Clinical investigation study to evaluate the consistency and reproducibility of two consecutive mosquito feeding assays in adults with varying *Plasmodium falciparum* gametocyte densities

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LIST OF ABBREVIATIONS

ACL-2	Arthropod Containment Level 2
AE	Adverse event
CDMS	Clinical Data Management System
cDNA	Complementary DNA
CFR	Code of Federal Regulations
Coartem®	Artemether/Lumefantrine
CRC	Clinical Research Center
CRF	Case Report Form
DBS	Dried blood spot
DMFA	Direct Membrane Feeding Assay
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
DSFA	Direct Skin Feeding Assay
ECCT	Expert Committee on Clinical Trials (ECCT)
EDTA	Ethylenediaminetetraacetic
ERC	Ethical Review Committee
GCP	Good Clinical Practice
GPS	Global Positioning System
HDSS	Health and Demographic Surveillance System
HIV	Human immunodeficiency virus
HRPO	Human Research Protections Office
HRPP	Human Research Protection Program
HSPB	Human Subject Protection Branch
IB	Investigator's Brochure
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Institutional Ethics Committee
IRB	Institutional Review Board
KEMRI	Kenya Medical Research Institute
MBF	Malaria Blood Film
MDC	Malaria Diagnostic Center
MDR	Malaria Drug Resistance
MoH	Ministry of Health
NIH	National Institutes of Health
ORP	Office of Research Protections
PBS	Phosphate Buffered Saline
P. falciparum	*Plasmodium falciparum*

Pf	*P. falciparum*
PATH REC	PATH's Research Ethics Committee
PCR	Polymerase chain reaction
PHI	Protected Health Information
PI	Principal Investigator
PII	Personal identifying information
QC	Quality Control
qPCR	Quantitative PCR
REC	Research Ethics Committee
RNA	Ribonucleic Acid
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse Transcriptase PCR
SAE	Serious Adverse Event
SERU	Scientific and Ethics Review Unit
SMFA	Standard Membrane Feeding Assay
SOP	Standard Operating Procedure
SSA	Sub-Saharan Africa
SSC	Scientific Steering Committee
TRIs	Transmission Reducing Interventions
UPIRTSO	Unanticipated Problem Involving Risk to Subjects or Others
US FDA	United States Food and Drug Administration
USAMRD-A	United States Army Medical Research Directorate - Africa
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

STATEMENT OF COMPLIANCE

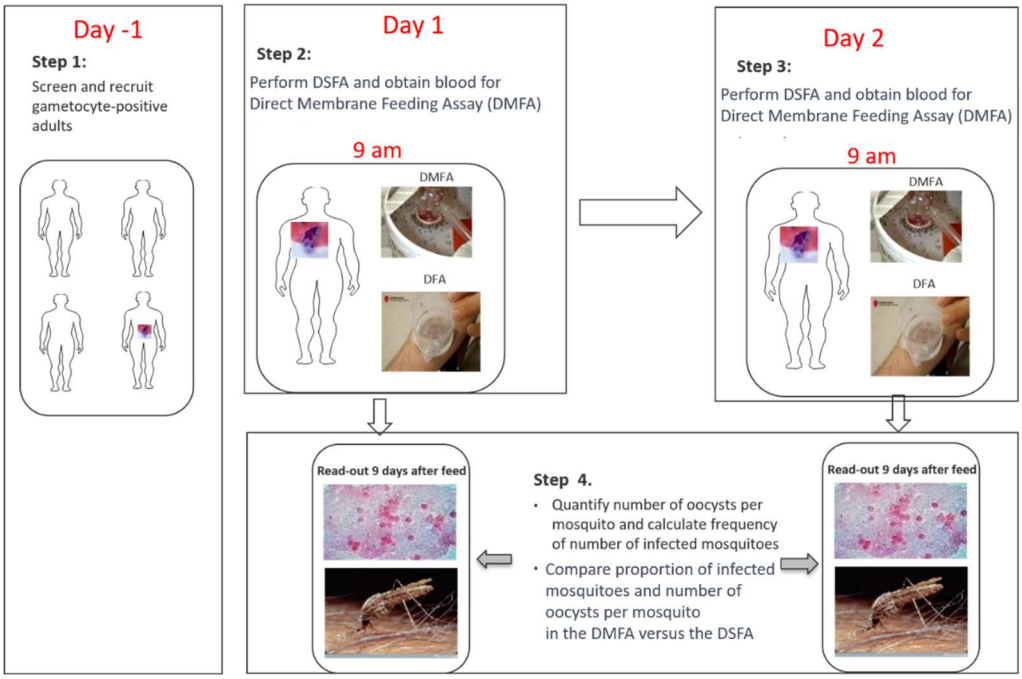
The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and ICH-GCP (E6) guidelines.

Site Investigator: Ben Andagalu
Title: Co-Director, Malaria Drug Resistance Laboratory
United States Army Medical Research Directorate/KEMRI

Signed:

Date:

PROTOCOL SUMMARY

Title	**Clinical investigation study to evaluate the consistency and reproducibility of two consecutive mosquito feeding assays in adults with varying *Plasmodium falciparum* gametocyte densities**
Design	The proposed trial design has been developed to assess the consistency and reproducibility of two consecutive direct skin feeding assays (DSFA) at 24-hour interval. The results will determine the type of pivotal trial design for a follow-on Phase 2b trial whose objective is to bridge the standard membrane feeding assay (SMFA) to the direct skin feeding assay (DSFA) and direct membrane feeding assay (DMFA) using a monoclonal antibody intervention, TB31F mAb, which interrupts transmission from human to mosquito. The results from this experimental medicine study will inform whether the preferred "Before-After" trial design in which each human volunteer serves as their own internal control can be utilized for a follow-on Phase 2b trial. The rationale for this experimental medicine trial is described in the background section of the protocol. Figure 1. Experimental medicine clinical study design  The sample size to be included in this study is shown below in Figure 2.

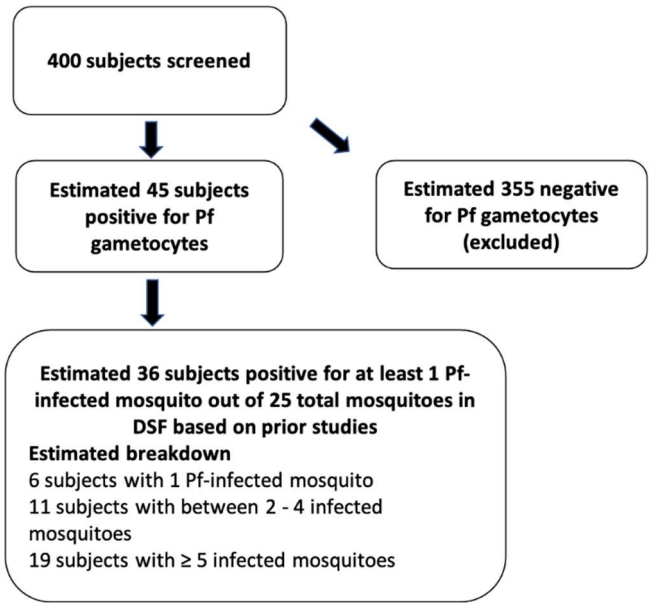
<table>
<tr><td></td><td> 

Figure 2. Estimated sample size screening and enrollment for experimental medicine trial.</td></tr>
<tr><td>Objectives</td><td>Primary Objective: We would like to better understand the relationship between parasite transmission from humans to mosquitoes both within a person and across persons by assessing the variation in the proportion of infected mosquitoes with at least one oocyst (oocyst prevalence) in DSFA and DMFA performed at two consecutive time points in the same human subject with P. falciparum gametocytemia using microscopy

Secondary Objectives:
1. To assess variation in oocyst density in DSFA and DMFA at two consecutive time points in same subject using microscopy.
2. To assess variation in the proportion of infected mosquitoes with at least one sporozoite (sporozoite prevalence) in DSFA and DMFA performed at two consecutive time points in the same human subject using microscopy
3. To assess the variation in sporozoite density in DSFA and DMFA at two consecutive time points in the same subject using microscopy.

Exploratory Objective:
1. To assess oocyst and sporozoite density in DSFA and DMFA at two consecutive time points in the same subject using molecular quantitative PCR (qPCR)
2. To summarize the relationships between DSFA and DMFA results for oocyst and sporozoite density</td></tr>
<tr><td>Endpoints</td><td>Primary Endpoint:</td></tr>
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	Oocyst prevalence in DSFA and DMFA in mid-guts of mosquitoes performed at two consecutive time points in the same human using microscopy **Secondary Endpoints:** 1. Oocyst density in DSFA and DMFA at two consecutive time points in same subject using microscopy. 2. Sporozoite density and prevalence in DSFA and DMFA performed at two consecutive time points in the same human subject using microscopy **Exploratory Endpoint:** Oocyst and sporozoite density in DSFA and DMFA at two consecutive time points using qPCR
Population	Adults 18 to 55 years old
Number of sites enrolling participants	One site; Kombewa Clinical Research Center, Kenya
Description of study agent	No investigational agent in this study
Study duration	Estimated 9 months from when the study opens to enrollment until completion of last subject last visit and study data analysis
Participant Duration	Estimated 4 days it will take for each individual participant to complete all participant visits

2. KEY ROLES

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Funder	PATH with support from the Bill and Melinda Gates Foundation

Dr. Ben Andagalu, Principal Investigator will be responsible for the overall conduct of the trial at this study site, administrative actions between USAMRD-A/K, Institutional Review Boards, and the sponsor,

oversight of field activities and patient care. He contributed to the protocol development and will contribute to the statistical analysis and writing of the scientific report(s).

All Clinical Investigators listed above will be responsible for the clinical conduct of the trial, the care provided to patients, oversight of field activities, and will contribute to the interpretation of data and analysis and writing of scientific report(s). Dr. Copeland also contributed to the protocol development.

3. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

3.1 Background information

3.1.1 Biological basis for transmission-reducing interventions

New tools are needed to accelerate the path toward eventual elimination of *Plasmodium falciparum* malaria. Interventions, such as vaccines, drugs, or biologics which can break the cycle of malaria parasite transmission between humans and mosquitoes, are viewed as being of particular importance in this regard and are endorsed by the WHO (https://www.who.int/immunization/sage/meetings/2013/april/7_Malaria_Vaccine_TRM_Final.pdf?ua=1). Such interventions harbor the potential to prevent mosquitoes becoming infected with parasites, even after feeding on infectious individuals, thereby breaking the cycle of transmission.

One of the more novel strategies being pursued is the interruption of transmission of malaria parasites through the mosquito vector, thereby reducing the number of secondary infections (4). The transmission of malaria requires the survival and development of *Plasmodium* parasites within the vertebrate host and the mosquito vector. Female anopheline mosquitoes ingest the sexual stage gametocytes when taking a blood meal from infectious vertebrate hosts. Within the mosquito's midgut, the parasite must overcome both the vector's innate defense mechanisms and the vertebrate host's immune mechanisms that include anti-parasite antibodies found within the blood meal following a mosquito bite (5). The conserved nature of the parasite's proteins expressed in the sexual stages, not under natural selective pressure, renders the sexual stages of the parasite attractive targets for transmission reducing interventions (TRIs).

TRIs interfere with this process of parasite transmission from the vertebrate host to the vector in various ways. Anti-malarial drugs could reduce gametocyte carriage or infectivity directly by killing circulating gametocytes in human blood, or human antibodies directed against gametocyte-specific surface proteins could prevent sporogonic development within the mosquito's midgut, preventing sporozoite formation.

3.1.2 Regulatory approaches for testing interventions that interrupt transmission

The absence of direct and immediate benefit to the recipient, and the complexity of the *Plasmodium falciparum* life cycle make clinical development of this class of interventions challenging. The regulatory pathway for interventions that interrupt malaria transmission is complex and challenging.

Since 2010, PATH has convened a series of workshops with malaria experts, policy makers, and regulators. The discussions in those meetings indicated that:

- There is no clear legal bar to approving a transmission-blocking intervention (such as a vaccine, drug, or biologic) that confers no direct benefit on recipients
- Regulations governing biological product licensing do not on their face require that a biological product confer direct clinical benefit on recipients. However, designing clinical studies for such vaccine may raise ethical issues which need to be appropriately addressed in clinical development of such product.
- Two Technical Consultation Groups (TCG) convened by PATH discussed the potential development pathways of a tool which interrupts malaria transmission. TCG-1 focused on a more conventional approach considering the pros and cons of a large single Phase 3 cluster randomized trial approval pathway, while TCG-2 focused on an Accelerated Approval pathway based on an analytically and biologically validated surrogate marker of clinical efficacy.
- A Type C meeting with the US FDA indicated that an accelerated approval pathway in which a biomarker endpoint of efficacy substitutes for a clinical endpoint of population-based efficacy may be acceptable. The US FDA may grant licensure on the basis of adequate and well-controlled clinical trials establishing that the biological product has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit. The advantages of such an approach includes feasibility, reduced trial costs, and shortened timelines to study outcomes.

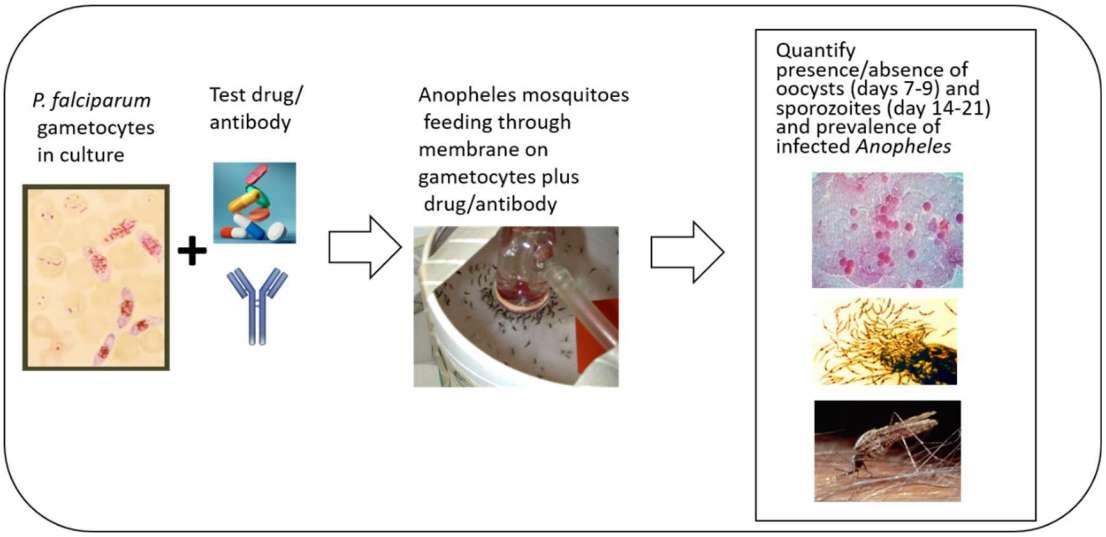
3.1.3 Accelerated approval using biomarker assay(s)

The development of an accelerated approval pathway (to be evaluated under future clinical trials) depends upon a biomarker assay that would reasonably likely predict clinical benefit. Assays that can accurately and quantitatively determine malaria transmissibility are therefore crucial in the evaluation of the efficacy of TRIs.

3.1.3.1 Standard Membrane Feeding Assay (SMFA)

The SMFA, which has been used by groups worldwide measures whether a drug or antibody added exogenously to an in vitro assay can completely block the development of *P. falciparum* malaria parasites in the *Anopheles* mosquito when quantified early as oocyst stage parasites (days 7 to 9), or later as sporozoites within the mosquito salivary glands (days 14-21). The SMFA is shown in the schematic below.

Figure 3: Standard Membrane Feeding Assay



The Standard Membrane Feeding Assay (SMFA) was developed from an experimental laboratory protocol that measures in vitro the inhibition of transmission of malaria parasites to their vectors (6,7) when a test drug, biologic, or antibody-containing sera are introduced. Although the SMFA assay is considered to be a surrogate assay that would recapitulate the activity as it would occur in vivo in a human host given the intervention, the assay does not directly involve the testing within a human volunteer provided a drug, biologic, or vaccine, and therefore direct linkage between the SFMA and an effect in vivo would need to be evaluated in future trials comparing and bridging the in vitro SMFA to assays that measure the same intervention in vivo (DSFA) described below. The present study does not include SMFA testing.

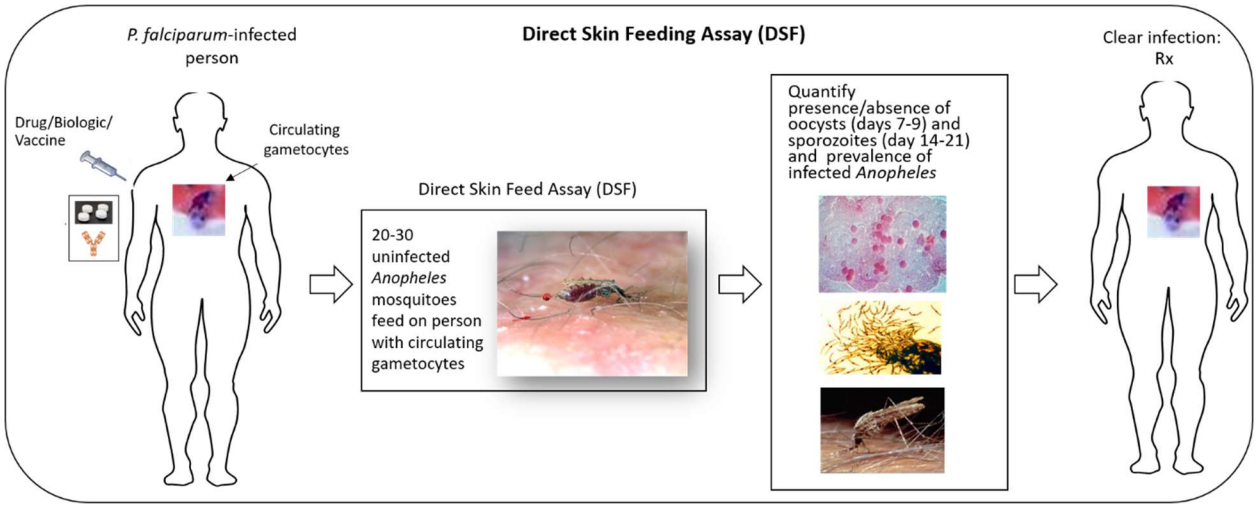
Briefly, laboratory-reared anopheline mosquitoes (typically *A. stephensi*) are permitted to feed on cultured *P. falciparum* gametocytes (NF54 isolate/3D7 strain) through a membrane. The fed mosquitoes are maintained in cages to permit any ingested parasite gametocytes to develop into oocysts. After approximately 7-9 days mosquitoes are dissected and the number of oocysts is used to report the success of infection and the percentage of parasite-infected mosquitoes are quantified. A portion of infected mosquitoes are also dissected between days 14 and 21 to assess salivary gland sporozoites which are the infective stages of the parasite following a mosquito bite which injects sporozoites into the skin thereby initiating the parasite's life cycle.

SMFA has been utilized widely to assess the transmission-blocking potential of test antibodies and anti-malarial drugs in preclinical and clinical vaccine development by mixing the cultured *P. falciparum* gametocytes with test antibodies/drugs and allowing *Anopheles* mosquitoes to feed. The transmission reducing activity of the test intervention is then calculated based on comparison of infection prevalence and oocyst density with that obtained in mosquitoes fed gametocytes mixed with control serum (8,9).

3.1.3.2 Direct Skin Feeding Assay (DSFA)

The DSFA has been developed to directly measure the transmissibility of parasites directly from human-to-mosquito without an in vitro membrane step as illustrated below. Although DSFA is biologically more relevant with regard to the functional activity that a drug/antibody has on malaria transmission, the assay is complex requiring human volunteers and the inherent variability in human hosts, parasite strains, and anopheline vector competencies rendering regulatory approval for such an approach unsustainable.

Figure 4: Direct Skin Feeding Assay

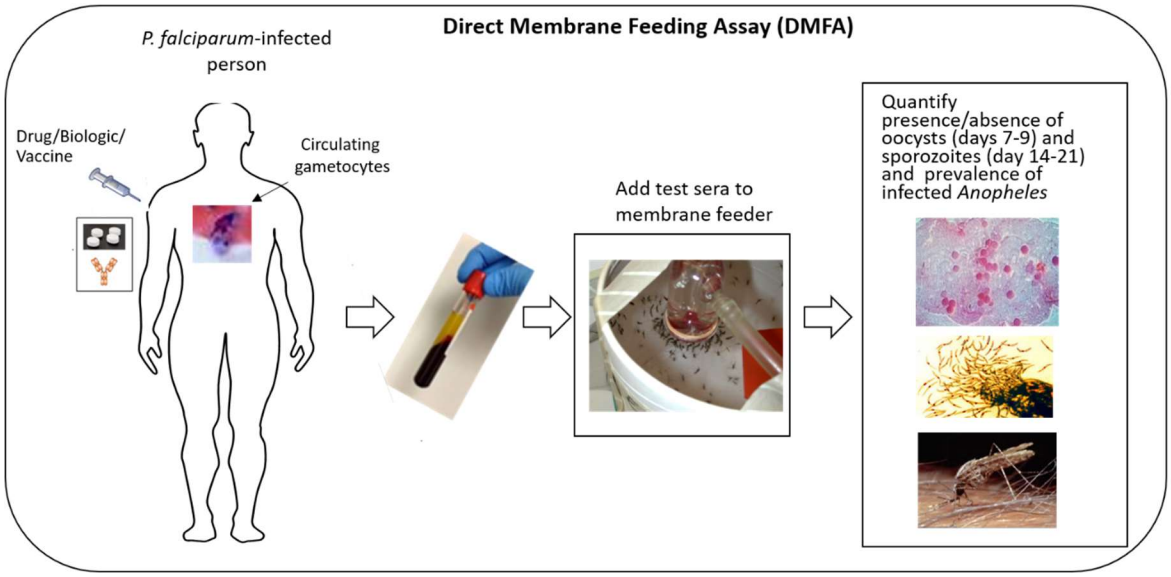


The DSFA is desirable since it closely mimics what would happen in nature, but also has important drawbacks: (i) the number of mosquitoes that can be fed on a human at any one time is restricted (~<30 per skin feed) by volunteer acceptability. (ii) it is difficult to quantify the number of gametocytes circulating in the human blood and in blood meal from a mosquito after they have fed on the human host. (iii) the DSFA may not be ethically used in certain age groups (i.e. young children) depending upon country-specific and community acceptability; (iv) the DSFA can be affected by inter-individual variation in innate attractiveness to mosquitoes; the DSFA is limited to geographical locations with a source of gametocyte-positive *P. falciparum*-infected persons and advanced laboratory and insectary support.

3.1.3.3 Direct Membrane Feeding Assay (DMFA)

A third type of assay named the Direct Membrane Feeding Assay (DMFA) is an assay that is positioned between the SMFA and DSFA. In the DMFA, instead of using cultured gametocytes of a single strain of *P. falciparum* as in the SMFA, venous blood samples from gametocytemic individuals previously administered with anti-malarial drugs or antibodies are placed into a membrane feeder and fed to mosquitoes. Such an assay illustrated below is performed in malaria endemic regions with direct access to naturally-infected gametocyte carriers and advanced laboratory support and insectary facilities. In the DMFA, oocysts in the mosquito midgut and sporozoites in the salivary glands are quantified.

Figure 5: Direct Membrane Feeding Assay



3.1.3.4 Biomarker validation for future clinical trial design

The search for a biomarker assay, such as the SMFA, will be the objective of a follow-on clinical trial. This experimental medicine protocol is focused on testing the reproducibility of two sequential direct skin feeding assays to inform trial design for future trials that assess biomarker feasibility. The section below describes how future trials will assess the feasibility of a biomarker SMFA to substitute for conventional trials that measure the interruption of malaria transmission.

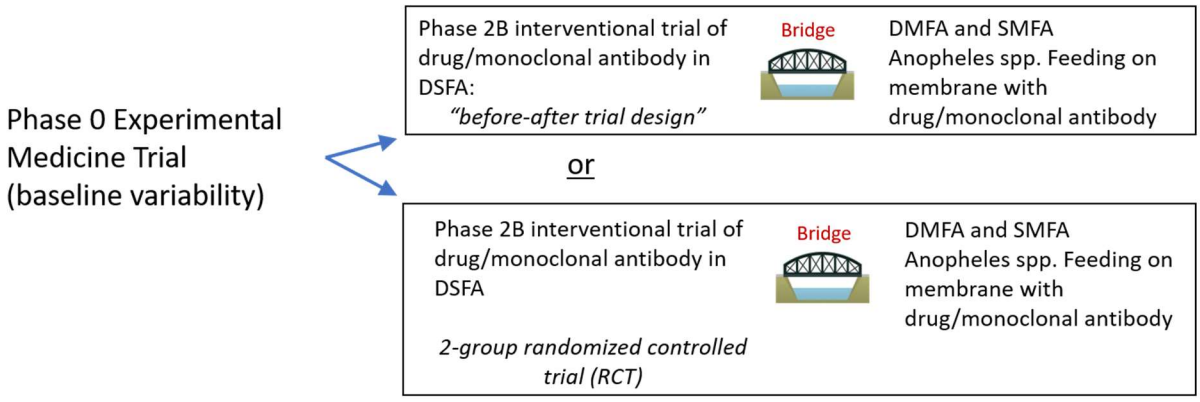
The SMFA, DMFA, DSFA have advantages and limitations. As stated previously, the SMFA is the gold standard used by multiple laboratories around the world. The assay is reproducible and robust. However, the SMFA is limited in terms of the number of malaria parasite strains and anopheline vector compatibility. Nevertheless, if the SMFA could recapitulate the transmission dynamics and functionality of the DSFA, the validation of the SMFA may meet the standards set by regulatory agencies for accelerated approval of new tools that interrupt malaria transmission as it does not include the requirement of using natural acquired *P. falciparum* parasites not naturally caught *Anopheles* species that transmit malaria.

DSFAs are quantitatively more sensitive than membrane feeding assays in detecting positive oocyst-infected *Anopheles* mosquitoes after having fed on low-density numbers of gametocytes in a feeding assay. The reasons for this are unknown and do not appear to be a function of sequestration of gametocytes in the skin of an infected person (unpublished data).

Our ultimate objective for evaluation of TRIs would be to substitute an in vivo DSFA for a surrogate in vitro SMFA assay. The suitability of the SMFA over the DSFA would need to be validated by bridging the two assays against each other. In order to evaluate these two assays against each other in a streamlined cost-effective manner, we have proposed a bridging study to be performed at a later date (not the subject of this experimental medicine trial), that capitalizes upon a single person serving as their own

internal control in a *"before-after"* study design. The quantitation of baseline variability in the current proposed trial would be used to inform trial design of a follow-on study.

Figure 6. Rationale of experimental medicine trial of baseline variability in transmission of parasites from human-to-mosquito



It is unknown whether the numbers of infected mosquitoes or the density of midgut oocysts are similar (i.e. variability) after having been fed on the same gametocyte-positive person at two consecutive short time intervals. This unknown is critical in our understanding and design of an interventional trial in which each human subject serves as their own internal control in a classic *"before-after"* trial intervention.

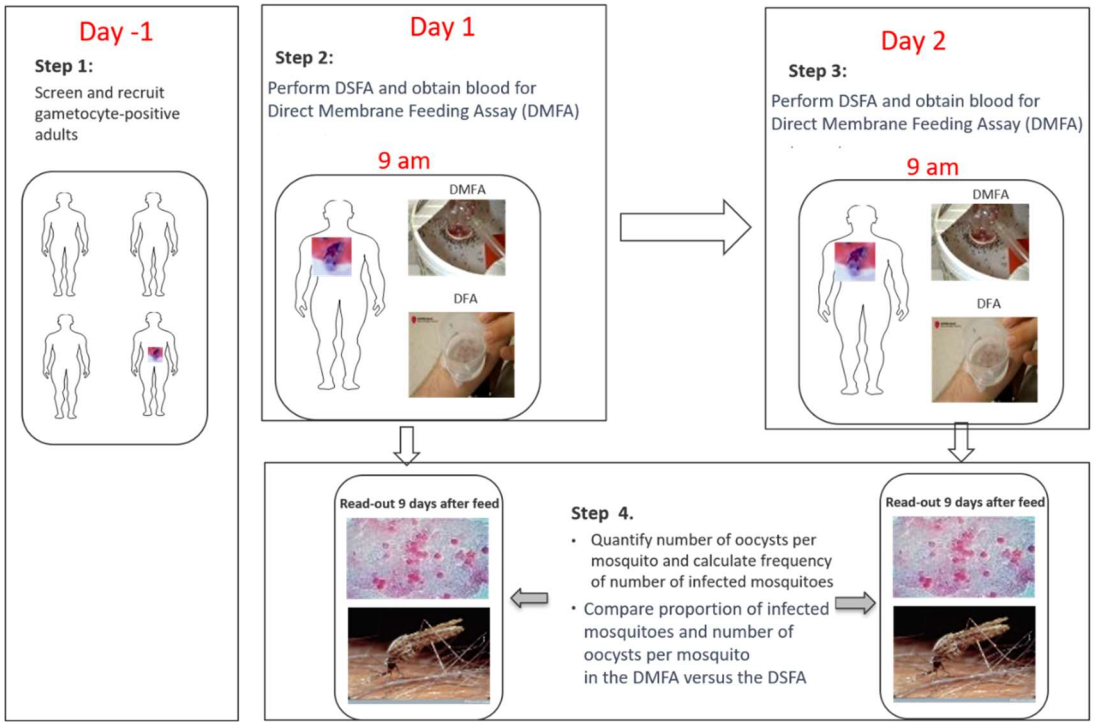
The "*before-after"* trial design depends upon the assumption that there is minimal variability in parasite-to-mosquito transmission dynamics in the same person when measured via DSFA at two time points separated by a 24-hour interval when no other intervention is applied (no TRI) and forms the hypothesis for carrying out the current experimental medicine trial.

3.2 Rationale for approach and hypothesis

It is crucial that variation in DSFA and DMFA be measured by quantifying the prevalence of infected mosquitoes and/or oocyst density in the mosquitoes at two sequential time points. The data obtained from this study will inform the methodology for future field studies of TRIs.

The illustration below depicts the strategy to determine variability in same person undergoing DSFA at two consecutive 24-hour intervals.

Figure 7: Schematic of study



The use of internal controls is a strategy that has been widely used in clinical trials. The advantages of this approach include the need for a smaller sample size to detect differences in efficacy of interventions. This experimental medicine trial proposes therefore to measure the baseline variation in the infectivity of mosquitoes in both the DSFA and DMFA when performed in the same individual at two consecutive time points.

The United States Army Medical Research Directorate – Africa (USAMRD-A) in Western Kenya has successfully piloted a platform within the Kombewa Clinical Research Center's (CRC) Health and Demographic Surveillance System (HDSS) study area that has been designed to study malaria transmission dynamics in a holoendemic setting. Study activities were executed under IRB-approved protocol between July 2015 and June 2016. The general objective was to monitor trends in *Plasmodium* spp. (asexual and sexual stage) carriage and transmissibility in a population exposed to high transmission through the seasonal variation of one calendar year. Among the specific study objectives was the need to approximate the infectiousness of the human population within the study area to mosquitoes by quantifying oocysts and sporozoites in lab-reared *Anopheles gambiae* mosquito midguts and salivary glands respectively after mosquito feeding assays. Over 1000 mosquito feeding assays (DFMA and/or DSFA) were performed during this study with high proportion of mosquitoes demonstrating positive midgut dissections and positive mosquito salivary gland dissections. The proposed experimental medicine trial will leverage the expertise that exists at USAMRD-A/K to ensure successful completion of the study objectives.

3.3 Potential Risks and Benefits

3.3.1 Known Potential Risks and Risk Management

3.3.1.1 Risks of Accidental Disclosure of Private Information

The study will be collecting private information and there is risk this could be exposed and reveal the status of the subject in the study or some other personally identifying information (PII) of protected health information (PHI). In order to ensure that all information collected on study volunteers is kept confidential, the following safeguards will be applied: Access to study files and personal information will be limited to study personnel, ethics committees, regulatory authorities, and sponsor. Study information will be kept in locked rooms when not in use. All information or samples that leave USAMRD-A will be labeled with a unique study identification number and have no PII. Any link between individual study identification number and an individual's PII (e.g., and individual's study file and associated documents) will be maintained at the USAMRD-A facilities in accordance with site standard operating procedures (SOPs) to maintain each individual's confidentiality.

3.3.1.2 Risks of Phlebotomy

Venipuncture is a routine clinical procedure the medical community commonly uses to obtain blood samples. Immediate complications may be slight pain during the entry of the needle into the skin, very rarely possible dizziness and syncope. Additionally, a hematoma may result from the venipuncture, but this has minimal risk. Late complications might include thrombosis of the vein due to trauma or infection. These complications are extremely rare. Participant monitoring, aseptic technique, including sterile disposable blood collection apparatus and adherence to standard medical precautions reduce any risk to a minimum. A credentialed phlebotomist or member of the clinical team experienced in venipuncture techniques will perform all venipunctures. The amount of blood to be taken for sampling will not be harmful to the subject's health.

3.3.1.3 Risks of Direct Skin Feeding Assay

DSFA exposes an individual to mosquito bites. In addition to mild expected discomfort from a bite itself and pruritus following the bite(s), there is also a risk of a local site reaction (e.g., allergic reaction; including pain, swelling, and erythema), secondary infection, or systemic allergic reaction (e.g., anaphylaxis). Mild local site reactions are common and can be treated with symptomatic medication as needed and subjects will be monitored for 30 minutes following DSFA for evidence of a severe systemic reaction and treated for any complications by the study team or with medical referral as needed. There is the additional risk of mosquitoes transmitting other infectious agents. This risk will be at the very minimum for this project since cow's blood is used to maintain the colonies and only new progenies of female mosquitoes will be used for skin feeding assays. Additionally, 10% of each new generation of female mosquitoes will be pooled and tested for presence of arboviruses by PCR at the USAMRD-A/K/KEMRI laboratories. In USAMRD-A/K's experience, there have been no known cases of the laboratory-reared mosquitoes transmitting other infectious agents.

3.3.1.4 Risks of receipt of Primaquine

Subjects who receive primaquine generally may experience the following adverse events:
Cardio-vascular: Cardiac arrhythmia and QT prolongation, mainly with high dose.
Gastrointestinal: Nausea, vomiting, epigastric distress, and abdominal pain.
Hematologic: Leukopenia, hemolytic anemia especially in G-6-PD deficient individuals and methemoglobinemia especially in NADH methemoglobin reductase deficient individuals.
Nervous system disorders: Dizziness.
Skin and subcutaneous tissue disorders: Rash maculopapular, pruritus.
Standard dose primaquine is contraindicated in subjects with severe glucose-6 phosphate dehydrogenase (G6PD) deficiency due to the risk of hemolytic anemia. However, a single low dose (15 mg primaquine base) is safe in individuals with G6PD deficiency and recommended by the WHO for the clearance of sexual stage gametocytes of *P. falciparum* without specific testing for G6PD adults (except in pregnant or breastfeeding women) (10). The side effects that may be experienced are mostly transient and usually do not require any specific treatment. The management of any persistent side effects is usually symptomatic.

3.3.1.5 Risks of Receipt of Artemether/Lumefantrine (Coartem®)

Adult subjects who receive Coartem® may experience the following adverse events:
Metabolism and nutrition disorders
Very common: Decreased appetite
Psychiatric disorders
Very common: Sleep disorder
Nervous system disorders
Very common: Headache, dizziness
Common: Clonus
Uncommon: Somnolence, hypoesthesia, ataxia
Cardiac disorders
Very common: Palpitations
Respiratory, thoracic and mediastinal disorders
Common: Cough
Gastrointestinal disorders
Very common: Vomiting, abdominal pain, nausea
Common: Diarrhea
Skin and subcutaneous tissue disorders
Common: Rash, pruritus
Uncommon: Urticaria
Musculoskeletal and connective tissue disorders
Very common: Arthralgia, myalgia
General disorders and administration site conditions
Very common: Asthenia, fatigue
Uncommon: Gait disturbance
Investigations
Uncommon: Electrocardiogram QT prolonged, liver function tests increased

Symptoms are generally mild and self-limited without any treatment required. To ensure compliance, subjects will receive the first dose of Coartem® under the supervision of a medical provider at the CRC and sent home with the remaining doses and instructions on how to complete the course. The management of any persistent side effects is usually symptomatic.

3.3.1.6 Risk of infectious mosquito bite to lab personnel

There is a small but potential risk of occupational exposure to the bite of an infectious *A. gambiae* mosquito that escapes from carton in insectary or during dissection procedures for determination of sporozoite prevalence. Laboratory personnel will be instructed to inform the PI upon a mosquito bite and every attempt will be made to capture and dissect or evaluate the mosquito for the presence of salivary gland sporozoites. Laboratory personnel will be counselled and followed up periodically for the emergence of blood stage parasitemia and treated for malaria according to the Kenya Ministry of Health National Guidelines for the Diagnosis, Treatment and Prevention of Malaria.

3.3.1.7 Other Health Risks of Participation in the Study

There is the risk of exposure to respiratory infections such as COVID-19 when potential participants visit health facilities. The Kenya Ministry of Health has put in place guidelines that help reduce the risk of exposure, such as screening all individuals presenting at health facilities, implementing physical distancing measures and ensuring healthcare workers have the appropriate personal protective equipment (PPE). The study team will follow local guidelines in order to minimize risk of exposure of both the participants and the study staff.

Briefly, staff will do self-screening for illness at home before they report on duty as per KEMRI guidelines, and will also be screened when they arrive at the duty station. Staff who are unwell will not be allowed to be on duty. Staff will use full PPE in accordance with WHO guidelines (mask, eye protection, disposable gown, gloves) when handling study participants. Staff will wash or sanitize hands before encounter, after any contact with the participant, and at the end of the encounter. Hand sanitizers and handwashing stations will be made available by the study. The staff will routinely disinfect the surfaces of the research center as well as the sample shipment boxes prior to their dispatch to the central lab. Central lab staff in full PPE will disinfect the surfaces of sample shipment boxes that are received from the study site prior to opening them. Central lab staff will work in full PPE whenever they are working with the samples.

Potential participants will be screened for symptoms of COVID-19 during the recruitment process. Those suspected to have COVID-19 will not be allowed to participate in the study. Participants who meet the case definitions of COVID-19 while in the study will not continue with study activities and will be advised to seek medical care as per Kenya Ministry of Health guidance. Study participants will sanitize hands upon entry and at exit from the research center and use face masks while at the center. Hand sanitizers and handwashing stations will be made available by the study. The study will also provide the participants with face masks.

The waiting areas for study participants as well as work stations for staff will be re-arranged to ensure physical distancing of 1.5 – 2 meters. It will be difficult to maintain physical distancing between participants and study staff during the execution of study procedures such as physical examination, measuring of vital signs and drawing of blood. For this reason, study staff will don full PPE, including face shields, while study participants will be provided with face masks.

Participants and staff suspected to have COVID-19 (meeting the case definitions of COVID-19 as per Kenya Ministry of Health guidelines) while at the study site will be kept in separate isolation rooms as they await to be processed as per Kenya Ministry of Health protocols on COVID-19. After they leave the site, study staff donned in full PPE, in accordance with Ministry of Health guidelines, will disinfect the study site, including all rooms they had visited.

While no other health risks are expected as part of participation in this study, if a participant is hurt as a direct result of participating in this study, the medical care will be provided by the study team at the respective research centers and the study will pay for the expenses. If the injury requires hospital admission, the participants will be admitted at the Kombewa Sub-County Hospital or the Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH), or another local area facility, as appropriate. If specialized treatment is required, they will be referred to relevant hospitals for management and the study will pay for medical expenses.

3.3.2 Known Potential Benefits

All volunteers for this study will receive the following benefits for their participation:

- All volunteers will undergo a medical examination at screening free of charge. All volunteers, whether accepted for enrolment into the trial or not, will benefit from this free health check-up. The results of all tests will be communicated to all volunteers. Where illnesses are newly diagnosed, a referral to an appropriate health provider will be made for the volunteer.

- For the short duration of their participation, subjects will receive free health care for acute conditions. In case of chronic conditions, subjects will be referred to appropriate health providers.

- All gametocytemic individuals will receive Coartem® and low dose primaquine therapy (excluding pregnant or breast-feeding women for the latter) for the clearance of their malaria infection as the conclusion of their participation in the study. This does not constitute standard of care since asymptomatic individuals do not typically receive malaria treatment. However, clearance of asymptomatic parasitemia is associated with significant health benefits (such as reduction of incidence of chronic anemia and reduction of ongoing malaria transmission).

4. OBJECTIVES AND PURPOSE

Primary Objective:
To assess variation in the proportion of infected mosquitoes with at least one oocyst (oocyst prevalence) in DSFA and DMFA performed at two consecutive time points in the same human subject with *P. falciparum* gametocytemia using microscopy

Secondary Objectives:

1. To assess variation in oocyst density in DSFA and DMFA at two consecutive time points in same subject using microscopy.
2. To assess variation in the proportion of infected mosquitoes with at least one sporozoite (sporozoite prevalence) in DSFA and DMFA performed at two consecutive time points in the same human subject using microscopy.

Exploratory Objectives:

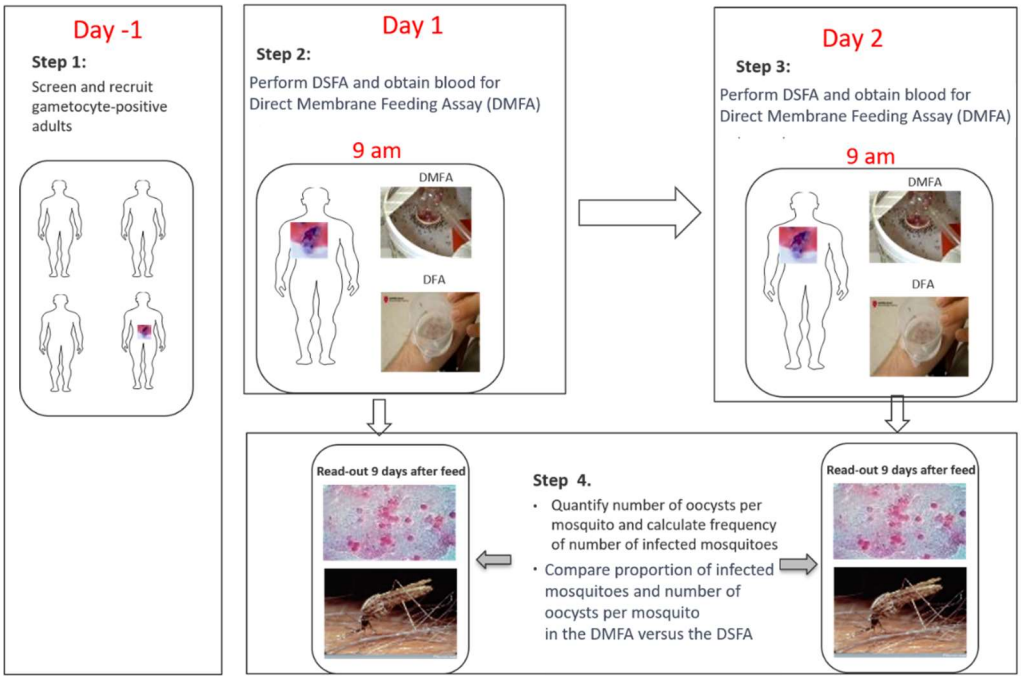
1. To assess oocyst and sporozoite density in DSFA and DMFA at two consecutive time points in the same subject using molecular quantitative PCR (qPCR).
2. To summarize the relationships between DSFA and DMFA results for oocyst and sporozoite density

5. STUDY DESIGN AND ENDPOINTS

5.1 Description of the Study Design

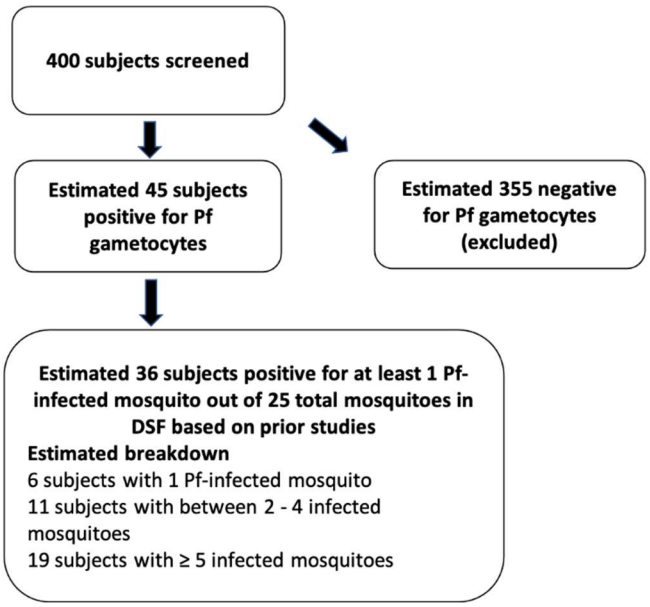
This is a clinical study with an entomological component. Study participants will be recruited to participate in mosquito feeding assays.
The proposed trial design has been developed to assess the consistency and reproducibility of two consecutive direct skin feeding assays (DSFA) at 24-hour interval. The results will determine the type of pivotal trial design for a follow-on Phase 2b trial whose objective is to bridge the standard membrane feeding assay (SMFA) to the direct skin feeding assay (DSFA) and direct membrane feeding assay (DMFA) using a monoclonal antibody intervention, TB31F mAb, which interrupts transmission from human to mosquito. The results from this experimental medicine study will inform whether the preferred "Before-After" trial design in which each human volunteer serves as their own internal control can be utilized for a follow-on Phase 2b trial.
The rationale for this experimental medicine trial is described in the background section of the protocol.

Figure 1. Experimental medicine clinical study design



The sample size to be included in this study is shown below in Figure 2.

Figure 2. Estimated sample size screening and enrollment for experimental medicine trial.



5.2 Study Endpoints

5.2.1 Primary Endpoint

The primary study endpoint will be oocyst prevalence in DSFA and DMFA in mid-guts of mosquitoes performed at two consecutive time points in the same human using microscopy. Oocyst prevalence is defined as the proportion of mosquitoes in a cup with at least one oocyst detected in the mid-gut among the mosquitoes (in the same cup) that underwent the feeding assays. The within and between subject variability will be described and the differences within and between DSFA and DMFA prevalence will be quantified.

5.2.2 Secondary Endpoints

1) Oocyst density in DSFA and DMFA at two consecutive time points in same subject using microscopy. Oocyst density is defined as the mean number of oocysts detected in infected mosquitoes that underwent feeding assays on the same subject. The variability and change in oocyst prevalence and density between the two consecutive feeds as measured by DSFA and DMFA will be determined.

2) Sporozoite prevalence and intensity
   a) Sporozoite prevalence in DSFA and DMFA performed at two consecutive time points in the same human subject using microscopy. Sporozoite prevalence is defined as the proportion of mosquitoes in a cup with at least one sporozoite detected in the salivary glands among the mosquitoes (in the same cup) that underwent feeding assays.
   b) Sporozoite density in DFSA and DFMA performed at two consecutive time points in the same human subject using microscopy. Sporozoite density is defined as the mean number of sporozoites detected in infected mosquitoes that underwent feeding assays

   The change in sporozoite prevalence and density between the 2 consecutive feeds will be determined.

5.2.3 Exploratory Endpoints

The exploratory endpoint will be oocyst and sporozoite density using qPCR and their comparison to data obtained through microscopy.

6. STUDY ENROLLMENT AND WITHDRAWAL

6.1 Participant Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Provision of signed or thumb printed and dated informed consent form
- Stated willingness to comply with all study procedures and availability for the duration of the study
- Male or female aged between 18 years and 55 years inclusive.
- Resident within the study area
- In good general health as evidenced by medical history and clinical examination before entering the study

- Ability to take oral Coartem and low-dose primaquine anti-malarials upon conclusion of day 2 (2nd direct skin feed) and be willing to adhere to the medication regimen
- For females, she must be of non-childbearing potential or use appropriate measures to prevent pregnancy for 30 days after receiving Coartem and primaquine. Non-childbearing potential means she is surgically sterilized or at least one year post-menopausal. Appropriate measures to prevent pregnancy include abstinence or adequate contraceptive precautions (i.e. intrauterine contraceptive device; oral contraceptives; diaphragm or condom in combination with contraceptive jelly, cream or foam; Norplant or Depo-Provera).
- For males, he must be willing to ensure that he does not get his partner(s) pregnant for at least 3 months after treatment with primaquine. Appropriate measures to prevent pregnancy include abstinence or adequate contraceptive precautions in either the participant or the partner.
- Positive for *P. falciparum* gametocytes as measured by PCR with cT value < 31.

6.2 Participant Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

- Presence of any signs or symptoms of malaria
- Presence of contraindications to administration of Coartem and primaquine as indicated in the respective drug package inserts
- History of severe allergic reactions to mosquito bites (other than pruritus and local swelling)
- Pregnant (i.e. a positive pregnancy test)
- Current or recent (within the preceding 2 weeks) use of antimalarial treatment
- Current participation in a malaria vaccine study
- Any other findings that the investigator feels would increase the risk of having an adverse outcome from participation in the trial.

6.3 Strategies for Recruitment and Retention

6.3.1 Community information

Community sensitization through public meetings (“barazas”) convened by project staff, whenever possible, will precede the actual human subject recruitment process. The community in which the study will take place will be informed about the nature and design of the study. Community leaders (chiefs, local village elders, and opinion leaders) and local health authorities will be formally briefed in their own language on the nature and purpose of the study. They will have the opportunity to ask questions of the PI or his designees. The community leaders can then disseminate the information within the community using their usual channels.

6.3.2 Recruitment

The target sample size for subjects with gametocytemia is approximately 45 individuals. Approximately 400 participants will be screened for gametocyte carriage by PCR to achieve the target sample size. Screening of subjects will continue until the desired sample size of gametocytemic individuals who undergo all study procedures is attained. Adults aged 18 – 55 years will be recruited from the villages in the Kombewa Health and Demographics Surveillance System (HDSS) consisting of half of Kisumu

West and all of Seme Sub-Counties of Kisumu County, Kenya. Informed consent will be obtained from all subjects. Language and illiteracy will not be impediments in the recruitment process, all briefings and explanations will be in the understandable language of the participant.

6.3.3 Reasons for Withdrawal or Termination

Subjects can leave the study at any time for any reason if they wish to do so without any penalty or loss of benefits to which they are otherwise entitled. Subjects can also be withdrawn from the study procedures at the discretion of the clinical investigator. The following reasons may lead to withdrawal of individual subjects:

- Withdrawal of informed consent by volunteer;
- Any serious adverse event;
- Any adverse event that, according to clinical judgment of the investigator, is considered as a definite contraindication to proceeding with the study procedures such as severe allergic reaction to mosquito bites from DSFA on day 1 of trial activities that would preclude DSFA on day 2.
- Volunteer non-compliance with study procedures

6.3.4 Handling of Participant withdrawals or termination

Screening of potential subjects will continue until the desired sample size of gametocytemic individuals who undergo all study procedures is attained. Subjects who are withdrawn or terminated will be replaced by recruiting new ones. If the reason for withdrawal or termination is a medical event, then the subjects will be referred for management at an appropriate Kenya Ministry of Health (MoH) treatment facility.

6.4 Premature Termination or Suspensions of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause as determined by the PATH, USAMRD-A, KEMRI or WRAIR. Written notification, documenting the reason for study suspension or termination, will be provided to the investigator, WRAIR IRB, and to Kenyan regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension. Clinical evaluation and management of emergent clinical malaria that results in early termination of and individual study participation will be done according to the Kenya MoH guidelines. In the case of consent withdrawal of participants who are known to harbor gametocytes, malaria treatment will be offered to clear any parasites that could be circulating within the participant. There will be no further evaluation of gametocyte negative participants or participants in whom gametocyte testing has not been carried out.

7. STUDY TREATMENT

There will be no investigational agents in this study. The only medications that will be used are antimalarial medications, Coartem® and primaquine provided upon completion of day 2 DSFA in order to clear any asexual and sexual stage parasites. Package inserts for Coartem® and primaquine will be provided to the ethical review committees.

7.1.1 Acquisition

Coartem® and primaquine will be procured by USAMRD-A from reputable vendors.

7.1.2 Product Storage and Handling

All anti-malarial medications should be stored below 30°C in the pharmacy at KCRC. Drugs will be provided to each subject upon completion of day 2 procedures.

7.1.3 Dosing and Administration

Refer to dosing and administration of Coartem® and primaquine in Table 1. The dosage for Coartem® and primaquine are as per the manufacturer's package insert. Coartem® is part of the usual treatment prescribed for malaria as per the Kenya standards of treatment for malaria. The addition of primaquine is not part of standard of care, however the participant will benefit from the reduction of gametocyte carriage as a result of primaquine administration. The administration of Primaquine and the first dose of Coartem will be done under direct observation by clinic staff. According to standard of care in Kenya for malaria treatment, doses of Coartem on days 2 and 3 will be taken by the subject as instructed by study staff. The KCRC Pharmacy will maintain accountability logs for dispensed drugs.

7.1.4 Justification for treatment of asymptomatic parasite carriers

Plasmodium spp. asymptomatic carriers, defined as individuals with detectable asexual or sexual parasites in their blood and an absence of any acute clinical symptoms of malaria, asymptomatic carriers are not yet targeted by national malaria intervention strategies in many malaria-endemic countries, including Kenya. Local malaria treatment guidelines are currently focused on the management of symptomatic malaria: the starting point for treatment algorithms is symptoms such as fever, with an emphasis on testing for malaria prior to treatment. Asymptomatic carriers do not seek treatment for their infection. They and, therefore, constitute a reservoir of parasites for maintaining the parasite life cycle and transmission by the anopheline vector and thus a real public-health risk. Studies have suggested that a proportion of individuals with asymptomatic blood-stage malaria infection may eventually become symptomatic, although reinfections or other causes of fever cannot always be ruled out. These asymptomatic parasites have also been linked with chronic anemia and co-infections with invasive bacteria. As such, the treatment of asymptomatic malaria carriers who will be identified in this study will have both individual and public health benefits. At the individual level, parasite clearance may reduce the risk of getting symptomatic malaria later on and may also reduce the risk of adverse health outcomes associated with parasite carriage, such as chronic anemia and co-infections with invasive bacteria. From a public health point of view, clearance of parasitemia may reduce the risk of malaria transmission to others, especially to other members of the household, some of whom may be susceptible to severe malaria. Good clinical judgment strongly encourages that persons who are asymptomatic and infected with malaria parasites when found to be positive by molecular diagnostic techniques be provided antimalarial medications to clear both the asexual parasites and sexual stage gametocytes.

Table 1. Anti-malarial Medications

Study Agents	Dosage forms and strength	Body weight	Day 1**** (AM)	Day 1 (+8hr)	Day 2 (AM)	Day 2 (PM)	Day 3 (AM)	Day 3 (PM)
COARTEM *	artemether (20 mg) and lumefantrine (120 mg)**	> 35 kg	4 tabs	4 tabs @ 8 hrs.	4 tabs	4 tabs	4 tabs	4 tabs
PRIMAQUINE***	primaquine phosphate tablets 26.3 mg primaquine phosphate (equivalent to 15 mg primaquine base)	> 35 kg	1 tab	-	-	-	-	-

* Coartem should be taken with food

** Coartem is also available as artemether (80mg) and lumefantrine (480mg) tablet for adults. The dosage of this strength is 1 tablet at each dosing time point.

***Primaquine may be taken with food

**** Refers to first day of drug taken after 2nd DSFA

8. STUDY PROCEDURES

8.1 Study procedures and evaluations

Table 2. Time and event

Procedures	Visit Day -1 to 0 (Screening)	Visit Day 1 (Baseline/Enrollment)	Visit Day 2 (Final)
Window (hours)	–	–	+/-3
Obtain informed consent of potential participant	●		
Obtain demographic information	●		
Obtain medical history	●		
Obtain medications history	●		
Perform medical examinations	●	●	●
Collect blood for gametocyte detection tests (2 mL) by PCR	●		
In females, urine pregnancy test	●		●
Verify inclusion/exclusion criteria	●	●	●
Verify gametocyte detection (RT-PCR) test results		●	
Record vital signs	●	●	●
Blood sample for serology – 5 mL*		●	
Blood sample for DMFA and microscopy (1ml)		●	●
DSFA		●	●
Admit the study participant for observation overnight		●	
Record local and unsolicited AEs as reported by participant or observed by investigator before DSFA and 30 minutes after DSFA		●	●
Administer low dose primaquine* and first dose of Coartem® and give remaining Coartem® dose with instructions			●

- Sera collected will be assayed in membrane feeding assay (risk mitigation) only if gametocyte-positive subject fails to transmit any parasites to mosquitoes in either the DSFA or DMFA performed either on day 1 or day 2. Sera will be destroyed before study close-out.

8.1.1 Study site

The study will be conducted within the Kisumu West and Seme sub-counties of Kisumu County, western Kenya. Study activities will be coordinated from the USAMRD-A/K Kombewa CRC located in Kombam village, Kombewa. The study site is located about 40 km west of Kisumu city, the administrative capital of Kisumu County. The study sub-counties cover an area of about 369 km^2 stretching on the North Eastern shores of Lake Victoria. The study area has been cartographically mapped by the USAMRD-A/K KCRC HDSS using Global Positioning System (GPS) technology. The HDSS is a longitudinal population registration system designed to track the evolving demographic and health status of the study populations over time. The HDSS population is monitored by field staff visiting each household bi-annually to capture health and demographic information (birth rates, death rates, causes of death, pregnancies, immunization status, in-and out-migrations, etc.). Various studies nested on this platform take advantage of the sampling frame inherent in the HDSS, whether at individual, household/compound or regional levels.

8.1.2 Recruitment, informed consent and enrolment

Field workers (who will include nurses and/or clinical officers) will approach potential participants within their homesteads and give them a brief overview of what this protocol intends to undertake and request them to undergo a formal consenting session using a standard recruitment script. A typical field team will have one nurse or clinical officer and one or two field assistants. Those potential subjects who accept will undergo a formal and private consenting process that will be conducted by the nurse or clinical officer. Only those who accept to participate (as evidenced by the signing or thumb printing of the informed consent form) will be considered for enrolment in the study. Privacy will be achieved by conducting the consenting sessions in a secluded area within the compound of a given homestead. In the event that the potential participant is illiterate, an individual not associated with the study will be identified from within the participant's village by the participant to act as an independent witness to the consenting process.

After informed consent is obtained, the field team will assign the participant a study identification number (study ID). The list of study IDs (format CV85###) available will be prepared by the study coordinator and availed to the field teams. As the field teams recruit participants, they would communicate with the coordinator so that double assignment of IDs does not occur in the event more than one field team is doing recruitment. The field team would then collect demographic data, a brief malaria history and assess eligibility. In addition to a general medical history, the clinician in the field team will specifically ask questions that would elucidate any contraindications to the administration of Primaquine and Coartem. The clinician will err on the side of caution whenever there is doubt regarding the presence or absence of a specific contraindication.

Women of child-bearing potential will have a pregnancy test done within the homestead. The nurse/clinical officer will instruct the participant on how to obtain the urine specimen and then will proceed to conduct the pregnancy test using a commercially available human chorionic gonadotropin (hCG) based test kit.

Individuals who meet eligibility criteria at this stage will have a blood sample collected for detection of gametocytemia.

8.1.3 Blood sample collection

The nurse or clinical officer in the field team will obtain 2ml of whole blood from participants. This will happen while the study team is still in the participant's homestead. Blood will be collected in a tube containing Ethylenediaminetetraacetic acid (EDTA) using the aseptic technique, placed in a sample

transportation box at +4 to +8^{0}C for shipment to the central Malaria Drug Resistance Laboratory (MDR) for the gametocyte detection assays by PCR. Blood sample collection will conclude the field visit. All study documents that will be filled out while in the field will be transported back to the KCRC. A signed copy of the ICF will be given to the study participant. The blood samples will be batched and transported to the MDR, to arrive within 6 hours of collection. In the event that the field team anticipates that the 6 hour window to ship the blood samples to MDR is closing in, the team will contact the transport dispatcher at the KCRC to pick up the sample transportation box from the field.

8.1.4 Enrolment of gametocytemic participants

Participants who are found to harbor gametocytes as per the PCR test (with a cT value of less than 31) will be enrolled to participate in the mosquito feeding assays. The MDR expects to turn around the results of the PCR tests within approximately 48 hours of sample collection. The results of the tests will be communicated to the study team in Kombewa, who will then contact the participants. For participants who do not have gametocytes detected, they will be informed of the results and the study team will explain to them that their participation has ended. Participants who are gametocytemic will be invited to participate in the mosquito feeding assays. Participants who are found to have very low density gametocytemia (PCR cT value of 31 and above) will not be invited to participate in mosquito feeding assays. They will, however, be offered treatment to clear their parasitemia as described in section 7 of this protocol. The team in Kombewa will facilitate the movement of the participants from their homesteads to the KEMRI/USAMRD-A facilities where the feeding assays will take place as described in section 8.1.5.

8.1.5 Mosquito feeding assays (DSFA & DMFA)

Mosquito feeding assays involve feeding unfed, sterile (pathogen-free), insectary-reared *Anopheles gambiae* mosquitoes on malaria infected humans for the purposes of obtaining oocyst and sporozoite counts in mosquito's midgut and salivary glands, respectively, thereby allowing an accurate evaluation of malaria transmission. The KEMRI/USAMRD-A/K entomology laboratory maintains a colony of *An. gambiae sensu stricto* (KISUMU strain) that were originally collected at Lwanda village, Siaya County in western Kenya. Mosquitoes are provided a 10% water infused sucrose meal daily, maintained at 30^o C (70-80% relative humidity), and reared under very stringent conditions. Cow's blood is used to maintain the colony, and only the progeny (3-5 day old female mosquitoes) are used for the feeding assays. Additionally, 10% of each new generation of mosquitoes are pooled and tested for arboviruses.
This study will conduct two types of mosquito feeding assays using study participants concurrently: Direct Skin Feeding Assay (DSFA) and Direct Membrane Feeding Assay (DMFA).

DMFA will be performed using the general procedures of Bousema et al. and DA et al. (13,14) for membrane feeding, with the notable exception that serum in collected subject blood samples will not be replaced with non-immune serum. Briefly, 0.12 mL of whole blood collected from study participants just prior to the feeding assays will be transferred to 2ml-capacity glass receptacles that comprise the artificial feeders warmed at 36-37 ^{0}C using a circulating water bath and provided to 3-5-day old starved female *An. gambiae sensu stricto* mosquitoes (KISUMU strain; n= 30 per cup; 2 cups per subject).

DSFA will be performed using the protocol described by Bonnet et al. and Bousema et al. (11,12) (studies successfully completed in Sub-Saharan Africa). Sixty sterile 3-5-day old *An. gambiae sensu*

stricto female mosquitoes (KISUMU strain) will be starved for five hours and transferred to a cardboard cup covered with netting prior to use. The mosquito containing cup will be placed on the calf or arm of each volunteer to feed for 15 minutes, after which antihistamine ointment will be applied to the volunteer's skin. (KISUMU strain; n=60 per cup; 1 cup per subject).

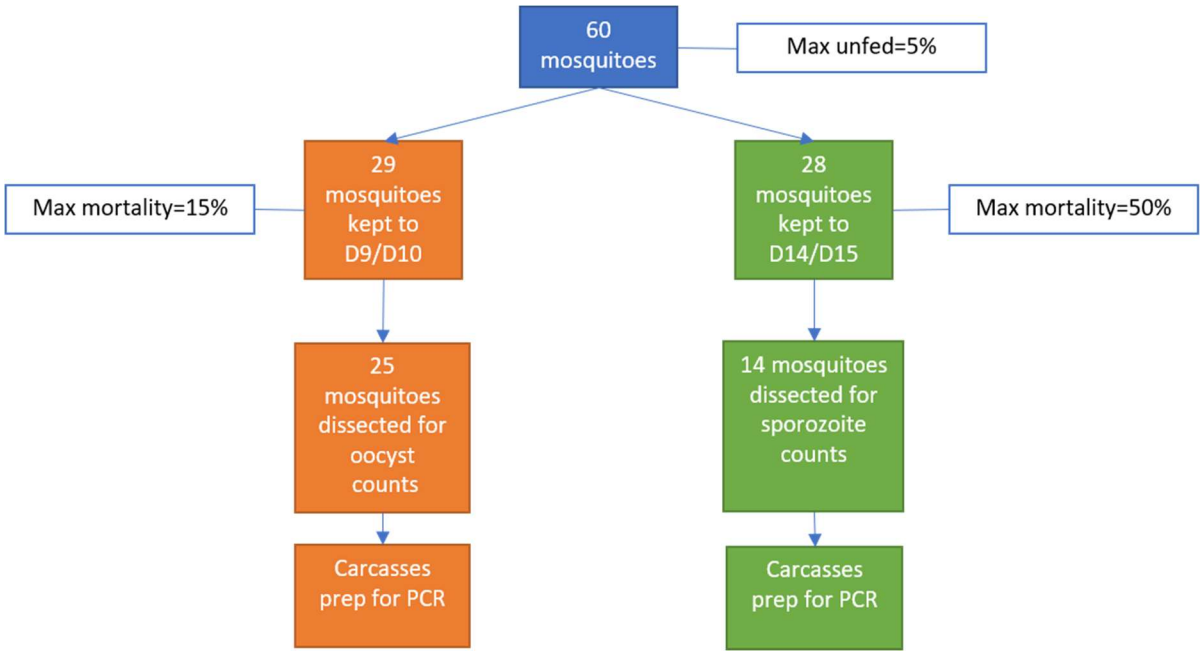
Day 1 procedures: Consenting human subjects identified as gametocytemic by RT-PCR will be transported to the KEMRI/USAMRD-A/K laboratory in Kisian within 48 hours of being identified in order to participate in the mosquito feeding assay. A blood sample (~ 1 mL) for DMFA will be collected from each subject (Table 2) prior to the DSFA. A blood sample (~ 5 mL) for serum collection will be collected and a membrane feeding assay may be performed (risk mitigation) only if gametocyte-positive subject fails to transmit any parasites to any mosquito in either the DSFA or DMFA on days 1 or day 2. Sera will be destroyed before study close-out. Upon completion of the first DSFA, the subjects will stay overnight under the supervision of KCRC staff at KCRC-affiliated district hospital. Subjects will not return to their home at the end of day 1 procedures.

Day 2 procedures: Subjects will be transported to the KEMRI/USAMRD-A/K laboratory in Kisian for the second DMFA and DSFA assay within +/- 3 hours of the time when the first DSFA was performed. The two sequential mosquito feeding DSFA and DMFA assays on day 1 and day 2) will be performed in all study participants within 24 hours (+/- 3 hours) to mimic a potential follow-on study design proposed to evaluate infectiousness of individuals before and after the administration of transmission-blocking intervention (drug and/or monoclonal antibody). A different skin site will be selected for DFSA on Day 2. The remainder of the blood sample collected for DMFA will be used to prepare malaria blood films (MBFs). Dried blood spots (DBS) for RT-PCR for *Plasmodium* detection will be collected and stored for a maximum of 30 days as a back-up diagnostic assay and then destroyed. There will be no future use archiving of biological samples.
Upon completion of day 2 of DSFA, subject will take day 1 of both Primaquine and Coartem anti-malarials. Coartem tablets for days 2 and 3 will be provided to subjects per standard operating procedures. Upon release from the clinic, the participation of each subject will be ended.

Mosquito and insectary procedures: All fed (and potentially infectious) mosquitoes will be held in locked environmental chambers located at the ACL-2 insectary at the USAMRD-A/K entomology facilities for security and optimum developmental conditions. Any unfed mosquitoes will be transferred to a second container and killed by placing in a -20^{o}C freezer for 48 hours prior to disposal.

Out of the 60 mosquitoes in each cup, it is estimated that approximately 5% will not feed. Therefore 29 will be held up to Day 9 or Day 10 post feed for oocyst count determination and the remaining 28 will be held up to Day 14 or Day 15 post feed for sporozoite count determination. The maximum anticipated mortality for mosquitoes held to D9/D10 is approximately 15%, while for those held to D14/D15 is approximately 50%. Thus, at least 25 mosquitoes will be available for oocyst counts and at least 15 for sporozoite counts as described in section 8.1.9.3 below. This is summarized in the figure below.

Figure 8: Disposition of mosquitoes after the feeding assays



8.1.6 Management of study participants

Subjects found to harbor gametocytes will be admitted for a period of approximately 24 hours to an observation ward at the Kombewa CRC or the Kombewa sub-county hospital located across the street from the CRC while awaiting their second feed as described above. While in the ward, they will be encouraged to dress in clothing that covers most of the skin so as to minimize contact with mosquitoes. In order to allow for the successful execution of the mosquito feeding assays, all gametocytemic participants will be offered antimalarial treatment only after completion of all feeding assays. A full course of artemether/lumefantrine 480/80mg (Coartem®) along with a single low dose of primaquine (15 mg primaquine base) will be administered as per the package insert – primaquine and the first dose of Coartem® will be witnessed by study staff. Primaquine will not be given to pregnant or breastfeeding women. Given that PCR testing tends to detect parasite levels much lower than the detection limit of standard clinical tests for malaria (RDT and microscopy), standard clinical testing will not be performed prior to administration of antimalarial treatment to clear parasitemia. Administration of primaquine to malaria patients is not part of standard care in Kenya. Primaquine, however, is specifically effective against gametocytes and therefore serves the purpose of ensuring clearance of circulating gametocytes in the participant. The administration of antimalarials to asymptomatic individuals is currently not part of standard of care in Kenya.

Gametocytemic participants who show signs of malaria while under observation will be managed in accordance to the Kenya MoH National Guidelines for the Diagnosis, Treatment and Prevention of Malaria. Such participants will not participate in further feeding assays and will be replaced until the desired sample size is achieved.

Study participants who withdraw consent while under observation and are still asymptomatic will be offered antimalarial treatment to clear parasitemia as described above prior to leaving the observation

ward. If they are symptomatic, they will be offered the choice to be managed by the study clinical team in accordance to the Kenya MoH National Guidelines for the Diagnosis, Treatment and Prevention of Malaria, or be referred to the Kenya Ministry of Health clinician for further management.

8.1.7 Standard of care study procedures

According to the Kenya MoH National Guidelines for the Diagnosis, Treatment and Prevention of Malaria, patients presenting with signs and symptoms of malaria will get tested for malaria (either by rapid diagnostic test or microscopy) and treatment offered immediately upon receiving the test results. Artemisinin based combination therapy (ACT) (for example artemether lumefantrine) would be typically prescribed for uncomplicated malaria, and intravenous artesunate would be prescribed for severe malaria. In certain cases, presumptive treatment of malaria is allowed. Additionally, drugs for alleviating symptoms, such as antipyretics for fever, are also typically prescribed.
For this study, asymptomatic participants will be recruited and tested for presence of gametocytes. They will receive malaria treatment as described in section 8.1.5.

8.1.8 Clinical laboratory procedures

A urine pregnancy test using a commercially available human chorionic gonadotropin (hCG) based test will be done in all females of child-bearing potential at screening and repeated no more than 24 hours prior to administration of primaquine, with all pregnant women being excluded. Clinical laboratory tests that are needed to guide subject treatment for emergent clinical malaria will be requested by the attending clinician in accordance to the Kenya MoH guidelines.

8.1.9 Other assays or procedures

8.1.9.1 Malaria blood film microscopy

Two glass microscope slides will be prepared by the project laboratory technician from the whole blood sample obtained from each volunteer at the screening visit, and prior to mosquito feeding assays in accordance to site SOPs, each with one thick and one thin blood film. The slides will be batched and read at a later date and thus will not influence the decision of whether to perform feeding assays or not. The primary purpose of collecting the slides is to classify gametocyte carriage as microscopic or sub-microscopic during data analysis. The slides will also be used as a quality control tool for the molecular assays described in section 8.1.8.2. An additional slide may be collected in an acutely ill subject to guide management – this slide will be read immediately, and the results shared with the study clinician.
A minimum of 200 microscopic fields will be examined to determine presence or absence of sexual (gametocytes) and asexual malaria parasites on the thick smear. If asexual parasites are found on the thick smear, the thin smear will be evaluated for *Plasmodium* speciation. If gametocytes are present, the male to female ratio will be determined. The findings of two expert microscopists will be considered. A third expert microscopist will be used to resolve any discrepant results.

8.1.9.2 *Plasmodium* detection molecular assays

Gametocyte RT-PCR will be performed overnight or on the day following blood collection on all eligible subjects that consent for the study and results will be used to guide mosquito feeding assays to be performed on gametocytemic individuals. Total nucleic acids (DNA/RNA) will be isolated from blood

samples and then PCR amplification of the *Plasmodium* 18s small subunit ribosomal RNA (rRNA) metabolic gene will be performed to detect *P. falciparum, P. ovale and P. malariae* parasites. For gametocyte RT-PCR, RNA will be reverse-transcribed and the resulting cDNA will be used to amplify gametocyte-specific gene targets. Among the markers that shall be used are *pfs16*, *pfs25* and *pfGMET.* For quantification, positive control standards with known densities will be included and used to generate a standard curve. Parasite density of unknown samples will then be extrapolated from the standard curve. These assays will specifically help quantify both parasitemia and gametocytemia below the current level of detection of microscopy. Participants who are found to have gametocytes by the RT-PCR will be invited to participate in mosquito feeding assays.

8.1.9.3 Oocyst and sporozoite detection and quantification

Approximately half of fed mosquitoes will be maintained for 9-10 days post feeding for detection and quantification of oocysts in the midgut. Briefly, midguts will be dissected from mosquitoes, stained with 1% mercurochrome and examined by microscopy. Oocyst numbers in each midgut will be recorded.

The remainder of fed mosquitoes will be maintained for 14-15 days post-feeding for detection and quantification of sporozoites. Briefly, salivary glands will be dissected from live mosquitoes submerged in phosphate-buffered saline (PBS) in order to visualize motile sporozoites by microscopy. Sporozoite prevalence will be recorded.

The dissected mosquitoes will be processed for detection and quantification of oocysts and sporozoites by molecular methods. After microscopy, slides containing midguts will be flooded with PBS to allow easy removal of coverslips, and excess mercurochrome will be removed by dragging the midgut gently through a clean PBS droplet. Mosquito carcasses will then be homogenized in glass grinding tubes with 180 µl of PBS and stored in 180 µL of DNA Tissue Lysis buffer at -80^{0}C in preparation for PCR analysis.

Nucleic acid extraction will be performed using commercial extraction kits following the manufacturer's instructions. *P. falciparum* parasite detection and quantification will be undertaken using the 18S rRNA gene target. To quantify the number of genomes detected in the mosquito midguts and salivary glands using the 18S qPCR assay, a standard curve generated using synthetic plasmid DNA will be used.

8.1.10 Specimen Preparation, Handling, and Storage

Whole blood collected in EDTA tubes from the field will be sent to the MDR central laboratory located on the KEMRI campus for processing (see section 8.1.3 for details). Specimens will be stored at KEMRI/USAMRD-A facilities. Filter paper and whole blood samples will be stored at -80^{0}C. DNA and RNA extracts will be stored at +4^{O}C if intended for immediate use or -20^{O}C if intended for near future use. The remaining DNA and RNA will be stored appropriately at -80^{0}C and retrieved whenever they need to be analyzed. Serum will be stored at -80^{0}C until required for analyses. At the end of the study, after all analyses have been completed, any remaining samples will be destroyed.

9. ASSESSMENT OF SAFETY

9.1 Adverse Events (AE)

Local solicited AEs, as defined in this protocol, include pruritus and erythema at the site of the mosquito bite, in a study participant following DSFA on days 1 and 2. Unsolicited AEs will be captured in study participants after informed consent is obtained and until the subject completes all study procedures.

9.2 Serious Adverse Event

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.3 Classification of An Adverse Event

For AEs, the following guidelines will be used to describe severity.

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events prevents a participant's usual daily activity.

9.4 Time Period and Frequency of Event Assessment and Follow-up

The investigator will record all reportable events with start dates occurring any time after informed consent is obtained until the last day of study participation. All AEs not meeting the criteria for severe adverse events (SAEs) will be captured on the appropriate case report form.

9.5 Reporting Procedures

9.5.1 Adverse event and Serious Adverse Event reporting

All serious adverse events (SAEs) will be promptly (within 48 hours) reported to the KEMRI SERU and to the WRAIR IRB. Prompt (within 48 hours) reports will be submitted to the KEMRI SERU by email (ERCAdmin@kemri.org; seru@kemri.org) and to the WRAIR IRB by phone (301) 319-9940, or by email (usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil), or by facsimile (301) 319-9961. The Principal Investigator will then submit written reports within 10 working days to the WRAIR IRB at the following address: Walter Reed Army Institute of Research, ATTN: Human Subject Protection Branch (HSPB), 503 Robert Grant Ave, Silver Spring, MD 20910.

Any SAEs or other events that meet PATH Research Ethics Committee's (REC) expedited reporting requirements will be promptly (within 24 hours) reported to the PATH Study Representative by email. The PATH Study Representative will then promptly (within 48 hours) submit the report to PATH REC using their online submission and reporting website (www.irbnet.org).

Follow up reports will be submitted as additional information becomes available. A summary of the non-serious adverse events and the SAEs (both related and unrelated) that occurred during the reporting period should also be included in the continuing review report to the KEMRI SERU, and the WRAIR IRB. The WRAIR HSPB will report SAEs to the USAMRMC ORP HRPO as per WRAIR SOP UWZ-C-636.

9.5.2 Unanticipated problems and UPIRTSOs

All non-serious unanticipated problems (events not involving risk to participants or others) will be reported in the continuing review/progress report to the WRAIR IRB and KEMRI SERU.

All serious unanticipated problems involving risk to participants or others should promptly (within 48 hours) reported to the WRAIR IRB and KEMRI SERU. The Principal Investigator will then submit a written report within 10 working days to the WRAIR IRB and KEMRI SERU.

Follow up reports should be submitted as soon as additional information becomes available. A summary of the serious unanticipated problems will also be included in the continuing review/progress report submitted to the WRAIR IRB and KEMRI SERU.

9.5.3 Pregnancies

Each pregnancy must be reported *promptly* **(within 48 hours of identification)** by email to the WRAIR IRB and KEMRI SERU.

9.5.4 Continuing Review/Progress Reports

The PI or designee will be responsible for submitting the required continuing review/progress reports and supporting documentation, as appropriate, to the WRAIR IRB and KEMRI SERU, allowing sufficient time for review and continuation determination prior the established continuing review/progress report date. A closeout report will be submitted at the end of the study upon completion of all the study activities or 5 years, whichever comes first.

9.5.5 Inspections and audits

Knowledge of any pending compliance inspections/visits by Office for Human Research Protections (OHRP) or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the KEMRI SERU (ERCAdmin@kemri.org; seru@kemri.org) and WRAIR IRB by phone (301) 319-9940, or by email (Usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil), or by facsimile (301) 319-9961. The WRAIR HSPB will report to USAMRDC ORP HRPO as per SOP UWS-HP-636.

9.5.6 Protocol deviations

Major protocol deviations are a subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being. The principal investigator will not implement any deviation from or changes to the protocol without the agreement of PATH and prior review from all IRBs expect where necessary to eliminate an immediate hazard to trial subjects or when the change involves only logistical or

administrative aspects of the trial. The PI will ensure that deviations from the protocol are documented and reported to PATH.

Major/significant deviations must be reported to the WRAIR IRB and KEMRI SERU within 48 hours of becoming aware of the event. A written report must be submitted within 10 working days of knowledge of the event to the WRAIR IRB and KEMRI SERU.

All deviations, including minor/non-significant deviations, will be reported to the WRAIR HSPB/IRB and KEMRI SERU in a cumulative summary report with each continuing review/progress report and with the study closeout report. These reports will include descriptions of the deviation, any actions taken in response to the deviation and an assessment of the impact of the deviation.

All subject-specific deviations from the protocol (e.g., failure to return for follow-up visits or blood collection within the time indicated in the protocol) are to be documented. The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or SSP. Action taken in response to the deviation, and the impact of the deviation will be assessed by the PI or sub investigator and recorded as Major or Minor as per the WRAIR HSPB definitions. Deviations will be reported in the continuing review/progress report to the WRAIR IRB and the closeout report.

Any protocol deviation that adversely affects the safety or rights of a subject or scientific integrity of the study, the deviation will be reported within 48 hours of recognizing the deviation, to the WRAIR IRB and KEMRI SERU with a written report submitted within 10 working days.

10. STATISTICAL CONSIDERATIONS

10.1 Overview and General Design

This is a single center and single group study designed to better understand the relationship between parasite transmission from humans to mosquitoes, within a person, across persons, and by both DSFA and DMFA performed at two consecutive time points.

10.2 Statistical Hypothesis

We are interested in assessing the equivalence of oocyst prevalence measured in the same subject undergoing DSFA at two consecutive 24-hour time periods. If the difference in oocyst prevalence measured on two consecutive days is represented as Δ, the null (H_0) and alternative (H_1) hypotheses of the primary efficacy endpoint analysis are as follows:

H0: $\Delta > \Delta_E$ or $\Delta < -_{\Delta E}$

H1: $-_{\Delta E} < \Delta < \Delta_E$

Where Δ¬E represents a value such that range that would support the use of a “before-after” trial design in a future mAb study.

10.3 Sample Size

This is a clinical investigation study where there is no prior knowledge related to the oocyst prevalence or density on a repeated DSFA or DMFA in a human subject. As such, the sample size chosen for this study was primarily based on logistical and budgetary considerations that would estimate the variability in DSFA. The results of this experimental medicine trial would be to ascertain the feasibility of using a paired "before-after" study design in same individual in a future trial when a TRI is introduced.

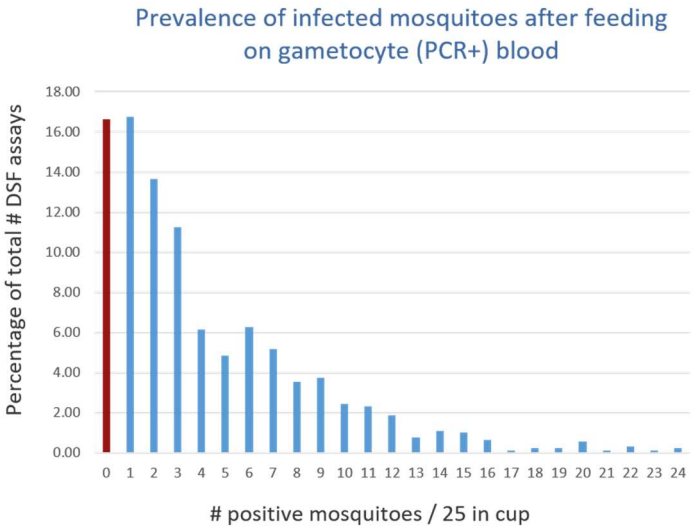
There are two unknowns and a major constraint in arriving at a meaningful sample size for the current trial challenging. First, there is no prior data in metrics of DSFA in same person at 2 consecutive time points. Secondly, there is a wide range of initial gametocyte densities in persons at baseline screening which may markedly influence the transmission and development of parasites from man-to-mosquito and detection of oocysts in the mosquito midgut 9 days after a blood meal.

Prior data obtained from the same site and same investigators in Kenya indicate that:

Approximately 25% of persons at any one time will have positive *P. falciparum* gametocytes by PCR

Of those that are positive *for P. falciparum* gametocytes and who have undergone DSFA, there will be a distribution of oocyst-positive mosquitoes (defined as positive event) detected 9 days after a mosquito feed as shown below.

Figure 9: Prevalence of infected mosquitos. Unpublished data from previous study

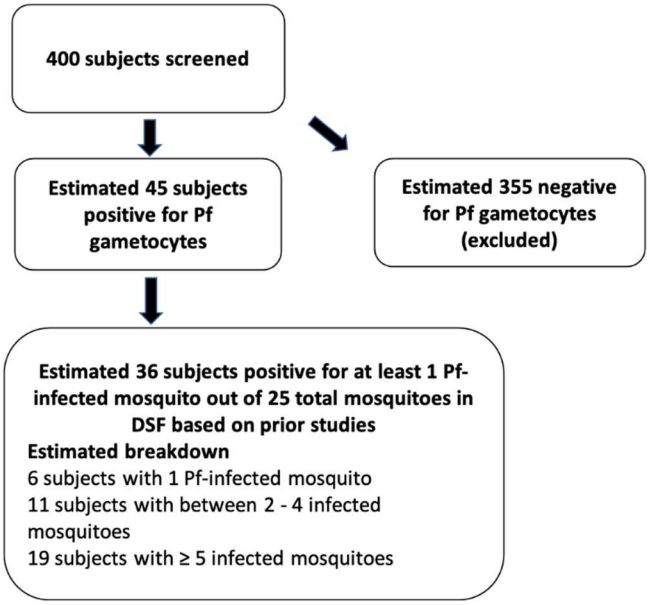


It is important to note that in approximately 16% of experiments with PCR-positive gametocytes, there will be no (zero) infected mosquitoes detected.

Greater than 50% of DSF assays have < than 5 infected mosquitoes out of 25 that were found to have oocysts detected in the midgut 9 days after a blood meal.

As the budget for the trial is constrained, we estimate a sample size below that will have 45 consented individuals out of 400 screened with positive *P. falciparum* gametocytes of whom will have an estimated transmissibility on day 1 indicated in the figure below. It is unknown whether the same level of transmissibility occurs on day 2 in the same person which is the subject of the proposed trial. The sample size proposed below provides for a reasonable and logistically feasible number of subjects (n=36) that will have two consecutive DSFA performed across a range of positive events.

Figure 10: Study sample size

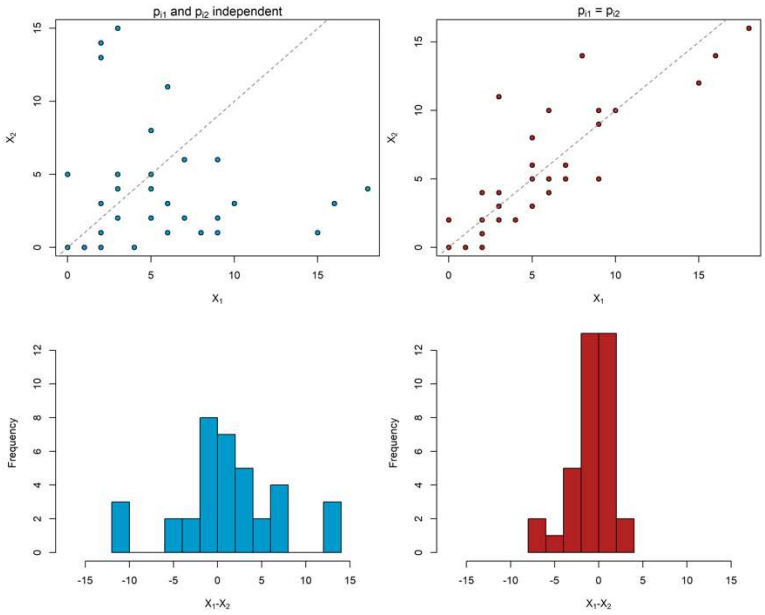


With these limitations in mind a simulation approach was used to understand the power to reject various equivalence null hypotheses ($-\Delta_E$, Δ_E) assuming either high correlation within subjects for consecutive DSFA or independence between measurements.

Briefly, we assume that the number of positive mosquitos for any DSFA is binomially distributed $x_{it} \sim Bin(25, p_{it})$ where $p_{it} \sim Beta(1.5,6)$. The parameters for the Beta distribution were selected to generate a distribution of positive mosquitos which achieves, approximately, the characteristics listed in points 3 and 4 above (16% with 0 positive mosquitos and 50% less than 5). For a single draw of $x_t = x_{1t},\ldots,x_{36t}$, the distribution of mosquitos looks similar to the data observed above.

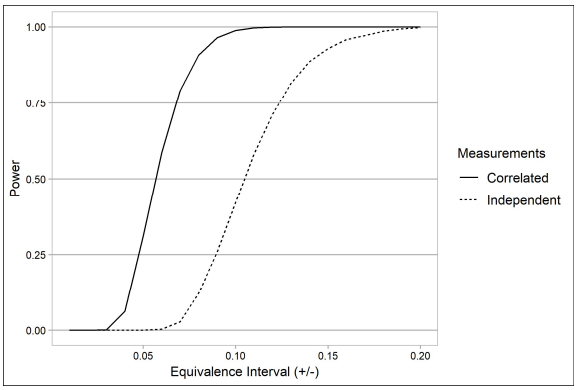
We considered two scenarios in our simulations. First, we assume that observations within person on consecutive days are independent. That is, $x_{i1} \sim Bin(25, p_{i1})$ and $x_{i2} \sim Bin(25, p_{i2})$ where p_{i1} and p_{i2} are independent draws from the Beta distribution. Second, we assume the underlying transmission probability within a person is constant for the two consecutive feeds and $x_{i1} \sim Bin(25, p_i)$ and $x_{i2} \sim Bin(25, p_i)$ where $p_i = p_{i1} = p_{i2}$. A simulated example for these two scenarios is shown in the figure below.

Figure 11. Simulating scenarios with independent daily transmission probabilities within subjects (left) or constant transmission probabilities over 24 hours within subjects (right).



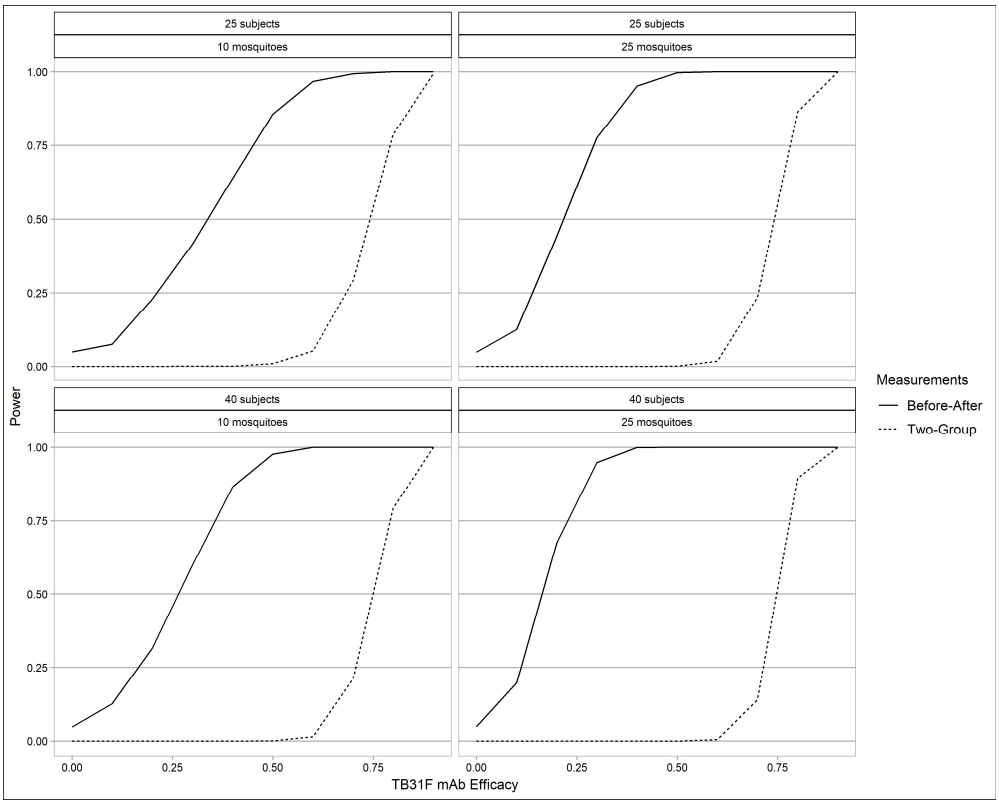
We simulated this experiment based on both of the scenarios and then used a paired t-test to test for equivalence in oocyst prevalence at various equivalence intervals ($-\Delta_E$, Δ_E). We find that with the current sample size of 36 subjects and 25 mosquitos per feeding assay, we will have 90% power to reject the null hypothesis that the difference of oocyst prevalence between consecutive feeding assays is greater than +/-7% for correlated data (scenario 2). However, the same equivalence interval (Δ_E=0.07) has only 4% power assuming independent data (scenario 1). Power for the equivalence test over a range of equivalence intervals and both scenarios are shown in the figure below.

Figure 12. Power calculation for equivalence test



The data collected in this study will help inform the design of follow on studies by informing parameter values of interest, such as the correlation between subsequent feeds, to assess the power to measure monoclonal antibody or drug efficacy with before-after or two-group designs under various samples sizes and efficacy assumptions. For example, in the figures below independent data has an average correlation of 0.0 and is compared with a t-test and correlated data has an average correlation of 0.5 and is tested with a paired t-test. Using the parameters of the simulated data above we can investigate the impact of sample size and design on the power to detect varying levels of efficacy. The real data from this study will help inform this parameter space.

Figure 13. Estimated power calculation for follow-on Phase 2b trial design



10.4 Definitions of Populations to be Analyzed

10.4.1 Enrolled population

All screened participants who provide informed consent.

10.4.2 Full analysis population

All participants in the enrolled population who participated in at least one direct skin feed.

10.4.3 Per Protocol population

All participants in the full analysis population who have no major protocol violations that are determined to potentially interfere with DSFA or DMFA results. This population will serve as the primary analysis population for the DSFA and DMFA assessments.

10.5 Analysis Methodology

All statistical methods shall be detailed in a statistical analysis plan (SAP) that will be finalized prior to database lock.

The number of subjects enrolled and having undergone DSFA will be summarized. Demographic data will be summarized by descriptive statistics. Except where otherwise indicated in this document or the SAP, summary statistics will be composed of the mean, standard deviation, 1st, 2nd, and 3rd quartile, and the minimum and maximum for continuous variables. For categorical variables, the count and proportion (using one digit beyond the decimal point) will be presented. Correlation coefficients will be used to summarize associations between measurements. Scatter plots and Bland-Altman plots will be used to graphically explore relationships between measurements. All study data will be presented in listings.

A full description of analyses will be described in the SAP, but brief descriptions have been provided in the subsequent sub-sections.

10.5.1 Analysis of Safety Data

All safety assessments will take place in the full analysis population. All subject-level percentages will be supplemented with two-sided 95% CIs computed via the Clopper-Pearson method. Summaries will include all events occurring on or after the date of first skin feed. Individual summaries (denominators for percentages) will be limited to the number of subjects within the appropriate analysis population with data available.

All unsolicited AEs, including serious and/or severe AEs will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA) version 20.1 or later. A summary table will be prepared for unsolicited AEs presenting incidence of any AE, any related AE, any serious AE, any severe AE, any AE of grade ≥2, any related AE of grade ≥2, and any AE leading to study withdrawal where a subject only contributes once.

10.5.2 Analysis of Primary Objectives

Oocyst prevalence

The variation in the proportion of infected mosquitoes with at least one oocyst (oocyst prevalence) in DSFA and DMFA performed at two consecutive time points in the same human subject will be assessed with the per protocol population. Summary statistics will be provided for each assay and by time point (first and second). Additionally, Pearson correlation coefficients and estimates of total variation decomposed by between and within person variation will be summarized for both DSFA and DMFA. A paired t-tests will be used to evaluate the equivalence hypothesis for DSFA and DMFA. Mean differences and the corresponding 95% confidence intervals (CIs) will be provided.

10.5.3 Analysis of Secondary Objectives

Oocyst density

The arithmetic mean oocyst density will be calculated for each subject by time point and assay. Distributions of subject-level means will be summarized by time point and assay. Medians will also be considered as a summary measure by subject. A generalized linear mixed model framework will be used

to incorporate the data from all mosquitos for each subject and summarize within and between person variability as well as summarize differences between time points. Variability and difference estimates (with 95% CIs) will be summarized for DSFA and DMFA. These analyses will be conducted on the per protocol population. A sensitivity analysis will be conducted using the full analysis population.

Sporozoite prevalence

The variation in the proportion of infected mosquitoes with at least one sporozoite in DSFA and DMFA performed at two consecutive time points in the same human subject will be assessed with the per protocol population. Summary statistics will be provided for each assay and by time point (first and second). Additionally, Pearson correlation coefficients and estimates of total variation decomposed by between and within person variation will be summarized for both DSFA and DMFA. A paired t-tests will be used to evaluate the equivalence for DSFA and DMFA. Mean differences and the corresponding 95% confidence intervals (CIs) will be provided.

Analysis of Exploratory Objectives

Oocyst and sporozoite density measured via qPCR

The arithmetic mean sporozoite and oocyst density as measured by qPCR will be calculated for each subject by time point and assay. Distributions of subject-level means will be summarized by time point and assay. Medians will also be considered as a summary measure by subject. A generalized linear mixed model framework will be used to incorporate the data from all mosquitos for each subject and summarize within and between person variability as well as summarize differences between time points. Variability and difference estimates (with 95% CIs) will be summarized for DSFA and DMFA. These analyses will be conducted on the per protocol population. A sensitivity analysis will be conducted using the full analysis population.

Assessing the relationship between oocyst and sporozoite density measured by DSFA and DMFA via microscopy and qPCR

Pearson correlation coefficients and Bland-Altman plots will be used to explore relationship between density measures as measured by DSFA and DMFA via microscopy and qPCR. The regression framework described above will be expanded to explore mean differences in measures. Estimated mean differences and corresponding 95% CIs will be reported. These analyses will be conducted on the per protocol population. A sensitivity analysis will be conducted using the full analysis population.

10.6 Handling of Dropouts and Missing Data

In this experimental medicine study, missing data will be assumed to be missing completely at random, and only observed data collected from participants and available in the appropriate study population will be used for analysis.

10.7 Timing of Analyses

Upon completion of the second DSFA and DMFA for the final participant, a topline analysis will be initiated to include the results of the primary and secondary oocyte prevalence and density results.

Following collection of the remainder of the data and database lock, all results will be described in a peer reviewed publication.

11. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The KCRC site will maintain appropriate medical and research records for this trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

12. QUALITY ASSURANCE AND QUALITY CONTROL

The KCRC site has SOPs for quality management that include staff training methods, product accountability records, specimen tracking logs, questionnaires, audio or video recordings.

13. ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Ethical Standard

This study will be conducted in accordance with the latest revision of the Declaration of Helsinki (2013), the Medical research Involving Human Subjects act (WMO), the ICH Good Clinical Practice, Department of Defense (DoD) requirements as outlined in 32 CFR 219, 45 CFR 46 and local rules of KEMRI SERU. The investigators are responsible for obtaining all relevant ethical approvals of the protocol and any subsequent amendments in compliance with local law before the start of the study.

13.2 Institutional Review Board

13.2.1 Ethics Approval

Submission of the protocol and supporting documents will occur in accordance with local regulatory requirements. KEMRI SERU is the IRB of record for the Kenyan Site, Kombewa in Kisumu. This protocol will be submitted to KEMRI Center Scientific Committee for review and approval. It will also be submitted to the KEMRI SERU where ethical aspects of the protocol will be reviewed before the protocol can be approved.

The Walter Reed Army Institute of Research IRB will also be involved in the approval process for this protocol. WRAIR IRB is the IRB of record for US Army Medical Research Directorate-Africa (USAMRD-A). For this reason, approval from the WRAIR IRB will be sought alongside the KEMRI SERU approval prior to implementation of the protocol or protocol amendments for this study.

PATH will delegate ethical review and oversight to KEMRI SERU.

13.2.2 Protocol Amendments

All amendments and modifications will be submitted to the WRAIR IRB and KEMRI SERU for review and approval. No changes in protocol conduct will be implemented until approval is obtained from the

WRAIR IRB and KEMRI SERU, as applicable, unless required to eliminate apparent immediate hazards to the study subjects. Amendments and modifications cannot be implemented until the WRAIR Commander's Memorandum has been issued. The WRAIR HSPB will submit amendments to USAMRMC ORP HRPO as per WRAIR SOP UWZ-C-636.

Non-significant amendments from the sponsor that are defined as amendments that do not affect the safety and wellbeing of the subjects at the site, and that are more administrative will also require both WRAIR IRB and KEMRI SERU approval prior to implementation.

13.3 Informed Consent Process

13.3.1 Consent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study procedures, and risks and benefits are given to the participant and written documentation of informed consent is required prior to starting the intervention.

13.3.2 Consent Procedures and Documentation

Discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant (or the consent witness) will be asked to read and review the document. The investigator or designee will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. Thereafter, signature of the written informed consent will be obtained from each participant who wants to participate in the study. The participant and the study staff conducting informed consent explanation will sign and date the consent form. The participant signature confirms that (s)he has understood the information. For illiterate individuals, the informed consent process will be conducted in the presence of a literate impartial witness selected by the participant but not a member of the CRC staff. The participant will thumb print the consent form and the witness will write the participant's name and sign and date the consent form. In case of any amendments to the protocol resulting in changes in study procedures, the participant will be informed, and additional informed consent will be obtained if necessary. In accordance with Good Clinical Practice, the volunteer may terminate participation in the study at any time for any reason without penalty. Additionally, if the participant is unable or unwilling to adhere to the protocol design, the investigator may terminate volunteer's participation. The study team will use the participant's preferred language usually English, Kiswahili or Luo, for informed consent. The participant will sign the informed consent document prior to any procedures being done specifically for the study. A copy of the informed consent document will be given to the participants for their records.

13.4 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence to the extent possible. No information

concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor. When the results of the research are published or discussed in conferences, no information or names will be used that would reveal identity. Authorized representatives of the Sponsor, KEMRI/Walter Reed Project (WRP), or representatives of the USAMRDC, as part of their responsibility to oversee the research and protect human subjects, are eligible to review records of individual subjects. As a result, they may see one or more names, but they will be bound by rules of confidentiality not to reveal anyone's identity to others. No names will be used in any report resulting from this study.

All study documentation containing personal information relating to study subjects like consent forms and other documents that might link subject ID with other subject personal details will be kept in a secure locked area with limited access at USAMRD-A/Kenya facilities. Such documentation will only be made available to authorized personnel. These study documents will be made available to the investigators, clinical personnel who require this information to treat the subject and the above-mentioned personnel for inspection or auditing reasons. In addition, access to subjects' medical records will be granted to representatives of the Sponsor, regulatory agencies and ethics committees for the purpose of validating data. All electronic data kept at the investigator's site will be kept secure. Computer access will only be made available to authorized personnel
The study participant's contact information will be securely stored at the study site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

13.4.1 Future Use of Stored Specimens

There will be no future use of stored specimens. Dried blood spots and sera collected from subjects will be destroyed at the end of the study.

13.5 Compensation

13.5.1 Compensation for injury

Participants will be insured against injury caused by the study according to legal requirements and compensation for research related injury, should it occur. If a participant suffers injury directly attributable to participation in this study, the participant is asked to contact the PI using the emergency contacts that will be provided on the consent forms. They will also be provided the contacts of KEMRI SERU secretariat in the event that they would like to speak to someone independent of the trial. Appropriate treatment during the trial will be provided by the site personnel**.**

13.5.2 Compensation for subject participation

Study participants will receive KSh. 1,000 for each day they participate in the feeding assays as compensation for time lost. A participant who participates on the first day only will receive KSh 1000, while a participant who participates for two days will receive KSh. 2000. Transport reimbursement will be provided separately if required (when unit transportation is not available) taking into consideration the average cost of travel to the clinic: they will receive between KSh. 500 and 1,000 for scheduled visits, and 300 and 500 Kenya Shillings for unscheduled visits as determined by the study team on a case by case basis.

14. DATA HANDLING AND RECORD KEEPING

Data collection is the responsibility of the clinical study staff at the site under the supervision of the site Principal Investigator (PI). The PI is responsible for assuring that the data collected is complete, legible, attributable, accurate, and recorded in a timely manner. Data recorded in the CRF should be consistent with the data recorded on the source documents. All source documents and laboratory reports must be reviewed by the site team, who will ensure that they are accurate and complete.

14.1 Data Collection and Management Responsibilities

14.1.1 Source Data

All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies). [ICH E6 section 1.51].

14.1.2 Source documents

Original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries of evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial are considered Source Documents. [ICH E6 section 1.52]

14.1.3 Data Capture Methods

Clinical data is recorded in source documents, should be completed in a neat, legible manner to ensure accurate interpretation of data. Source documentation supporting the CRF data should document the dates and details of study procedures and subject status. The PI will ensure that all information in the CRFs and all source documents that support the data collected from each subject are maintained in a secure area and treated as confidential material.

The CRFs will be developed by local staff at KCRC and data entered into the database by KCRC staff.

14.1.4 Data Storage

All study documentation containing personal information relating to study subjects like consent forms and other documents that might link subject ID with other subject personal details will be kept in a secure locked area with limited access at the KCRC. Such documentation will only be made available to authorized personnel. These study documents will be made available to the investigators, clinical personnel who require this information to treat the subjects and the above-mentioned personnel for inspection or auditing reasons. In addition, access to subjects' medical records will be granted to representatives of the Sponsor, regulatory agencies, ethics committees, and the USAMRDC for the purpose of validating data.

14.2 Study Records Retention and Disposal

Study documents should be retained for the duration required by PATH, applicable local regulations, and in according to U.S. DoD requirements, in a safe and secure facility. No records will be destroyed without the written consent of PATH. Upon study completion, all records will be stored safely at one of USAMRD-A's archiving facilities or at a secure contracted storage facility for at least 5 years. If storage at an alternative facility is considered, this will be documented and the documentation will be filed in the study's regulatory file. Should longer storage be recommended by the sponsor or relevant authorities, the site will contact the reviewing IRBs for consideration and approval. Study files can be stored either as paper or digitally. Digitization, if done, will consist of the scanning of all paper records according to site SOPs to ensure accurate and complete representation of the same. This may be done by the site staff of a contracted third-party. All digitized records will be maintained on a secure, password protected computer system with access limited to those with access to study as previously listed in this protocol as well as USAMRD-A's archivist(s) and IT personnel who will maintain the database. Upon completion of digitization of study records, and receipt of a sponsor's approval, paper copies may be destroyed by incineration according to site SOPs.

Paper or digitized records may be disposed of after storage duration has been met (5 years or as otherwise specified as above) and with the sponsor's approval. Any remaining paper records will be incinerated and/or digitized records will be securely deleted. The final disposal of any remaining paper or digitized records will be witnessed by a representative of USAMRD-A's regulatory affairs department and will be documented. The documentation of records disposal will be provided to the sponsor and a copy will be stored at the Regulatory Affairs department.

14.3 Publication and Data Sharing Policy

Public availability of data at the end of this trial will be posted on a recognized clinical trial registry such as www.clinicaltrials.gov.

This study will comply with PATH policy, which ensures that the public has open access to the published results of Gates Foundation funded research, as well as US DoD publication and data sharing policies. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication.
The first publication or disclosure of study results shall be a complete publication or disclosure coordinated by PATH.

15. KEY PERSONNEL ROLES

Dr. Ben Andagalu, Principal Investigator: will be responsible for the overall conduct of the trial at this study site, administrative actions between USAMRD-A/K, Institutional Review Boards, and the sponsor, oversight of field activities and patient care. He contributed to the protocol development and will contribute to the statistical analysis and writing of the scientific report(s).

Kombewa CRC Sub Investigators: will be responsible for the clinical conduct of the trial, the care provided to patients, oversight of field activities. They contributed to the protocol development and will contribute to the statistical analysis and writing of scientific report(s).

MDR/MDC/Entomology laboratory Sub Investigators: will be responsible for the execution of the various research assays of the trial. They contributed to the development of the protocol and will contribute to the statistical analysis and writing of the scientific report(s).

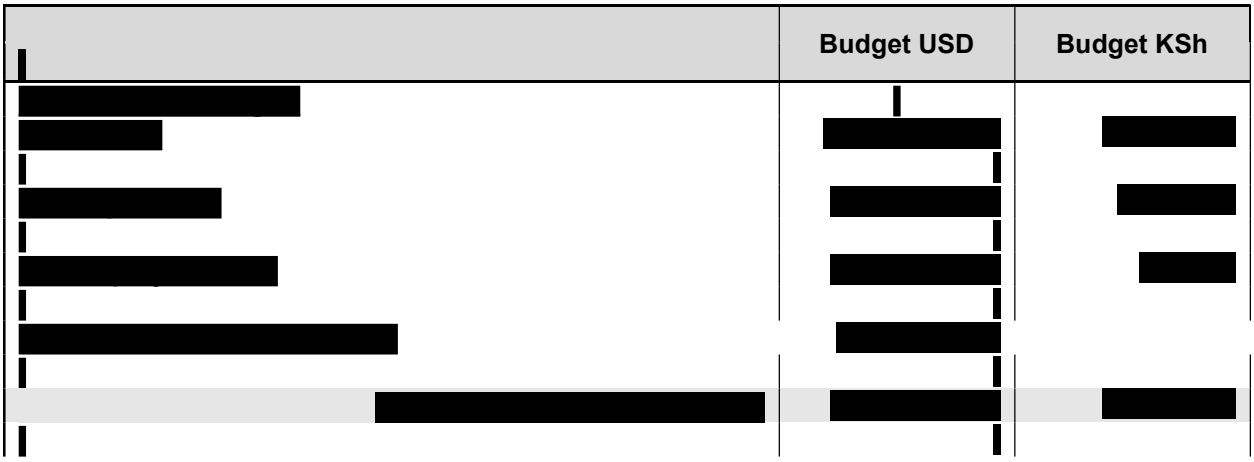
Study pharmacist: will be responsible for the accountability of medications used in the study – including dispensing and maintenance of accountability logs

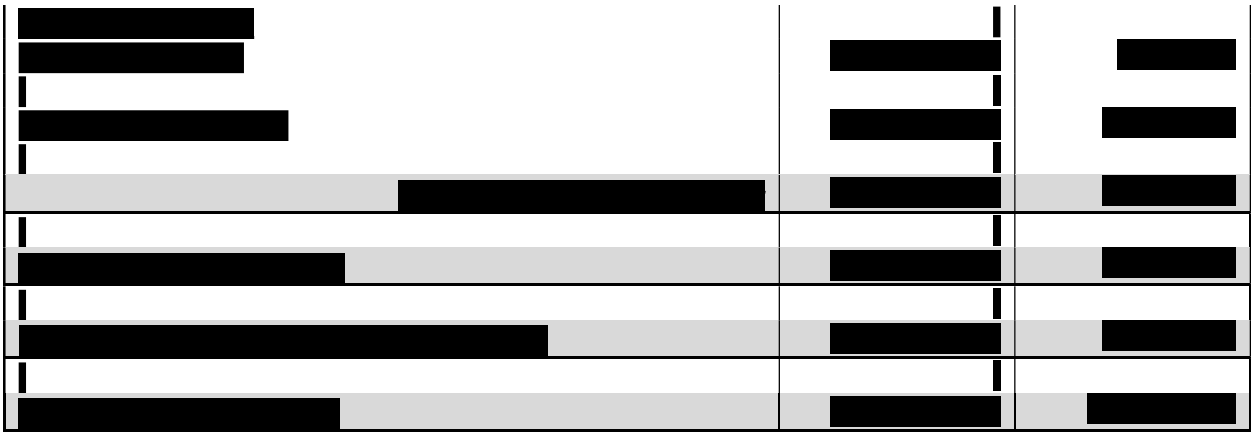
Study clinical team (clinical investigators, nurses and clinical officers): will be responsible for recruitment and administering consent procedures

16. MILITARY RELEVANCE

US Service Members conduct operations in regions of the world where they are at risk of malaria acquisition. Acquiring malaria is significantly detrimental to individual Service Members and their units resulting in lost duty hours, increased utilization of medical resources in the field (often in austere settings), potential requirement for medical evacuation, risk of death to individual Service Members, and risk to a unit's mission if a significant proportion or key individuals become ill. As such medical countermeasures to malaria are essential in malaria endemic regions and include effective treatment, effective prophylaxis, and immunoprophylaxis (such as a vaccine). Additionally, efforts to decrease or eliminate malaria transmission in endemic regions (such as transmission blocking interventions) would also support the Warfighter through decreasing or eliminating the risk of malaria in a given region. Efforts such as this which are aimed at facilitating the development of transmission blocking interventions contribute to the medical readiness of US Service Members indirectly. While it is unlikely that an agent that would be tested with assays such as the DMFA would be directly used in a Service Member, the overall effort is directed at interventions that do contribute to the health and readiness of our Service Members, thus giving it relevance to the US Military. Additionally, this supports the efforts of the US Africa Command as their major lines of effort include building sustainable partnerships, improving the health infrastructure of the countries in their Area of Responsibility (AOR), and "setting the theatre" (in which, decreased malaria risk is directly relevant to Service Members deploying to the AOR).

17. BUDGET





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