Comparative study of commercially available typhoid point of care tests to benchmark current and emerging tools

Short title:

Commercial Typhoid tests validation

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Table of Contents

List of Abbreviations and Acronyms

Abbreviation/acronym	Meaning
CRF	Case Report Form
EDC	Electronic Data Capture
EDL	Essential Diagnostic List
FIND	Foundation for Innovative New Diagnostics
GCLP	Good Clinical Laboratory Practice
GCP	Good Clinical Practice
IAF	Informed Assent Form
ICF	Informed Consent Form
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISF	Investigator Site File
IVD	In vitro Diagnostic
LMIC	Low and Middle Income Countries
PCR	Polymerase Chain reaction
PI	Principal Investigator
RDT	Rapid Diagnostic Test
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
WHO	World Health Organisation

ABSTRACT

Typhoid fever is an enteric bacterial infection caused by *Salmonella enterica* serovar Typhi (Salmonella Typhi; S. Typhi). It is an important infectious disease in low- and middle-income countries with over 10.9 million new cases worldwide and 116.8 thousand death in 2017. South Asia and Sub Saharan Africa are the most affected areas of the world. Typhoid fever is common in areas with inadequate sanitation and hyegine. In routine practice, diagnosis of typhoid fever is rarely confirmed as diagnostic tests are unavailable or have limited diagnostic accuracy. Blood culture is the commonest reference standard test but has a lower sensitivity. Alternatives to those methods exist but their performance is poor. The Widal test is still used but as it is based on cross-reactive antigens, it lacks sensitivity and specificity. Clinician often use rapid diagnostic tests to diagnose typhoid. A number of typhoid fever RDTs are commercially available but performance data are not available or not consistent from a study to another. This prospective, multicentre, cross-sectional study will be carried out in 3 hospitals of Nairobi, Kenya. 2000 clinically suspected typhoid cases will be enrolled in this study, blood culture as well as serum for RDT will be received. All typhoid positive and equal typhoid negative serum will be tested for investigational RDTs.

This collaborative study between KEMRI and FIND will systematically compare different point of care typhoid tests currently available in the market against the same set of reference standard.The knowledge gained from this trial may benefit health providers' by providing information on diagnostic accuracy of current typhoid test and to decide on utility of these commercial tests. The result obtained from this trial will also be made available to help inform Ministry of Health in Kenya and the WHO Essential Diagnostic list (EDL) and stakeholder decision making more broadly.

LAY SUMMARY

Typhoid fever bacterial disease caused by *Salmonella* Typhi. It is one of the most common serious bacterial diseases in the developing world, with an estimated 10.9 million people contracting the disease worldwide and 116.8 thousand people dying of the disease in 2017. Culture method using blood and bone marrow samples are considered as the benchmark for teting for typhoid disease. Those methods are however time consuming, requires drawing blood from the body, and requires adequate infrastructure and trained staff that are not available in most of areas where the disease is very common locally. There are other methods

but their performance is poor. The Widal test is a cheap test that is widely used despite its high rate of giving test results which wrongly indicates that typhoid is present. Rapid Diagnostic tests (RDTs) have been developed and are available in the market but their performance data are not available or not consistent from one study to another. Therefore, this study will aim to perform a direct comparison of typhoid tests currently in the market to help inform the Ministry of Health in Kenya and the WHO Essential Diagnostic list (EDL) and stakeholder decision making more broadly.

INTRODUCTION/BACKGROUND

Typhoid is an enteric disease caused by the bacterium *Salmonella* Typhi; it is estimated that 11 to 20 million people contract typhoid each year and 128 000 to 161 000 die from the disease; children under 5 are at higher risk of contracting the disease (1).

Typhoid endemic areas are located mainly in South Asia and Sub-Saharan Africa and is transmitted through contaminated water and food (2). The disease is treatable with a specific regimen of antibiotics, however antimicrobial resistance has been reported in several countries, particularly in Pakistan (3). Several vaccines have been developed but their uptake has been low, partly due to limited information on the exact burden of the disease in endemic countries.

The symptoms of typhoid are similar to other undifferentiated febrile illnesses and typhoid can be mistaken with vector borne febrile illnesses such as scrub typhus (4).

Blood and bone marrow cultures are considered the gold standard for the diagnosis of typhoid. Those methods require specific infrastructure and skilled staff that are not always available in LMICs and are not adequate for rapid patient management. In addition, although very specific, blood culture sensitivity is impacted by misuse of antibiotics that lower the bacterial load to undetectable levels in patients' blood (5).

PCR assay for diagnosis of typhoid fever has been used in many studies. Lower concentration for bacterial load in peripheral blood in typhoid fever results in lower sensitivity of PCR (6). Currently there is no validated PCR test in common use, only in-house system which are open to differing interpretation and none would meet the rigors of quality control. (7)

As a consequence, alternatives to blood culture have been used in LMIC. The Widal test is the most used test despite a low performance (sensitivity range: 57-34%; specificity range: 43-83 %;(4) reported in several studies. Other options in typhoid diagnosis are rapid diagnostic tests; among them, three tests (Typhidot, Tubex and Test-it™ Typhoid IgM) have been evaluated in several studies. It has been reported a variability of tests performance in different studies and according to the geographical regions (6). Variability in the test performance reported so far in the literature has hampered WHO to recommend any of these

rapid tests in the EDL. FIND in collaboration with international typhoid experts developed a target product profile outlining the ideal characteristics of point of care tests. As part of this activity it became apparent that no quality data are available that systematically compare all available commercially point of care tests against the same set of reference standards used in multiple populations (e.g. Africa vs Asia). These lack of benchmarking data significantly impedes health providers' ability to decide on the utility of commercial tests in different settings, ultimately restricting use and access. Further the lack of well characterized samples reduces the ability for targeted innovation in the typhoid space.

PROBLEM STATEMENT

Differentiating typhoid infection from other cause of fever in endemic areas is a diagnostic challenge. Although commercial point of care rapid diagnostic tests for enteric fever are available as alternatives to the current reference standard test of blood or bone marrow culture or to the widely used Widal test, their diagnostic accuracy is unclear.

JUSTIFICATION FOR THE STUDY

The gold standard test to confirm typhoid fever is through isolating *Salmonella* Typhi from blood or bone marrow culture. However, this method takes several days to get the test result. In addition, due to misuse of antibiotics and the biology of the pathogens that renders the bacterial load difficult to detect in blood, the blood culture method may give a false negative test result even though the person has typhoid fever. Besides, the blood culture also requires adequate infrastructure and trained manpower that are not easily available in most of endemic areas such as Kenya (5).

A cost-effective alternative to the culture method using blood /bone marrow sample is Widal test which is widely being used in endemic areas. However, published reports show that Widal test has low sensitivity and specificity (10). Other alternatives are Rapd Diagnostic Tests (RDTs) which are easy to use and deliver quick results. There are number of commercially available RDTs for typhoid fever but their performance data are not available or not consistent from a study to another (7). Foundation for Innovative New Diagnostics (FIND) in collaboration with international typhoid experts developed a target product profile outlining the ideal characteristics of point of care tests. As part of this activity it became apparent that no quality data are available that systematically compare all available commercially point of care tests against the same set of reference standards used in multiple populations (e.g. Africa vs Asia). This lack of benchmarking data significantly impedes health provider's ability to decide on the utility of commercial tests in different settings, ultimately restricting use and access. Furthermore, the lack of well characterized samples reduces the ability for targeted innovation in the typhoid space. A second gap that was

identified was the lack of a simple well-performing gold standard suggesting latent class modelling as a solution used for other pathogens with an imperfect gold standard (8).
Therefore, this study will aim to perform a head to head comparison of typhoid tests currently available in the market in two different and geographically distant countries and simultaneously develop a sample set that can be used in future evaluations of emerging technologies and/or to support innovative test development. The knowledge gained from this trial may benefit society by providing information on diagnostic accuracy of currently available point of care typhoid tests. Further the data obtained in this trial will be made available to WHO to support the EDL and stakeholder decision making more broadly.

TRIAL OBJECTIVES AND ENDPOINTS

General Objective

To compare commercially available typhoid point of care tests (RDTs) against a defined reference standard of blood culture and simultaneously develop a sample set to evaluate emerging technologies and/or to support innovation test development.

Primary Objective

1.1. To evaluate different RDTs that are commercially available internationally for detecting antigens or antibodies to *Salmonella* Typhi and use Blood culture as standard for comparison.

1.2. Establish a biorepository of well characterized specimen collection that can be used to evaluate emerging tests.

Secondary Objectives

2.1 Evaluation of operational characteristics (invalid and indeterminate rates) of Typhoid RDTs

Primary Endpoint:

1.1 Point estimates of sensitivity and specificity for each test, with 95% confidence interval, using blood culture as reference standard.\

1.2 Establishment of a biorepository of well characterized specimen collection at the site available for future assessments of emerging technologies

Secondary Endpoints:

2.1 Estimates of operational characteristics of different RDTs based on quantitative assessment including invalid and indeterminate rates

DESIGNS AND METHODOLOGY

Trial Site

The study will take place in three hospitals in Nairobi County, found in Sub-Saharan Africa (Kenya). The three hospitals in Nairobi are Mbagathi County Hospital, Mbagathi Road; Medical Missionaries of Mary, Reuben Centre Hospital in Mukuru Slum, Embakasi and City Council Clinic in Mukuru Slum, Embakasi.

Justification for selectig the the three study hospitals: Mbagathi County Hospital, Mbagathi Road draws majority of its patients from Kibera informal settlement (slum), an area with high burden of bacterial infections due to poor sanitation and limited resources. Kibera is a large, densely populated (77,000 persons/km^2) urban settlement situated within Nairobi, a city with >4 million inhabitants. It is characterized by poor sanitation evidenced by excreta and garbage in footpaths and open sewers. Water supply in Kibera is largely unregulated and food vendors trade outdoors under minimal hygiene standards (Njuguna, 2013).

Medical Missionaries of Mary, Reuben Centre Hospital and City Council Clinic are located with andserves Mukuru informal settlement (slum) population. Maukuru slum also suffers from high bacterial disease burden due to poor sanitation and limited resources. Mukuru slum (20 km East of Nairobi) is home to a population of over 150,000 in an area approximately 2km^2(population density, 75,000/km^2) [Kenya Population Census, 2010] living in congested housing and under poor sanitary conditions. The majority of the populations work in nearby industrial areas.

Study Design

This is a prospective multicentre diagnostic accuracy study using collected blood and/or serum samples that will be conducted in three hospitals at Nairobi, Kenya. Participants will be recruited when they present to three hospitals where the study will take place. Clinical officers appointed in the 3 hospitals will collect blood samples from individuals older than eight years presenting with fever because most documented typhoid fever cases involve school-aged children and young adults. The samples will be transported in cold chain to the Centre for Microbiology Research (CMR), KEMRI laboratories where they will be processed using blood culture. Once culture results are available, investigational RDTs will be performed on all blood culture positive and equal culture of negative serum.

Trial Population

The trial population will be composed of adults and children suspected of typhoid between 8-65 years of age because most documented typhoid fever cases involve school-aged children and young adults. Participants will be recruited in 3 hospitals in Nairobi, Kenya.

Inclusion Criteria

Participants are eligible to be included in the Trial only if all of the following inclusion criteria apply:

- Individuals aged 8 years of age to 65 years of age
- History of fever or axillary temperature of >37.5^{0}Cfor at least 3 consecutive days within the last 7 days prior to enrolment
- Clinical suspicion of enteric fever
- One of the following scenarios:
    - Presents to outpatient department or Emergency Department
    - Admitted to hospital within last 12 hours
- Able and willing to provide informed consent (and assent when required)

Exclusion Criteria

Participants are excluded from the Trial if any of the following exclusion criteria apply:

- Unwillingness to participate in the study
- Inability to provide the required volume of blood
- Unwillingness to provide blood
- Known non-infectious / Non typhoid Infectious causes of fever or other alternate diagnosis of fever

Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently entered in the trial. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Studies (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information including demography, and screen failure details (i.e. eligibility criteria, participation refusal, site operational

issues/facilities issues, other) will be recorded in a dedicated Screening Log. Moreover, individuals who are considered screen failures may not be rescreened.

Participant Discontinuation/Withdrawal from the Trial

A participant may withdraw voluntarily from trial participation at any time without any impact on their care or treatment.

A participant may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance, or administrative reasons.

If the participant withdraws consent for disclosure of future information, the study may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the trial, he/she may request destruction of any samples taken and not tested

The investigator must document the withdrawal of participant and the reason for withdrawal using a Note to File (attached to the Informed Consent Form in the participant's file at the site)

Sample Size

The reported values for prevalence of Salmonella Typhi positive patients in Kenya is 5% (Data from personal communication with Prof. Sam Kariuki Co-PI). The reported values of sensitivity and specificity of diagnostic tests are generally low, (Review article on typhoid RDTs summarizes TUBEX test from IDL Biotech has sensitivity of 78% and specificity of 87%, Test™ It from LifeAssay has sensitivity of 69% and specificity of 90%, Typhidot from Reszon Diagnostic has sensitivity of 78% and specificity of 77%.(9) Similarly reported sensitivity and specificity of SD Bioline Salmonella Typhi IgG/IgM from Abbot and Typhoid IgG/IgM Combo rapid test from CTK Biotech are 69%/76% and 40-79%/68-79% respectively (15)). For the purpose of determining the sample size, sensitivity and specificity were both assumed to be 50%, which corresponds to the scenario that requires the highest number of samples to estimate sensitivity and specificity. Based on this hypothesis, and on the feasibility of recruitment at the sites of enrolment, it was decided to set the sample size in order to recruit at least 100 culture positive and culture negative samples in Kenya. These sample sizes will allow to estimate the sensitivity of the test with 80% power to detect a 95% confidence interval of width of +/- 14% in Kenya (11). Based on the prevalence mentioned above, this number of positive samples requires the enrolment of at least 2,000 participants in Kenya (at a prevalence of 5%). From a similar study carried out by an MSc student in our lab that we supervised (*Performance of tubex® tf IGM antibody test against culture in typhoid fever detection in Nairobi county, MSc Thesis by Walter Kipngetich Lelei, JKUAT, 2019*), the average number of patients presenting with febrile illnesses at Mbagathi County Hospital was 20 per day, using this as a basis and considering their relatively smaller sizes, we estimate that for Medical Missionaries of Mary, Reuben Centre Hospital and City Council Clinic, we will have 10 and 5 patients presenting per day in Medical Missionaries of Mary, Reuben Centre Hospital and City Council Clinic respectively. We plan to sample for only 3 days in a week (Mon-Wed) due to the observed trend of high patient inflow on these days from Walter

Lelei's MSc work (total 35 patients per day, 420 per month). This works out to a minimum of 2,520 which is above the sample size of 2,000 participants that we are targeting.

TRIAL PROCEDURES

Trial procedures are summarized in Study flow in Figure 1. Protocol waivers or exemptions are not allowed.

Adherence to the trial design requirements, including those specified in the study flow (Figure 1) is essential and required for trial conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable. All enrolled participants will be registered on an enrolment form.

Procedures conducted as part of the participant's routine clinical management and obtained before signing of the Informed Consent Form (ICF) and Informed Assent Form (IAF) may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria.

Specimen Collection, Handling, Transport and Storage

Procedures for specimen collection, handling, transport, and storage will be described in detail in the Trial Manual provided by FIND to the site. Blood will be collected by venepuncture following national guidelines on drawing blood. The maximum amount of blood collected from each participant in the trial, including any extra assessments that may be required, will not exceed 14 mL. Repeat may be taken at the time of enrolment for safety reasons or for technical issues with the samples. Blood will be handled as follows:

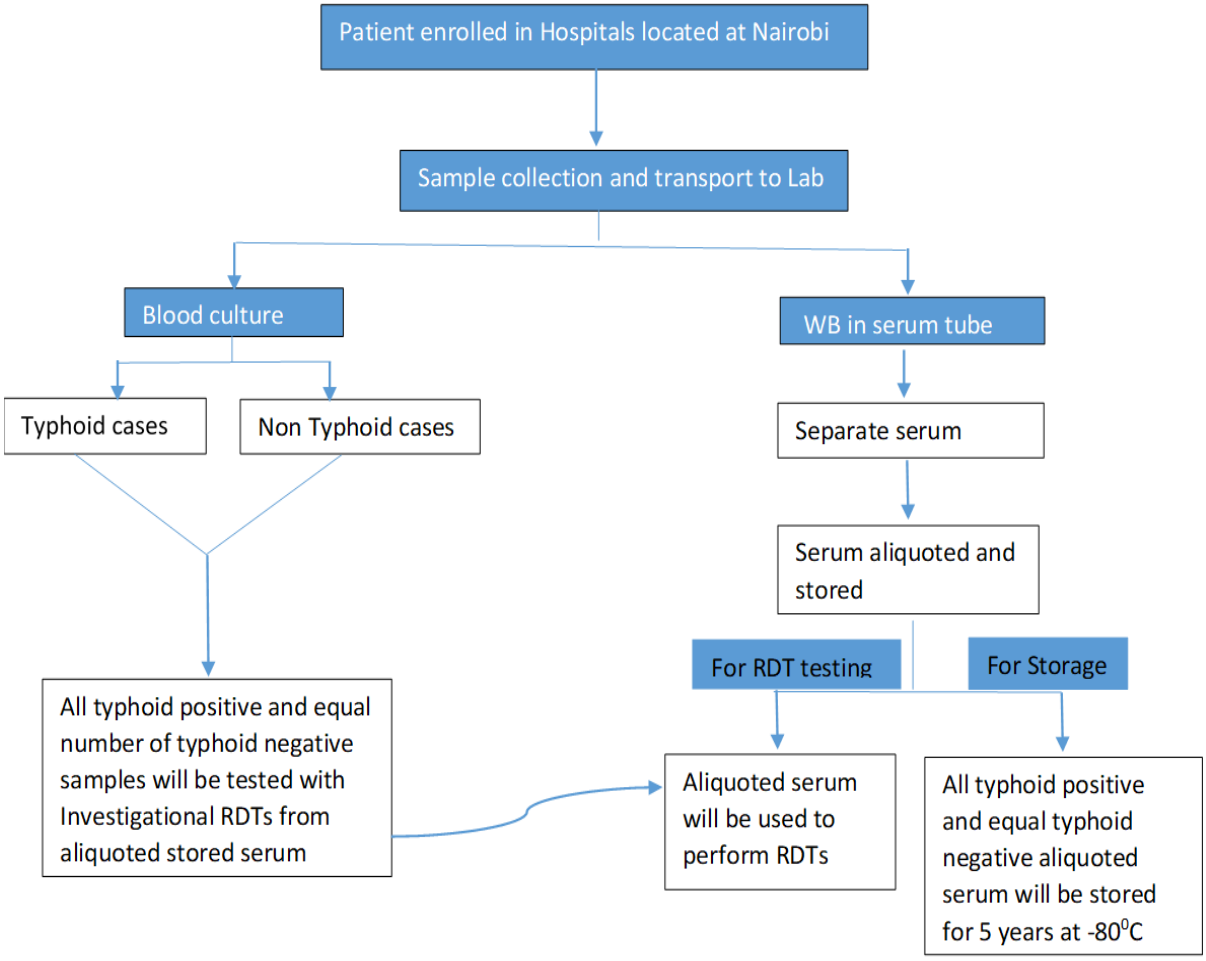
*Table 1***:** *Amount of blood to be collected*

Samples For	**Amount of sample in adults (>14 years) (14ml- 10+4ml)**	**Amount of sample in children (8-14 years) (7ml-3.5+3.5 ml)**
Blood Culture	1 x 10 ml whole blood	1 x 3.5 ml whole blood
Serum	1 X 4 ml whole blood	1 X 3 ml whole blood

Study workflow

The study flow is shown in Figure 1.

Figure 1: Study flow



Consenting patients will be enrolled in the study based on eligibility criteria. Blood will then be obtained from the patients by venepuncture method. The blood samples will be collected in blood culture bottles and in serum tubes. The volume of blood from the patient will follow the guidelines in **Table 1**. After collection at study hospitals, samples will be transported to the CMR, KEMRI laboratories in cold boxes every day and will be processed within 24 hours maximum. Samples will be stored at 4°C at CMR laboratories until further processing.

All samples preparations (blood culture and serum preparation) will be conducted in parallel. Blood culture will be done using an automated blood culture system (BD BACTEC 9050). BACTEC vials will be processed as per manufacturer's instructions. Bottles will be incubated until signal-positive or till the end of day 5, whichever is earlier. When positive signal is

observed, bottles are unloaded from instrument and Gram's stain and cultures and biochemical tests to identify *Salmonella* Typhi will be performed using standard microbiological procedures. Patients will be classified as typhoid or non-typhoid cases according to the blood culture results. However, since the results of the blood culture may take several days to be available, serum will be separated from all whole blood samples and will be aliquoted. It is expected that at least 100 blood culture positive and 100 blood culture negative samples will be tested with index tests and will be kept for long term storage.

Testing with the index tests will be performed in batch at the end of each month while the remaining volumes of the selected sera will be kept at -80°C. Depending on the initial volume that will differ between adult and children samples, 3 to 6 aliquots will be kept at -80°C for long term storage and will be used for future evaluation of diagnostic tests.

Investigational products

Reference standard tests to identify *Salmonella* Typhi:

All blood samples collected from study participants will undergo blood culture.

- Blood culture: Blood culture is considered as gold standard for the diagnosis of typhoid fever. Blood culture is a routine process for diagnosis of typhoid fever.

Index tests:

Index tests are RDTs that will be performed according to manufacturer's instructions by trained laboratory personnel. The readers of the index tests and reference standard test will be blinded to the results of the other tests.

Typhoid RDTs for inclusion in the study were selected by the study team based on a combination of the following criteria:

- RDT is CE-marked
- Published independent evaluation data
- International availability
- Volume of samples
- Price of the test
- Turnaround time

RDTs will be procured by FIND and shipped to the study sites in Kenya. There are no financial ties of any nature with the manufacutrers. All selected RDTs which will be included in the study are shown in Table 2.

Table: 2 Lists of RDTs

1	SD Bioline Salmonella Typhi IgG/IgM Fast
2	Typhidot Rapid IgG/IgM combo test
3	TUBEX TF
4	Typhoid IgG/IgM Combo Rapid Test CE
5	Enterocheck WB
6	Test-it™ Typhoid IgM
7	Typhoid IgG/IgM Rapid Test Cassette
8	S.typhi-S.paratyphi "A" Direct Antigen Detection
9	DiaquickS.typhi IgG/IgM Ab
10	Diaquick S. typhi/paratyphi Ag cassette
11	Widal*

*Widal test is included as index test because it is widely used for decision making of typhoid diagnosis and treatment.

Specificity and sensitivity of only few RDTs are currently available in studies but varies from one study to another and from one geographical region to another. Overall TUBEX test from IDL Biotech has sensitivity of 78% and specificity of 87%, Test™ It from LifeAssay has sensitivity of 69% and specificity of 90%, Typhidot from Reszon Diagnostic has sensitivity of 78% and specificity of 77%.(9) Similarly reported sensitivity and specificity of SD Bioline Salmonella Typhi IgG/IgM from Abbott and Typhoid IgG/IgM Combo rapid test from CTK Biotech are 69%/76% and 40-79%/68-79% respectively. Detail information on each RDTs with package inserts are available.

DATA MANAGEMENT

Data handling and record keeping

All participant data relating to the trial will be recorded in source documents and transcribed on to a paper Case Report Form (CRF) and/or an electronic CRF by trial site staff. Data will then be entered from the paper CRF into FIND's online clinical trials platform (OpenClinica Enterprise Edition *version 4.0*). Data will be verifiedto ensure they are accurate, correct and consistent with the source documents. CRF will be electronically signed by the study team on site. Data will be cleaned of errors by the study team throughout the trial as it is captured electronically.

Records and documents, including signed ICFs, IAFs (when required) pertaining to the conduct of this trial will be retained by the study team for 5 years after trial completion.

Source Data and Source Documents

Source documents will provide evidence for the existence of the participant and substantiate the integrity of the data collected. The study team will maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial participants. The study team will request previous medical records or reports (if available). Source documents or certified copies will be filed at the study site.

Participant data will consist of medical history, clinical examination, laboratory testing. Observations from the clinical examination will be recorded directly in the paper CRF and/or the eCRF. A paper CRF, which mirrors the design of the electronic CRF, may be used for ease of data entry by non-clinical and non-laboratory staff. The CRF will consist of some data that has been entered directly (e.g. source data) and some data that has been transcribed from other sources as detailed in the table 3. Any source data directly entered into the CRF will be clear and accurate, and will be signed and dated by the person who generated the data. Changes to source data will be traceable i.e. dated, initialed and explained (if necessary) and will not obscure the original entry.

Table 3: Source data definition and record

Type of source data	Original place of entry
Demographics	Paper CRFs
Medical history	Medical records or Paper CRFs
Specimen collecting time and date	Clinical officers record
Specimen time and date of receipt at the lab	Paper CRFs or eCRFs
Lab results	Lab notebook or LIMS

Other unidentified source data will be described in the Site Initiation Visit Report. Lab staff authorized by the PI will be trained and given a unique password to enter lab data originally recorded in laboratory notebooks or Laboratory Information Management System directly into the electronic CRF, thereby avoiding time consuming transcription on to a paper CRF first.

The investigator will maintain a delegation log with roles and responsibilities related to the management of records. Additionally, the investigator will ensure adequate training of research team involved on data collection. The investigator will also be responsible for internal validation of data.

The study team will permit trial-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to participant medical records and source documents used for this trial.

Data Management

Details of the data collection and management will be described in the data management plan and in accordance with ICH GCP principles safeguarding the confidentiality of participants' information**.**

Each enrolled participant will be assigned a unique study participant ID number used to identify samples and information. The use of an anonymous participant ID will ensure participant's confidentiality. To ensure that each clinical sample can be linked to clinical and laboratory results and tracked effectively from the collection site to the end-user, all lab request forms, sample collection pots, tubes and aliquots will be labelled using the participant's ID using a set of barcode labels provided by FIND.

Each set will be composed by the label types, according to the number and type of specimens collected at each site as described in Table 4**.** Labels will contain readable text and a barcode:

Table 4: Composition of Barcode labels

Label Type	Content	Text	Barcode	Use
Core label	• Study ID • Site ID • Participant ID	Yes	Yes	Place on Information sheet, CRF, enrolment form and lab results form
Specimen label	• Study ID • Site ID • Participant ID • Event ID • Specimen type	Yes	Yes	Place on blood culture bottle and serum tubes
Aliquot label	• Study ID • Site ID • Participant ID • Event ID • Specimen type • Aliquot ID	Yes	Yes	Place on tubes containing serum for testing of investigational RDTs and for long-term storage of serum

Data Management procedures, including the setup of the database, programming edit and range checks and querying, are described in the Data Management Plan.

Whenever possible clinical data and laboratory results will be captured directly onto electronic CRFdesigned by FIND/KEMRI in the OpenClinica EDC system. Site staff will be responsible for entering their data from a paper CRF into OpenClinica where direct capture into EDC is not possible. Test results will be exported from the devices when possible and

electronic files will be transferred to FIND for further analysis. FIND will setup a secure data transfer system such as a File Transfer Protocol (FTP) server. Details will be provided in the Trial Manual.
The site will be provided with individual password-protected accounts to access OpenClinica, following a training session given by FIND, either on site or remotely sharing screen through Skype or any other similar system.

No information concerning the trial or the data generated from the trial will be released to any unauthorized third party without prior written approval of the study team.

Statistical Analysis Plan

The statistical analysis plan (SAP) will be developed and finalized before the start of enrolment and will describe in detail the methodology used to evaluate each of the endpoints. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

- **Efficacy Analyses**

Endpoint	Statistical Analysis Methods
1.1	Point estimates of sensitivity and specificity for each test with 95% confidence interval, using Blood culture as reference standard.
1.2	Establish a biorepository of well characterized specimen collection that can be used to evaluate emerging tests.
2.2	Evaluation of operational characteristics of different RDTs including invalid and indeterminate rates.

Analysis datasets

The following datasets will be defined:

- ITT (Intention-To-Test) all subjects successfully enrolled in the study
- MITT (Modified-Intention-To-Test) all subjects in ITT for whom at least one test result is available
- PP (Per-Protocol) all subjects in ITT for whom results for all tests are available (complying with the protocol)

Description of statistical methods

General approach

Estimates of accuracy

Table 5 summarizes the way results of diagnostic tests are reported when two tests are compared. Based on the definitions in the table, the following values are defined:

Sensitivity = TP / (TP + FN)

Specificity = TN / (FP + TN)

For each point estimate, a 95% confidence interval will be derived based on the Wilson's score method. Missing and invalid results (e.g. the results for one specific test for a given participant) will not be imputed and will be removed from the analysis.

Table 5 Definition of test results

		Reference Standard Outcome		
Test result		Reference positive	Reference negative	Total
	Test result Positive	TP	FP	PP = TP + FP
	Test result negative	FN	TN	PN = FN + TN
	Total	P = TP + FN	N = FP + TN	P + N

TP = true positives, TN = true negatives, FP = false positives, FN = false negatives, P = positive, N = negative

Analysis of the primary outcome(s)

Outcome 1.1
This outcome will be evaluated with the methodology described in section 4.1.1, using using blood culture as reference standard, on the PP population, for each test included in the trial.
Outcome 1.2
This outcome does not require any statistical analysis.

Analysis of the secondary outcome(s)

Outcome 2.1
The number of indeterminate and invalid test results will be reported for each RDT, together with the relative percentage over the total number of tests performed.

Baseline descriptive statistics

Descriptive statistics tables will be generated to summarize the characteristics of the participants in the PP and ITT populations, per.
The number of participants included and excluded will be reported, overall and for each clinical site and test. Among the included samples, the information will be broken down by the following variables:
Socio-demographics:

•Age (<11, 12:17, >17)
•Gender (female, male)
•Clinical site
Sample characterization:
•Reference test positive or negative
Results will be reported either in absolute numbers (e.g. number of subjects in a group) or summarized by mean, median, standard deviation, minimum, maximum and quartiles.

Details on the methodology will be provided in the SAP.

QUALITY MANAGEMENT

Quality Management for this trial consists of Quality Control activities, training and capacity building, as well as the use of Standard Operating Procedures, Work Instructions, Tools and Templates.

Training on the protocol, GCP and the use of the IVDs and laboratory tests will be provided by the study team. A Laboratory Manual, which describes all of the sample testing procedures, will be provided by the study team prior to the commencement of the Trial. Training on the EDC system will be provided by FIND Data Management prior to first participant enrolment.

Quality control (study monitoring)

Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly. The study team will perform risk based monitoring of this trial, and associated Quality Control checks, as described in the Monitoring Plan. Independent trial monitors will perform source data review and source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the trial is being conducted in accordance with the currently approved protocol and any other trial agreements, ICH GCP, and all applicable regulatory requirements.

Quality Assurance (auditing)

As part of routine Quality Assurance, the study team will conduct an audit of the study sites.

Trial site closure

The study team reserves the right to close the trial site or terminate the trial at any time for any reason at its sole discretion, provided there is reasonable cause and sufficient notice is given in advance to stakeholders of the intended termination. Investigational sites will be closed upon trial completion. A trial site is considered closed when all required documents and trial supplies have been collected and participant samples aliquoted and stored on site.

PROJECT TIME FRAME

Approximately six months from the time the site receives IEC Approval and is authorized to start enrolment. The table 7summarizes the estimated time frame for the main activities.

Table 7: Time frame for main activities

Months	1-3	4	5	6	7	8	9	10	11	12	13
Proposal Development	x										
Ethical Approval		x	x								
Purchase and Delivery of Supplies			x	x							
Sample collection and processing					x	x	x	x	x	X	
Sample testing					x	x	x	x	x	X	
Data entry and data analysis										X	x
Report entry											x

In addition, a subset of serum samples will be stored at KEMRI at -80°C for future evaluation of diagnostic tests.

ETHICAL CONSIDERATIONS

Regulatory and Ethics Approvals

This trial will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki
- Applicable Good Clinical Practice Guidelines: ICH GCP E6 (R2)

- Applicable laws and regulations

The protocol, protocol amendments, ICF, IAF and other relevant documents (e.g. advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the trial is initiated. A copy of the IRB/IEC approval letter will be filed in the investigator site file. Any substantial amendments to the protocol will require IRB/IEC approval before implementation of changes made to the trial design, except for changes necessary to eliminate an immediate hazard to trial participants. Protocol amendments restricted to clerical edits only will be provided to the trial sites and submitted to the IRB/IEC for informational purposes.

The investigator will be responsible for the following:

- Providing written summaries of the status of the trial to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the Trial at the site and adherence to requirements of ICH guidelines, the IRB/IEC, the WHO Good Clinical Laboratory Practice (GCLP), and with applicable national regulations.

Informed Consent Process

The investigator or his/her representative will explain the nature of the trial to the participant or his/her legally authorized representative in a language understandable to him/her and answer all questions about the trial.

The investigator or his/her representative will inform the participants that their participation is voluntary. Participants or their legally authorized representative will be required to sign and date a statement of informed consent that meets the requirements of ICH GCP E6 R2 guidelines where applicable, and of the IRB/IEC or study center.

For children less than 18 years old, parental consent will be obtained, with assent from children (13 – 17 years) before enrollment. If the child agrees to participate, he/she will be asked to sign an Informed Assent Form. In all children, any opposition, resistance or protest to study procedures will be discussed with the parents/legally authorized representative to assess whether the behaviour is merely an expression of the anticipated but acceptable

burden, or is reason for concern on study continuation. When the analysis concludes that these are expressions of dissent, it will be respected.

Before enrolling the participant in the trial a written informed consent will be obtained and ample time will be given to the participant to decide to participate in the trial. The date the written consent was obtained (as well as the time, ideally) will be recorded. The authorized person obtaining the informed consent will also sign and date the Informed Consent Form (ICF).

It will be mandatory for illiterate participants to provide a thumbprint on the ICF or IAF and the ICF or IAF signed and dated by an impartial witness.

A copy of the ICF(s) and IAF will be given to the participant or the participant's legally authorized representative.

Participants who are rescreened are required to sign a new ICF and IAF (when required).

The ICF and IAF will contain a separate section that addresses the use of remaining samples for future evaluation studies of typhoid or other febrile illnesses tests. The investigator or authorized designee will explain to each participant the objectives of keeping those samples for future use. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period.

Benefit/Risk Assessment and Safety Monitoring

The in-vitro diagnostic tests (IVD) under investigation are considered low risk to Trial participants since only venepuncture will be performed. Foreseeable adverse events associated with venepuncture are mild, temporary discomfort at the venepuncture site, bruising, and phlebitis. Very rarely, patients may experience vaso-vagal syndrome during phlebotomy. Vaso-vagal reactions may include diaphoresis, nausea, syncope and rarely fainting.

To minimize adverse events, all phlebotomy will be performed either by study clinical officers trained in phlebotomy, or by trained phlebotomists. In case of adverse event and SAE during phlebotomy, standard medical care will be provided to participants and the treating team as well as investigators will be informed and the event reported to the hospital management, PI and documented in the study record.

Handling and manipulation of pathogenic samples will be performed according to the local biosafety requirements to further minimize risk of contamination for the technical personnel and the environment. Given the nature of the study, termination due to safety or other reasons is not anticipated.

Blood culture is recommended diagnostic test for typhoid fever in Kenya, so the report of blood culture, one of the reference test will be provided to clinician for further clinical management of participants, all other index tests will not be used for the purposes of clinical management, and participants will be treated in accordance with national guidelines. The blood culture results from CMR-KEMRI labs will be available after an average of 5 days and will be sent to the clinician caring the participating patient All participants enrolled in the trial will receive standard-of-care health services from the health facility. All trial activities will be conducted by trained trial personnel.

Knowledge gained from this trial may benefit society by providing information on the diagnostic accuracy of current typhoid tests. Data obtained in this trial will be made available to the WHO Essential Diagnostic list working group.

Given the minimal risks associated with this trial and the potential benefits to society, the benefits outweigh the risks. As for any clinical trial, there is a possibility of unknown and unforeseen risk; yet this possibility is slim for this trial. If unforeseen risks are identified during the trial, FIND, trial partners, Institutional Review Boards (IRB)/ Independent Ethics Committees (IEC), and trial participants will be provided with relevant information.

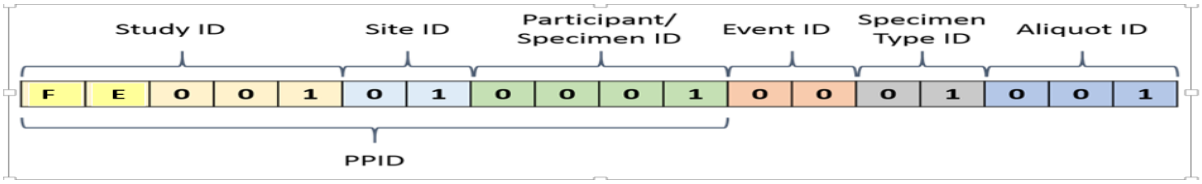
Data Protection

Participants will be assigned a unique identifier generated by the study team. Any participant records or datasets will contain the identifier only; participant names or any information which would make the participant identifiable will not be used in any way.

The participant will be informed that his/her personal trial-related data will be shared by the study sponsor, FIND in accordance with local data protection law. The level of disclosure will also be explained to the participant.

The participant will be informed that his/her medical records may be examined by quality assurance auditors or other authorized personnel appointed by the study team, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

An example of the Participant ID is shown below.



EXPECTED APPLICATION OF THE RESULTS

As mentioned earlier, blood and bone marrow cultures are the gold standard for diagnosis of typhoid. Alternatives diagnostic tests that are easier to use, faster and cheaper are required. Numerous alternative tests have been developed such as RDTs or PCR and are currently available in the market. However data on their performance are limited mainly because evaluation studies are commonly focusing on three tests (Tubex, Typhidot and Test-It).When an alternative test is evaluated, comparison of its performance to other tests may not be possible since the reference tests used may differ between studies: while blood and bone marrow cultures are the gold standards, the poorly performant Widal test is sometimes used as the reference standard. This lack of harmonization in the study protocols negatively impact the identification of promising typhoid diagnostic tests and the generalization of performance data in different geographical areas of the world. In addition, access to samples from typhoid patients clearly hampers the evaluation of new typhoid tests.

This collaborative study between KEMRI and FIND will systematically compare different point of care typhoid tests currently available in the market against the same set of reference standard in two geographically distant typhoid endemic areas in different continents (Asia and Africa). The knowledge gained from this trial may benefit health providers' by providing information on diagnostic accuracy of current typhoid test and to decide on utility of these commercial tests. The result obtained from this trial will also be made available to help inform the WHO Essential Diagnostic list (EDL) and stakeholder decision making more broadly.

As a part of the study a biorepository will be established at site with well characterized samples which can be used in future evaluations of emerging technologies and/or to support innovative test development.

WASTE MANAGEMENT AND BIOSAFETY OF THE STUDY

The blood samples that are to be used in this study have the potential to cause human infections. Measures will be put in place to ensure the safe handling of the blood samples so as to prevent infection to the study personnel and the community as well. The measure will include strict adherence of lab SoPs on waste management and biosafety, ensuring that only qualified personnel will be allowed to handle the blood samples, provision and use of suitable personal protective equipment (PPE) which is properly stored, cleaned as required, replaced when defective and separated from normal clothing, the study team will excersise safe collection, storage and disposal of biological waste from the study, including the use of secure and identifiable containers, after suitable treatment where appropriate as per the lab SOPs and safe handling and transport of a biological agents for disposal.

STUDY LIMITATIONS

Due to limited funding, the study will cover only three hospitals in 1 County of the 47 Counties in Kenya, thus leaving out 46 other Counties.

ROLES OF INVESTIGATORS:

Dr. Robert Onsare: Overal implementation of the project including samples collection, lab processing and data analysis.

Elizabeth Ndegwa: Elizabeth is a registrerd clinical officer and will provide clinical guidance in interaction with patients.

Prof. Sam Kariuki: Will play an advsisory role in all aspects of the project.

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BUDGET SUMMARY

	Unit	Unit Cost	Cost (EUR)
Personal Cost			
Project supervisor (Manager), PhD-KEMRI Staff monthly salary: 2900 euros; gratuity:0, period: 9 months			8,700
3 Clinical Officers, Diploma (From Hospitals) - To give 55% monthly salary: 440 euros; gratuity: 0			7,920
2 Laboratory Technologists, BSc (From KEMRI) - To give 65% effort monthly salary: 440 euros; gratuity:0			7,800
Field Driver - KEMRI (70% effort)			5,208
Sub-Total			**29,628**
Blood Culture/Enrichment media/ reagents for blood samples (2,400 samples)			
Columbia blood agar – 500g pack	5	136.7	683.5
MacCONKEY agar -500g pack	5	136.7	683.5
Sulphur, Indole Motility media – 500g pack	1	122.1	122.1
Urea agar and supplement - 500g pack @ 172.1x1pc =172.1	1	172.1	172.1
TSI	1	172.1	172.1
Simon citrate – 500g pack	1	122.5	122.5
Glass test tubes	3000	0.3	900
Glass slides and cover slips (100 per unit)	30	25	750
Petri dishes –3500 pcs			1000
Disposable sterile wire loops	20,000	0.21	2310
Cotton wool- 500g pack	10	4.4	44.2
Syringes and needles (10ml) 100pc per unit	60	4	240
Serology antiseras: - Polyvalent	2	215.1	430.2
-Monovalent	10	215.1	2151
Biohazard bags (Red, Yellow, Black) – 50 pcs	48	187	8976
Sharps disposal containers	12	821.45	9857.4
Emersion oil	1	500	500
Aerobic Blood Culture bottles, 3000 pieces,50 pcs per unit =19,200	60	320	19200
Sub-Total			**48,315**
Consumables for archiving isolates, 600 samples			
Sarstedtet Freezing vials	750	0.25	187.5
10 by 10 freezer boxes	7	21	147
Sub-Total			**335**
Sample transport and Field trips			
Fuel for the field vehicle			1,608
Vehicle service and Maintenance			3,192
Sub-Total			**4,800**
Miscellaneous			
Office and lab Supplies & materials			6,000
Physical protective Equipment (PPE) for the laboratory personnel (Gloves, dust coats, face masks and Eye goggles)			1428
Sub-Total			**7,428**
Total Cost			**90,505**

Indirect Cost (15%)			**13,575.765**
Grand Total			**104,081**

Budget Justification

Personal Costs:

The Project supervisor (Manager) is the Principal Investigator of the trial, he will supervise and coordinate field and laboratory staff in sampling activities for 9 months.

Three Clinical officersfrom the sampling sites will be involved in the trial. They are currently working in those sites and part of their time will be dedicated to participant screening, consenting and blood drawing.

Two full timeLaboratory technologist will be involved in the trial. They are currently working in KEMRI. They will process samples for blood culture, PCR and aliquot the serum for storage. Once the culture report comes they will also perform RDTs in stored samples in batches

A designated KEMRI driver will be responsible for transportation of the staff to study sites and samples from study sites to laboratory at KEMRI.

Blood Culture, Enrichment media and Reagents for Blood sample

Blood culture is one of the reference tests of this study. This includes cost for aerobic blood culture bottles with enrichment media for blood culture. Once the blood culture is positive, cost of biochemical media and reagents to identify Salmonella Typhi is included in this section.

Consumables for archiving isolates:

The consumables for archiving serum samples of 100 blood culture positive and equal number of negative cases, each sample will be stored in 4 freezing vials which will be stored at KEMRI for 5 years. These samples will be used for future evaluation of new typhoid diagnostics.

Sample transport and field trips:

Fund to support daily transport of sample after collection from sites to KEMRI laboratory

Miscellaneous costs:

This includes cost of office and laboratory supplies and personal protective equipment for laboratory staffs.

APPENDIX 1: CASE REPORT FORMS

CASE REPORT FORM-CLINIC V1.0
Commercial typhoid test validation

GENERAL INFORMATION		
1. Date of enrolment (DD/MM/YYYY)	__ __ / __ __ / __ __ __ __	2. Site ☐Mbagathi County Hospital ☐Mukuru Kwa Reuben Missionary Clinic ☐City Council Clinic
3. Place of enrolment: ☐OPD ☐Inpatient ☐Emergency ward		

INCLUSION CRITERIA (Answers to questions 1-5must be **YES** for the participant to be eligible.)		
YES	**NO**	
		1. Aged 8 years to 65 years?
❑	❑	2. History of fever or axillary temperature of >37.5^{0}C for at least 3 consecutive days within the last 7 days prior to enrolment?
❑	❑	3. Clinical suspicion of enteric fever?
❑	❑	4. A signed informed consent and/or assent by participant and/or parent/legal guardian?
		5. One of the following conditions:
❑	❑	a. Presents to outpatient department or Emergency department?
❑	❑	b. Admitted to hospital within 12 hours?

EXCLUSION CRITERIA		
YES	**NO**	
❑	❑	1. Unwillingness to participate in the study
❑	❑	2. Known non-infectious / non typhoid Infectious causes of fever or other alternate

		diagnosis of fever
❑	❑	3. Inability to provide the required volume of blood
❑	❑	4. Unwillingness to provide blood

PARTICIPANT DEMOGRAPHICS			
	Date of birth (DD/MM/YYYY):	__ __ / __ __ / __ __ __ __	
	Gender:	❑Female	❑Male

CURRENT MEDICAL STATUS		
VITAL SIGNS		
Temperature (°C)	☐☐.☐ ❑ Axillary ❑ Oral ❑ Ear ❑ Skin	
Any clinical symptoms at the time of screening?	❑ Yes	❑No
If YES, check which ones (can be more than one):	❑Abdominal pain	
	❑Nausea	
	❑Fatigue	
	❑ Cough	
	❑ Headache	
	❑Diarrhea	
	❑ Constipation	
	❑ Vomiting	
	❑ Rash	
	❑ Conjunctival Redness	
	☐ Other *(specify)* _____ _____	

PAST MEDICAL STATUS		
Do you have history of any of the following in last 6 months?	❑ Yes	❑No
If YES, check which ones (can be more than one):	❑Tuberculosis	
	❑Typhoid	
	❑Dengue	
	❑ Malaria	
	❑ Scrub Typhus	
	☐ Other *(specify)* _____ _____	

TREATMENT HISTORY		
1. Has the subject taken antibiotics since the symptoms started?	❑ Yes	❑No

If YES, check which ones (can be more than one): Treatment start date: __ __/__ __/__ __ __ __ (DD/MM/YY) Treatment end date: __ __/__ __/__ __ __ __ (DD/MM/YY)	❑Ciprofloxacin ❑Ofloxacin ❑Levofloxacin
	❑ Cefixime
	❑ Amoxycillin
	❑ Amoxycillin/clavulanate
	❑ Azithromycin
	❑ Other *(specify)* _____ _____
2. Has the subject taken any other treatment?	❑ Yes ❑No
If YES, check which ones (can be more than one):	❑ Antimalarial
	❑ Antipyretic
	❑ Other*(specify)* _____ _____

VACCINATION HISTORY	
Has the subject received a typhoid vaccine? (Single dose TVC vaccine)	❑ Yes ❑No ❑Don't know If yes, when (DD/MM/YYYY): __ __/__ __/__ __ __ __

PRESUMED DIAGNOSIS			
Presumed diagnosis by the clinician:	❑Enteric fever	❑ Dengue	❑Other: _____

BLOOD COLLECTION

1. Blood sample drawn ❑YES ❑NO
2. Blood sample draw date (DD/MM/YYYY):__ __/ __ __/__ __ __ __ at (24h00) __ __: __ __
3. If not blood drawn, why? ❑Patient Refused ❑Supply shortage ❑Unable/difficult to draw

Comments	Date (DD/MM/YY)	Initials	Signature
	__ __/ __ __/ __ __	__ __ __	
	__ __/ __ __/ __ __	__ __ __	
	__ __/ __ __/ __ __	__ __ __	

Data entry agent initials: _ _ _

Date of completion of data entry (DD/MM/YYYY): __ __/__ __/__ __ __ __

Investigator's Signature: _____

Date of signature (DD/MM/YYYY): __ __/__ __/__ __ __ __

CASE REPORT FORM-Blood culture V1.0
Commercial typhoid test validation

REFERENCE TESTING BY BLOOD CULTURE		
1. Technical operator initials: _ _ _		
2. Aerobic blood culture performed (If no, put an explanation in comments) ❑Yes ❑ No		
3. Aerobic blood culture	Incubation date (DD/MM/YYYY): __ __/__ __/__ __ __ __ Time (24h00): __ __: __ __	❑Positive ❑ Negative ❑Contamination If Positive: Date (DD/MM/YYYY):__ __/__ __/__ __ __ __ Time (24h00): __ __: __ __
4. Salmonella Typhi		❑Positive ❑ Negative
5. Other clinically relevant growth		_____
6. Contaminants growth		_____
7. *Data reported to patients*		❑ *Yes, Date* (DD/MM/YYYY): __ __/__ __/__ __ __ __ ❑ *No, why:* _____

Comments	**Date (DD/MM/YY)**	**Initials**	**Signature**
	__ __ / __ __ / __ __	__ __ __	
	__ __ / __ __ / __ __	__ __ __	
	__ __ / __ __ / __ __	__ __ __	

Data entry agent initials: _ _ _

Date of completion of data entry (DD/MM/YYYY): __ __/__ __/__ __ __ __

Investigator's Signature: _____

Date of signature (DD/MM/YYYY): __ __/__ __/__ __ __ __

CASE REPORT FORM-Serum preparation V1.0
Commercial typhoid test validation

SERUM PREPARATION	
1. Serum prepared	❑Yes, if yes, Date (DD/MM/YYYY): __ __ /__ __/__ __ __ __ ❑ No, if no Why:
If yes at 1. Serum stored?	❑Yes If yes, ____ aliquots stored Storage Location: ❑ No, if No Why: _____

Comments	**Date (DD/MM/YY)**	**Initials**	**Signature**
	__ __/__ __/__ __	__ __ __	
	__ __/__ __/__ __	__ __ __	
	__ __/__ __/__ __	__ __ __	

Data entry agent initials: _ _ _

Date of completion of data entry: __ __/__ __/__ __ __

Investigator's Signature: _____

Date of signature (DD/MM/YYYY): __ __/__ __/__ __ __ __

CASE REPORT FORM-RDTsV1.0
Commercial typhoid test validation

A. TYPHOID RDTs EVALUATION				
Test name	**Test Results**			
		Date (DD/MM/YYYY)	Results	Comments
Widal Test	Initial Test Result	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
Typhidot Rapid IgG/IgM combo test	Initial Test Result (IgG)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results (IgG)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	

	Initial Test Result (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
TUBEX TF	Initial Test Result	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
SD Bioline SALMONELLA TYPHI IgG/IgM Fast	Initial Test Result (IgG)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results (IgG)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Initial Test Result (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg	

			❑Inv	
Typhoid IgG/IgM Combo Rapid test CE	Initial Test Result (IgG)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results) IgG	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Initial Test Result (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
Enterocheck WB	Initial Test Result	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
Test it™ Typhoid IgM	Initial Test Result	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	

	Repeat testing if invalid results	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
Typhoid IgG/IgM Rapid Test Casette	Initial Test Result (IgG)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results) IgG	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Initial Test Result (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
S.Typhi-S.Paratyphi "A" Direct Antigen Detection	Initial Test Result	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
DiaquickS.Typhi IgG/IgM Ab	Initial Test Result (IgG)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg	

			❑Inv	
	Repeat testing if invalid results (IgG)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Initial Test Result (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results (IgM)	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
DiaquickS.typhi/ paratyphi Ag cassette	Initial Test Result	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
	Repeat testing if invalid results	__ __/__ __/__ __ __ __	❑Pos ❑ Neg ❑Inv	
If any of the RDTs not done, put an explanation in comments below.				

Comments	Date (DD/MM/YY)	Initials	Signature
	__ __ / __ __ / __ __	__ __ __	
	__ __ / __ __ / __ __	__ __ __	

	__ __/__ __/__ __	__ __ __	

Data entry agent initials: _ _ _

Date of completion of data entry (DD/MM/YYYY): __ __/__ __/__ __ __ __

Investigator's Signature: _____

Date of signature (DD/MM/YYYY): __ __/__ __/__ __ __ __

APPENDIX 2: Consent and assent forms

(a)(i) Adult Informed Consent Form: English Version

Version 2.0 of 11 August 2020

Study Title: Comparative study of commercially available typhoid point of care tests to benchmark current and emerging tools

Study Centre: KEMRI **Study Site:**

Principal Investigator: Dr. Robert Onsare

Sponsor: FIND

Language: English

This Informed Consent Formhastwoparts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

Part 1: Information sheet:

Introduction

Dear Sir/madam,

Good day, we are a group of researchers from the Kenya Medical Research Institute (KEMRI), Nairobi. The study is supported by the Foundation for Innovative New Diagnostics (FIND), a charity working to develop new ways of diagnosing sick people having fever to understand the cause of their illness. FIND works together with researchers, scientists, governments and companies to develop affordable and easy to use tests for diagnosing typhoid and other febrile illnesses. We are doing a study on typhoid, a disease that is common in this area and is caused by germs transmitted by eating contaminated food and taking contaminated drinks and can lead to complications if it is not treated. The symptoms of typhoid are however similar to those from other diseases caused by other bacteria, parasites or viruses, and which must be treated with different treatments than typhoid. It is therefore important to correctly diagnose and understand the cause of the fever symptoms, to give the correct treatment and help you to recover from your disease. Such a diagnosis is not easy, and we are conducting the study to try to improve it. You have fever or you had fever within the last 7days, and we suspect that you might suffer from typhoid. This is why we are asking you if you would like to participate in the study. Participation is voluntary, and you have the right to refuse. Before you decide you may take a moment to talk to your family or friends about the study. I will now give you some more information about the study. If my words are not clear, please ask me to stop and I will take time to explain. If you have questions later, you can ask me or the other study staff, at any time.

Purpose of the study

It is very common that patients have fever symptoms like you. Unfortunately, it is difficult to quickly and easily identify the cause of these symptoms. In some cases, the fever can be due to typhoid which can be treated with antibiotics. Such antibiotics should however not be used if your fever is caused by a viral or parasitic infection. Currently typhoid is diagnosed by blood culture which takes 5 to 7 days to get result. Doctor has to wait for 5-7 days to know if the fever is due to typhoid and to decide of the best treatment.

Rapid Diagnostic Tests are other tests that may be used to diagnose quickly and easily typhoid. However, there are several ones and nobody knows for sure which test is the best to diagnosetyphoid. So, the reason why we are doing this study is to find out which of these Rapid Diagnostic Tests works better.

The study will last for about 12 months, however, for you as an individual, your time in the study will be from when you accept to be part of the study and sign this form until you have given a sample of your blood. This should take more than 1 or 2 hours for you. We will need to collect samples from a maximum of 4,900 patients (adults and children) in 2 different countries in Pakistan and Kenya.

Study Procedures

If you agree to participate in the study, we will ask you some questions about your healthand any treatments that you might have taken recently. Then, we will take 14ml (3 table spoon)/7ml of blood from your vein.

It is important that you understand that blood will be collected from your vein for routine diagnosis whether you decide to be in the study or not. You will get the same treatment, whether you decide to be in the study or not. For all patients, we will collect blood. The only difference, if you are in the study, is the volume of blood to be collected from your vein. The following table gives you an idea of the differences.

Type of Sample	In the study	Not in the study
Blood from your vein	14 mL of blood (3 tea spoon) (> 14 years) 7 mL of blood (1 and ½ tea spoon) (8-14 years)	10 mL of blood for blood culture (2 tea spoon) 3.5 mL of blood for blood culture (1/2 tea spoon)

If you decide to be part of the study, some of your blood will be used to perform blood culture. Another part of your blood will be used with the rapid diagnostic tests. The leftover venous blood will be kept for 5 years at the KEMRI in Nairobi, Kenya, for future research on infectious diseases. If you would like these leftovers to be destroyed at the end of the study, please inform the study doctor. We will seek permission from the Ethics committee from KEMRI to use your sample for future studies (the Ethics committee is a group of people who review studies to ensure that your rights are protected).

What are the risks and benefits?

This study may or may not lead to a direct benefit to you. The tests and the blood culture will be free of charge for you. The study will be beneficial for your community as a whole. In fact, we hope it will help doctors worldwide to better diagnose typhoid and give the right treatment to sick people.

Your participation in this study has little risks for you; the only difference with usual care is that we will take more blood from your vein. You may feel the pain from the needle stick. It may cause discomfort or a small bruise as with any other blood test that requires a venous blood sample. A new needle will be used for each participant, so there is no risk of transmitting infection.

Follow-up and treatment

This study is only about diagnostic tests and we will not test any new drug or other therapy. If you are diagnosed with typhoid from blood culture you will be treated following the current standard treatment in Kenya. If your blood culture for typhoid is negative, you will be treated following the national guidelines for management of non-typhoid febrile illness. We will not use other test results to diagnose you. If considered necessary by the doctor, you will be referred to the appropriate health facility and treated according to standard of care available in Kenya.

What are the costs?

You will not receive money from KEMRI or FIND or any other person as a result of the use of your blood in medical research or testing.

You will not be paid to take part in the study; however, we will make sure that you don't have to pay additional costs from your participation. All diagnostic tests for this study will be free of charge.

Payment for injury

It is unlikely that you will suffer any physical harm caused by one of our sample collection procedures. Should this ever occur, you will be treated and fairly compensated for it according to local laws and any insurance policy in effect. You agree to give away to KEMRI and FIND, for free, any property rights you might have in your bodily fluids and any medical, scientific or commercial products such as drugs or diagnostics or other inventions created through the study or use of those fluids. Your participation in this study is a free gift in the spirit of human kindness to help future generations fight illness and remain healthy.

How will confidentiality be respected?

We will not share any of your personal information outside of the KEMRI study team. Your name will not be mentioned on any sample, or on the data collected during the study. You will be given a unique number, which will be used to identify the samples and data collected. If the results of this study get published in a scientific journal, your name will not appear on the publication. All members of the research team commit to protect the confidentiality of the information you provide. The members of the Ethics Committee, auditors, and Sponsor's representatives (FIND study monitors and auditors) may access your personal information,

however, all these people have to respect the confidentiality, and your personal information will not be revealed publicly.

Your participation is entirely voluntary

It is your choice to decide whether you want to be in the study or not. Whatever your decision is, all the diagnostic tests will be provided free of cost to you. Also, the treatment you will receive will not be affected by your decision to be in the study or not. Finally, you can decide to be in the study, and later on change your mind. This decision will not affect the quality of your care. Just tell me, you don't need to provide any reason for this. During this study, if new information becomes available that may affect your willingness to continue the study, we will tell you. If you decide to continue in the study, we will ask you to sign a new consent form with the new information.

The use of samples for any future studies will require the approval of an ethics committee (a group of people who review studies to ensure that your rights are protected).

Whom to contact in case of problem or question?

Deciding whether or not to participate in this study is completely voluntary. Refusal to participate will not adversely affect you in any way, and if you choose to discontinue your participation at any time there will be no penalty.

You can contact Dr. Robert Onsare, Tel.+254 20 2723006, Email: RSOnsare@kemri.org, at the Centre for Microbiology Research (CMR), KEMRI Nairobi in case you: (i) have any more questions or (ii) feel that you might have been harmed by being in the study.

Should you have any questions about your rights as a research participant, please contact **the Secretary, Scientific and Ethics Review Unit (S.E.R.U) of the Kenya Medical Research Institute (KEMRI) Tel. 2722541 ext. 3307/ 0717719477; P.O Box 54840-00200, Nairobi-Kenya, E-mail: SERU@kemri.org.**

Part 2: Consent Form

- **For ADULT, literate patients**

I have read the Participant Information Sheet concerning this study or have understood the verbal explanation and I understand what will be required of me if I take part in this study.

I understand that the blood I will provide will be tested for typhoid and that they will be stored in freezers in a safe and secure facility for up to 5 years in KEMRI, Nairobi, Kenya. After that time they will be destroyed.

I understand that the stored fluids will belong to KEMRI &FIND. I understand that KEMRI &FIND will use these fluids to develop or evaluate new tests to diagnose typhoid and possibly other illnesses. I understand that other researchers and other companies may want to use these fluids for other medical research, testing, and product development and I agree to such use. I understand that I will not receive money from FIND or any other company as a result of the use of my fluids in medical research or testing and I give away for free any property rights I might have in my bodily fluids and any medical, scientific or commercial products or inventions created through the study or use of those fluids. My participation in this study is a free gift in the spirit of human kindness to help future generations fight illness and remain healthy.

I understand that study staff, representatives from KEMRI, FIND, members of the ethics committee overseeing this study and the regulatory authority (ies) will be given access to my medical records so they can verify what was done and look at the data. In signing this, I authorize access to my medical records.

I understand that I may drop out of this study at any time, for any reason, without penalty and without any loss of medical care. I have read the patient information sheet, or it has been read to me, and I have understood the purpose of the study, the procedure to be conducted, and the risks and benefits related to my participation. I have had the opportunity to ask questions and all have been answered to my satisfaction. I consent voluntarily to participate as a participant in this study and:

☐ I agree that part of my samples get stored for future research on infectious diseases

☐ I don't agree that part of my samples get stored

Print Name of Participant (Last name/Firstname):_____

Signature of Participant _____

Date _____

Day/month/year

Part 3: For witnesses of ADULT, illiterate participants:

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

☐ The participant agrees that part of his/her samples get stored for future research on infectious diseases diagnosis

☐ The participant does not agree that part of his/her samples get stored

Print name of witness (Last name/First name)_____ AND Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Sehemuya 3: Kwa Mashaidi wa Watu Wazima Wasiojua Kusoma au Kuandika

Nimeshuhudia usomaji sahihi wa fomu ya idhini kwa mshiriki, na mtu huyu amepata nafasi ya kuuliza maswali. Ninadhibitisha kuwa mtu huyu, ametoa idhini kwa hiari.

☐ Mshiriki anakubali kwamba, sehemu ya sampuli zake, zitahifadhiwa kwa utafiti wa siku zijazo kwenye utambuzi wa magonjwa ya kuambukiza.

☐ Mshiriki hakubali kuwa sehemu ya sampuli zake zitahifadhiwa.

Chapisho la Jina la Shahidi (Jina la Mwisho/Jina la Kwanza) _____

Saini kwa kutumia kidole cha Gumba

Saini ya Shahidi _____

Tarehe _____

Siku/Mwezi/Mwaka _____

Part 4: Statement by the researcher/person taking consent

I, the undersigned, have defined and explained to the participant in a language he/she understands, the procedures of this study, its aims and the risks and benefits associated with his/her participation. I have informed the participant that confidentiality will be preserved, that he/she is free to withdraw from the trial without affecting the care he/she will receive at the hospital. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Print Name of Researcher/person taking the consent (Last name/First name)

_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Sehemu ya 4: Taarifa ya Mtafiti/ Mtu anaye chukua idhini

Nimeelezea mshiriki kwa kutumia lugha anayeelewa, taratibu za utafiti, malengo, faida na hatari zinazohusiana na ushiriki wake. Nimemwelezea mshiriki kuwa, usiri utahifadhiwa na kwamba yuko huru kujiondoa kwenye utafiti huu wakati wowote bila kuathiri matibabu na utunzaji atakaoupokea hospitalini. Ninathibitisha kuwa, mshiriki alipewa nafasi ya kuuliza maswali juu ya utafiti na maswali yote yaliyoulizwa na mshiriki yamejibiwa kwa usahihi na kwa uwezo wangu wote. Ninathibitisha kuwa mtu huyu, hajalazimishwa kutoa idhini na idhini imetolewa kwa hiari.

Nakala ya fomu hii ya kufahamisha imetolewa kwa mshiriki.

Chapisho la Jina la Mtafiti/Mtu anayepokea fomu ya idhini hii (Jina la Mwisho/Jina la Kwanza)

_____

Saini ya Mtafiti _____

Tarehe _____

Siku/Mwezi/Mwaka _____

2 (b) (i) Children 13-17 years Informed Assent Form: English Version

Version 2.0 of 11 August 2020

Study Title: Comparative study of commercially available typhoid point of care tests to benchmark current and emerging tools

Study Centre: KEMRI Kenya **Study Site:**

Principal Investigator:

Sponsor: FIND

Language: English

This Informed Consent Formhastwoparts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

Part 1: Information sheet:

Introduction

Hi, my name is_____ and I work for Kenya Medical Research Institute (KEMRI). My job is to study a disease called typhoid in Kenya. We are doing a new study in typhoid. We want to find better methods to identify the cause of the disease in people who, like you, have fever or had fever within the last 7 days. I am going to give you information and invite you to be part of a research study. You can choose whether or not you want to participate. We have discussed this research with your parent(s)/guardian and they know that we are also asking you for your agreement. If you decide to be in the study, your parent(s)/guardian also have to agree. But if you do not wish to take part in the study, you do not have to, even if your parents have agreed.

You may take a moment to think before you decide, and you may discuss anything in this form with your parents or anyone else you feel comfortable talking to. You can decide whether to participate or not after you have talked it over. There may be some words you don't understand or things that you want me to explain more about because you are interested or worried. Please ask me to stop at any time and I will take time to explain.

Why are we doing this research?

We want to find better and faster ways to understand which type of germ makes adults and children sick with Typhoid. There are several Rapid Diagnostic Tests available in market which could be used to identify typhoid, to help the doctor decide which treatment he or she should give you. We don't know however if these rapid tests works well. We therefore need to evaluate it before we can recommend its use throughout Kenya.

Why am I asking you to be involved?

We are conducting the research in both adults and children. In Kenya, children of your age often have fever with typhoid, and today we have little and time-consuming means to figure out whether fever is due to typhoid or not. If we want to use already available Rapid Diagnostics Tests to get better and quick result then we need to make sure that it works for children as well as for adults.

Do you have to be involved in the research?

You don't have to be in this research if you don't want to be. It's up to you. If you decide not to be in the research, it's okay and nothing changes. This is still your hospital or health center, everything stays the same as before. Even if you say "yes" now, you can change your mind later and it's still okay.

What is going to happen to you?

If you agree to participate in the study, after you visit the doctor and suspected to have typhoid we will ask you some questions about your health. Then, we will take 7ml (in age group 8-14years) (3 table spoon) and 14 mL (in >14 years of age) of blood from your vein, out of which 3.5ml (in 8-14 years of age) and 10m (in >14 years of age) will be used to perform blood culture, which is a routine procedure to diagnose typhoid fever. Addition to this 3.5ml (in 8-14 years of age) and 4ml (in >14 years of age) of blood (app 1 table spoon) will be taken to do other tests to diagnose typhoid.

It is important that you understand that blood will be collected from your vein for routine diagnosis whether you decide to be in the study or not. You will get the same treatment, whether you decide to be in the study or not. For all patients, we will collect blood. The only difference, if you are in the study, is the volume of blood to be collected from your vein. The following table gives you an overview of the differences.

Type of Sample	In the study	Not in the study
Blood from your vein	14 mL of blood (3 table spoon) (> 14 years) 7 mL of blood (1 and ½ table spoon) (8-14 years)	10 mL of blood for blood culture (2 table spoon) 3.5 mL of blood for blood culture (1/2 table spoon)

If you decide to be part of the study, the samples will be used to perform the routine diagnostic tests and the other tests that are necessary for the study. The leftover venous blood will be kept for 5 years at the KEMRI in Nairobi, Kenya, for future research on infectious diseases. If you would like these leftovers to be destroyed at the end of the study, please inform the study doctor. We will seek permission from the Ethics committee from KEMRI to use your sample for future studies (the Ethics committee is a group of people who review studies to ensure that your rights are protected).

Is this research bad or dangerous?

There is little difference between the study and what usually happens to children with fever when they get to the hospital or the health center. You may feel the pain from the needle stick. It may cause discomfort or a small bruise as with any other blood test that requires blood to be taken from the arm. A new needle will be used for each patient, so there is no risk of transmitting a disease.

Is there anything good that will happen to you?

This study may or may not lead to a direct benefit to you. The tests and the blood culture will be free of charge for you. The study will be beneficial for your community as a whole. In fact, we hope it will help doctors in worldwide to better diagnose typhoid and give the right treatment to sick people.

Your participation in this study has little risks for you; the only difference with usual care is that we will take more blood from your vein. You may feel the pain from the needle stick. It may cause discomfort or a small bruise as with any other blood test that requires a venous blood sample. A new needle will be used for each participant, so there is no risk of transmitting infection

There will be no reward for taking part in the study.

Will everybody know about it?

We will not tell other people that you are in this research and we won't share information about you to anyone who does not work in the research study.

Can you choose not to be in the research?

There is no obligation for you to be in this research. No one will be mad or disappointed with you if you say no. It's your choice. You can think about it and tell us later if you want. You can say "yes" now and change your mind later and it will still be okay. This decision will not affect the quality of your care. Just tell me or the study doctor, you don't need to give any justification for this.

Whatever your decision is, all the diagnostic tests will be provided free of cost to you. Also, the treatment the doctor will give you will be the same if you are in the study and if you are not.

Who can you talk to or ask question to?

You can ask me questions now or later. I have written a number and address where you can reach us and discuss any study related issues or, if you are nearby, you can come and see us. If you want to talk to someone else like your teacher or Doctor or your auntie, that's okay too. You can also talk to the Doctor regarding issues about the study.

Whom to contact in case of problem or question?

You may contactDr. Robert Onsare, Tel.+254 20 2723006, Email: RSOnsare@kemri.org, at the Centre for Microbiology Research, KEMRI Nairobi in case you: (i) If you have any problem or question related to the study or (ii) feel that you might have been harmed by being in the study.

Should you have any questions about your rights as a research participant, please contact **the Secretary, Scientific and Ethics Review Unit (S.E.R.U) of the Kenya Medical Research Institute (KEMRI) Tel. 2722541 ext. 3307/ 0717719477; P.O Box 54840-00200, Nairobi-Kenya, E-mail: SERU@kemri.org.**