ fourfold rise in IgG titres titres
SUSPECTED RESPIRATORY INFECTION		
BACTERIAL	*S. pneumoniae*	Positive *S. pneumoniae* RDT on urine
	Streptococcal tonsillitis	Positive Group A Streptococcus RDT
NON-BACTERIAL	Viral respiratory infection	Positive PCR on throat swab for: •Influenza A virus (Flu A) •Influenza B virus (Flu B) •Respiratory syncytial virus A (RSV A) •Respiratory syncytial virus B (RSV B) •Parainfluenza virus 1 (PIV 1) •Parainfluenza virus 2 (PIV 2) •Parainfluenza virus 3 (PIV 3) •Parainfluenza virus 4 (PIV 4) •Metapneumovirus (MPV) •Adenovirus (AdV) **or** Positive PCR on throat swab AND (white cell count ≥10x10^9/L OR lymphocytes ≥4x10^9/L): •Coronavirus NL63 (CoV NL63) •Coronavirus 229E (CoV 229E) •Coronavirus OC43 (CoV OC43) •Coronavirus HKU (CoV HKU) •Bocavirus (HBOV) •Enterovirus (HEV)
SUSPECTED GASTRO-INTESTINAL INFECTION		
BACTERIAL	Bacterial gastroenteritis	Positive stool culture for *Clostridium difficile, Salmonella* serovars, *Bacillus cereus, Campylobacter* spp, *Yersinia enterocolitica, Vibrio* spp, *Aeromonas* spp
NON-BACTERIAL	Amoebic or parasitic gastroenteritis	*Entamoeba histolytica, Ascari lumbricoids, Trichuris trichuria, Taenia* species or hookworm species on direct stool examination
	Viral gastroenteritis	Positive rotavirus/adenovirus RDT
SUSPECTED URINARY TRACT INFECTION		
BACTERIAL	Urinary tract infection	Positive leukocytes or nitrites on urine dipstick AND positive urine culture with non-contaminant bacteria other than mixed flora
SUSPECTED SKIN/JOINT/MUCOSAL INFECTION		
BACTERIAL	Significant skin/joint/ear infection	Gram stain positive for gram-positive OR gram-negative OR rods OR cocci OR positive culture from aspirate or discharge
NON-BACTERIAL	Significant skin/joint/ear infection	Gram stain positive for fungus OR parasite
SUSPECTED CENTRAL NERVOUS SYSTEM INFECTION		
BACTERIAL	Meningitis	Gram stain for gram-negative intracellular diplococci OR gram-positive diplococci OR gram-negative rods OR culture-positive for *Neisseria meningitides, Streptococcus pneumoniae, Streptococcus agalactiae, Haemophilus influenzae* or other bacteria causing neonatal meningitis OR *S. pneumonia* positive RDT in cerebrospinal fluid (CSF)
NON-BACTERIAL	Meningitis	Positive cryptococcal antigen RDT OR *Cryptococcus neoformans* observed in CSF culture OR yeast or *Trypanosoma* in Gram stain
NO FOCUS		
BACTERIAL	Syphilis	Positive syphilis RDT
NON-BACTERIAL	Cryptococcal disease	Positive cryptococcal antigen RDT

Figure 3 Microbiological criteria used to differentiate bacterial versus non-bacterial causes of AFI. Tests that were performed but do not appear in the figure were not considered for the electronic classification step. However, all test results were communicated to the clinical panel reviewers.

Cases that could not be assigned in the first step were converted into a summary case file that included the patient's history, clinical data and laboratory findings (online supplemental appendix 4). All case files were reviewed by a panel of three clinicians who were independent from the study and possessed at least 5 years' relevant experience in the geographical area of the study site concerned. Each clinical panel member reviewed all patient files, blinded to the assessment results of other members, and assigned them to one of the three overarching categories: bacterial infection, non-bacterial infection or undetermined cause of fever. Their adjudications were compared and, depending on the level of agreement between each clinical reviewer, the cases were

classified into a final category (figure 2). For patients with AFI where two of the three panel members gave a classification of 'bacterial' or 'non-bacterial', these patients were considered to have 'probable bacterial infection' or 'probable non-bacterial infection', respectively, for analysis purposes; the analyses were then performed both with and without these cases included in the bacterial and non-bacterial classification.

Sample collection

BFF-Dx provided a unique opportunity to establish a sample collection of extensively characterised biological samples from patients with febrile illness from different settings in Africa and South America. Samples were processed and aliquoted within 8 hours of sample collection and stored onsite at −80°C until they could be shipped, on dry ice, to a central location (ZeptoMetrix, Franklin, Massachusetts, USA). The samples, together with information regarding sample types, volumes and numbers of related aliquots are available on request to product developers and researchers (https://www.finddx.org/specimen-bank/specimens-fev/); this sample collection will allow for further comparative analyses.

Biomarker tests and analysis

Previously identified, promising host fever biomarkers[18] were selected to be part of an initial panel for evaluation (online supplemental appendix 5). Qualitative biomarker data will be analysed using standard two-by-two tables to assess the sensitivity, specificity and negative and positive predictive values for bacterial infections, based on local disease prevalence. Receiver operating characteristic (ROC) analysis will be carried out using the quantitative biomarker data to assess various diagnostic characteristics (area under the curve, sensitivity and specificity) at different cut-off points. The ROC analysis will be used to determine the optimal cut-off values for the various biomarkers in the different study settings. Detailed results of this biomarker analysis, from both individual and combined cohorts, will be made available in forthcoming publications.

BENEFITS OF THE BIOMARKER FOR FEVER-DIAGNOSTIC STUDY

BFF-Dx is one of the largest studies ever undertaken of fever biomarkers in patients with non-severe AFI in outpatient settings in LMICs. It involved extensive laboratory testing and an aetiologic classification system applied to more than 1900 individuals from enrolment centres in three countries across two continents. Of particular importance was the need to identify biomarkers that could be used to distinguish bacterial from non-bacterial AFIs in the large proportion of patients that presents at health facilities. It was essential that this distinction was valid among outpatients without severe illness, who comprise the majority population in outpatient settings in LMICs. One of the problems previously encountered when evaluating host fever biomarkers has been the lack of comparable reference tests to enable comparative analyses of biomarkers.[18] BFF-Dx affords a uniform recruitment and analysis protocol that allows direct comparisons to be made among various cohorts from very different settings. A further longer-term benefit arising from BFF-Dx is the sample collection we have established; these samples are available to researchers and companies beyond those already collaborating with FIND. These samples, together with their related clinical and microbiological data, are providing unrivalled opportunities for the identification of novel diagnostic targets and for advancing the development and evaluation of new diagnostic tests intended to guide the management of patients with AFIs. BFF-Dx has also enhanced local knowledge among our collaborators, revealing the circulation of previously undocumented pathogens and helping health professionals to improve estimates of the causes of fever in their local areas. The study data constitute a valuable ongoing resource for local teams and will contribute to efforts aimed at improving local research and planning activities. Collaborating colleagues from the study sites report that the multidisciplinary nature of BFF-Dx has led to improvements in several aspects of their work, including the coordination of sample dispatch, processes for collecting results from multiple laboratories and forging new links with laboratory and clinical teams. The study has also contributed to scientific capacity building, both through the provision of laboratory equipment and storage capacity and by facilitating local PhD studies into multiple aspects of AFIs.

LIMITATIONS

Despite our best efforts, there were several challenges and limitations associated with BFF-Dx. First, not having a control group meant we had no baseline data for the biomarkers or for the carriage of respiratory pathogens in the healthy population. Second, considering that patients with severe illness were excluded from the study, the inclusion of patients with central nervous system (CNS) symptoms should have been removed from the study design. In the event, however, no patients with CNS symptoms were recruited to the study from any of the three enrolment sites, confirming that all patients with severe symptoms were excluded. Third, the studies in Brazil and Gabon lasted for less than 1 year; therefore, any seasonal effects on the causes of fever in these locations could not be fully observed. Fourth, while we anticipate that the epidemiology of AFIs in Brazil, Gabon and Malawi will be broadly representative, pathogens that are geographically focal, especially in Asia, will not be represented in the samples we collected. Fifth, no perfect method exists for classifying AFI cases into those of bacterial or non-bacterial aetiology. When all other factors were taken into consideration, the approach we adopted was the most appropriate for determining bacterial/non-bacterial cases. For example, we could have chosen to classify AFIs of bacterial origin as only those cases that were microbiologically confirmed. However, given the

limited percentage of microbiologically confirmed cases obtained even in the most comprehensive aetiological studies,[10 24] restricting the analysis to this subgroup would not have truly represented the intended-use population for which the new test was expected to be used. Sixth, in the Malawi study, fever at presentation was an inclusion criterion, and not history of fever, as patients were not likely to self-medicate with antipyretics, as was the case in Gabon and Brazil. However, as several fever-causing infections present with intermittent fever, we consider that history of fever in the last 7 days should be part of the systematic inclusion criteria of any such study. Finally, the overall classification process may have been refined and improved by adding additional tests and parameters or expanding the clinical panel. However, technical solutions, financial resources and local capacities were limited, and the project methodology was designed to make the best use of available resources.

CONCLUSION

This study and related activities (eg, systematic reviews, TPP development, technology reviews), along with the use of biomarkers to guide evidence-based decision making, were initiated 5 years ago as part of a concerted effort by the global health community to reduce the overuse of antibiotics and to curb the threat posed by antimicrobial resistance.[14] Now, we have completed one of the most extensive studies ever designed to address this very specific challenge. Despite the study's limitations, we believe that the approach adopted and the outputs achieved (from a standardised methodology to a sample collection), which have been made openly available, can move the dial on ambitious goals such as improving patient care and reducing the overuse of antibiotics worldwide. Therefore, BFF-Dx provides a positive exemplar for global collaborations that aim to improve healthcare for all in response to ongoing public health challenges.

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Contributors SD, BTT, WR, SG, AMS, STA, MML and TS contributed expertise for the clinical, epidemiological and diagnostic activities. SO and AM led the development of data management processes and the statistical analysis plan. VH, CE and SD contributed specialist expertise for laboratory and diagnostic activities. In Brazil, AMS contributed to adapting the protocol, reference testing and translation. In Gabon, STA and MML contributed to adapting the protocol and translations. In Malawi, SG contributed to adapting the protocol and the data management plan. VH, CE and SD drafted the publication. All authors contributed to the design and development of the BFF-Dx protocol and/or subsequent implementation, analysis and/or publication input. All authors critically revised the manuscript for important intellectual content, read and approved the final version and agreed to its submission.

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Version 07MAR19

Place barcode label here

Clinic name: _____ *Participant ID: FIND 00104__ __/__ __ __ __*

Case Report Form – Clinic

ELIGIBILITY

1.	Age between 2 and 17 years old	❑YES	❑NO
2.	Temperature of ≥ 38°C (oral or ear)/temperature of ≥ 37.5°C (axillary or skin) at initial evaluation or within 6 hours of arrival to the hospital or history of fever within 7 days.	❑YES	❑NO
3.	Less than 7 days of symptoms	❑YES	❑NO
4.	Participant has no severe/life threatening illness*	❑YES	❑NO
5.	Availability for a follow-up visit, if required	❑YES	❑NO

** based on clinician assessment or the presence of any general signs of critical illness as defined by WHO guidelines (for children: extensive vomiting, active seizure or recent history of seizures, altered mentation, inability to feed, or any of the severe IMNCI classifications; for adults: impending airway obstruction, central cyanosis, severe respiratory distress, feeble pulse, active seizure or recent history of seizures, or unconsciousness)*

STUDY INCLUSION

6.	Based on the answers above is the participant eligible for the study?#	❑YES	❑NO	
7.	Did the parent consent for the child to participate in the study?	❑YES	❑NO	
8.	Did the adolescent (13-17 years old) give an assent to participate in the study?	❑YES	❑NO	❑N/A

 # to be eligible, answers to Q1 to Q8 should all be "yes"

DEMOGRAPHIC INFORMATION

9.	Date of enrolment:	__ __(dd)/__ __(mm)/__ __ __ __(yyyy)				
10.	Sex:	❑Male	❑Female			
11.	Place of enrolment:	❑OPD	❑Inpatient	❑Health Center		
12.	Date of birth:	__ __(dd)/__ __(mm)/__ __ __ __(yyyy)			Age (years)	
13.	Is the participant pregnant ***N/A for male***	❑ Yes	❑ No	❑N/A		

**Offer test if requested*

CLINICAL HISTORY

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Place barcode label here

Clinic name: _____ *Participant ID: FIND 00104__ __/__ __ __ __*

Tick all symptoms present as a part of current episode and estimate duration for each.

	SYMPTOMS	RESPONSE			DURATION (in days)		
14.	Duration of illness				☐☐		
15.	Fever (days)	❑YES	❑NO		☐☐		
16.	Redness of the eyes	❑YES	❑NO		☐☐		
17.	Eye discharge	❑YES	❑NO		☐☐		
18.	Sore Throat	❑YES	❑NO	❑UNKNOWN	☐☐		
19.	Ear discharge	❑YES	❑NO		☐☐		
20.	Swelling behind the ear	❑YES	❑NO		☐☐		
21.	Sneezing and rhinorrhoea	❑YES	❑NO		☐☐		
22.	Postnasal drip	❑YES	❑NO		☐☐		
23.	Cough	❑YES	❑NO		❑<2 weeks	❑<2 months	❑≥2 months
24.	Chest pain	❑YES	❑NO	❑Unknown	❑<2 weeks	❑<2 months	❑≥2 months
25.	Diarrhoea	❑YES	❑NO		☐☐		
26.	Vomiting	❑YES	❑NO		☐☐		
27.	Pain while swallowing	❑YES	❑NO	❑UNKNOWN	☐☐		
28.	Abdominal pain	❑YES	❑NO	❑UNKNOWN	☐☐		
29.	Dysuria	❑YES	❑NO	❑UNKNOWN	☐☐		
30.	Urinary frequency or urgency	❑YES	❑NO	❑UNKNOWN	☐☐		
31.	Rash	❑YES	❑NO		☐☐		
32.	Headache	❑YES	❑NO	❑UNKNOWN	☐☐		
33.	Neck stiffness	❑YES	❑NO	❑UNKNOWN	☐☐		
34.	Photophobia	❑YES	❑NO	❑UNKNOWN	☐☐		
35.	Joint pain or swelling	❑YES	❑NO	❑UNKNOWN	☐☐		
36.	Other (please specify)	❑YES	❑NO				
37.	_____				☐☐		
38.	_____				☐☐		

all yes must have duration

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Case Report Form Clinic
Biomarker evaluation study – AF_01_P08800-00
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Clinic name: _____ *Participant ID: FIND 00104__ __/__ __ __ __*

TREATMENT HISTORY

39.	Has the participant taken antibiotics? ❑YES ❑NO ❑Don't know	If Yes:	40. Treatment start date: __ __/__ __/__ __ __ __ ❑Don't know 41. Treatment end date: __ __/__ __/__ __ __ __ ❑Don't know
42.	Has the participant taken antipyretics ❑YES ❑NO ❑Don't know	If yes	43. Treatment start date: __ __/__ __/__ __ __ __ ❑Don't know 44. Treatment end date: __ __/__ __/__ __ __ __ ❑Don't know
45.	Has the participant taken any other treatment? ❑YES ❑NO ❑Don't know	46. If Yes (tick one or several): ❑Antimalarial ❑Antipyretic ❑Other, specify:	

PAST MEDICAL HISTORY

47.	Does the participant have a chronic disease: ❑YES ❑NO ❑ Don't know	48. If Yes (tick one or several): ❑DM ❑HIV ❑TB ❑Other chronic diseases, specify:

if all yes must have follow up questions answered

VACCINATION HISTORY

49.	Has the participant been vaccinated according to EPI?	❑Completed vaccination	❑Partially vaccinated
		❑ Not vaccinated	❑Don't know

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Clinic name: _____ *Participant ID: FIND 00104__ __/__ __ __ __*

PHYSICAL EXAMINATION

VITAL SIGNS

50. **GENERAL APPEARANCE** ***all questions must have response recorded***

- ❑ Not ill — *Healthy and strong impression throughout examination*
- ❑ Moderately ill — *Some impairment of activities, mostly self-sufficient but clearly symptomatic*
- ❑ Acutely ill — *Unable to carry out usual activities, visibly distressed, high fever, prostrated*
- ❑ Chronically ill — *Prominent facial bones (for adults), Emaciated with bone and skin appearance*

51.	Temperature (°C)	❑ Axillary ❑ Oral ❑ Ear ❑ Skin
52.	Respiratory rate (per minute)	
53.	Pulse rate (per minute)	
54.	Blood pressure (mmHg)	_____

ANTHROPOMETRY

55.	Weight (Kg)	
56.	Height (cm)	
57.	Mid upper arm circumference (cm) *(optional)*	
58.	Peripheral signs of malnutrition *(tick one or several)*	❑No signs ❑Hair colour change ❑Oedema ❑Skin lesions

SYSTEMIC EXAMINATION		If Yes, tick one or several:		
59. ***HEENT***	❑Yes ❑No	❑Pharyngeal erythema ❑Pharyngeal enlargement ❑Conjunctival exudate	❑Conjunctival redness ❑Pain and swelling around teeth	
60. ***Lungs***	❑Yes ❑No	❑ Fast breathing ❑ Decreased air entry ❑Retractions	❑Dullness ❑Crepitation ❑Chest in drawing	❑Other, Specify:
61. ***Heart***	❑Yes ❑No	❑Tachycardia	❑Ejection murmur	❑Other, Specify:
62. ***Abdomen***	❑Yes ❑No	❑Tenderness ❑Hepatomegaly	❑Splenomegaly ❑Fluid Collection	❑Other, specify:
63. ***Genitourinary***	❑Yes ❑No	❑Costovertebral angle tenderness	❑Other, specify:	
64. ***Nervous System***	❑Yes ❑No	❑Positive meningeal signs ❑Focal neurologic deficit	❑Other, Specify:	

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Clinic name: _____ *Participant ID: FIND 00104__ __/__ __ __ __*

65. ***Integumentary***	❑Yes ❑No	❑Maculopapular ❑Cellulitis/abscess >5mm ❑Other, specify: ❑Impetigo ❑Dermatovesicular rash
66. ***Lymphadenopathy***	❑Yes ❑No	If yes, specify location: _____ size:_____ mm
67. ***Joint Swelling***	❑Yes ❑No	If yes, specify location: _____
68. ***Other findings***	❑Yes ❑No	❑ If yes, specify:_____

If yes follow up questions must be answered

RAPID TESTS

69. Strep A RDT with Ths002	❑Positive ❑ Negative ❑Invalid ❑N/A
70. Malaria RDT	❑Pf positive ❑Pan positive ❑ Negative ❑Invalid
71. CRP/Malaria RDT	❑Pf positive ❑Pan positive ❑ Negative ❑Invalid ❑CRP positive ❑ CRP Negative ❑ CRP Invalid

70-71 must be done for all patients

CHEST X-RAY

72.	Chest X-Ray performed	❑YES ❑NO ❑N/A		
73.	Date :	__ __(dd)/__ __(mm)/__ __ __ __(yyyy)		
74.	Normal	❑YES ❑NO		
75.	Localization of abnormality (optional) *(tick one or several)*	❑Left upper zone ❑Right upper zone ❑Diffuse	❑Left mid zone ❑Right mid zone	❑Left lower zone ❑Right lower zone
76.	Picture (optional) *(tick one or several)*	❑Infiltrate consolidation ❑Mediastinal/hilar lymphadenopathy	❑Cavitary lesion ❑Micronodules (Miliary)	❑Tuberculoma ❑Pleural effusion
77.	Principal conclusion: *(tick one only)*	❑Bacterial pneumonia likely ❑Other: _____	❑Pneumonia or atypical TB	❑Pneumonia unlikely, TB likely

If yes for question 72, 73-77 must be completed

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Clinic name: _____ *Participant ID: FIND 00104__ __/__ __ __ __*

PRESUMED DIAGNOSIS TREATMENT

78. Presumed diagnosis by the clinician: *(tick one only)*	❑ Bacterial infection ❑ Viral infection ❑ Malarial infection ❑ Parasitic infection ❑ Multiple infection ❑ Don't know		❑ Non-infectious illness, specify: ❑ Other, specify:
79. Hospitalization?	❑ Yes	❑No	❑Don't know
80. Treatment Prescribed:	❑ Yes	❑No	❑Don't know

81. If Yes, specify treatment: *(tick one or several)*

If Antibiotics, tick the box:

❑Penicillin
❑Cloxacillin
❑Ampicillin
❑Amoxi/clavulan
❑Ceftriaxon
❑Gentamycin
❑Doxycyclin
❑Ciprofloxacin
❑Chloramphenicol
❑Clindamycin
❑Erythromycin
❑Cotrimoxazole
❑Azithomycin
❑Tetracyclin
❑Cefoxitin

❑ Supportive care
❑ Antimalarial, specify:
❑ Antiviral, specify:
❑ Other, specify:

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Clinic name: _____ *Participant ID: FIND 00104__ __/__ __ __ __*

82.	Withdrawal or early exclusion from study	❑ Yes ❑No If Yes, specify reason:

Comments: _____

_____

Investigator's Signature: _____ Date completion: __ __/__ __/__ __ __ __

First data entry: _____ Date completion: __ __/__ __/__ __ __ __

Second data entry: _____ _____ Date completion: __ __/__ __/__ __ __ __

❑ **Copy CRF sent** Date: __ __/__ __/__ __ __ __

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Participant ID: FIND 00104 __ __/__ __ __ __

Investigator initials_____

Patient Age in years_____ Sample volume collected EDTA_____

Clinical laboratory CRF enrolment visit

Investigator: Please "standard panel" will be run for all participants
Transporter: Please check all documents and confirm receipt of samples as requested
Lab scientist: 1. Please tick/note the results at the appropriate place.

INVESTIGATOR REQUEST		**TRANSPORTATION CHECK**	**BARCODE**	
STANDARD PANEL	☒	**1 EDTA tube**	**ED WB COL002**	☐
NO FOCUS PANEL	☐	**Same EDTA tube**		

2. If patient is HIV+ve by RDT add NO FOCUS panel RDT testing

Laboratory tests	**Result**
HIV RDT*** If HIV $^{+ve}$ complete NO FOCUS panel RDTs**	❑Positive ❑ Negative ❑Invalid
Malaria Microscopy results **reader 1** Reader _____	❑Positive ❑Pf ❑Po ❑PM ❑Negative Density____para/µL
	❑Positive ❑Pf ❑Po ❑PM ❑Negative Density____para/µL
Malaria Microscopy results **reader 2** Reader _____	❑Positive ❑Pf ❑Po ❑PM ❑Negative Density____para/µL
	❑Positive ❑Pf ❑Po ❑PM ❑Negative Density____para/µL
Malaria Microscopy results **reader 3** Reader _____	❑Positive ❑Pf ❑Po ❑PM ❑Negative Density____para/µL
	❑Positive ❑Pf ❑Po ❑PM ❑Negative Density____para/µL
Haematology full blood count	WBC(x10^3/µL):_____ Hct(%):____ LY(%):____NEU(%)____ (*optional*):_____
NO FOCUS if HIV +ve ☐	
No focus panel	❑Done ❑Not done
Cryptococcus	❑Positive ❑ Negative ❑Invalid
Syphilis	❑Positive ❑ Negative ❑Invalid

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Participant ID: FIND 00104 __ __/__ __ __ __

Comments: _____

Laboratory scientist name: _____ Date completion: __ __/__ __/__ __ __ __

Final data entry: _____ Date completion: __ __/__ __/__ __ __ __

❑ **Copy CRF sent** Date: __ __/__ __/__ __ __ __

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Clinic name: _____ *Participant ID: FIND 00104* __ __/__ __ __ __

Case Report Form – Follow up

Treatment History between Initial Evaluation

1. Has the participant taken antibiotics? ❑NO ❑Don't know	❑YES → If yes specify _ _ _ _ _	2. Treatment start date: __ __/__ __/__ __ __ __	❑Don't know
		3. Treatment end date: __ __/__ __/__ __ __ __	❑Don't know
		4. Participant was considered cured:	❑YES ❑NO
5. Has the participant taken any other treatment? ❑NO ❑Don't know	❑YES →	6. ❑Antimalarial ❑Antipyretic	❑Other, specify:

Follow up Clinical Assessment

7.	Has the fever gone ?	❑YES	❑NO	❑Don't know
8.	If yes to #5, how many days after initiation of treatment?	_____		
9.	Are there any additional symptoms?	❑YES	❑NO	❑Don't know
10	If yes, what is the type of symptoms? ❑ Respiratory ❑ Gastrointestinal ❑Fever without focus ❑Rash ❑ Urinary tract ❑ Arthritis ❑ Other, please specify:_____			

Final Clinical Diagnosis

11	Presumptive Diagnosis:	▪ Bacterial infection ▪ Viral infection ▪ Parasitic infection ▪ Multiple infection	❑ Non-infectious illness, specify: ❑ Other, specify: ❑ Don't know
12	Date Diagnosis (dd/mm/yyyy):	__ __/__ __/__ __ __ __	
13	Patient found:	❑ Alive ❑ Dead	Note: _____

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Place barcode label here

Clinic name: _____ *Participant ID: FIND 00104* __ __/__ __ __ __

Investigator's Signature: _____ Date completion: __ __/__ __/__ __ __ __

First data entry: _____ Date completion: __ __/__ __/__ __ __ __

Second data entry: _____ _____ Date completion: __ __/__ __/__ __ __ __

FIND – Biomarker evaluation study / HOF_01_P08800-00
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Participant ID: FIND 00104 __ __/__ __ __ ____

Place barcode label here

Microbiology Laboratory CRF enrolment visit

Investigator initials: _____

Investigator: Please tick/mark the required tests on the form, "standard panel" will be run for all participants.
Transporter: Please check all documents and confirm receipt of samples as requested
Lab scientist: Please confirm receipt of samples and tick/note the results at the appropriate place

INVESTIGATOR REQUEST		**TRANSPORTATION CHECK**		**BARCODE**	
STANDARD PANEL	☒	**Blood culture bottle *1**		**BCCOL001**	☐
Urine for Storage	☒	**Container**		**U001**	☐
URINARY PANEL*	☐	**Urine sample**		**UCOL001**	☐
STOOL PANEL~	☐	**Stool sample * 1 – split in parasitology**		**Patient ID only**	☐
CNS PANEL	☐	**CSF sample**		**CSF001**	☐
SKIN/JOINT/ASPIRATE	☐	**Other sample/S**		**OT**	☐
Transported by			**Received by**		

INVESTIGATOR REQUEST		**TRANSPORTATION CHECK**		**BARCODE**	
RESPIRATORY PANEL	☐	**Urine**			☐
Transported by			**Received by**		

Laboratory tests	**Results**
STANDARD PANEL	**Time and date of blood collection:** **Tubes collected:** Aerobic ❑
Blood culture	❑ Positive ❑ Negative ❑ Contamination
	If culture positive, specify Gram staining results: ❑ Gram positive ❑ Gram negative ❑ Rods ❑ Cocci ❑ No pathogen observed, ❑ Pathogen isolated Pathogen: ❑ E.coli ❑ kleb pneu ❑ Staph aur ❑ Salmonella Other: _____

DIARRHEAL PANEL	**Time and date of stool collection:**
Faeces culture	Pathogen isolated: ❑ No ❑ Yes specify_____

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Participant ID: FIND 00104 __ __/__ __ __ ____

Place barcode label here

URINARY PANEL	**Aliquot 2 tubes of 1 mL and store at -80°C (research lab), ensure collection of at least 40ml if additional tests required** **1 Urine sample** ☐	**U COL001** ☐
Urine dipstick (combu 9)	WBC: ❑Positive ❑ Negative ❑ Invalid Nitrites: ❑Positive ❑ Negative ❑ Invalid	
Urine Culture	❑ Positive ❑ Negative ❑ Contamination If positive specify pathogen isolated ❑ E.coli❑ Proteus❑ Pseudo ❑ Entero ❑ Staph ❑ Strep ❑ S.saprophyticus ❑ Other _____	
RESPIRATORY PANEL ☐	**Use urine for this panel**	
S. pneumoniae RDT (urine)	❑Positive ❑ Negative ❑Invalid	
CNS PANEL ☐	**Time and date of CSF collection:**	
CSF Examination	Grossly looks: ❑Crystal clear ❑Turbid ❑Bloody Cells (per mm3):_____ Neutrophil (%):_____ Protein:_____ mg/dL Glucose:_____ mg/dL	
Cryptococcus RDT (CSF)	❑Positive ❑ Negative ❑Invalid	
S. pneumoniae RDT (CSF)	❑Positive ❑ Negative ❑Invalid	
Gram stain	❑ Not done ❑ Pathogen observed ❑ No pathogen observed If pathogen observed *(tick one of several):* ❑ Gram neg intracellular diplococci ❑Gram pos diplococci ❑ Gram neg rods ❑ Yeast ❑ Other, specify:_____	
Culture	Pathogen isolated: ❑No ❑ ,Neis men ❑,Strep Pn ❑,Strep Aga ❑, Cypto ❑,Other ❑ specify _____	
SKIN/JOINT/ASPIRATE ☐	**Time and date of sample collection:** **Type of sample collected:**	
Gram stain	❑ Not done ❑ Pathogen observed ❑ No pathogen observed If pathogen observed *(tick one as needed):* ❑ Gram pos ❑ Gram neg ❑ Rods ❑ Cocci ❑ Yeast ❑ Other, specify:_____	
Culture	Pathogen isolated:	

Comments:_____

Laboratory scientist: _____ Date completion: __ __/__ __/__ __ __ _

Final data entry: _____ Date completion: __ __/__ __/__ __ __ __

❑ **Copy CRF released to Data** Date: __ __/__ __/__ __ __ __

FIND – Biomarker evaluation study / HOF_01_P08800-00
Version 07MAR19
Participant ID: FIND 00104 __ __/__ __ __ ____

Place barcode label here

Biobank Storage

Samples for biobanking	Vol	Barcode ID	Freezer box name and number	position
Urine biobanking 1	1ml	U001	FIND Urine biobanking	
Urine biobanking 2	1ml	U002	FIND Urine biobanking	

PS: Take samples to research laboratory freezer and attach this part of the CRF to the Research CRF.

Comments: _____

Laboratory scientist: _____ Date completion: __ __/__ __/__ __ __ _

Final data entry: _____ Date completion: __ __/__ __/__ __ __ __

❑ **Copy CRF released to Data** Date: __ __/__ __/__ __ __ __

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Place barcode label here

Participant ID: FIND 00104 __ __/__ __ __ __

Investigator initials_____

Parasitology laboratory CRF enrolment visit

Transporter: Please check all documents and confirm receipt of samples as requested, sign form
Lab scientist: Please sign form on receipt of correct samples
Tick/note the results at the appropriate place.

INVESTIGATOR REQUEST		**TRANSPORTATION CHECK**	**BARCODE**	
Stool Panel	☐	**Note: Stool sample to be split in Parasitology and sent to microbiology**	**STCOL001**	☐
Urinary Panel	☐	**Urine to be sent from microbiology laboratory (if applicable)**	**Patient ID (barcode not required)**	☐
Transported by:			**Received by:**	

DIARRHEAL PANEL	**Time and date of stool collection:**
Rotavirus/adenovirus RDT	❑Adenovirus Positive ❑Rotavirus Positive ❑ Negative ❑Invalid
Appearance of faeces	❑ Bloody ❑ Rice water ❑ Hard stool ❑ Don't know ❑ Watery ❑ Green watery ❑ Other, specify:_____
Microscopy	❑ Not done ❑ Pathogen observed ❑ No pathogen observed If pathogen observed *(tick all that apply):* ❑ Ascari lumbricoids ❑ Trichuris trichuria ❑ strongyloides species ❑ Hookworm species ❑ protozoa spp ❑ Other, specify:_____

Unary PANEL	**Time and date of stool collection:**
Microscopy	❑ Not done ❑ Pathogen confirmed ❑ No pathogen observed If other pathogen observed specify:_____

* *if suspicion of schistosomiasis*

Comments: _____

Laboratory scientist name: _____ Date completion: __ __/__ __/__ __ __ __

Final data entry: _____ Date completion: __ __/__ __/__ __ __ __

❑ **Copy CRF sent** Date: __ __/__ __/__ __ __ _

Supplementary Appendix 2

List of test panels with request criteria, sample types, and variations by site

Panel	**Sample type**	**Test**	**Brazil**	**Gabon**	**Malawi**
Standard	EDTA-whole blood	Malaria	BIOLINE Malaria Ag Pf/Pan (Alere/SD, South Korea)		
		HIV	HIV 1/2 (BIOCON)	VIKIA HIV 1/2 (bioMérieux, France)	Alere Determine™ HIV–1/2 Ag/Ab Combo and Uni-Gold HIV 1/2
		Typhoid	None	Blood culture	TyphiDot (Biodiagnostic Research Sdn. Bhd, Selangor Darul Ehsan, Malaysia)
	EDTA-plasma	*Rickettsia* spp.	PCR	Vircell ELISA (IgM/IgG)	
		Leptospira spp.	Microscopic agglutination test (MAT)	SERION VIRION ELISA (IgM/IgG)	
		Chikungunya virus	PCR; Chembio DPP RDT (IgM/IgG) and EUROIMMUN ELISA (IgM/IgG)	EUROIMMUN ELISA (IgM/IgG)	
		Dengue virus	PCR; Chembio DPP RDT and EUROIMMUN	EUROIMMUN ELISA (IgM/IgG andNS1)	

			ELISA (IgM/IgG)	
		Zika virus	PCR; Chembio DPP RDT and EUROIMMUN ELISA (IgM/IgG)	EUROIMMUN ELISA (IgM/IgG)
No focus	EDTA-whole blood	Syphilis	Syphilis test SD Bioline 3.0 (Alere/SD, South Korea)	
		Cryptococcus	CrAg LFA, (IMMY, USA)	
Respiratory	Oropharyngeal swab	Group A *Streptococcus* RDT	In-Line Strep A test (Quidel, USA)	
	Oropharyngeal swab	Multiplex PCR for respiratory pathogens	Fast Track Diagnostics Respiratory Pathogens 21	Seegene Allplex™ Respiratory Panel Assays 1 to 4
	Urine	Rapid urinary antigen test for *Streptococcus pneumoniae*	BinaxNOW® *S. pneumoniae* Antigen Card (Alere, South Korea)	
Stool	Stool	Rotavirus and adenovirus	VIKIA (BioMerieux, France)	
Urinary	Urine	Dipstick, white blood cells, and protein	Urilab H10 (Urilab Systems-	Combur 10-Test (Roche	Urilab H10 (Urilab Systems-

			Diagnostics, India)	Diagnostics, Switzerland)	Diagnostics, India)
Central nervous system	Cerebrospinal fluid	Cryptococcus	CrAg LFA, (IMMY, USA)		

Abbreviations RDT: rapid diagnostic test; PCR: polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay; IgM: immunoglobulin M; IgG: immunoglobulin G; NS1: non-structural protein 1; MAT: microscopic agglutination test

Supplementary Appendix 3

List of samples collected and relevant transport and storage conditions

Specimen	**Transport**	**Temperature**	**Time from collection to storage or testing**	**Purpose**
Whole blood in an EDTA tube	Local Courier	8ºC	Max 8 hrs	Local testing and long-term storage
Whole blood in an RNA PAXgene tube	Local Courier	8ºC	2 hrs	Aliquoting and storage
Whole blood in a plain tube	Local Courier	8ºC	Max 8 hrs	Local testing, long-term serum storage
Whole blood in a lithium heparin tube	Local Courier	8ºC	Exactly 60 mins	Activation of HNL
Aliquoted serum	World Courier	-20ºC	Max 8 hrs	Local serology testing
	World Courier	-80ºC	Max 8 hrs	Specimen bank
Aliquoted plasma	World Courier	-20ºC	Max 8 hrs from collection	Local serology testing
	World Courier	-80ºC	Max 8 hrs from collection	Specimen bank

Activated heparin plasma		-20ºC	Directly after activation	Local ELISA testing
	World Courier	-80ºC	Directly after activation	Specimen bank
PAXgene RNase-free aliquots	World Courier	-80ºC, RNase-free cryotubes	10 × 1 ml aliquots after incubation for 2 hrs	Specimen bank
Urine	Local Courier	4-8ºC	Max 4 hrs	Local testing
	World Courier	-80ºC	Max 4 hrs	Specimen bank
Stool	Local Courier	4-8ºC	Max 4 hrs	Local testing
Aspirate	Local Courier	4-8ºC	Max 4 hrs	Local testing
Cerebrospinal fluid	Priority Local Courier	Ambient temperature	Max 4 hrs	Local testing
Oropharyngeal swab	Local Courier	8ºC	Max 4 hrs	Local testing
	World Courier	-20ºC, stored in transport media	Max 4 hrs	Reference testing

Patient report for FE001010893

FIND

23 January 2019

1. Clinical Data

Demographic Information

- Age: 59
- Gender: Male

Symptoms

- Fever duration: 2 days
- Other symptoms: Headache,Joint pain or swelling

Vaccination History

- Vaccination status: Completed vaccination

Physical Examination

Vital signs measurement

- Temperature: 37.9°C
- Respiratory rate: 24pm
- Pulse rate: 103pm
- Blood pressure: 136/77

Anthropometry

- Weight: 54kg
- Height: 168cm
- Mid upper arm circumference: 250mm
- Peripheral signs of malnutrition: No sign

Treatment prescribed:

- Treatment: Antibiotics: CIPROFLAXIN - Other

2. Laboratory Data

Standard Panel

- RDT results: Malaria RDT negative, HIV RDT negative, Typhoid IgM RDT positive
- Whole White Blood Cell count(10ˆ3/ul): 11.1 - Neutrophil (% of WBC): 67.568 - Lymphocyte (% of WBC): 26.126 - Hematocrit (% of Whole Red Blood Cell count): 43.6

Serology results by ELISA

Chikungunya

- Baseline sample: IgG Positive
- Baseline sample: IgM Negative

Escadafal C, *et al. BMJ Global Health* 2020; 5:e003141. doi: 10.1136/bmjgh-2020-003141

Dengue

- Baseline sample: NS1-Antigen Negative
- Baseline sample: IgG Positive
- Baseline sample: IgM Negative

Leptospirosis

- Baseline sample: IgG Negative
- Baseline sample: IgM Negative
- Follow-up sample IgG Negative
- Follow-up sample IgM Negative

Rickettsia

- Baseline sample: IgG Positive
- Baseline sample: IgM Negative
- Follow-up sample IgG Negative
- Follow-up sample IgM Negative

Zika

- Baseline sample: IgG Positive
- Baseline sample: IgM Negative

3. Follow-up Data

Treatment at Follow-up

Antibiotics

- Antibiotics: Yes
- Start date: 2018-03-22
- End date: 2018-03-26
- Cured: Yes

Final clinical diagnosis

Presumptive diagnosis: Viral Inf

Escadafal C, *et al. BMJ Global Health* 2020; 5:e003141. doi: 10.1136/bmjgh-2020-003141

Supplementary Appendix 5

Initial list of selected biomarkers to be evaluated

Biomarker	Assay (Manufacturer)	Sample	Reference
HNL	HNL ELISA prototype (Philips/University of Uppsala)	Activated heparin plasma	Venge *et al.,* 2015[1]
MxA + CRP	FebriDx® (RPS Diagnostics)	Fresh EDTA-whole blood via fingerprick	Sambursky *et al.,*2015[2]
CRP + TRAIL + IL-10	ImmunoDx or ImmunoXpert (MeMed) (offsite using bio-banked samples in 2018)	EDTA-plasma	Oved *et al.,* 2015[3]
CHI3L1	ELISA (R&D Systems)	EDTA-plasma	Erdman *et al.,* 2015[4]
HBP	ELISA (Axis-Shield)	EDTA-plasma	Kapasi *et al.,* 2016[5]
CRP	CRP NycoCard with NycoReader II (Alere)	EDTA-plasma	n.a.
PCT	ELISA (Abcam)	EDTA-plasma	n.a.

n.a., not applicable

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