Human Subjects Protocol

Title:	Feasibility of daily dried blood spot sampling to evaluate the natural history of low-density *Plasmodium* infections
Protocol No.:	MMDL-001
Version number and date:	Version 1.1 (05MAR2020)
Principal Investigator:	Sean C. Murphy, MD/PhD
Sponsor:	U.S. National Institutes of Health

Protocol Version Log

Version	Date	Comments
1.0		Original version submitted to NARC
1.1	05MAR2020	Resubmission to NARC that addresses several requested changes including: • Additional information about the at home and in clinic blood draw procedures • Additional measures in place to monitor for possible tetanus infection and response • Changed the word “trial” to “study” throughout • Removed redundancies between inclusion and exclusion criteria • Clarified points about study protocols and analyses.

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Protocol Signatures

By my signature below, I agree to conduct the study according to this protocol version as approved by my IRB of record, in accordance with applicable IRB requirements, Good Clinical Practice guidelines, and federal and local regulations. I will not modify the protocol without first obtaining an IRB-approved amendment and new protocol version unless it is necessary to protect the health and welfare of study subjects.

Sean C. Murphy

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Investigator Name

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Thomas G. Egwang

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Investigator Name

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Investigator Signature Date

Protocol Synopsis

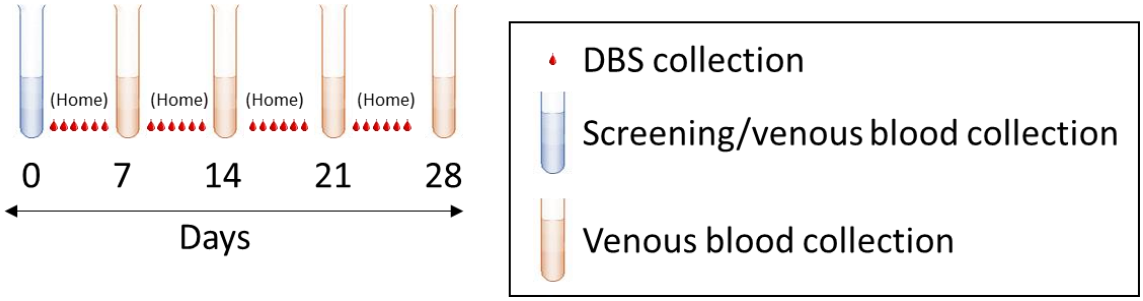
Protocol MMDL-001 Synopsis	
Protocol Title	Feasibility of Daily Dried Blood Spot Collection to Study the Natural History of Asymptomatic Malaria Infection to Inform Malaria Elimination
IND Number	N/A
Study Objectives	**Primary Objective:** • To determine the feasibility of daily at-home dried blood spot collections as a malaria epidemiology investigative tool. • To quantify the daily 18S rRNA and gametocyte mRNA-based parasite density profiles of asymptomatic Ugandan adults and children (aged 8-17 years old) over a 28-day (+/- 2 days) period. **Secondary Objectives:** • To evaluate the quality of human ribosomal RNA collected using daily, at-home blood spot collections. • To determine the prevalence of asymptomatic malaria infection in persons living in the Katakwi District. • To quantify the proportion of individuals with asymptomatic malaria infection whose parasite densities follow each of the four profiles over the 28-day (+/- 2 days) period: (1) continually decrease (2) continually increase, (3) remain constant, (4) oscillate. • To quantify the proportion of asymptomatic infection individuals that progress to symptomatic malarial disease over the course of the study period. • To quantify the correlation between parasite and gametocyte densities during the 28-day (+/- 2 days) sampling period. **Exploratory Objectives:** • To determine covariates associated with different parasite density profiles. • To utilize the at-home collected blood spots to assess multiplicity of infection and related epidemiological parameters. • To identify genetic signatures in the parasite and/or human host associated with asymptomatic infection • To evaluate other immune responses to better understand malaria immunology
Study Subjects	A total of 100 adult and 30 pediatric (ages 8-17 years old) study subjects who show no signs of clinical malaria and are RDT-negative at enrollment will be recruited into the study.
Key Inclusion Criteria	• Males and female children aged 8-17 years old (with assent procedures) and male and female adults 18-60 years • Asymptomatic for Grade 2 or higher malaria-related signs and symptoms and afebrile (<38.0°C) at baseline • Lives in one of the seven villages in Katakwi District, Uganda • Does not plan to move out of the study area for one month following enrollment. • Willing to self-collect a daily blood spot sample for 28-days (+/- 2 days).

	• Willing and able to return to the study clinic on a weekly basis to turn in DBS cards and provide 5 mL (adult) or 1 mL (child) of blood through venous draw at each visit. • Reliable access to the clinical sites and availability to participate for duration of study. • Able to fully understand the implications of study participation and provide informed consent. • Able to provide assent (for child participants) • Agreement to come to the study clinic if study participant experiences febrile illness or prick site infection during the study period. • Agreement not to take anti-malarial medications unless through the Malaria Clinic at St. Anne Health Center III. • Agreement not to take other medications without informing the study team. • Absence of any significant chronic disease.
Key Exclusion Criteria	• Being pregnant at enrollment or planning to get pregnant during the study period. • Currently taking antimalarial treatment(s). • RDT-positive at screening • Any other finding that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety, or a subject's ability to give informed consent, or increase the risk of having an adverse outcome from participating in the study.
Overview of Study Design	This is an exploratory, single-arm study designed to longitudinally collect blood from asymptomatic adults aged 18-60 years old and children aged 8-17 years old in Uganda for the purposes of evaluating this blood collection approach and for studying the behavior of *Plasmodium* infections over time using sensitive molecular diagnostics. The purpose of this research is to evaluate the feasibility of the collection procedure in the community and use the data collected to better understand the natural history of the silent (no fever or signs) and invisible (undetectable) malaria (asymptomatic malaria) in asymptomatic persons, including how parasite densities change over time. Volunteers will be recruited to the study following community introductions and informational meetings. The study aims to recruit 100 healthy adults 18-60 years old and 30 children aged 8-17 years old. After screening and consenting, participants will undergo an enrollment intake examination. Basic demographic and health data will be taken on all study participants, including history of antimalarial use, bednet ownership and use, age, sex assigned at birth, area of residence, proximity to a swamp, season, height, weight, temperature, and hemoglobin levels in order to capture possible risk factors for asymptomatic malaria, use of malaria prevention strategies, and illnesses in the previous two weeks. At enrollment, all participants with no signs of clinical malaria infection will be tested onsite with a Rapid Diagnostic Test by fingerstick blood

	collection. The RDT will be selected that detects both *P. falciparum* histidine rich protein 2 (PfHRP2) and *Plasmodium* lactate dehydrogenase (pLDH). Prospective participants who are RDT-positive will be referred to the Malaria Clinic at St. Anne Health Center III to determine if they should be treated or not and such persons will not continue in the study. Eligible, RDT-negative persons will be enrolled in the full study and will receive a dried blood spot (DBS) collection training session at the enrollment visit. They will also have 5 mL (adult) or 1 mL (child) of blood drawn from a vein, which will be stored for testing at a later date. At this visit, all study participants will be trained in self-collection of DBS samples including collection of a Day 0 blood spot by fingerstick. After training, participants will be given DBS cards for the first week and will be sent home with the cards, storage materials, and study-related information. During the first week, participants will collect daily DBS samples on their cards at home and will store them in gas-impermeable plastic bags with desiccant provided by the site. Automatic self-retracting lancets (prickers) will be used for collection and used prickers will be stored in a sharps container provided by the site. Participants will return to the study site weekly (Days 7 (+/- 2 d), 14(+/- 2 d), 21(+/- 2 d), and 28(+/- 2 d)) to submit DBS cards and used prickers and receive new ones, receive additional training on DBS collection (if needed), answer a few questions about their health and malaria prevention habits in the previous week, have their temperature taken, and have a 5 mL (adult) or 1 mL (child) blood draw which will be stored and tested at a later date. DBS and blood samples will be preserved in order to perform *Plasmodium* 18S rRNA RT-PCR, gametocyte mRNA RT-PCR, and human 18S rRNA RT-PCR at the University of Washington. At any time during the study, any febrile participant will be offered an RDT on demand, and RDT-positive persons will be treated according to national treatment protocols, and participation in the study would end. Persons who are febrile and RDT-negative will be further evaluated by the clinic staff through additional tests and evaluations. Any person deemed to have malaria by clinic staff will also be treated as per national guidelines and will be discharged from the study.
Intervention Regimen	There is no comparative intervention being tested in this protocol.
Study Endpoints	**Primary endpoints** • Number of completed at-home blood spot collections. • Number of completed in-clinic visit blood collections. • *Plasmodium* 18S rRNA biomarker test results on pooled blood spots from all RDT-negative participants • *Plasmodium* 18S rRNA biomarker test results on individual blood spots from all persons with positive pooled blood spots

	Secondary endpoints • Comparison of blood spot vs. venous blood Plasmodium 18S rRNA biomarker test results • Kinetic analysis of infections by biomarker testing over 28-day (+/-2 d) period • Human 18S rRNA as a measure of sample quality • Gametocyte-specific RT-PCR testing on blood spots as a surrogate of gametocyte carriage
Safety Monitoring	Participants with symptoms of malaria who test positive for malaria infection by RDT or other evaluation will be treated with an anti-malarial agent according to national guidelines. Any severe cases of malaria will be treated at the referral hospital, Katakwi General Hospital.
Study Duration:	Participation in the study will last for one month. During the study, participation will consist of five visits to the study clinic (days 0, 7 (+/-2d), 14(+/-2d), 21(+/-2d), and 28(+/-2d)), and daily at-home self-collection of DBS.
Study Sites:	St. Anne Health Centre III, Katakwi District, Uganda

Study Schema



Each test tube represents a clinic visit day with a venous blood collection. Each red blood drop represents a day for at-home collected dried blood spots.

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Abbreviations and Acronyms

AE	adverse event
C	Celsius
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CRF	case report form
d	day
DBS	dried blood spot
ELISA	enzyme linked immunosorbent assay
F	Fahrenheit
FDA	Food and Drug Administration
HIPAA	Health Insurance Portability Authorization Act
HRP2	Histidine rich protein 2
IC	informed consent
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
Kg	Kilogram
LDH	Lactate dehydrogenase
LLIN	Long-lasting insecticidal net
mL	Milliliter
MMDL	Molecular Malaria Diagnostic Laboratory
mRNA	Messenger RNA
P.	*Plasmodium*
PCR	polymerase chain reaction
Pf	*Plasmodium falciparum*
qRT-PCR	quantitative reverse-transcription polymerase chain reaction
RBC	red blood cell
RDT	rapid diagnostic test
RNA	ribonucleic acid
rRNA	Ribosomal RNA
SOP	Standard Operating Procedure
UNCST	Uganda National Council for Science and Technology
UW	University of Washington
WAC	Washington Administrative Code
WBC	white blood cell
WHO	World Health Organization

1.0 Background and Rationale

1.1 Burden of Disease

Malaria represents a major public health problem worldwide, causing significant morbidity and mortality in malaria-endemic regions and in previously unexposed persons including travelers and military personnel. Transmission occurs throughout tropical Africa, Asia, Oceania and Latin America as well as in some subtropical areas. Malaria parasite causes an estimated 200-500 million clinical cases annually resulting in approximately 0.5 million deaths, mostly in children [1]. Such statistics may be actually underestimated since studies from Ghana and elsewhere indicate that for every patient with clinically-diagnosed malarial febrile illness seen in health facilities, 4-5 episodes go undiagnosed in the community [2]. Beyond morbidity and mortality, malaria also levels an enormous and poorly understood economic burden on affected regions [3]. Malaria also remains a major public health threat to non-immune individuals, including travelers and military personnel. Increasing parasite drug resistance and mosquito insecticide resistance highlight the importance of developing an effective vaccine as a major global health priority [4].

1.2 Rationale for the Study

Malaria is the most prevalent and deadly human parasitic disease. To stem the worldwide impact of this devastating disease, a safe and broadly effective malaria vaccine and improved antimalarial therapeutics are urgently required. Current diagnostic tools like microscopy and rapid diagnostic tests (RDT) fail to detect a disproportionately large number of malaria infections with low parasite densities in endemic regions. Since the advent of more sensitive techniques, asymptomatic carriers have been revealed in endemic regions. **Such asymptomatic carriers contribute to ongoing transmission, but their exact role is poorly understood.** Asymptomatic carriers can be divided into two categories: RDT-positive and RDT-negative *but* ultrasensitive molecular test-positive. This study aims to test the utility of daily dried blood spot (DBS) collections tested by ultrasensitive quantitative reverse transcription polymerase chain reaction (qRT-PCR) test to better understand the dynamics of low-density parasite carriers over time.

A recent meta-analysis compared lateral flow RDTs to DNA PCR-based tests [5]. The RDT limit of detection (LoD) was 100-200 parasites/µL compared to 1-5 parasites/µL for the PCRs. On average, RDTs detected less than half of all PCR-detectable *Plasmodium falciparum* infections in cross-sectional population surveys [5]. RDTs detected the fewest number of PCR-positive infections in adults, suggesting that this age category comprises the highest proportion of low-density carriers, especially in low transmission settings. In areas where PCR prevalence was 5-20%, ***87% of all infections in individuals >15 years were undetectable by RDT***. Most would also be undetectable by the newly reported highly-sensitive RDT (HS-RDT, LOD ~1/µL) [6].

While widely accepted that low-density carriers exist, their contribution to ongoing transmission is less clear. **Most studies of low-density carriers are cross-sectional, such that samples are only collected at a single point in time. Therefore, there is limited information as to whether measured parasite densities will later increase to densities high enough to cause clinical symptoms or be detected by standard field tests, or if these infections are clearing and therefore do not contribute significantly to transmission.** The literature contains very few reports of longitudinal sampling. A 1997 study reported microscopy-derived parasite densities daily for 20

children in a holoendemic site in Tanzania and found 100-1000-fold variation in densities between days [7] – this study did not apply sensitive, quantitative molecular tools since they were not available at that time. A 2000 study in Mali collected three daily DBS samples from men over 13 days and found ≥100-fold variations in parasite densities within a 6-hour period [8]. In Mozambique, analysis of parasite densities collected at seven time points over 28 days in a cohort of asymptomatic men revealed that following an initial decrease in parasite densities, parasite densities continued to vary in some individuals over the 28-day period [9]. A recent study in a low transmission setting in Vietnam also showed that parasite densities in asymptomatic carriers oscillate over time [10]. In these studies, samples were collected in clinical settings, requiring a large amount of human resources and placing additional burdens on subjects to visit the clinic each day, which can lead to attrition. In the study in Mozambique noted above, 23% of participants missed one or more follow-up visits. New techniques that are low in cost, minimally invasive, and relatively easy to perform are needed in order to better understand the natural history of asymptomatic low-density *Plasmodium* infections in endemic areas.

Since there is limited data on the natural history of asymptomatic infections that would allow researchers to determine the contribution of low-density carriers in cross-sectional studies, there is no clear mandate to use more sensitive tests to identify and treat such persons. A recent publication by Drakeley and colleagues [11] described several theoretical trajectories of asymptomatic infection (**Fig. 1A**), which differentially affect our understanding of asymptomatic infection, carriage, and clearance. Several factors may contribute to such fluctuations including synchronized parasite sequestration, immune evasion, and superinfection by new strains. The Drakeley team identified several gaps in asymptomatic malaria research and three key metrics that need to be determined to understand how asymptomatic infections contribute to ongoing transmission:

(i) the proportion of asymptomatic individuals that later develop symptoms and seek treatment;
(ii) the distribution of the duration of asymptomatic infections in populations; and
(iii) the relative infectivity of asymptomatic infections.

There is a paucity of studies of the natural history of infection of asymptomatic malaria using sensitive, quantitative molecular techniques. As noted above, most information on asymptomatic infections comes from cross-sectional studies, which offer no insight as to the dynamics of the infection. Asymptomatic infections are difficult to study because they require frequent sampling, sensitive diagnostics, and the ability to differentiate between new and existing infections. Recognizing such problems, we and several other investigators are attempting to provide answers to the key epidemiological questions. In a recent study in Uganda, where transmission is high, blood was collected every two months for six months from 300 asymptomatic individuals [12]. The rate of co-infection was high in this population, with an average of 19 different strains found in children aged 5-9 years. Duration of infection was also highest in this age group, with a mean infection duration of 319 days. In the aforementioned low transmission setting in Vietnam [10], infections cleared more rapidly - ~20% of individuals cleared their infection within one month, and clearance followed a logarithmic decline for up to 24 months. However, in the Vietnamese study, it was not always clear if the first detected infection was newly acquired or pre-existing. This same study showed that parasite densities oscillate over time, and it was not uncommon for extremely low-density infections to evolve into higher densities. Our University of Washington laboratory also evaluated parasite densities in asymptomatic, HIV-positive malaria carriers and found parasite

densities to be highly variable over the month-long testing period (**Fig. 1B**). In some participants, densities oscillated between low (<10 parasites/µL) and high densities (>1,000 parasites/µL) in a period of two weeks (unpublished results). Daily sampling of children in Tanzania revealed oscillation patterns of infection on a daily basis, with some children dropping to undetectable levels on one day followed by >100 parasites/µL the next, but assessments were by microscopy, so little is known about the dynamics of infection at lower densities [7].

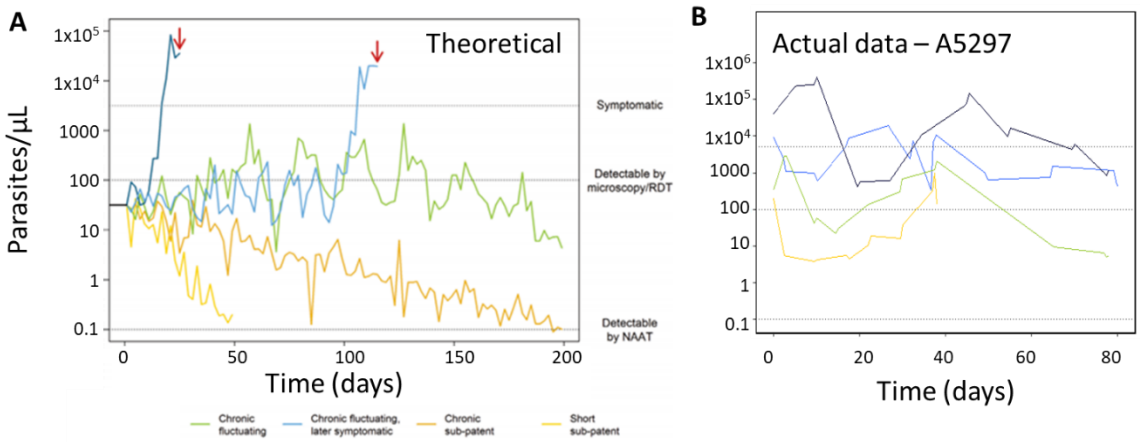

Figure 1. Hypothetical and actual data on asymptomatic *Plasmodium* infections in field settings. (**A**) Four hypothetical infection trajectories from [11]. (**B**) Actual infection trajectories from four HIV-positive, asymptomatically *Plasmodium*-infected persons as tested by qRT-PCR at the UW.

In studies that collected longitudinal data to study the natural history of infection, participants were selected that were positive by either RDT or microscopy at enrollment. Therefore, the individuals enrolled in these studies were not reflective of the low-density carriers that would be missed by control efforts currently utilizing RDTs and microscopy as diagnostic tools. **This study is designed to sample individuals who are not positive by RDT at baseline, and then use pooled qRT-PCR sampling to cost-effectively identify low-density carriers in the population to evaluate the dynamics of these infections over a 28-day period**.

The infectivity of asymptomatic persons needs to be more clearly understood, and several approaches are being used worldwide. A study in a low transmission setting in Ethiopia estimated that asymptomatic sub-patent *P. falciparum* infections contributed ~30% of the infectious reservoir [13]. In Burkina Faso, 32% of children with submicroscopic gametocytemia (average 7.9 gametocytes/µL) were infectious to mosquitoes [14]. Conversely, a study in Cambodia showed that none of their asymptomatic carriers infected mosquitoes, independent of the presence of gametocytes [15]. This study did not present asexual parasite or gametocyte densities, so it is difficult to compare with the Ethiopian and Burkina Faso studies to understand the critical differences. While there is no absolute threshold for infectivity, several studies have shown that low density gametocyte carriers can infect mosquitoes [14, 16, 17] including when just 1 gametocyte/µL is present [16]. The infectiousness of an individual rises with increased gametocytemia [14, 16, 17], and overall parasite densities are highly correlated with gametocyte densities [16, 18]. A recent systematic review of studies on infectiousness concluded that individuals with sub-patent infections are ~1/3 as

infectious as patent infections [19], a not insignificant number that suggests that sub-patent infections pose a real threat to elimination efforts. Here, we will estimate the infectivity potential of individuals in our study using parasite and gametocyte densities using published data to correlate between these values and infectivity. Since we will have daily parasite and gametocyte densities, we can estimate the potential contribution of an individual over time, given varying profiles of parasite kinetics. It is not feasible to directly measure infectivity using standard membrane feeding assays (SMFA) in this study.

2.0 Study Design

2.1 Primary Objectives

- To determine the feasibility of daily at-home dried blood spot collections as a malaria epidemiology investigative tool.
- To quantify the daily 18S rRNA and gametocyte mRNA-based parasite density profiles of asymptomatic Ugandan adults and children (aged 8-17 years old) over a 28-day (+/- 2 d) period.

2.2 Secondary Objectives

- To evaluate the quality of human ribosomal RNA collected using daily, at-home blood spot collections.
- To quantify the proportion of low-density asymptomatic infections in the study population.
- To quantify the proportion of individuals with asymptomatic malaria infection whose parasite densities follow each of the four profiles over the 28-day (+/-2 d) period: (1) continually decrease (2) continually increase, (3) remain constant, (4) oscillate.
- To quantify the proportion of asymptomatic infection individuals that progress to symptomatic malarial disease over the course of the study period.
- To quantify the correlation between parasite and gametocyte densities during the 28-day (+/-2 d) sampling period.

2.3 Exploratory Objectives

- To determine covariates associated with different parasite density profiles.
- To utilize the at-home collected blood spots to assess multiplicity of infection and related epidemiological parameters.
- To identify genetic signatures in the parasite and/or human host associated with asymptomatic infection
- To evaluate other immune responses to better understand malaria immunology

3.0 Subject Selection and Withdrawal

3.1 Inclusion Criteria

1) Male and female children aged 8-17 years old (with assent procedures) and male and female adults 18-60 years
2) Asymptomatic for Grade 2 or higher malaria-related signs and symptoms and afebrile (<38.0°C) at baseline
3) Lives in one of the seven villages in Osuk subcounty, Katakwi District, Uganda

4) Does not plan to move out of the study area for one month following enrollment.
5) Willing to self-collect a daily blood spot sample for 28-days (+/-2 d).
6) Willing and able to return to the study clinic on a weekly basis to turn in DBS cards and provide 5 mL (adult) or 1 mL (child) of blood through venous draw at each visit.
7) Reliable access to the clinical sites and availability to participate for duration of study.
8) Able to fully understand the implications of study participation and provide informed consent.
9) Able to provide assent (for child participants)
10) Agreement to come to the study clinic if study participant experiences febrile illness during the study period.
11) Agreement not to take antimalarial medications from outside the study.
12) Agreement not to take any additional medications without informing study staff.
13) Absence of any major chronic disease
14) Ability and willingness to donate 25 mL (adult) or 5 mL (child) of blood for qRT-PCR malaria parasite testing and have a finger stick for dried blood spot collection

3.2 Exclusion Criteria

1) Being pregnant at enrollment or planning to get pregnant during the study period.
2) Currently taking antimalarial treatment(s).
3) RDT-positive at enrollment
4) Any other finding that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety, or a subject's ability to give informed consent, or increase the risk of having an adverse outcome from participating in the study.

3.3 Subject Recruitment and Screening

A total of 100 adult subjects aged 18-60 years old and 30 pediatric subjects aged 8-17 years old meeting eligibility criteria will be included in the study. Subjects will be recruited from the St. Anne Health Center III catchment area through a variety of Institutional Review Board (IRB)-approved messaging and outreach methods. In addition, the study team will employ existing relationships with the Malaria Clinic as part of the recruitment strategy. Recruitment and screening activities outlined in Section 5.0 will take place at St. Anne Health Center III (Clinic site) Malaria Clinic, Katakwi District, Northeastern Uganda.

3.4 Early Withdrawal and Termination of Subjects

A subject may voluntarily withdraw consent (or assent in the case of children) for study participation at any time. A participant's voluntary withdrawal from the study will not affect future enrollment in other St. Anne Health Center III studies or for routine health care at the clinic.

Events that may lead to early termination of an enrolled participant include:

1) Subject withdraws consent or assent, refuses further participation or is unable to comply with study procedures.
2) Subject relocates to an area away from the clinic area and follow-up is not possible.
3) The Investigator determines that the subject is lost to follow-up.
4) The subject develops a symptomatic malaria infection.
5) Site investigator and/or clinic staff determine that the subject should be withdrawn from the study without his/her consent due to concerns that continued participation may present a risk to the health/safety of the subject, fellow participants or study staff.
6) The Investigator decides that the subject should be withdrawn or the study is stopped for any reason.
7) Subject develops an infection at the prick site.

4.0 Clinical facilities and resources

St. Anne Health Center III in Katakwi District, Northeastern Uganda, will be the study site. St. Anne has an antenatal clinic (> 150 monthly visits), labor ward (> 50 monthly deliveries), and a childhood immunization clinic (> 200 immunization visits monthly). In addition, there is a pharmacy, a laboratory with capacity to perform malaria microscopy and rapid diagnostic tests and other diagnoses including HIV/AIDS. Patients are treated by clinical officers, medical officers, nurses, and mid-wives. Within St. Anne, Med Biotech Laboratories (MBL) operates a 24-hour dedicated Malaria Clinic within premises provided by the Health Center. The Malaria Clinic has a waiting/reception area which can accommodate more than 50 study participants daily, a clinical consultation room, a well-equipped laboratory and phlebotomy room, a nurses' room, and a pharmacy with lockable metal cabinet for study files. Medical records are locked up in a strong metal cabinet in the pharmacy. A qualified and licensed study physician works closely with the hospital staff. He is aided by a clinical officer seconded by St Anne and four licensed nurses. Finally, the lab coordinator supervises three technicians who provide laboratory support to the malaria clinic. Infants who are judged by the clinician to be severely ill are referred to St. Anne or Katakwi General Hospital (KGH) for clinical management (St. Anne and KGH) and blood transfusion in case of severe anemia (KGH). In the event that transfer to KGH is required, the Malaria Clinic has a study vehicle that can transport patients.

5.0 Study Procedures

5.1 Screening

Prospective participants who respond to flyers or other study materials will be asked their age and whether they have recently had a malaria infection. Potentially eligible persons will be recruited to come to the clinic for an in-person screening/enrollment visit.

5.2 Informed Consent

Informed consent encompasses all written or verbal study information the St. Anne Health Center III study staff provide to the subject, before and during the study. All informed consent discussions will be documented by the study staff in the subject's source documentation. Consent discussions include, but are not limited to, background on malaria and rationale for this study, an overview of the study design, study procedures, requirements for participation in the study, and risks and benefits to the subject.

The initial informed consent process will take place at the screening visit, which is conducted by nursing and research study staff. Participants will be offered a copy of the screening consent at the screening/enrollment visit and will have the opportunity to take the consent home to read thoroughly at their own pace. Screening may occur over several visits or can be conducted at the same time as study enrollment. The screening/enrollment visit, including informed consent, screening questions, and if a participant signs a consent (and assent for pediatric participants) at that time, RDT test for malaria infection, finger stick training for dried blood spot collection, and venous blood draw, will last 1-2 hours.

Written informed consent will be obtained only by study staff trained in the protocol and designated on the study signature log, using the protocol-specific Informed Consent Form approved by the local IRB/Independent Ethics Committee (IEC) and developed and administered in accordance with St. Anne Health Center III policies and procedures and local IRB/IEC requirements.

Subjects will be provided with a copy of all consent forms that they sign. The original signed and dated copy will be kept on file in their study binder, stored in locked, limited access cabinets within the study site.

5.3 Assent (for pediatric participants)

Participants aged 8-17 years old are required to have consent from one parent (using a form specific for parents of child-aged participants) as well as assent of the child. For the assent process, the purpose of the study is explained to the child in age-appropriate terms, following the separate Assent document, including what will happen to them in terms of procedures if they decide to be in the study. The children are allowed to ask questions and they are told that they do not need to be in the study, even if a parent has consented for them to be in the study. The children are also reminded that they can change their mind at any time. The assent of the child will be verbally confirmed at each study visit and will be documented in the chart. Assent must be affirmative; failure to disagree will not be considered evidence of assent.

5.4 Assessment of Understanding

To ensure the participant (or parent of the participant) fully comprehends key concepts related to the study and to highlight areas that may need additional discussion or clarification, an Assessment of Understanding will be administered to the participant (or parent of the pediatric participant) after signing the informed consent. All incorrect responses will be reviewed with the participant or parent of the participant, and he or she must verbalize understanding of all incorrect responses. Those who fail to answer all questions correctly will have a discussion with the investigator to ensure understanding. Once participants or parents of participants have proven they understand the answers to all questions in the quiz correctly, they may then complete the screening and enrollment. At the discretion of the Investigator, any subject whose comprehension is questionable, regardless of score, may be excluded from enrollment. Discussions of understanding will be documented in the subject's source documents.

5.5 Enrollment

Subjects may be enrolled on the same day as the informed consent/assent procedures. Upon enrollment, consented participants will provide demographic and health data including history of antimalarial use, bednet ownership and use, age, sex, area of residence, proximity to a swamp, season, height, weight, malaria related symptoms including temperature (Appendix A), and hemoglobin levels in order to capture possible risk factors for asymptomatic malaria, use of malaria prevention strategies, and illnesses in the previous two weeks.

All consented/assented afebrile (<37.5 °C) participants without Grade 2 or higher signs or symptoms of clinical malaria infection will be tested onsite with a Rapid Diagnostic Test by fingerstick blood collection. The RDT will be selected that detects both *P. falciparum* histidine rich protein 2 (PfHRP2) and *P. falciparum* lactate dehydrogenase (pLDH). Prospective participants who are RDT-positive will be referred to the malaria clinic at St. Anne Health Center III to determine if they should be treated or not and such persons will not continue in the study.

Eligible, RDT-negative persons will be enrolled in the full study. They will have 5 mL (adult) or 1 mL (child) of blood drawn from a vein, which will be stored for testing at a later date. All study participants will be trained in self-collection of DBS samples and will collect a Day 0 blood spot by fingerstick. After training, participants will be given DBS cards for the first week and will be sent home with the cards, storage materials, and study-related information.

5.6 Blood Collection

As noted in Section 5.1, a fingerstick blood collection will be initially used on Visit 1 (Day 0) to perform the RDT required for eligibility assessment. Thereafter, a venous blood sample of 5 mL (adult) or 1 mL (child) will be drawn from RDT-negative persons at Visit 1 (Day 0) and an additional fingerstick will be performed for DBS collection as part of the DBS training.

5.6.1. Dried Blood Spot Collection Procedure

Dried blood spots (DBS) will be collected by fingerstick. The participant's finger will be disinfected with an alcohol swab. After waiting a few seconds for alcohol to dry, the person performing the fingerstick will quickly pierce the skin using a self-retracting sterile automatic pricker designed for DBS collection. Two 50 microliter blood spots will be placed on a standard DBS card, by saturating the entire circle on the provided card. The

finger will be swabbed again, and the participant will be offered a bandage. Cards will be dried and then they will be stored in gas-impermeable bags with desiccant at room temperature. During clinic visits, the DBS will be collected by study staff. DBS will be collected by fingerstick at home on Days 1-6 (+/-2 d), 8-13 (+/-2 d), 15-20 (+/-2 d), and 22-27 (+/-2 d), and will not be overseen by study staff on these days.

5.6.2. Venous Blood Collection

An additional 5 mL (adult) or 1 mL (child) of venous blood will be obtained by conventional venipuncture in the clinic at Visits 2 (Day 7 (+/-2 d)), 3 (Day 14 (+/-2 d)), 4 (Day 21 (+/-2 d)) and 5 (Day 28 (+/-2 d)) for later testing for malaria by qRT-PCR. Blood will be drawn by a trained study team member. In brief, the skin around the vein where blood will be drawn will be cleaned with an alcohol wipe. Then a tourniquet will be placed on the participant's arm approximately 5-10 cm above the site of venous puncture. Study staff will collect into an EDTA anticoagulant tube. When the required amount of blood is collected, the tourniquet will be removed, the needle withdrawn, and the participant will be asked to apply pressure to the puncture site using gauze or a cotton swab. A bandage will be applied if desired by the participant.

The blood will be handled and aliquotted as specified in Section 5.10 – Specimen Handling and Storage.

Blood collection will be performed if clinically indicated for symptomatic patients following local and national guidelines for malaria diagnosis and treatment.

5.7 Treatment Intervention

No study intervention is being tested in this protocol. If a participant is febrile (≥37.5°C) and/or symptomatic with Grade 2 or higher malaria-related signs and symptoms and is positive for malaria infection by RDT, the participant will be referred to the Malaria Clinic for routine diagnosis and treatment. Such participants will end their participation in the study. Their samples may still be included in the testing process at the discretion of the investigator even though the participant will have no further involvement in the study.

5.8 *Plasmodium* 18S rRNA qRT-PCR, gametocyte qRT-PCR and human 18S rRNA qRT-PCR

While detection of parasites on thick blood smears remains the most common primary endpoint for malaria trials, nucleic acid-based methods have been increasingly used to replace blood smear testing. Nucleic acid-based methods have significantly increased sensitivity for detection of *P. falciparum* blood-stage infection approaching 20 parasites/mL, often 2-4 days earlier than by paired thick blood smears in human challenge studies [20, 21]. Quantification of parasite density also allows for evaluation of parasite growth curves. The blood sample collection and handling protocol recommended by the University of Washington (UW) testing laboratory will be used to guide whole blood sample collection for this study.

The assay to be utilized on pooled samples is a validated, laboratory-developed third-generation quantitative RT-PCR assay for *Plasmodium* 18S rRNA [21] or an updated version if validated improvements in the assay are implemented by the MMDL. The internally-controlled assay has undergone extensive validation studies, has been used in five previous Seattle Malaria Clinical Trial Center (MCTC) clinical trials and has been submitted to the FDA through the Drug Development Tool Biomarker Qualification pathway. The assay has been evaluated by the UW laboratory for accuracy, correlation,

agreement, precision, analytical sensitivity, analytical specificity (interferences), reportable range and carryover. This test was also amongst the highest performing assays utilized by seven Controlled Human Malaria Infection (CHMI) centers in a recent external quality assurance comparison of nucleic acid-based tests [22]. The test is enrolled in ongoing quality assurance through the World Health Organization's Malaria Molecular EQA Program.

Deconvoluted samples will be subjected to a modified *Plasmodium* 18S rRNA assay multiplexed with the *P. falciparum* gametocyte-specific qRT-PCR targeting a spliced gametocyte-specific mRNA target [23].

For non-*P. falciparum* species, the pan-*Plasmodium* 18S rRNA amplicon will be sequenced using Sanger sequencing methods to determine the species.

Human 18S rRNA RT-PCR will be performed using a commercially-available primer/probe set.

5.9 Exploratory Assays

The following exploratory analyses *may* be conducted at completion of the study:

- Measurement of anti-sporozoite and anti-blood stage antibody responses by ELISA
- Nucleic acid sequencing to identify genetic traits of the parasites and/or human host associated with asymptomatic infections.
  (Samples for Exploratory Studies will be prepared and stored using standard methods. Additional assays may be applied to samples to evaluate other immune responses to better understand malaria immunology.)

5.10 Specimen Handling and Storage

Specimens will be collected and transported to the appropriate laboratory per standard Med Biotech Laboratories (MBL) and UW MMDL procedures for handling transport of biological specimens and sample collection recorded on the appropriate case report form (CRF). Universal precautions will be followed at all times during collection, handling, and processing of biological specimens. Handling and disposal of bio-hazardous materials will observe St. Anne Health Center III/MBL and University of Washington environmental health and safety policies and Occupational Health and Safety Administration regulations.

Venous blood samples collected in the clinic for qRT-PCR and plasma will be collected into EDTA tubes, labeled with the subject identification code and the time and date, and transported to the clinic laboratory for aliquoting and stabilization. Aliquots of of whole blood will be placed into bioMerieux NucliSENS lysis buffer to stabilize the RNA and then such lysed samples will be frozen at ≤-65°C. The remaining whole blood will be centrifuged to isolate plasma, which will also be aliquotted and stored at ≤-65°C. Samples intended for qRT-PCR analyses will later be shipped to the University of Washington on dry ice for evaluation. Backup lysed samples and all plasma samples will be stored locally until needed for future testing. No private health information will be released.

DBS cards collected in the clinic and at home will be labeled with the patient identifier code and the collection dates. DBS cards will be stored in gas-impermeable bags with desiccant during the at-home stage, while on-site in Uganda, and after transfer to the

University of Washington. DBS cards can be stored at room temperature throughout the collection, storage, and transfer process. All DBS cards will be transferred to the University of Washington and all will be entirely consumed in the primary testing process. No private health information will be released with the DBS.

5.11 Safety Monitoring and Adverse Events

Participants will be assessed for signs and symptoms of malaria (listed in Appendix A) at screening and again if they present with malaria-related symptoms. Such participants will be referred to the Malaria Clinic for standard care and will be discharged from the study if they test RDT-positive with symptomatic infection.

Participants' fingerstick and antecubital blood draw sites will be assessed at each weekly study visits. Any adverse events observed during the study that were not present at baseline will be recorded in the study record. In particular, study staff will examine for signs of localized infection (redness, erythema, non-healing wound, draining fluid), pain, bruising, or hematoma formation. Participants will also be asked at each clinic visit whether they wish to continue in the study or if there are any aspects of the study collection processes that are intolerable.

Once per week, a village health team worker will visit the participant at home to check for any signs of infection. If infection is suspected, the village health team worker will immediately refer the participant to the clinic for care.

Treatment of any adverse event is at the discretion of the Investigator in accordance with Good Clinical Practice. Any treatment should be documented in the subject case record and the appropriate Case Report Form (CRF).

The Investigator is responsible for reporting serious adverse events and unanticipated problems involving risk to human subjects or others to the IRB of record, according to its policy and applicable regulations.

6.0 Data Handling and Record Management

6.1 Source Documentation

All information gathered as part of this study, biological sampling data, and adverse events will be collected and recorded on source documents in the subject's medical record. A file containing all the source documents will be maintained for each study subject at the study site, and it will include completed case report forms, laboratory and clinical findings, signed and dated consent forms and other documents, and any medical records. Source documents will be kept in locked cabinets with access limited to authorized study staff. Upon request of any monitor, IRB, or other regulatory authority, the Investigator/Institution will make available for direct access all requested study-related records.

6.2 Confidentiality

All laboratory specimens, reports, study data collection, process and administrative forms will be identified by coded number to maintain subject confidentiality. Forms, lists, logbooks, appointment books, and any other listings that link volunteer ID numbers to other identifying information will be stored in a locked file in an area with limited access or if electronic format, in a password protected file with access limited to authorized study staff. Subject's study information will not be released without the written

permission of the subject, except as necessary for monitoring by a monitor, Institutional Review Board, or regulatory authorities as required by law.

6.3 Required Records

Essential documents are documents that individually and collectively permit evaluation of the conduct of a study and meet regulatory requirements. The following documents are required and will be maintained by study staff:

1) Curricula vitae and licenses, if applicable, for all study personnel
2) IRB approvals (protocol, amendments, informed consents, advertising), progress reports and correspondence
3) Source documents (including signed informed consent forms) and completed case report forms (including query resolutions)
4) Laboratory documentation (certification and normal ranges)
5) Protocol-specific training records
6) Logs (Delegation of Responsibilities/Signature Log, Subject Screening/Enrollment Logs, Subject Identification Codes)
7) Serious Adverse Event Reports
8) Correspondence, Notes to File, telephone contact reports, protocol deviation reports
9) Manuals (e.g., study binder, laboratory, pharmacy)

6.4 Protocol Deviations

A protocol deviation is defined as any occurrence involving a procedure that did not follow the study protocol, applicable procedures, and/or regulatory requirements. The noncompliance may be either on the part of the subject, the Investigator, or the study staff.

It is the responsibility of the Investigator and study staff to use continuous vigilance to identify and report deviations according to local IRB requirements. All staff involved in the conduct of a study with human subjects shall be aware of the specific protocol requirements for completing study visits and notify the Principal Investigator in the event of any breach of protocol as soon as it is discovered. All deviations from the protocol must be addressed in the subject's source documentation. As applicable, the study staff will develop and promptly implement corrective actions in response to any deviation.

7.0 Potential Risks and Benefits

7.1 Risks from Phlebotomy and Finger Sticks

Phlebotomy and finger sticks carry a minimal risk of minor discomfort and the possibility of bruising at the site of the needle or automatic lancet (pricker) puncture and, rarely, the possibility of infection and/or clot formation at the needle or automatic pricker puncture site. There is a small risk of tetanus if the prick site becomes infected. Potential risks will be minimized by inquiring as to previous history of problems associated with routine blood draw, including degree of light headedness and fainting, and use of sterile technique and restricting needle or lancet movement. Participants will be monitored for infection at weekly clinic visits, as well as through a once weekly home visit by a village health team worker.

7.2 Risks to the Community

Malaria is endemic in this part of Uganda. Participants enrolled in this study will be RDT-negative and will not be tested by qRT-PCR until the conclusion of the study. As such, these participants may harbor low density infections below the limit of RDT detection. Since treatment will be provided for anyone who presents with a symptomatic infection in accordance with national guidelines, the plan to not perform qRT-PCR in real time will not differ from the normal experience of people in this community.

The data from this study will be aggregated, discussed, and published in a manner that will not individually identify individual subjects.

7.3 Benefits of Participation

Participation in the study is voluntary. There is no direct benefit to the subjects for participating in the study; however, the information gained from this study may help reveal how malaria transmission is occurring in endemic regions.

If a participant has a previously undiagnosed malaria infection, he or she will be tested at enrollment or upon symptomatic presentation and will be referred for definitive treatment to cure that infection, which will reduce his/her risk of malarial disease in the future.

8.0 Statistical Considerations

8.1 Sample Size Considerations

- The study is powered to detect a prevalence of asymptomatic infection of 40% given a catchment population of 2000 individuals, 9% precision, 95% confidence, and 80% power. This requires 108 individuals, adjusted to 130 for loss to follow-up. Thus, the study will recruit 100 adults and 30 children (8-17 years) to measure compliance with high precision (70±9% if true proportion is 70% compliant)**.**

8.2 Study Endpoints & Analytical Plans

- Categorical variable defining compliance of each individual during the study
    - The number of DBS collected will be enumerated, and participants will be classified as excellent (26-28 samples), good (20-25 samples), fair (15-19 samples), or poor (<15 samples) compliers over the study period. Excellent and good compliers will be combined for the main analysis.
    - To assess the feasibility of the daily DBS collection, the proportion of individuals who were excellent or good compliers (≥20 samples collected) over the study period will be calculated. Patterns of compliance will be summarized and presented as descriptive statistics on the number and proportion of individuals who missed >3 days of sampling in a row, individuals who missed >7 consecutive days of sampling, or who discontinued sampling after 1, 2, or 3 weeks of study time. Results for the entire study population will be summarized in aggregate and separately for adults and children. For adults, we will also disaggregate by sex.
- Quantity of human rRNA in each sample indicated by qRT-PCR
    - Number of days since the sample was placed on the DBS, with the first collected sample of the week being assigned a 7, and the last sample collected a 0.

    - To assess DBS quality, we will use a linear regression model with random effects to estimate changes in human rRNA C_T by day for a subset of samples positive for *Plasmodium* 18S rRNA.
- Daily quantification of asexual stage parasites and gametocytes, expressed as parasites/µL of blood
    - Pooled samples from individual participants will be initially tested as an indicator of "any low-density infection"
    - All daily samples will be tested for pool-positive individuals.
    - To characterize patterns of natural history of infection in individuals found to be low-density parasite carriers by pooled sampling, individual samples will be tested and daily fluctuations in carriage of positive individuals will be plotted. Based on the plots, individuals will be characterized into four possible different patterns of carriage: (i) continually increasing over time; (ii) continually decreasing over time; (iii) oscillating with increasing and decreasing densities; and (iv) continually decreasing densities over time.
    - Proportions of each of the four carriage patterns will be summarized, and profiles of the individuals within each type of pattern will be presented, based on age, sex, and other characteristics collected during the baseline survey. Among any individuals who clear their infection, median time to clearance will also be summarized.
    - To evaluate clearance of low-density infections over time, Kaplan-Meier survival analysis will be performed to calculate the half-life probability of a low-density infection. This calculation will define the outcome as failure to reduce parasite density by at least 50% of the first recorded value at each day. For individuals who were not qRT-PCR positive on the first day of the study, but became positive at a subsequent time point, only samples from the time they were positive will be included in the analysis (left-censoring of data).
    - The infectivity of positive individuals will be estimated using gametocyte densities at each time point, paired with literature reported estimates of the expected proportion of infected mosquitoes at such densities[24]. The infectiousness of an individual will be estimated at each time point, and then overall infectious potential during the 28-day study period will be estimated by multiplying each daily value together.

9.0 Ethics and Responsibility

9.1 Quality Control and Quality Assurance

Study activities will take place in accordance with the Good Clinical Practices, Good Clinical Laboratory Practices, and site-specific standard operating procedures prescribed by UNCST guidelines on research involving human subjects.

Self-collected dried blood spots (DBS) will be reviewed by the study team during weekly visits. Any issues with quality of the collection will be addressed directly with the participant, and retraining will be performed, if needed. Study staff will verify that the participant's Study ID number matches the ID number on the blood spots and collection cards before storing them.

Study data will be collected on paper Case Report Forms at the Study site, and then input into an electronic online database platform. All data will be double-entered by two

independent staff members, and any discrepancies will be flagged and resolved. In addition, separate quality assurance of the electronic database will be conducted by team members of the University of Washington, who will look for inconsistencies in the data and create queries for the study team.

All blood samples will be labeled and stored as specified in Section 5.10 – Specimen Handling and Storage

9.2 Investigator Responsibility

The Investigator will conduct the study according to the IRB approved protocol/amendments and government regulations and will only make changes after notifying the IRB or to eliminate an immediate hazard in order to protect the safety, rights or welfare of subjects. All deviations from/changes in the protocol, unanticipated problems and changes in the Investigator will be documented and reported to the Sponsor and IRB, as required by their policies and procedures.

The Investigator will personally conduct or supervise the study. If the Investigator delegates responsibilities to study staff, s/he will ensure that they will be informed about the protocol and their study-related duties and functions. Training of staff should also include training on the protocol, regulatory requirements and Good Clinical Practices. The training and delegation of responsibilities will be documented on the appropriate form(s). The Investigator will maintain adequate and accurate records documenting the conduct of the study as described in Section 5.0. These records will be available for inspection by Sponsor representatives, regulatory authorities and the IRB. As part of the conduct of the study, the Investigator is required to provide periodic reports to the IRB, per its' policies and procedures.

The Investigator will obtain informed consent from subjects or subjects' legally authorized representative, using an IRB approved consent form. The consent process is described in Section 4.2.

The Investigator will report all adverse events to the IRB as described in Section 4.11.

9.3 Compensation

Participants (or their families for pediatric participants) will be compensated for time and travel to participate in this study. Subjects will receive $10 for the screening/enrollment visit (Visit 1). Compensation for subsequent visits 2, 3, 4, and 5 that include blood draw will be $5 plus $5 for each week's completed DBS card ($10 per week between visit and DBS card return). If a subject withdraws from the study, payment will be made based on the last completed visit.

9.4 Special Populations

This study is considered to be 'Research involving greater than minimal risk and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition'.

In addition to healthy adults, this study will enroll healthy children aged 8-17 years old. Based on local laws and practices, pediatric participants must have consent from one parent or legal guardian and the child's assent in order to be enrolled. Ongoing assent must be reconfirmed at each in-person visit.

10.0 Institutional Review Board

10.1 Approval and Continuing Review

The Investigator must obtain approval of the protocol, amendments, informed consent forms, recruitment and advertising materials from a duly constituted IRB and comply with its policies, procedures and conditions. The Investigator may not start the study or make changes to the protocol without prior approval from the IRB, unless it must be done to protect the safety of a subject. IRB approval to conduct the research must be obtained prior to the start of the study or prior to implementation of protocol amendments. Annual re-approval of the study is required, if applicable by study duration. The Investigator is also responsible for obtaining approval of any additional documents designated by the IRB.

10.2 Protocol Modifications

In the event of a protocol modification, the informed consent must also be revised, if applicable, and submitted for approval to ensure alignment with any amendment to the protocol. Any subject already enrolled in the study will be informed about the revision and may be asked to sign the revised informed consent. In this case, a copy of the revised, signed, and dated informed consent will be given to the subject. All original versions of the informed consent will be retained in the protocol regulatory file.

10.3 Reporting

The Investigator will provide periodic progress reports to the IRB according to its policies and procedures for its continuing review of the research and report adverse events and unanticipated problems involving risk to subjects or others.

10.4 Subject Confidentiality and Privacy

Confidentiality must be maintained and private information must be secured for subjects in a research study both during and after study period. Documentation, data, and all other information generated for a subject will be held in strict confidence whether the information was obtained verbally, recorded on paper, or in electronic files. This confidence extends to cover testing of biological samples in addition to the clinical information. The study database will identify study subjects only by a study identification number and will not contain identifying information such as name, address, national identification number, medical record number, or personal contact information. No identifying subject information concerning the study or the data will be released to any unauthorized third party without prior written approval of the subject except as necessary for monitoring by the IRB, UNCST or the US FDA or as required by law or in the case of an emergency, to facilitate medical care. Subjects will be made aware of the occasions when information may be released without their consent. Subject records will be stored in locked files. Only authorized individuals will have access to source documents that contain subject identifying information.

10.5 Research-Related Injury

If a subject becomes ill or injured as a direct result of study participation and requires additional medical evaluation or referral for treatment, the participant will cared for at the Malaria Clinic, of referred to St. Anne Health Center III, or Katakwi District Hospital for further care. The cost associated with care for any study related injury (i.e. localized infection, bruising, bleeding) will be covered by study and be of no cost to the participant. In addition, the study will provide free treatment for any case of malaria that develops in a study participant during their participation in the study. Uncomplicated malaria will be

treated directly at the Malaria Clinic, and complicated cases will be referred to St. Anne Health Center III or Katakwi General Hospital. Med Bio Laboratories (MBL) has a Memorandum of Understanding (MoU) with both of these reference clinics so that study subjects will be treated at no cost to the participant. There are no funds to pay subjects for a non-research-related injury. In the event of a non-study-related illness or injury, study staff will refer the participant to the proper channels to receive additional care under the procedures of the area.

11.0 Publication and/or Presentation Policy

Following completion of the study, it is anticipated that the results of the study will be presented to the scientific community via oral presentations and written publications. Any proposed publication or presentation will be governed by University of Washington and Med Biotech Laboratories Publication Policies and any proposed presentation, abstract, or manuscript will be made available for review by the Sponsor or any involved author(s) prior to submission.

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Appendix A: Toxicity Grading Scale for Malaria Signs and Symptoms Solicited Systemic Adverse Reactions

MALARIA SIGNS AND SYMPTOMS SYSTEMIC SOLICITED SIGNS/SYMPTOMS				
ADVERSE EVENT	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Fever (°C)* (°F)*	38.0 – 38.6 100.4 – 101.5	38.7 – 39.3 101.6 – 102.7	39.4 – 40.5 102.9 – 104.9	>40.5 >105
Chills/Rigors	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	NA
Headache	No or minimal interference with usual activities	Repeated use of non-narcotic pain reliever > 24 hours or greater than minimal (some) interference with activities	Inability to perform usual activities; any use of narcotic pain reliever	ER visit or hospitalization
Fatigue/malaise	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Myalgia (excluding low back pain)	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Low back pain	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Anorexia	Transient (< 24 hours) or intermittent anorexia with no or minimal interference with oral intake	Persistent anorexia resulting in decreased oral intake for 24 – 48 hours	Persistent anorexia resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated	Life-threatening consequences (e.g., hypotensive shock)

MALARIA SIGNS AND SYMPTOMS SYSTEMIC SOLICITED SIGNS/SYMPTOMS (continued)				
ADVERSE EVENT	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Vomiting	No or minimal interference with usual activities and/or 1-2 episodes/24 hours	Greater than minimal interference with usual activities and/or >2 episodes/24 hours; no or mild dehydration	Inability to perform usual activities, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools or <400 gms/24 hours	4-5 loose stools or 400 – 800 gms/24 hours	6 or more watery stools or >800 gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Abdominal pain	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Arthralgia	Joint pain causing no or minimal interference with usual activities	Joint pain causing greater than minimal interference with usual activities	Joint pain causing inability to perform usual activities	Disabling joint pain causing inability to perform basic self-care
Chest Pain (non-musculoskeletal)	Transient (< 24 hours) or intermittent chest pain with no or minimal interference	Persistent chest pain resulting in greater than minimal interference with usual activities	Persistent chest pain resulting in inability to perform usual activities secondary to chest pain	ER visit or hospitalization