STUDY PROTOCOL

FIEBRE: Febrile illness evaluation in a broad range of endemicities

Version and date: 4.0 / 28 February 2019

Trial sites: Inpatient and outpatient health facilities in Lao PDR, Malawi, Mozambique, Myanmar, and Zimbabwe

STUDY SPONSOR: London School of Hygiene & Tropical Medicine

SPONSOR ADDRESS: Keppel St, London, WC1E 7HT, United Kingdom

LSHTM ethics reference: 14538

This protocol describes the FIEBRE study and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres entering participants for the first time are advised to contact the trials centre to confirm they have the most recent version.

Problems relating to this trial should be referred, in the first instance, to the study coordination centre.

This trial will adhere to the principles outlined in the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations.

MAIN CONTACTS:

Study Management Group

REDACTED FOR PUBLICLY POSTED PROTOCOL

Sponsor: London School of Hygiene & Tropical Medicine is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance and Integrity Office: London School of Hygiene & Tropical Medicine, Keppel Street, London, WC1E 7HT, UK

Contents

1 GENERAL INFORMATION

Investigators (in alphabetical order by family name):

REDACTED FOR PUBLICLY POSTED PROTOCOL

2 STUDY OVERVIEW AND PROTOCOL SUMMARY

Study population: Patients (inpatient and outpatient) of all ages two months and older who present with fever to selected health facilities in sub-Saharan Africa and Southeast Asia, and community controls.

Number of Sites: Five; in 1) Lao PDR, 2) Malawi, 3) Mozambique, 4) Myanmar, and 5) Zimbabwe.

Study duration: 12 months of participant recruitment at each site; to be extended at individual sites if required to meet sample size target.

General objectives: To identify the causes of fever, and the antimicrobial susceptibility of bacterial pathogens causing fever, in low- and middle-income settings where few data are available.

Specific primary objectives:

1) To determine the treatable and/or preventable causes of fever in children and adults presenting as outpatients, and among those admitted to hospitals, in areas represented by the study sites;
2) To determine how fever aetiology varies according to patient age, geographical area, local malaria and HIV prevalence, and other risk factors;
3) To determine the prevalence and spectrum of antimicrobial resistance among bacteria identified in clinical specimens from febrile patients.

Specific secondary objectives:

1) To generate data on incidence of specific infections in study site catchment areas, and therefore contribute key data on disease burden for some infections that are not counted in current “global burden of disease” estimates;
2) To build an archive of well-characterised and geographically diverse biological samples from well-characterised clinical phenotypes for use in evaluation of new diagnostic and prognostic tests, and in identification of human- and pathogen-related biomarkers that may improve case management strategies;
3) To evaluate available biomarker assays to assess their performance and potential utility in fever case management in the study areas;
4) To generate data to inform the development of new fever case management algorithms which may be evaluated in future studies.

3 BACKGROUND AND RATIONALE

3.1 Background Information

3.1.1 Current status of fever case diagnosis and management in malaria-endemic areas

Fever is one of the most common symptoms leading to health care seeking and hospital admission in sub-Saharan Africa and Asia.[1,2] Current WHO algorithms for the primary care level do not provide comprehensive guidance to clinicians for the management of non-malarial fevers. If the malaria test is negative, the patient is classified as "Fever: no malaria" in the Integrated Management of Childhood Illness (IMCI) guidelines[3] or in the Integrated Management of Adolescent and Adult Illness (IMAI) guidelines[4] and advice is given to "treat according to the apparent cause of fever." Many febrile illnesses present with non-specific symptoms and signs, and the current recommendations often result in treatable diseases being left untreated or treated with inappropriate antimicrobials, on the one hand, and over-treatment of self-limiting conditions with antimicrobials on the other, with important implications for the development of antimicrobial resistance.[5,6]

3.1.2 Current knowledge of causes of fever in malaria-endemic areas

Little is known about the non-malarial causes of fever in many parts of the world,[7] so there is sparse evidence on which to base empirical treatment guidelines for febrile patients testing negative for malaria. A few studies provide some indication of the clinical spectrum of febrile illness,[8,9] but these studies were often disease specific, for example focussing on urinary tract infections in Nigeria[10] or arboviruses in Asia.[11]

Members of our group have recently reviewed[7] and mapped[12] the currently available data on causes of fever in patients attending health facilities in sub-Saharan Africa and Southeast Asia. In addition, a few studies specifically designed to look at aetiologies of fever have been recently published.[2,13-16] While the results are useful within the specific study areas, the study designs were heterogeneous – with differences in patient age, type of health facility, seasons covered, inclusion criteria, and study design – making it difficult to compare findings across sites and produce a clear picture of the causes of fever in each age group and at each level of care. In addition, there is heterogeneity in laboratory case definitions used; use of diagnostic tests that are not sufficiently validated or not widely accepted; and lack of control groups, preventing calculation of attributable fractions.

In particular, there is very little information on incidence for many of the infections thought to be clinically important at the FIEBRE study sites, and consequently little information on which to base estimates of burden of disease, or to guide empirical therapy or control measures.[17]

3.1.3 Fever case management and antimicrobial resistance

The ability to differentiate between bacterial and viral infections could have a major global impact on antimicrobial resistance by limiting the unnecessary use of antimicrobials. The WHO convened a meeting of experts in September 2015, including members of our group, on biomarkers to discriminate bacterial from other infectious causes of fever.[18] The conclusion of the meeting was that the evaluation of biomarker tests to distinguish between bacterial causes of fever requiring antimicrobial treatment, and self-limiting viral infections, in low-income settings was a very high priority.

3.2 Significance and Scientific Rationale

This study, titled Febrile Illness Evaluation in a Broad Range of Endemicities (FIEBRE), is proposed to help to fill key information gaps by means of a multi-site investigation, using consensus clinical, laboratory, and social science protocols, in regions with a high burden of infectious disease from which few or no data are available.

We will conduct a study on causes of febrile illness, and antibacterial resistance, at five sites in sub-Saharan Africa and Southeast Asia. We will focus on detecting infections that are treatable (e.g. with specific antimicrobials) and/or preventable (e.g. with vaccination or vector-control approaches). We will use a common design, with harmonised inclusion criteria, case definitions, laboratory procedures, and result interpretation. This approach will generate reliable and comparable data that can contribute to updated recommendations on the clinical management and prevention of febrile illnesses, adapted to local contexts. The results of this study will provide data to support more reliable estimates of the incidence and, in turn, burden of disease, and updated evidence-based algorithms for the management of febrile illness; and will provide a basis for future evaluation of novel diagnostics, and rational approaches to disease surveillance.

We will study adults and children, and both outpatients and inpatients from the same facilities or catchment areas. Because the vast majority of patients attending health facilities with fever are not admitted to hospital, data on outpatients are essential to guide the development of clinical management guidelines including strategies to improve targeting of antimicrobials. The goal of the study in outpatients is to identify treatable and preventable causes of fever, with a view to optimising appropriate antimicrobial prescribing to reduce the spread of antimicrobial resistance, while ensuring the best possible clinical outcomes. It is also critical to understand the causes of severe illness and death to design empiric treatment regimens that are effective, so the inclusion of inpatients is essential. The goal of the inpatient study is similar, with a view to reducing the case fatality ratio by improving the matching of antimicrobials with the aetiology of severe disease.[19]

Control participants will be enrolled from the communities surrounding participating health care facilities for two reasons. First, interpreting the results of some diagnostic tests requires knowledge of background prevalence of infection or colonization in the study population. Community controls will provide samples to estimate this background for each study site. Second, a healthcare utilisation survey (focusing on fever episodes and treatment seeking) administered to control participants will allow us to estimate the incidence and burden of specific causes of fever in the study settings.

This study provides a unique opportunity to collect and store biomedical samples with data from a large and well-characterised group of febrile patients and controls from representative settings in sub-Saharan Africa and Southeast Asia. The samples will be useful for identification of novel diagnostic targets, and for development and evaluation of new point-of-care diagnostic tests intended to guide the management of febrile patients. New tests could include those that predict severity of illness, detect specific infections, and/or differentiate between bacterial and viral infections, as identified as a high priority at a WHO meeting of experts convened in 2015.[18] Informed consent will be sought for study participation, and for storage and future use of data and biological samples.

For clinicians to be able to appropriately use new knowledge on aetiologies of fever, diagnostic tests, and antimicrobial resistance data to manage febrile patients, research evidence must be integrated into clear guidelines. Implementation of clinical algorithms,

such as IMCI or integrated Community Case Management (iCCM), has shown that health care workers are able to adopt and apply recommendations when reasonable support is provided.[20] The design and support for these guidelines must take into account end users' perceptions and patients' preferences in order to ensure good adherence and optimise impact. These issues will be addressed in qualitative work conducted alongside and linked to the FIEBRE study, which will be presented in a separate social science research protocol.

4 STUDY OBJECTIVES

4.1 General objective

To identify the causes of fever, and the antimicrobial susceptibility of bacterial pathogens causing fever, in low- and middle-income country settings where few data are available.

4.2 Specific primary objectives

1) To determine the treatable and/or preventable causes of fever in children and adults presenting as outpatients, and among those admitted to hospitals, in areas represented by the study sites;
2) To determine how fever aetiology varies according to patient age, geographical area, local malaria and HIV prevalence, and other risk factors;
3) To determine the prevalence and spectrum of antimicrobial resistance among bacterial pathogens identified in clinical specimens from febrile patients.

4.3 Specific secondary objectives:

1) To generate data on incidence of specific infections in study site catchment areas, and therefore contribute key data on disease burden for some infections that are not counted in current "global burden of disease" estimates;
2) To build an archive of well-characterised and geographically diverse biological samples from well-characterised clinical phenotypes for use in evaluation of new diagnostic and prognostic tests, and in identification of human- and pathogen-related biomarkers that may improve case management strategies;
3) To evaluate available biomarker assays to assess their performance and potential utility in fever case management in the study areas;
4) To generate data to inform the development of new fever case management algorithms which may be evaluated in future studies.

5 SUMMARY OF STUDY DESIGN

FIEBRE is a study of febrile illness in people aged two months and older residing at one of five sites (three sites in sub-Saharan Africa, two Southeast Asia). Patients who present with fever at the selected facilities will be recruited (Day 0) if they or their guardians/caregivers (in the case of minors) provide written informed consent. Study staff will take a targeted illness and exposure history, and perform a physical examination. Pharyngeal swabs and a venous blood sample will be collected from all participants; a urine sample will be collected from selected participants. Tests for malaria and for HIV (at sites where HIV prevalence exceeds 1% in the general adult population, for patients not already known to be infected), serum cryptococcal antigen (CrAg) and urine lipoarabinomannan (uLAM, a marker of active tuberculosis infection) point-of-care tests (for subsets of patients), and blood cultures will be performed on site; bacteria and fungi isolated from clinical specimens will be identified and tested for antimicrobial susceptibility.

Study patients will be managed by the clinical staff responsible for usual patient care at each study site, according to local standard of care. Results of malaria tests, HIV tests, CrAg and uLAM tests, blood and urine cultures, and antimicrobial susceptibility testing will be provided to the clinical staff as soon as available. Serology and other laboratory tests will be performed at internationally recognised reference laboratories. At Day 28 after enrolment, study patients will be asked to provide a further blood sample for serology and clinical outcome will be evaluated.

Recruitment to the study will be over a 12-month period to ensure that seasonal variations in causes of fever are captured. Background prevalence of specific infections in the study population at each site will be estimated with blood and pharyngeal samples from community controls. In addition, control participants will be surveyed to obtain representative data about treatment seeking and medicines use. By combining data on causes of fever at study sites with the estimate of the proportion of patients with fever seeking care at those facilities, we will estimate the incidence of common infections in the study areas, in order to contribute to efforts to define burden of disease.[21-24] A separate protocol will be prepared and submitted for ethics approval describing in-depth qualitative

research on fever management and local concepts related to antimicrobials, to be conducted in the same study areas.

6 STUDY POPULATION AND SELECTION CRITERIA

Participants will be patients who present with fever for health care at the selected health care facilities. Patients of all ages two months and older will be eligible for enrolment. Study sites include outpatient and inpatient facilities in Laos, Malawi, Mozambique, Myanmar, and Zimbabwe. Patients will be recruited if they fulfil all of the following criteria (see also section 7.2 on participant enrolment, and Screening and Enrolment Forms [Appendix C]):

1) Tympanic or axillary temperature ≥37.5°C at presentation;
2) Not having been hospitalized or having undergone surgery in the previous month;
3) Age ≥2 months (two months or older);
4) For outpatients, residence (at the time of enrolment) within the defined catchment area around the health facility (see section 7.5.1);
5) For outpatients aged ≥15 years, absence of symptoms of lower respiratory infection and of diarrhoeal diseases as defined by:
    a) cough AND ≥1 of the following: cough productive of green/yellow sputum, or haemoptysis;
    b) ≥3 loose stools within the previous 24 hours;
6) For outpatients aged ≥2 months to <15 years, absence of symptoms of diarrhoeal diseases as defined by: ≥3 loose stools within the previous 24 hours;
7) Willingness and ability to provide demographic and clinical information, and clinical samples, at the time of enrolment and 28 days later;
8) Provision of written informed consent for adult participants; or for children, provision of written consent from a parent/guardian and assent from the child (according to local regulations and practices at each study site).

Controls will be community members in the study site health facilities' catchment areas, frequency matched to participants by age and season (see section 7.5 for details of selection and study activities for community controls).

7 METHODS AND STUDY PROCEDURES

7.1 Study sites and personnel

7.1.1 Selection of study sites

The study will be conducted at five sites, all of which have high mortality in children under age five years, quality-assured microbiology laboratories, little or no published data on causes of fever, and suitably qualified research teams and capacity: 1) Phonhong Vientiane Province, Lao Peoples' Democratic Republic; 2) Chikwawa, Malawi; 3) Yangon, Myanmar; 4) Manhiça, Mozambique; and 5) Harare, Zimbabwe. These sites have been selected because they fulfil the above criteria, and because there is substantial across-site variation in the prevalence of HIV and malaria (based on available data) and other endemic infectious diseases.

Within each participating country, the health facilities for this study will be selected on the basis of the following characteristics: interest and capacity of local researchers and public health authorities; accessibility from the collaborating research team's base; interest and capacity of the health facility staff; and patient numbers adequate to allow enrolment of the target sample size over the period of 12 months (to be extended at individual sites if required to meet sample size target). On the basis of current information and preliminary agreements, selected sites include: in Lao PDR, Phonhong Vientiane Provincial Hospital in Vientiane Province; in Malawi, Chikwawa District Hospital; in Mozambique, Manhiça's District Hospital (MDH) in Manhiça, and peripheral health posts of Maragra, Malavele, Palmeiras, Ilha Josina Machel, and Taninga; in Myanmar, Thanlyin District Hospital; in Zimbabwe, Harare and Parirenyatwa Hospital and two primary care clinics in Harare City. If for unforeseen reasons we are unable to conduct the evaluation at the specific facilities listed, alternative sites will be chosen to preserve the representative range of geography and epidemiology.

7.1.2 Personnel conducting protocol activities

For the purposes of this study, routine clinical care will be provided by the staff of participating health facilities, i.e., health workers who are employed by the health facility. Such health workers typically include physicians, clinical officers, nurses, laboratory

technologists or technicians, and other similar cadres of health care staff. In this protocol, these personnel are referred to as “health facility staff.”

Study-specific activities will be conducted by personnel hired for the purposes of the research study, i.e., individuals recruited by the organisation coordinating the study site. In this protocol, these personnel are referred to as “study staff.” Study staff typically will be individuals with clinical, laboratory, and/or social science training and with prior experience in clinical and/or social science research. Study staff will be trained in the protocol and relevant study procedures prior to the start of the study, and health facility staff will be informed of the study. At each site study staff will work with health facility staff to achieve the study goals while ensuring smooth continuation of care.

7.2 Study enrolment

7.2.1 Selection of patients for recruitment and enrolment

At each site, patients (or parents/guardians on behalf of minors) will be invited to participate in the study when they present with fever to the participating health facilities for care. Health centre staff will work with study staff to identify patients who present with fever. Study staff will screen and enrol participants according to the selection criteria listed in section 6. A total of 2400 patients will be enrolled at each site (see sample size calculations in section 11.3), with approximately half being outpatients and half inpatients. If a patient is enrolled as an outpatient but deteriorates clinically and is admitted to hospital within <24 hours after enrolment, the patient will be re-classified as an inpatient for study purposes. If a patient is enrolled as an outpatient and then admitted ≥24 hours after enrolment, s/he will remain listed as an outpatient for study data purposes.

Since the causes of fever are likely to be different in children and adults, enrolment will be stratified so that participants are enrolled in two age groups of approximately equal numbers: children aged two months (≥ 2 months) to <15 years, and those aged 15 years and older. In addition, so that results accurately represent the patient population at each site, site-specific recruitment strategies will be developed to achieve enrolment of a random sample of patients presenting throughout the day and week (including those presenting after hours or at weekends, who may come from areas further from the health facility, and/or have more severe presenting symptoms) and over the course of the 12-month

recruitment period. In each of the four categories defined by age and in-/outpatient status, 12 consecutive patients per week will be recruited. The day of the week that recruitment starts will be rotated. There may be slight variations on this strategy due to site specific constraints. The total number of patients presenting to the hospital with fever each day (or week), including those not in the study, over the course of the study period will be recorded.

Patients who are not enrolled in the study will be managed according to routine practice by health facility staff. For those patients who are screened and excluded, the reason for exclusion will be recorded, along with the patient's age, sex, and location of usual residence. At each site where HIV testing will be performed (i.e., where HIV prevalence in the general adult population is >1%), HIV counselling and testing is linked to an established treatment program. Other acute or chronic conditions are managed on-site as capacity allows, or referred to a higher level of the health care system according to local guidelines.

7.2.2 Provision of written informed consent and assent

Written informed consent forms and information sheets on study participation (Appendices A and B) will be provided for each adult participant (over the age of majority according to local regulations for each site) or parent/guardian (for younger participants) at the time of enrolment. A trained study staff member will conduct individual screening interviews and informed consent discussions with potential study participants and parents/guardians. Informed consent will be conducted in the potential participant/parent/guardian's preferred language, with the assistance of a translator if necessary. Consent forms, approved by all relevant ethical review boards, will be available in English (Laos, Malawi, Myanmar, Zimbabwe) or Portuguese (Mozambique) and in the local language/s for each site.

If an adult or parent/guardian consents to participation but is unable to write, her/his fingerprint will be substituted for a signature, and a signature from an impartial adult witness to the informed consent procedures will be obtained. For participants under the age of adulthood at each site, country-specific guidelines on the inclusion of minors in research (including guidelines related to consent from a parent or guardian, and/or emancipated minors) will be followed. In particular, in addition to parent/guardian consent, assent will be sought for participants under the adult age, according to local regulations and recommendations for each study site. If a patient is severely ill and unable to give consent or

assent at the time of enrolment, their next of kin (parent or guardian in the case of a minor, spouse in the case of married individuals, etc, will be asked to provide informed consent on behalf of the patient. If/when the participant regains capacity to make their own decisions, they will be asked to confirm or withdraw their informed consent, with care taken to ensure they understand that they are free to withdraw from the study without risk of jeopardizing their access to current or future routine health care.

Potential participants and/or their parents/guardians will have the opportunity to ask questions and discuss details of the study with study staff, and are free to ask further questions at any time during or after completion of the study activities. If the participant meets all study enrolment criteria, s/he will be enrolled (Appendix C). At enrolment, each participant's contact information (telephone number and place of residence) will be recorded. This information will be kept in locked files, separately from study data files, until completion of reference lab testing (section 7.6) so that participants can be contacted if needed for clinical or study follow-up purposes.

7.3 Study activities and data collection at the time of patient enrolment

7.3.1 Clinical and demographic data collection

At enrolment of each participant, study staff will collect basic demographic data (age, sex, place of residence, ethnicity) and information on the history of the present illness (including presence and duration of symptoms, current and past health history, immunization and recent treatment history, recent specific exposures). A study staff clinician will perform a physical examination including symptoms and signs for assigning a standardized severity score (e.g., FEAST Paediatric Emergency Triage [PET][25] or Lambaréné Organ Dysfunction Score [LODS][26,27] for children aged <15 years and quick Sequential Organ Failure Assessment [qSOFA][28-30] or another "universal vital assessment" [UVA][31] score for older patients; see sample data collection form, Appendix D).

7.3.2 Laboratory sample collection and processing

Study staff will collect a nasopharyngeal swab and a venous blood sample from each participant for use in study laboratory procedures. Where permitted by ethics approvals, an oropharyngeal swab will also be obtained to increase the yield of possible respiratory pathogens. In addition, a urine sample will be collected from patients aged ≥2 months and

<2 years, and from patients of any age who have pain on micturition, frequent micturition, suprapubic tenderness, or costovertebral angle tenderness. A urine sample will also be collected from HIV-positive inpatients (and HIV-positive outpatients, at sites where resources allow) for uLAM testing. Study staff will prepare the samples and conduct the diagnostic tests described. All other care will be provided by health facility staff.

The FIEBRE study will collect clinical samples for two purposes: for assays that are of immediate clinical benefit to patient care (malaria RDT, HIV testing, serum CrAg, uLAM, blood and urine cultures), and for research purposes. Minimum whole blood volumes needed for tests of immediate benefit to patient care are: approximately 50 uL for malaria and HIV rapid tests; approximately 10 mL for patients aged ≥15 years and 4 mL for patients <15 years for blood culture for bacteria and fungi; plus 5 mL for mycobacterial blood culture in patients ≥15 years where community HIV prevalence is >1%. In addition, approximately 4 mL of serum (i.e. 8 mL of whole blood), and if possible buffy coat from an EDTA sample, are needed for reference lab testing for infectious diseases (serologies and nucleic acid assays); 250 uL of plasma (i.e. 0.5 mL whole blood) for assays of immune and inflammatory biomarkers; and 2.5 mL of whole blood for additional RNA testing (in a subset of adult participants only; see just below).

For the FIEBRE study, actual blood sampling volumes for young children will be based on body weight up to 7 kg, according to the chart below (Table 1) adapted from various clinical and research guidelines,[32] which suggests 2 mL per kg body weight, or 2.5% of estimated total blood volume by body weight, within a 24-hour period as a standard guideline for ill patients. The blood volume to be sampled refers to the total volume that can be drawn for both clinical care and research use. For example, a 2-kg patient could provide a maximum of 5 mL of blood; for a 5-kg patient, 10 mL; and for a 7-kg patient, 14 mL. For the FIEBRE study, weight-based guidelines will be used for all patients ≤7 kg.

Table 1. Maximum total blood draw volumes by body weight

Body weight (kg)	**Total blood volume (mL)**	**Max allowable blood volume drawn in 24-hour period*** (2.5% of total blood volume in mL)
1	100	2.5
2	200	5

3	240	6
4	320	8
5	400	10
6	480	12
7	560	14

* The total blood volume listed refers to the total blood volume that can be drawn for both clinical care and research procedures.[32]

For patients with body weight >7 kg and aged <15 years, the maximum amount of blood drawn will be 15 mL. For patients aged ≥ 15 years, the maximum amount of blood drawn will be 21 mL at sites where HIV prevalence in the general population is ≤1%, and 26 mL at sites where HIV prevalence in the general population is >1% (to allow 5 mL of blood for mycobacterial blood culture). In a randomly selected subset of 180 patients aged ≥15 years at each site, an additional 2.5 mL whole blood will be drawn for storage in PAXgene tubes (containing RNA stabilization solution) for eventual RNA assays; this means that for this subset of patients, the total volume drawn will be 28.5 mL at African sites, and 23.5 mL at Asian sites. (The number of samples collected in PAXgene tubes is limited for budgetary reasons.)

For patients from whom the full volume of blood cannot be obtained for any reason, the available sample will be prioritized as follows: 1) tests performed on-site that are important for clinical management (blood culture, malaria test, HIV test); 2) reference laboratory assays; 3) biomarker assays; 4) aliquots for sample archive and future research use.

If a patient shows clinical signs of severe illness (dehydration, pallor indicating anaemia, hypotension), blood sampling will be discussed with the treating clinician/s and minimized if deemed clinically necessary. In all cases the safety and appropriate care of the patient will be prioritised. For example, adequate volume for blood cultures which will inform clinical care will be prioritized over other study samples.

According to routine practice at each study site, for each participant, a malaria rapid diagnostic test (RDT) or blood smear for routine light microscopy will be performed on site; and an HIV RDT will be performed at sites where community HIV prevalence exceeds 1% in the general adult population (see section 7.6, Specific laboratory assessments). Blood culture

bottles will be inoculated, and bottle weight recorded before and after inoculation to estimate the volume of blood cultured. Blood culture bottles will be incubated at collaborating laboratories for each clinical site. Where obtained, urine samples will be tested with a dipstick for nitrites and leukocyte esterase; samples positive for either will be incubated for culture at collaborating laboratories for each clinical site. Urine samples from HIV-positive patients will be tested for LAM. Bacteria and fungi isolated from clinical specimens will be identified and tested for antimicrobial susceptibility. Any microorganisms isolated will be cryopreserved and shipped to an international reference laboratory for confirmation of identification and antimicrobial susceptibilities; blood samples for each participant will be aliquoted, stored, and shipped to internationally recognised reference laboratories for additional tests (see section 7.6). All personnel will use universal precautions when handling blood samples.

7.3.3 Provision of routine outpatient and inpatient care

Symptoms and signs at the time of enrolment and subsequently will be investigated and managed according to the local standard of care by the health facility staff providing care. Study staff will collect data on symptoms and signs and any treatments given on Day 0. For hospitalized patients, on the day of discharge study staff will record length of stay, condition on discharge, and any treatments administered during hospitalization including antimicrobials. The standard of care for any medical problem that exceeds the capacity of the participating centre is to refer to a higher level of care. As clearly described during the informed consent discussion, treatment and referral will be done at the discretion of the health facility staff.

Results of malaria tests, HIV RDT and CrAg and uLAM point-of-care tests (where performed), blood cultures, and antimicrobial susceptibility profiles of bloodstream and urine isolates will be provided as soon as available to the health facility staff responsible for the management of each patient. Results of serological and molecular tests performed at international reference laboratories will only be available some months after patients are recruited. Some of these tests may diagnose infections that would benefit from appropriate treatment even months later (e.g., serological assays for brucellosis and Q fever). During the informed consent process, study participants will be informed of this possibility, and will be asked to provide their contact details (to be kept in a locked database separate from the study results

databases) so that study staff can notify them of positive reference laboratory results which may benefit from treatment (e.g., brucellosis, Q fever) and if appropriate, prescribe treatment.

Study staff will be available to offer advice to health facility clinicians on interpretation of and appropriate response to test results. If the results of antimicrobial susceptibility testing find evidence of bacterial pathogens that are resistant to all locally available antimicrobials, study staff and investigators will work with local and national health authorities to make available appropriate alternatives.

7.3.4 Scheduling of follow-up visit

All participants will be asked to return to the study site for one follow-up visit 28 days after enrolment. If the visit cannot occur on day 28, another date from day 26 to day 48, inclusive, will be acceptable. For any intervening care-seeking, participants will also be encouraged to return to the study site if feasible. Each participant will be given a card with her/his study identification number and the follow-up study visit date, and on which to record any care or treatment that is received in the interim. At sites where child health records are in routine use, data on interim care will be extracted from these records.

Study staff will attempt to minimize loss-to-follow-up by: telephoning participants/parents/guardians in advance of their scheduled follow-up visit to remind them of the appointment; reimbursing participants' transport costs for study visits; and visiting at home participants who do not keep their follow-up appointment (with appropriate permission obtained at the time of informed consent).

7.4 Study activities for patients at 28-day follow-up visit

7.4.1 Clinical data collection

At each participant's day 28 follow-up visit, study staff will record the clinical outcome of the illness (see sample data collection form, Appendix D). Participants who do not return for the scheduled visit will be followed up at home or by mobile telephone. If the participant reports symptoms at the time of follow-up, s/he will be referred to health facility staff for further investigation and care.

It is recognised that a poor clinical outcome (e.g., permanent disability, death) associated with a health condition may lead to negative and stressful emotions for the participant, those close to her/him, and involved health care providers. In the unfortunate event of a poor clinical outcome, study staff will attempt to collect relevant data in a manner that is sensitive, respectful of the participant's dignity, context-appropriate, and that presents the least inconvenience possible to the participant and her/his family. These ideas, potential scenarios and appropriate methods will be discussed with study staff and health facility staff during pre-study training, and will be based on existing guidance to the extent available.[33]

7.4.2 Laboratory sample collection

At each participant's 28-day follow-up visit venous blood will be taken for serology testing. Weight-based guidelines (section 7.3.2) will be used for patients ≤7 kg. For patients with body weight >7 kg and aged <5 years, the maximum amount of blood drawn will be 5 mL. At study sites where ethical and regulatory authorities permit, for patients with body weight >7 kg and aged ≥5 years and <15 years, the maximum amount of blood drawn will be 10 mL; otherwise the maximum volume for this population will also be 5 mL. For patients aged ≥ 15 years, the maximum amount of blood drawn will be 10 mL. Samples will be aliquoted, stored and shipped to internationally recognised reference laboratories for additional tests (see section 7.6 below).

7.5 Community controls

Interpreting the results of some serological assays and pharyngeal swab assays requires knowledge of background prevalence of infection or colonization in the study population. To address this, 600 community controls (section 11.3 sample size calculations), frequency matched by age, sex, month of enrolment (to address seasonality), and geographical area to the cases, will be selected from the community living in the catchment area of the health facility for each site. No controls will be specifically recruited for the inpatient population, who may come from a wider geographic area. However, an analysis comparing controls with our sample of in-patients who do come from the area from which controls were selected, will be carried out.

7.5.1 Selection and recruitment of community controls

To define the catchment area for outpatients and community controls, existing data and pilot data from each site will be combined to define an area around the study facility in which approximately 80% of outpatients live. Residing within this area will be a selection criterion for FIEBRE outpatients (section 6). Community controls will be recruited within this area, individually matched to participating outpatients.

During each month of the study at each site, half of the outpatients will be selected at random (or by taking every second outpatient in chronological order of presentation). For each of these a community control will be found matched on age (within bands: ≥2 to <6 months, ≥6 to <12 months, ≥1 to <3 years, ≥3 to <5 years, in 5 year bands up to age 24, and in 10 year bands at older ages), sex and place of residence. At sites where the study team's capacity enables them to recruit a larger number of controls, controls will be matched to a larger proportion of enrolled outpatients. For example, if capacity allows, one control will be found for every outpatient; or controls will be found for more than half of the outpatients, e.g. for a random sample of three-quarters of enrolled outpatients (or by selecting every second, third, and fourth outpatient, in chronological order of presentation). This approach will allow us to enhance the precision of estimates for specific primary objective 1 and specific secondary objective 1 (see also section 11.1 below on quantitative outcome measures and analysis).

Community controls will be recruited and enrolled if they, or their parents/guardians, provide informed consent.

7.5.2 Informed consent of community controls

Written informed consent and assent will be obtained for community controls in the same way as described in section 7.2.2 for case/patient participants (Appendices A and B, information sheet and informed consent form for community controls).

7.5.3 Sample collection for community controls

Diagnostic testing will be identical for controls and cases, with three exceptions: blood cultures will not be taken from controls, as the diagnostic yield is likely to be close to zero in this group; convalescent sera will not be collected from controls, as loss-to-follow-up is likely to be so high as to render results unusable; and urine will not be collected from controls.

Venous blood will be drawn from control participants as follows: Weight-based guidelines (section 7.3.2) will be used for controls ≤7 kg. For patients with body weight >7 kg and aged <15 years, the maximum amount of blood drawn will be 5 mL. At study sites where ethical and regulatory authorities permit, for controls with body weight >7 kg and aged ≥5 years and <15 years, the maximum amount of blood drawn will be 10 mL; otherwise the maximum volume for this population will also be 5 mL. For participants aged ≥15 years, the maximum amount of blood drawn will be 10 mL. In a randomly selected subset of 20 controls aged ≥15 years at each site, an additional 2.5 mL whole blood will be drawn for storage in PAXgene tubes (containing RNA stabilization solution) for eventual RNA assays; this means that for this subset of control participants, the total volume drawn will be 12.5 mL. (The number of samples collected in PAXgene tubes is limited for budgetary reasons.)

If the malaria RDT is positive for a control participant, antimalarial treatment will be offered according to national guidelines.

7.5.4 Other data collection for community controls, and estimation of population fever incidence and aetiologies

When a control participant provides blood and pharyngeal samples, study staff will collect basic demographic data (age, sex, place of residence, ethnicity). They will be asked if they have a fever and, if they answer yes, their tympanic temperature will be recorded.

In addition, questionnaires will be administered to the community control participants in order to capture more representative data about treatment seeking and medicine (primarily antimicrobial) use. To increase the statistical power of this health care utilisation survey, as for other recent studies that estimate incidence and "burden" of infections,[21,34] for each control participant, the participant (or head of household or health care decision-maker if the participant is a child) also will be asked about basic demographic information (date of birth or age, and gender) and treatment seeking practices for each household member. This health care utilisation survey will provide an estimate of the period prevalence of fever as well as the proportion of persons with fever in the community who present to the study enrolment sites for care. The fraction of persons with fever presenting to a study site will be used to calculate multipliers to estimate the population-based incidence of fever overall, and the incidence of specific causes of fever in the catchment area of study health care

facilities. This "multiplier" method is well established[1,22] and is widely used for estimating the incidence of febrile illnesses including but not limited to brucellosis,[35] leptospirosis,[21] and typhoid fever.[36] The multiplier method requires an estimate of the total number of cases of fever that present to our study sites. The multiplier method also requires an estimate of the proportion of cases of fever in the population who do not present to the study hospital, which will be obtained through the healthcare utilisation survey among controls. It will also require an estimate of the catchment population for each site, or if that is not possible, for a defined population around each site (which may be smaller than the total catchment area). In the latter case, only heath care utilisation data from controls and their household members in the defined population will be used for the estimate of the population incidence of fever in the defined population.

7.6 Specific laboratory assessments

Laboratory assessments focus on detecting infections that are treatable and/or preventable. The same pathogens will be sought in samples from all sites. Pathogens to be sought include: blood parasites; bacterial, mycobacterial, and fungal (including *Cryptococcus* spp.) bloodstream infections; typhus group and spotted fever group *Rickettsia* spp.; *Orientia tsutsugamushi; Coxiella burnetii*; *Leptospira* spp., *Brucella* spp.; relapsing fever; *Leishmania* spp; and arboviruses including dengue, chikungunya, Zika, and Japanese encephalitis virus.

Appendix E lists the target pathogens, with relevant assays and reference laboratories, and the case definitions used to identify causes of fever. All of the reference laboratories listed are internationally recognized centres of excellence, and all have agreed to test FIEBRE samples as described. If for any reason the labs' capacities or agreements change in the future, alternative reference laboratories will be identified to maintain the highest possible standards for testing of FIEBRE samples. Laboratory procedures are summarized in this protocol section and in Appendix E; further details for each assay and procedure will be described in specific SOPs.

7.6.1 Malaria rapid diagnostic tests (RDTs)

All study participants will be tested for malaria at the time of enrolment with a standard antigen-detecting RDT and a blood film. The following criteria will be considered in selecting

malaria RDTs for use at study sites: national guidelines for each site, target antigen depending on local malaria epidemiology (e.g., histidine-rich protein 2 [HRP2] for sites where *P. falciparum* predominates, with or without a vivax-specific or pan-specific *Plasmodium* lactate dehydrogenase [pLDH] band), and high performance in WHO-FIND standardized product testing.[37] Malaria RDTs will be obtained directly from their manufacturers, will be centrally procured, and a sample will be sent for lot testing in a WHO-approved laboratory before use. RDTs will be performed according to manufacturer instructions. Study staff responsible for performing, interpreting and recording malaria RDT results will be trained accordingly before the study begins.

7.6.2 Microscopy for malaria and other blood pathogens

If blood smear microscopy is routinely performed for clinical care at a study site, for all patients participating in the study a blood smear will be prepared and read according to local standards for this purpose. All study participants who test positive for malaria, by local microscopy or RDT, will receive appropriate antimalarial treatment according to national malaria guidelines.

In addition, for study purposes, for all participants (patients and controls) at the time of enrolment a thick and thin blood smear will be prepared, fixed and stained according to standard SOPs. These smears will be read at the study site by expert microscopists for presence or absence, density and species of *Plasmodium*; and also for non-malaria blood parasites and *Borrelia* spp. Smears will be read independently by two expert microscopists, with discordant results resolved by a third expert reader. In addition, a 10% sample of smears from each site will be shipped to the Liverpool School of Tropical Medicine (Liverpool, United Kingdom) for quality assurance according to WHO malaria microscopy standards.[38]

7.6.3 HIV testing

At study sites where HIV prevalence is >1% in the general adult population (Malawi, Mozambique, and Zimbabwe), for patients of all ages whose status is not already documented, HIV testing will be performed according to national guidelines using a point-of-care test. If a study participant is ≥18 months and has a positive HIV antibody test, the positive result will be recorded in study data and no further testing will be performed. If a

positive result is obtained in a participant <18 months, confirmatory testing will be performed. Participants who are diagnosed as HIV positive during the study will be linked to counselling and care according to local guidelines and standards.

7.6.3.1 Serum cryptococcal antigen (CrAg) testing

A serum CrAg point-of-care test will be performed for all inpatients, and for HIV-positive outpatients. Test results will be made available in real time to health care providers for use in treatment decision-making.

7.6.3.2 Urinary lipoarabinomannan (uLAM) testing

At sites where HIV testing is performed, a urine sample will be collected from HIV-positive inpatients (and from HIV-positive outpatients, at sites where resources allow) for uLAM testing, as a point-of-care test (in addition to mycobacterial culture) for active tuberculosis infection. Test results will be made available in real time to health care providers for use in treatment decision-making.

7.6.4 Bacterial and fungal culture and susceptibility testing

At each site, blood culture media will be inoculated using sterile technique and according to standardized SOPs. Blood culture bottles will be incubated and those with bacterial or fungal growth will be detected either visually or by an automated system. Microorganism identification and antimicrobial susceptibility testing will be performed on site according to internationally recognised SOPs and guidelines.

Mycobacterial blood cultures will be performed for HIV-positive inpatients (and for HIV-positive outpatients at sites where resources allow) aged 15 years and older, at sites where HIV prevalence is >1% in the general adult population (Malawi, Mozambique, Zimbabwe).

In addition, for culture-positive samples, cryopreserved isolates of bacteria and fungi will be shipped on dry ice to the University of Otago and Southern Community Laboratories (Dunedin, New Zealand; national reference laboratory) for confirmation of identification and of antimicrobial susceptibility to international standards. For mycobacteria, the same approach will be followed with cryopreserved isolates shipped to the National and Supranational Reference Mycobacterium Laboratory in Borstel, Germany.

7.6.5 Pharyngeal swabs

Pharyngeal swabs will be collected by study staff trained in the appropriate techniques, and placed in dry sterile tubes for storage and transport. The swabs will be shipped to Micropathology Ltd, in Canterbury, UK, where they will be tested for influenza A/B, respiratory syncytial virus (RSV) and other respiratory pathogens by nucleic acid amplification techniques.

7.6.6 Urine samples

A urine sample will be collected from participants younger than two years, and from older patients who have symptoms and/or signs consistent with urinary tract infection (suprapubic pain or tenderness, frequent or painful micturition, costovertebral angle tenderness). Study staff will perform a urine dipstick, and samples that are positive for nitrate and/or leukocyte esterase will be cultured; any bacterial or fungal pathogens will be identified and tested for antimicrobial susceptibility (as for blood in section 7.6.4).

7.6.7 Sample storage and shipping

Whole blood samples from each participant will be used to prepare six dried blood spots (DBS) of 10 microlitres each on filter paper and stored for malaria PCR and biomarker testing (see 7.6.10).

Serum, EDTA plasma, buffy coat where obtainable, and red blood cell pellet will be separated on-site and stored at -20°C for a maximum of one month before being transferred to a -80°C freezer.

A proportion of blood samples will be stored at -80°C at each site as back-up and for any future research use as allowed by the study protocol and informed consent documents. Aliquots of whole blood and acute and convalescent sera from study patients, whole blood and serum from controls, and pharyngeal swabs from all participants will be shipped on dry ice to LSHTM in two batches from each site at approximately the study mid-point, and after completion of participant follow-up. Samples will be shipped with an international courier that is certified to ship biological samples, and that has an excellent reputation and extensive experience in this regard.

After receipt at LSHTM, blood aliquots, pharyngeal swabs, and microbial isolates will be stored at -80°C and shipped in batches to international reference laboratories for further testing for the diagnosis of specific infections (sections 7.6.8 and 7.6.9). Internationally recognized reference laboratories for each pathogen of interest have been identified and engaged to provide gold-standard results for detection of infectious aetiologies. For standardization, samples from all study sites will be tested at the same reference laboratories.

Aliquots of serum, blood, DBS, and pharyngeal samples will be archived at -80°C at a purpose-designed archive housed at LSHTM for future use (see section 7.7).

7.6.8 Quality assurance for laboratory procedures at study sites

A common quality management system will be implemented at all study sites, including the following components: SOP for each procedure performed; bench job aids; internal and external quality control procedures; internal audits; supervision visits and monitoring of quality indicators. A central quality management team with international experience, including senior clinicians and laboratory scientists, will help to ensure that all laboratory procedures are performed according to SOPs at all sites and with high quality standards.

7.6.9 Serological and molecular testing at reference laboratories

Serologies and molecular tests will be performed on acute sera (day 0) from case and control participants, and serologies will be performed on convalescent sera (day 28) from case participants, at collaborating international reference laboratories as follows:

- Mahidol Oxford Tropical Medicine Research Unit (MORU; Bangkok, Thailand) and Australian Rickettsial Reference Laboratory (Geelong, Australia) – immunofluorescence antibody (IFA) tests, and if budget allows nucleic acid amplification test (NAAT; using buffy coat where obtainable for *Rickettsia/Orientia* spp assays;), for: *Rickettsia* spp (spotted fever group and typhus group), *Coxiella burnetii* (Q fever), and *Orientia tsutsugamushi* (scrub typhus).
- UK National Brucellosis Reference Unit (Liverpool, United Kingdom) – *Brucella* enzyme immunoassays (EIA) and microscopic agglutination tests (MAT).

- Queensland Health Leptospirosis Reference Laboratory (Brisbane, Australia) – *Leptospira* microagglutination test.
- London School of Hygiene & Tropical Medicine (London, UK) – direct agglutination test (DAT) for anti-*Leishmania* antibodies, with assistance from the Department of Clinical Parasitology, Hospital for Tropical Diseases, London, UK.
- French National Centre of Reference of Arboviruses (Marseille, France) – ELISA, plaque reduction and neutralization tests for: chikungunya, dengue, Japanese encephalitis, Zika, and other relevant arboviruses.
- Micropathology Ltd (Canterbury, UK) – NAAT for influenza viruses and respiratory syncytial virus; the multiplex assay also detects human metapneumovirus, parainfluenza virus types 1, 2,3 &4, adenoviruses, rhinoviruses, enterovirus, parechovirus, bocavirus, *Mycoplasma pneumoniae*, and other potential respiratory pathogens.
- MiraVista Diagnostics (Indianapolis, USA) – serum *Histoplasma* and *Cryptococcus* antigens (including external quality assurance of serum CrAg point-of-care test results for those samples collected after implementation of CrAg testing at sites) (day 0 sample only).

7.6.10 Biomarker assays

In addition to archiving samples at LSHTM for future biomarker investigations, a limited set of assays will be carried out as a part of FIEBRE study activities. These will include the biomarkers in Table 2 that have been identified in previous studies as potentially useful in diagnosing bacterial infection and/or as prognosticators of illness severity. Of note, the identification and investigation of biomarkers of infection is a fast moving field and novel biomarkers likely will be identified by the global scientific community during the time period of this study. Therefore, once FIEBRE samples are available, we will review and revise (if necessary) the list of biomarkers to be tested to prioritize assays that are most likely to lead to public health benefit in fever case management for patient populations typified by FIEBRE participants.

Table 2. Biomarkers identified as potentially useful in distinguishing bacterial from non-bacterial infections, or in predicting illness severity

Biomarker/ test	Supportive evidence from Africa/Asia	Sample requirements and test method
C-reactive protein (CRP)	Variable performance from a multitude of studies, including Asia and to a lesser extent SSA[39,40]	10ul EDTA plasma; Luminex assay to be performed at LSHTM
Triggering receptor expressed on myeloid cells (sTREM-1)	Published data[39,41,42]; study of ~2000 febrile children in Uganda (under review, Kain et al)	100ul EDTA plasma; Luminex assays to be performed at LSHTM
Angiopoietin-Tie-2	Some supportive data from Malawi[43,44]; study of ~2000 febrile children in Uganda (under review)	
Interleukin 6 (IL-6)	Study of ~2000 febrile children in Uganda (under review; published data[39]	
soluble fms-like tyrosine kinase-1 (sFlt1)	Study of ~2000 febrile children in Uganda (under review)	
Soluble tumor necrosis factor receptor-1 (sTNFR1)	Study of ~2000 febrile children in Uganda (under review)	
Heparin binding protein (HBP)	No data from LMICs. Primarily validated as marker of severity and organ dysfunction[39,45,46]	

In total 250 µl of EDTA plasma will be aliquoted and stored for these assays from all study participants from the day 0 blood draw, including patients and controls. In addition, 250 µl will be aliquoted and stored from each patient's day 28 blood draw. Comparing biomarker levels on day 0 and day 28 will provide a better understanding of their kinetics, as previous studies suggest that patients in whom some biomarkers fail to normalize could be at higher risk of severe outcome.[44,47,48]

The biomarker assays will be carried out at LSHTM where patient samples will be archived. The diagnostic and prognostic value of these biomarkers will be assessed to determine their utility alone and in combination in differentiating between bacterial and non-bacterial causes of illness, and in predicting severe outcomes using mortality and severity scores as endpoints.

Biomarker assay results will not be used in clinical management of FIEBRE study participants.

7.7 Sample archive

Informed consent (Appendices A and B) will be sought from study participants or parents/guardians at recruitment for the future use of their biomedical samples and anonymized data, including in the development and evaluation of new diagnostic tests. The consent process for future use of samples and data will be conducted at the same time as consent for study participation, but separate consent documents will be signed (or finger-printed), i.e. one for study participation, and one for storage and future use of samples. A proportion of blood, serum and/or DBS samples from participants at each site will be stored at the respective sites (section 7.6.7). The remaining sample archive will be housed at LSHTM in London, United Kingdom, in a facility with experienced personnel and appropriately monitored -80°C freezers and other relevant equipment. Samples will be made available for diagnostics researchers and developers for the evaluation of new point-of-care diagnostic tests intended to guide the management of febrile patients. Access to the FIEBRE archive will follow a formal process of application to the FIEBRE consortium. If commercial companies wish to access FIEBRE samples and data for the purpose of evaluating new diagnostic or biomarker assays, they will seek formal approval from the FIEBRE consortium and from an independent committee chaired by Professor Rosanna Peeling, Director of the International Diagnostics Centre at LSHTM, and including independent senior scientists as well as lay members. Intellectual property rights will be agreed between requestors and the LSHTM legal department.

8 SAFETY CONSIDERATIONS AND REPORTING

8.1 Potential risks

Risks associated with participation in this study are minimal. For participating patients, risks are essentially no greater than they would be for routine health care at the participating health facilities: To the routine evaluation and management of febrile outpatients and inpatients, this study adds the collection of venous blood according to standard guidelines for clinical research (section 7.3.2), a negligible medical risk. For control participants, risks are primarily due to collection of venous blood without a clinical indication. Physical discomfort, transient bleeding, and bruising may result when blood is obtained by venepuncture. Aseptic technique and universal precautions against body fluid exposures will be practiced in obtaining blood samples. Some participants will present for care with serious illness; however, all participants will be managed by qualified clinical staff according to national standards of care. In addition, the study will provide good-quality diagnostic

information in real time, which has the potential to enhance the quality of care provided. Loss of privacy is a potential risk associated with participation in any research project. Potential for loss of confidentiality will be minimized by use of coded study numbers on data collection forms and laboratory samples, and all data will be stored in locked filing cabinets and password-protected computer files.

8.2 Known potential benefits

All participants will be provided with routine health care services according to the local standard of care. In addition, the study will provide good-quality diagnostic information, which has the potential to enhance the quality of care provided. In particular, blood culture results may be life-saving. Participants may also benefit from the knowledge that they are assisting in work of potential value to fever case management and health care in settings of which their site is representative.

8.3 Governance, data safety and monitoring

The study will be overseen by a committee comprising the co-investigators, a group that includes a full-time project manager, a full-time senior clinician and study coordinator, and a number of senior clinicians, laboratory scientists, a statistician and dedicated data manager. A full-time study coordinator will be recruited at each study site. Senior scientists at the international reference laboratories will provide inputs to and oversight of relevant study activities.

Co-investigators met face-to-face in May 2017 to agree on details of the study protocol, including data and specimen collection and other logistics. The co-investigators will meet by conference call every three months for the duration of the study, or more frequently if unforeseen issues arise that need to be addressed. Face-to-face meetings of co-investigators are planned approximately annually; and a meeting will be convened once study results are available, to agree on plans for publication and dissemination.

In addition, an External Advisory Committee of three to five senior scientists will provide independent monitoring of the study's progress and integrity of the data.

8.4 Definitions

Adverse event (AE): Any untoward medical occurrence in a patient or study participant

Serious adverse event (SAE): Any untoward medical occurrence that:

- results in death;
- is life-threatening;
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- consists of a congenital anomaly or birth defect.

Other "important medical events" may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

8.5 Reporting Procedures

Any questions concerning adverse event reporting will be directed to the Chief Investigator in the first instance.

8.5.1 Non-serious AEs

All AEs reported at the Day 28 follow-up visit will be recorded as a matter of routine, in the participant's medical records where appropriate, and summarized on study CRFs.

8.5.2 Serious AEs

Approximately half of all patients enrolled in the FIEBRE study will be inpatients, and therefore already potentially seriously ill. Serious Adverse Events (SAEs) will be considered to be hospitalization of a patient enrolled as an outpatient; prolonged hospitalization of inpatient participants; persistent or significant disability/incapacity at the time of study discharge; or death. SAEs will be reported to the Study Coordination Centre within 24 hours of the local site being made aware of the event.

An SAE form will be completed and submitted to the Study Coordination Centre with as much detail as available at that time. If awaiting further details, a follow up SAE report should be submitted promptly upon receipt of any outstanding information.

9 FOLLOW-UP

No formal study follow-up is planned for participants after the completion of study activities, as described in sections 7.2, 7.3, 7.4 and 7.5, with one exception: As in sections

7.2.2 and 7.3.3, patients for whom serology results are positive for infections that may benefit from treatment even months after blood samples are obtained (e.g. brucellosis, Q fever) will be contacted to assist with obtaining treatment, and to provide advice on infection prevention and control if relevant and appropriate.

10 DATA MANAGEMENT

10.1 Data quality assurance and monitoring

Study staff will be trained on the study protocol prior to the start of study activities at each site. Data collection forms will be reviewed by the Study Coordinators and Principal Investigators (PIs) for completeness and accuracy. PIs from the different countries will maintain regular contact to ensure consistency in protocol implementation at each site, and PIs will meet regularly with study staff at their site/s to ensure consistency in the collection of data. PIs will participate in regular study group meetings to assess progress of the study, address any difficulties with the RDTs or protocol, and provide feedback to members of the study group. A 2-week pilot period will be conducted before beginning the study at each site, which will allow the investigators and study personnel to identify and resolve potential logistical and technological problems prior to beginning data collection. Standardized protocols and SOPs will be followed for quality control/quality assurance of clinical evaluations, biological sample procurement and preparation, and all laboratory procedures.

10.2 Records

All participants will be identified throughout the study by a unique identifying number (UID) that will be assigned at recruitment using uniquely numbered and barcoded consent forms (see Figure A). Hospital clinical case records will be linked to the data set via the paper consent form, the hard copy of which will be included in the patient's clinical record files at the health facility providing care. The case recruitment form will include a step to photograph a region of the consent form that contains patient details, mark of consent and unique identifier number. Persistent links between personally identifying information and research data will be required for the duration of the study as some results from reference laboratories have long term health implications to the patient and these will need to be reported back to clinicians leading patient care. Access to images of consent forms and personally identifying data will be restricted on a need-to-know basis and will never be revealed to anyone outside the study team.

Urithishano na muachano wa viumbe hai (Genetics) unavyoathiriwa na kovu linalosababishwa na ugonjwa wa kikope.

KEMRI / LSHTM Trachoma Research Programme
London School of Hygiene & Tropical Medicine

Figure A. Unique Identifier Assignment: In this example from another study, the unique QR code is used to digitally link the consent form to the data record.

All data will be backed up regularly to an off-site secure facility. Primary data outputs will be in XML format. For backup purposes, we will routinely make copies of all case files in a human readable PDF report format. Data requiring forwarding or reverting back to clinicians will be transmitted as PDF reports through encrypted channels.

Electronic data will be stored on password-protected and encrypted media. Access to the records will be limited to study staff. The investigators and study staff will allow all requested monitoring visits, audits or reviews by relevant ethical review boards.

All data entry errors will be reported using a separate ODK form. The original data files will not be manipulated in any way at any point during or after the study. Error corrections to the data set will be performed during the final data analysis using a traceable, reproducible and fully open, script based approach. The analysis script will be annotated to specifically reference each change made to the data, referring to the unique timestamps of each error monitoring form.

10.3 Data management

Clinical data will be collected using Open Data Kit (ODK [https://opendatakit.org]) and Android devices. Electronic data entry quality will be ensured by real-time error capture, internal validation, consistency checks and stringent formatting constraints. Completed data forms will be securely transferred by direct internet connection to a 256 bit SSL encrypted and fire-walled ODK server, which is hosted at LSHTM (www.odk.lshtm.ac.uk). Data connections with the encrypted server can be made over mobile connections and data are transferred automatically to the server whenever an internet access point is available. This allows continuous backup of data and quality monitoring in real time.

Data from each site will be accessible to investigators responsible for that site throughout the study. Anonymised quantitative data will be held for sharing as original databases stored with a soft copy of the fully annotated questionnaires and the STATA scripts files used for recoding and analysis. Personal identifiers, such as names, will not be held, with ID numbers used instead. Details of how to access the data will be published with each study publication, and will be provided to any *bona fide* researcher requesting access. The LSHTM Data Compass repository will also enable access to repository contents through a searchable index.

The qualitative data will be made available in second-order formats to protect the confidentiality of participants. Making full interview transcripts or fully transcribed field notes publicly available would risk participant identification and could hamper participation in the study. Second-order summaries of both the observational data and interviews will be prepared in the form of daily contact summaries that are anonymised and therefore sharable. This process will be described to participants at the point of consent.

The LSHTM Research Data Management Support Service, through the LSHTM institutional Research Data Repository, will store off-site backups indefinitely. Microorganism isolates, DBS, residual frozen blood and serum samples, and residual pharyngeal samples will be archived at LSHTM, providing that there is institutional support to maintain quality management systems for archiving and retrieval, power backup and -80°C freezer capacity.

10.4 Immediate and long-term use of the data

Data collected in this study will be compiled, analysed, and made available to collaborating partners and to ministries of health in participating countries, and will be prepared for publication in open-access peer-reviewed journals. Data may be made available to research students in participating countries and at participating institutions for educational use. The investigators will comply with international standards and guidelines regarding open access to research data. Study protocols, SOPs and data collection tools will be made freely available on a dedicated study website. Upon completion of the study, all study documents and record forms will be filed and stored at study sites for at least 10 years. Electronic data records will be stored indefinitely by investigator/s in research facility systems. Results of the study may be publicized in the future by collaborating partners and stakeholders as part of public health education efforts and control programs for infectious diseases and use of antimicrobial medicines (see also section 14).

11 DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Quantitative data analysis will be performed with Stata and other software packages.

11.1 Quantitative outcome measures and analysis

Listed by study objective:

1) To determine the treatable and/or preventable causes of fever in children and adults presenting as outpatients, and among those admitted to hospitals, in areas represented by the study sites;

For outpatients the attributable fraction (AF) will be calculated for each pathogen or group of pathogens. This will be done separately for each site and age stratum. For each group of patients, the odds ratio (OR) for the association between each pathogen and fever will be calculated by comparing cases and controls. Logistic regression will be used to calculate the odds ratio. In order to take the matched design into account, strata based on geographical location and season will be defined at each site and the analysis will be adjusted for age, sex and stratum. Since cases and controls are recruited at a constant rate throughout the year but fever incidence is likely to vary by season, the analysis will be weighted by the proportion of patients included in the study of the total number of cases seen at the study

site in the given season. AF will then be calculated as $AF_A = p_A(1-OR_A)$. Confidence intervals will be calculated in an appropriate way, e.g. bootstrapping or delta method.

For inpatients, first the distribution of diagnoses in each site will be tabulated for all in-patients in the study. Then a second analysis to calculate AFs will be carried out for in-patients, but this will be restricted to inpatients who come from the same geographical area as the controls. The calculation of AFs for inpatients may be dropped if insufficient numbers of in-patients come from the same area as the controls.

2) To determine how fever aetiology varies according to patient age, level of the health system, geographical area, local malaria and HIV prevalence, and other risk factors;

Stratified analyses will be carried out as above to estimate the AF for sub-groups of patients.

3) To determine the prevalence and spectrum of antimicrobial resistance among bacterial pathogens identified in clinical specimens from febrile patients;

At each site the proportion of bacterial pathogens with antimicrobial resistance defined by standardized criteria will be calculated. The proportion of common organisms demonstrating resistance to a standard panel of antibiotics will be reported.

Specific secondary objectives:

1) To generate data on incidence of specific infections among study participants, and therefore contribute key data on disease burden for some infections that are not counted in current "global burden of disease" estimates;

For an area whose population size is known, a health utilization survey will be used to estimate *r*, the proportion of fevers for which treatment was sought at a study centre. The total number of cases seen at the study site from the defined population will be multiplied by 1/*r*, to obtain the total cases in a year from the defined population. This will be divided by the size of the population to estimate incidence. Incidence of fever caused by specific pathogens will be calculated by multiplying the incidence by the AF for that pathogen.

3) Evaluation of available biomarker assays to assess their performance and potential utility in fever case management in the study areas;

The sensitivity, specificity, and positive and negative predictive value of each biomarker will be estimated.

4) To generate data to support the development of new fever case management algorithms which may be evaluated in future studies;

The association between the presence of pathogens with predefined clinical and other variables will be examined.

11.2 Sample size considerations

Any single pathogen or aetiology is assumed to be likely to be rare in the study populations.[2,14,15] To identify causes of fever, a sample size of 600 patients per group will enable estimation of the prevalence of an infection whose true prevalence is 5% with a precision of +/- 1.7% with 95% confidence, and to estimate the prevalence of an infection whose true prevalence is 1% with a precision of +/- 0.8% (see Table 3).

Table 3. Sample size estimates for varied infection prevalences

Number of participants	Prevalence	Confidence interval
600	5%	+/- 1.7
600	2%	+/- 1.1
600	1%	+/- 0.7

Since blood cultures and convalescent samples will not be taken from community controls, the prevalence of respiratory viruses (e.g., influenza and RSV) detected by polymerase chain reaction (PCR), and of baseline seropositivity to other pathogens, will be compared in cases and controls. The prevalence of respiratory viruses, and of seropositivity to common causes of fever, is assumed to be approximately 5% in the general population. A sample of 600 outpatients and 300 controls will provide >90% power to show a significant difference between a prevalence of 12% in cases and 5% in controls. Therefore, 600 febrile patients will be enrolled in each of four analysis groups (children aged ≥2 months to <15 years as inpatients and outpatients, and inpatients and outpatients aged ≥15 years), for a total of 2400 patients per site; plus 300 controls in each of the two age groups at each site (total 600).

12 QUALITY ASSURANCE

Quality control and assurance of all study procedures and will be conducted according to SOPs and as briefly described in sections 7.6.7, 7.6.8, 7.6.9, and 10.1. To ensure the quality and integrity of data, and the safety of participants, an External Advisory Committee will be contracted for the duration of the study as in section 8.3.

13 EXPECTED OUTCOMES OF THE STUDY

This study is expected to provide a clearer picture of the causes of febrile illness in different geographical settings and populations, and comparable data on local perceptions and management of fevers. It will give insight into the aetiologies that contribute to similar proportions of fever cases worldwide, and those which are geography-, season-, or population-specific. It will make use of and inform already well-developed public health networks that deal with big databases. This will allow more precise mapping of the causes of fever, which will be used to develop evidence-based algorithms for the management of

febrile illnesses in children and adults, and to inform rational surveillance efforts in the future. The data generated by this study to support updated, evidence-based guidelines for fever case management have the potential to safely improve targeting of antimicrobial prescription for outpatients.

In addition, this study provides a unique opportunity to develop a first-of-kind archive, by collecting and storing biomedical samples from a large and well-characterised group of febrile patients and controls from diverse locations in tropical settings. Use of the archive is expected to lead to development and evaluation of new point-of-care diagnostic tests, which are expected to allow improved fever case management, as well as safe reduction of unnecessary antimicrobial use and therefore reduction of antimicrobial resistance (see also sections 7.5 and 7.7).

14 DISSEMINATION OF RESULTS AND PUBLICATION POLICY

A high degree of public engagement is an integral part of the research plan for this study. Stakeholders include research participants, clinicians, policy-makers, and the general public. In the first year of the study meetings will be held with community leaders and with the public at each site to inform local communities about the aims of the study and the methods to be used. Seminars will be organised at participating health facilities to inform medical and nursing staff about the study before it begins. The social science component of the study (protocol to be presented separately) will involve ethnographic observations and key informant interviews with members of the community and medical and nursing staff, which will provide further opportunities for public engagement. A public engagement event will be held at each study site at the end of the project to disseminate the results to the communities who participated in the study.

In addition, investigators and study staff will engage with policy makers and the research community from the outset of study preparation. The study will be registered on www.clinicaltrials.gov, www.isrctn.com, www.pactr.org, and http://www.anzctr.org.au/. Investigators will take advantage of national and international networks to ensure that policy makers at various levels are aware of the study, and that the results are made available to them as soon as possible. When final results are available, feedback and dissemination meetings will be held at each site both for medical, nursing and laboratory

staff, and for the communities who participated in the study. Press releases will be arranged to publicise study results through the press office at LSHTM and other participating institutions, and to engage with local media at each study site to ensure that the results of these studies are widely disseminated.

Results from this study will be prepared for publication in open-access peer-reviewed journals as soon after study completion as possible.

15 DURATION OF THE STUDY

The duration of participant recruitment and follow-up is expected to take approximately 12 months from the start of study activities at each site as shown in Table 4, depending on rates of enrolment. The recruitment period will be extended at individual sites if required to meet sample size target.

Table 4. Proposed study timeline at each study site from the time of protocol approval

Activity	Mo 1-3	Mo 4-6	Mo 7-9	Mo 10-12	Mo 13-15	Mo 16-24
Study/site prep, training						
Pilot, begin enrolment						
Enrol to sample size						
Participant follow-up						
Reference lab test results						
Data cleaning, analysis						
Report writing, results dissemination						

16 ANTICIPATED POTENTIAL PROBLEMS

Table 5. Potential study problems and proposed solutions

Potential problem	Proposed solution
Delayed start of study activities due to delays in ethics approvals, procurement, other logistic impediments	1) Work with relevant personnel/agencies, local and national authorities as needed to minimize impact on project timelines 2) If necessary discuss with DFID re: adjustment of deliverables timeline
Insufficient sample size (for cases and/or controls), or slower than expected enrolment	1) Work with local community leaders, health facility staff and other relevant personnel to identify and address possible causes 2) Increase number of clinical sites (within the same geographical/population area)
High (>15%) loss to follow-up	1) Reinforce importance of full follow-up in informed consent discussion and at study visits 2) Increase home visit staff
Adverse events and/or protocol violations	1) Report to appropriate ethical committee and EOC 2) Prevention as appropriate through protocol amendment and/or personnel training
Loss of or damage to blood samples and isolates during storage or transport	1) Minimize risk with use of good-quality equipment, SOPs and training at sites, and use of well-established international courier 2) Reserve a portion of each sample at study site in addition to LSHTM archive

17 ETHICAL CONSIDERATIONS AND PARTICIPANT CONFIDENTIALITY

Ethical approval will be sought from national/institutional review committees in all participating countries. Written informed consent will be obtained from all participants as described in section 7.2. Participants will be identified by coded study numbers in all data collection forms and electronic databases. No individual identities will be used in any reports or publications resulting from the study. Only study staff and study investigators will have access to the information collected, for purposes of data entry and analysis. At the time of informed consent for study participation, participants will be informed that participation in a research study may involve a loss of privacy; however, only study personnel will have access to the information collected.

18 SPONSOR

London School of Hygiene & Tropical Medicine (LSHTM) will act as the main sponsor for this study. Delegated responsibilities will be assigned locally.

19 FUNDING

The UK Department for International Development (DFID) are funding this study through a grant to LSHTM. Activities at study sites and reference laboratories are funded through contracts established between LSHTM and collaborating institutions.

20 AUDITS AND INSPECTIONS

The study may be subject audit by the London School of Hygiene & Tropical Medicine under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to Good Clinical Practice.

REFERENCES

1. Crump JA, Kirk MD. Estimating the Burden of Febrile Illnesses. *PLoS Negl Trop Dis* 2015; **9**(12): e0004040.
2. Mayxay M, Castonguay-Vanier J, Chansamouth V, et al. Causes of non-malarial fever in Laos: a prospective study. *Lancet Glob Health* 2013; **1**(1): e46-54.
3. World Health Organization. Integrated Management of Childhood Illnesses (IMCI) chart booklet. 2014. 978 92 4 150682 3. http://www.who.int/maternal_child_adolescent/documents/IMCI_chartbooklet/en/.
4. World Health Organization. Integrated management of adolescent and adult illness (IMAI): interim guidelines for first-level facility health workers at health centre and district outpatient clinic: acute care. 2009. 978 92 4 159787 6. http://www.who.int/hiv/pub/imai/primary_acute/en/.
5. D'Acremont V, Kahama-Maro J, Swai N, Mtasiwa D, Genton B, Lengeler C. Reduction of anti-malarial consumption after rapid diagnostic tests implementation in Dar es Salaam: a before-after and cluster randomized controlled study. *Malar J* 2011; **10**: 107.
6. Hopkins H, Bruxvoort KJ, Cairns ME, et al. Impact of introduction of rapid diagnostic tests for malaria on antibiotic prescribing: analysis of observational and randomised studies in public and private healthcare settings. *BMJ* 2017; **356**: j1054.
7. Prasad N, Murdoch DR, Reyburn H, Crump JA. Etiology of Severe Febrile Illness in Low- and Middle-Income Countries: A Systematic Review. *PLoS One* 2015; **10**(6): e0127962.
8. Animut A, Mekonnen Y, Shimelis D, Ephraim E. Febrile illnesses of different etiology among outpatients in four health centers in Northwestern Ethiopia. *Jpn J Infect Dis* 2009; **62**(2): 107-10.
9. Njama-Meya D, Clark TD, Nzarubara B, Staedke S, Kamya MR, Dorsey G. Treatment of malaria restricted to laboratory-confirmed cases: a prospective cohort study in Ugandan children. *Malar J* 2007; **6**: 7.
10. Rabasa AI, Gofama MM. Urinary tract infection in febrile children in Maiduguri north eastern Nigeria. *Niger J Clin Pract* 2009; **12**(2): 124-7.
11. Capeding MR, Chua MN, Hadinegoro SR, et al. Dengue and other common causes of acute febrile illness in Asia: an active surveillance study in children. *PLoS Negl Trop Dis* 2013; **7**(7): e2331.
12. Non-malarial febrile illness map. Infectious Diseases Data Observatory (IDDO); 2017. http://www.wwarn.org/surveyor/NMFIv2/#/0.
13. Chheng K, Carter MJ, Emary K, et al. A prospective study of the causes of febrile illness requiring hospitalization in children in Cambodia. *PLoS One* 2013; **8**(4): e60634.
14. Crump JA, Morrissey AB, Nicholson WL, et al. Etiology of severe non-malaria febrile illness in Northern Tanzania: a prospective cohort study. *PLoS Negl Trop Dis* 2013; **7**(7): e2324.
15. D'Acremont V, Kilowoko M, Kyungu E, et al. Beyond malaria--causes of fever in outpatient Tanzanian children. *N Engl J Med* 2014; **370**(9): 809-17.
16. Mueller TC, Siv S, Khim N, et al. Acute undifferentiated febrile illness in rural Cambodia: a 3-year prospective observational study. *PLoS One* 2014; **9**(4): e95868.

17. GBD Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388**(10053): 1459-544.
18. Dittrich S, Tadesse BT, Moussy F, et al. Target Product Profile for a Diagnostic Assay to Differentiate between Bacterial and Non-Bacterial Infections and Reduce Antimicrobial Overuse in Resource-Limited Settings: An Expert Consensus. *PLoS One* 2016; **11**(8): e0161721.
19. Kelly H, Cowling BJ. Case fatality: rate, ratio, or risk? *Epidemiology* 2013; **24**(4): 622-3.
20. Gouws E, Bryce J, Habicht JP, et al. Improving antimicrobial use among health workers in first-level facilities: results from the multi-country evaluation of the Integrated Management of Childhood Illness strategy. *Bull World Health Organ* 2004; **82**(7): 509-15.
21. Biggs HM, Hertz JT, Munishi OM, et al. Estimating leptospirosis incidence using hospital-based surveillance and a population-based health care utilization survey in Tanzania. *PLoS Negl Trop Dis* 2013; **7**(12): e2589.
22. Crump JA, Youssef FG, Luby SP, et al. Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. *Emerg Infect Dis* 2003; **9**(5): 539-44.
23. Panzner U, Pak GD, Aaby P, et al. Utilization of Healthcare in the Typhoid Fever Surveillance in Africa Program. *Clin Infect Dis* 2016; **62 Suppl 1**: S56-68.
24. Paul RC, Rahman M, Gurley ES, et al. A novel low-cost approach to estimate the incidence of Japanese encephalitis in the catchment area of three hospitals in Bangladesh. *Am J Trop Med Hyg* 2011; **85**(2): 379-85.
25. George EC, Walker AS, Kiguli S, et al. Predicting mortality in sick African children: the FEAST Paediatric Emergency Triage (PET) Score. *BMC Med* 2015; **13**: 174.
26. Conroy AL, Hawkes M, Hayford K, et al. Prospective validation of pediatric disease severity scores to predict mortality in Ugandan children presenting with malaria and non-malaria febrile illness. *Crit Care* 2015; **19**: 47.
27. Helbok R, Kendjo E, Issifou S, et al. The Lambarene Organ Dysfunction Score (LODS) is a simple clinical predictor of fatal malaria in African children. *J Infect Dis* 2009; **200**(12): 1834-41.
28. Freund Y, Lemachatti N, Krastinova E, et al. Prognostic Accuracy of Sepsis-3 Criteria for In-Hospital Mortality Among Patients With Suspected Infection Presenting to the Emergency Department. *JAMA* 2017; **317**(3): 301-8.
29. Wang JY, Chen YX, Guo SB, Mei X, Yang P. Predictive performance of quick Sepsis-related Organ Failure Assessment for mortality and ICU admission in patients with infection at the ED. *Am J Emerg Med* 2016; **34**(9): 1788-93.
30. Ranzani OT, Prina E, Menendez R, et al. New Sepsis Definition (Sepsis-3) and Community-acquired Pneumonia Mortality: A Validation and Clinical Decision-making Study. *Am J Respir Crit Care Med* 2017.
31. Moore CC, Hazard R, Saulters KJ, et al. Derivation and validation of a universal vital assessment (UVA) score: a tool for predicting mortality in adult hospitalised patients in sub-Saharan Africa. *BMJ Global Health* 2017; **2**(2).
32. North Shore-LIJ Health System. Human Subject Protection Program Guidance Document: Maximum blood draw limits (v 15Jan2013). 2013. www.feinsteininstitute.org/wp-content/uploads/2013/02/Maximum-Blood-Draw-Limits.pdf.
33. (CIOMS) CfIOoMS. International Ethical Guidelines for Health-related Research Involving Humans. In: World Health Organization, editor. Geneva, Switzerland; 2016. 978-

929036088-9. https://cioms.ch/shop/product/international-ethical-guidelines-for-health-related-research-involving-humans/.

34. Marks F, von Kalckreuth V, Aaby P, et al. Incidence of invasive salmonella disease in sub-Saharan Africa: a multicentre population-based surveillance study. *Lancet Glob Health* 2017; **5**(3): e310-e23.
35. Gargouri N, Walke H, Belbeisi A, et al. Estimated burden of human Salmonella, Shigella, and Brucella infections in Jordan, 2003-2004. *Foodborne Pathog Dis* 2009; **6**(4): 481-6.
36. von Kalckreuth V, Konings F, Aaby P, et al. The Typhoid Fever Surveillance in Africa Program (TSAP): Clinical, Diagnostic, and Epidemiological Methodologies. *Clin Infect Dis* 2016; **62 Suppl 1**: S9-S16.
37. World Health Organization. WHO-FIND malaria RDT evaluation programme. 10 July 2017 2017. http://www.who.int/malaria/areas/diagnosis/rapid-diagnostic-tests/rdt-evaluation-programme/en/ (accessed 7 Sept 2017.
38. World Health Organization. Malaria microscopy quality assurance manual – Ver. 2. 2016. 978 92 4 154939 4. http://www.who.int/malaria/publications/atoz/9789241549394/en/.
39. Kapasi AJ, Dittrich S, Gonzalez IJ, Rodwell TC. Host Biomarkers for Distinguishing Bacterial from Non-Bacterial Causes of Acute Febrile Illness: A Comprehensive Review. *PLoS One* 2016; **11**(8): e0160278.
40. Lubell Y, Blacksell SD, Dunachie S, et al. Performance of C-reactive protein and procalcitonin to distinguish viral from bacterial and malarial causes of fever in Southeast Asia. *BMC Infect Dis* 2015; **15**: 511.
41. Chen HL, Hung CH, Tseng HI, Yang RC. Soluble form of triggering receptor expressed on myeloid cells-1 (sTREM-1) as a diagnostic marker of serious bacterial infection in febrile infants less than three months of age. *Jpn J Infect Dis* 2008; **61**(1): 31-5.
42. Jiyong J, Tiancha H, Wei C, Huahao S. Diagnostic value of the soluble triggering receptor expressed on myeloid cells-1 in bacterial infection: a meta-analysis. *Intensive Care Med* 2009; **35**(4): 587-95.
43. Mankhambo LA, Banda DL, Group IPDS, et al. The role of angiogenic factors in predicting clinical outcome in severe bacterial infection in Malawian children. *Crit Care* 2010; **14**(3): R91.
44. Ricciuto DR, dos Santos CC, Hawkes M, et al. Angiopoietin-1 and angiopoietin-2 as clinically informative prognostic biomarkers of morbidity and mortality in severe sepsis. *Crit Care Med* 2011; **39**(4): 702-10.
45. Fisher J, Linder A. Heparin-binding protein: a key player in the pathophysiology of organ dysfunction in sepsis. *J Intern Med* 2017; **281**(6): 562-74.
46. Linder A, Arnold R, Boyd JH, et al. Heparin-Binding Protein Measurement Improves the Prediction of Severe Infection With Organ Dysfunction in the Emergency Department. *Crit Care Med* 2015; **43**(11): 2378-86.
47. Conroy AL, Hawkes M, McDonald CR, et al. Host Biomarkers Are Associated With Response to Therapy and Long-Term Mortality in Pediatric Severe Malaria. *Open Forum Infect Dis* 2016; **3**(3): ofw134.
48. Mikacenic C, Hahn WO, Price BL, et al. Biomarkers of Endothelial Activation Are Associated with Poor Outcome in Critical Illness. *PLoS One* 2015; **10**(10): e0141251.