Preliminary Evaluation of Dynamics of Subclinical Malaria

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Statement of Compliance

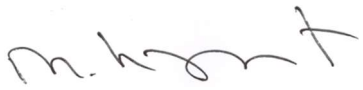
The study described in this protocol will be conducted in compliance with the protocol, the International Conference on Harmonization Good Clinical Practice E6, the applicable regulatory requirements (US 21 CFR Part 50-Protection of Human Subjects and Part 56-Institutional Review Boards, US 45 CFR 46), and the applicable rules and regulations of Myanmar, China, and Bangladesh.

The IRBs of the Department of Medical Research (FWA00018816), Defence Services Medical Research Centre (FWA00023030), Duke University Health System (FWA00009025), National Institute of Parasitic Diseases China CDC (FWA00022996), and icddr,b (FWA00001468) will review and approve the protocol prior to study start. Documentation of the approval by these bodies will be kept in the PI's study file and Sponsor's regulatory files.

SIGNATURE PAGE

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 applicable US federal regulations and ICH guidelines.

Site Investigator:

Signed:  Date: 19 Feb 2018

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SUPPLEMENTS/APPENDICES

List of Abbreviations

ACT	Artemisinin-based combination treatment	ISM	Independent Safety Monitor
CDASH	Clinical Data Acquisition Standards Harmonization	LoD	Limit of detection
CDISC	Clinical Data Interchange Standards Consortium	MDA	Mass drug administration
CFR	Code of Federal Regulations	MOHS	Myanmar Ministry of Health & Sports
CIOMS	Council for International Organizations of Medical Sciences	MOP	Manual of Procedures
CRF	Case Report Form	N	Number of subjects
DBS	Dried blood spots	NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
DGHI	Duke Global Health Institute	NIH	National Institutes of Health
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH	NIPD	National Institute of Parasitic Diseases, China Center of Disease Control (CDC)
DMR	Department of Medical Research, Myanmar MOHS	NMCP	National Malaria Control Programme
DSMB	Data and Safety Monitoring Board	NNT	Number needed to treat
DSMRC	Defence Services Medical Research Centre, Myanmar Ministry of Defence	OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
ERC	Ethics Review Committee	OHRP	Office for Human Research Protections
FWA	Federal-Wide Assurance	ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
GCP	Good Clinical Practice	PI	Principal Investigator
GMS	Greater Mekong Sub-Region	RDT	Rapid diagnostic test for malaria
HIPAA	Health Insurance Portability and Accountability Act	RIP	Research information portal

List of Abbreviations

icddr, b	International Centre for Diarrhoeal Disease Research, Bangladesh	sFTP	Secure file transfer protocol
ICF	Informed Consent Form	SMC	Safety Monitoring Committee
ICH	International Conference on Harmonisation	SOP	Standard Operating Procedure
IEC	Independent or Institutional Ethics Committee	usPCR	Ultrasensitive polymerase chain reaction
IRB	Institutional Review Board	WHO	World Health Organization

PROTOCOL SUMMARY

Title:	Preliminary Study of Subclinical Malaria
Population:	Up to 6,000 residents of all ages in villages, refugee camps, military bases, and work sites, including children, pregnant women, and the elderly; the number of participants screened to achieve enrollment ceiling is not known
Number of Sites:	Up to 6 (each with at least 2 villages, towns, unions, refugee camps or plantations, or a single military base)
Study Duration:	Up to 1.5 years
Subject Duration:	Up to four months (military population may be under observation up to 4-6 weeks)
Ethical Oversight:	Ethics Review Committee (ERC), Department of Medical Research (DMR), Myanmar Ministry of Health and Sports (MoHS)
	Institutional Review Board (IRB), Defence Services Medical Research Centre (DSMRC), Myanmar Ministry of Defence
	IRB, International Centre for Diarrhoeal Disease Research (icddr,b), Bangladesh
	IRB, National Institute of Parasitic Diseases (NIPD), Chinese Center for Disease Control and Prevention (China CDC)
	IRB, Duke University Health System

Study Objectives:

Primary objective:

Make a preliminary assessment of the dynamics of subclinical malaria detected by ultrasensitive PCR to guide the design of a matched cohort study of subclinical malaria in Myanmar and along its borders with China and Bangladesh

(*The objective of the subsequent matched cohort study, to be reviewed as a separate protocol, will be to compare the incidence of clinical malaria and malaria transmission capacity, in participants with and without subclinical malaria*.)

Secondary and exploratory objectives:

1. Within usPCR+ and usPCR- cohorts, test for association between demographic and other risk factors (e.g. occupation, travel) and incidence of clinical malaria and transmission capacity
2. Collect preliminary data to estimate the history of exposure to malaria parasite and mosquito vector species by serological profiling
3. Collect preliminary data to develop a model system to predict future malaria risks using geospatial and satellite data on environmental conditions, patterns of parasite migration and human mobility, field data on vector abundance and malaria burden over time at study sites

1. KEY ROLES

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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

2.1.1. Malaria, antimalarial drug resistance, and malaria eradication

Malaria is caused by a mosquito-borne, protozoan parasite belonging to the genus *Plasmodium*. Of the five species of *Plasmodium* that infect humans, *P. falciparum* and *P. vivax* are responsible for the majority of morbidity and death. The malaria burden is greatest in sub-Saharan Africa, with a high mortality in children under five years of age. In Asia, malaria endemicity is lower, but it presents a major public health problem, affecting people of all ages due to a lack of the acquired immunity that can be developed through the continuous, heavy exposure more typical of Africa. *P. falciparum* is susceptible to genetic polymorphisms associated with antimalarial drug resistance, and Southeast Asia has been the epicenter of repeated emergences of drug resistant malaria [1]. Malaria can be cured completely if treated with effective drugs. Artemisinin-based combination therapies (ACTs) are the first line malaria treatment globally. The widespread implementation of highly efficacious ACTs has contributed to large reductions in the malaria burden in many parts of the world, inspiring a renewed hope of global malaria eradication [2].

2.1.2. Myanmar is the frontier of artemisinin resistance and a crucial battleground for malaria elimination

Southeast Asia has become a focus in the war against malaria after the emergence of artemisinin resistant falciparum malaria. Myanmar, the emerging democracy formerly known as Burma, has the heaviest malaria burden in the region, with more cases and deaths than the rest of the region combined [3]. Artemisinin resistance first emerged in Cambodia [4-6] and has been documented by us and others along the borders of Myanmar with Thailand [7, 8] and China [9], in central Myanmar, and in southern Laos and southern Vietnam [10]. Although artemisinin resistance is manifested chiefly by delayed parasite clearance so long as the ACT partner drug is effective, with the new emergence of resistance to ACT partner drugs treatment failure now occurs in up to 46% of people treated with ACTs in western Cambodia [11], raising the specter of untreatable malaria, and threatening prospects for eradication [12]. The populations currently impacted by artemisinin resistance are those living in the six Greater Mekong Sub-Region (GMS) countries: Cambodia, Vietnam, Laos, Thailand, China's Yunnan Province, and Myanmar (Figure 1). Importantly, Myanmar shares borders with Bangladesh and India, two countries with no evidence yet of confirmed artemisinin resistance, but both with large populations at risk of malaria and travel links to Africa.

Figure 1. Map of the Greater Mekong Subregion.

The initial response to artemisinin resistance was to try to contain it by eliminating falciparum malaria from western Cambodia and erecting a "firewall" to block its spread. The containment strategy, led by the World Health Organization (WHO), the Global

Fund to Fight AIDS, Tuberculosis and Malaria, and regional governments, was predicated on the assumption—based on past patterns of spreading drug resistance—that a "wave" of resistance was spreading westward from Cambodia toward Myanmar [10]. The Myanmar Artemisinin Resistance Containment zone was launched along the Thailand-Myanmar border where diagnosis, treatment and prevention were scaled up [13] and pilot mass drug administration (MDA) schemes were implemented from across the border in Thailand into Myanmar's Kayin [14], as well as in Mon and Tanintharyi States (unpublished).

Using genomic epidemiology approaches, we recently demonstrated that while artemisinin resistant parasites have indeed spread between Cambodia and southern Vietnam, the resistant parasites initially present in Myanmar emerged *de novo* there [15]. This unexpected finding—which was immediately shared with Myanmar's National Malaria Control Program (NMCP), other country partners, and the WHO—challenged the notion that erecting a firewall along Myanmar's eastern border could stop the spread of resistance: if resistance can pop up independently, it will be impossible to contain. An urgent technical expert review [16] led the WHO to abandon the containment strategy and to instead launch a regional malaria elimination campaign [17, 18]. Myanmar endorsed the goal of malaria elimination by 2030 at the East Asia Summit in November 2015, and a costed national malaria elimination plan for Myanmar was finalized in 2016.

2.2. Scientific Rationale

2.2.1. Risks of Asymptomatic or Subclinical Malaria

Myanmar's NMCP currently stratifies malaria risk at the township level, based on ecological risk factors for malaria transmission, reported clinical cases, and presence of or proximity to artemisinin resistance. This stratification scheme, though practical, does not account for heterogeneous malaria risk or distinguish between locally acquired and imported malaria, resulting in inefficient targeting of interventions. Moreover, malaria elimination may require eradicating all parasites from all infected persons, not just those with clinical symptoms [19]. If clinical malaria represents the "tip of the iceberg", understanding the rest of the iceberg—whether it poses risk or even benefit to infected individuals, and how it contributes to transmission in different epidemiological settings—will be important for malaria control and elimination.

"Asymptomatic"—or more accurately, *subclinical* malaria—has long been recognized in semi-immune Africans [20]. New "ultrasensitive" molecular tests [21-23] have recently revealed unexpectedly high rates of subclinical malaria in Southeast Asia, where long held dogma had it that all infections quickly give rise to symptoms in presumably non-immune people. Some argue that "asymptomatic" infections are both harmful and infectious and must be treated [24]. The WHO recently endorsed MDA for malaria elimination in Southeast Asia [18], and increasingly large scale MDA schemes are being undertaken based on subclinical malaria prevalence as measured by ultrasensitive tests [14]. However, no previous studies have assessed the clinical risks or transmission potential of the extremely low-density malaria infections detected by these tests.

Using an ultrasensitive reverse transcription polymerase chain reaction (usPCR) assay that we developed [22], we are finding that the prevalence of malaria at sites within and bordering Myanmar is highly heterogeneous. Villages with high prevalence—including some MDA sites—are often in close proximity to villages with little or no malaria. While our usPCR test

reliably detects *P. falciparum* and *P. vivax* at parasite densities below 16 parasites/mL, these very low-density infections are difficult to further characterize using currently available molecular and *in vitro* methods, precisely because the parasite densities are so low—up to 10,000-fold lower than those detected by rapid diagnostic tests (RDTs) and ten to 100-fold lower than conventional PCR (Figure 2).

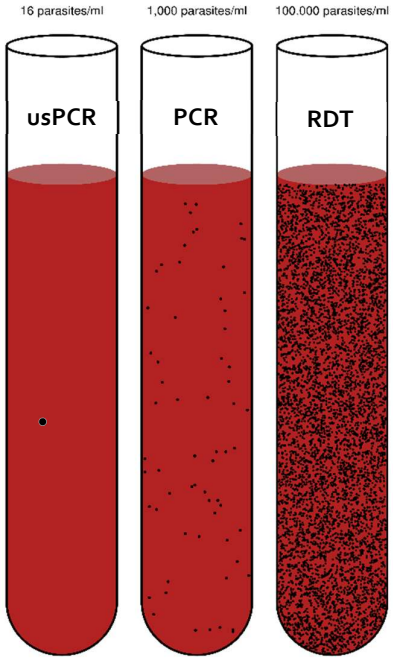


Figure 2. Representation of density of malaria infections detected by our finger-stick usPCR (left), standard PCR (center) and RDTs (right). Black dots represent lower limit of number of parasites detected in an equal volume of blood.

Clinical risk or benefit. A recent review concluded that "'asymptomatic malaria' is a chronic and debilitating condition that should be treated" [24]. However, most of the cited studies of "asymptomatic" or subclinical malaria infection used microscopy or rapid diagnostic tests for malaria (RDTs), which have lower limits of detection (LoD) of about 100 parasites/μL (or 10^5/mL). More recently studies have used standard PCR, with an LoD of about 1000-5000 parasites/mL [25]. In contrast, a high-volume intravenous usPCR assay has an LoD of 22 parasites/mL [21] and the LoD of a finger-stick assay that we developed and are using (Figure 2) is <16 parasites/mL [22]. Although studies are conflicting as to whether subclinical malaria at parasite densities detected by conventional methods is protective or harmful [26, 27], no previous studies have assessed the clinical risks or transmission potential of ultralow density subclinical malaria infections.

The ability to detect these ultralow parasite densities is unprecedented, and for the first time it will be possible to study how long these infections last and whether they increase the risk of clinical malaria illness in the infected individual. Alternatively, ultralow density malaria infection could represent a co-adaptation of host and parasite that optimize transmission while minimizing symptoms [28].

Transmission potential. It has been argued that malaria elimination requires eradicating all parasites from all infected persons, not just those causing symptoms [19]. Subclinical malaria has been known to be a transmission reservoir in Africa since the 19th century [20, 29], and 40 years ago surveys in Bangladesh found up to 47.3% of people of all ages to be infected with falciparum and/or vivax malaria at the end of the transmission season. These studies used microscopy to detect much higher parasite densities than those detected along the Thailand-Myanmar border by usPCR to guide MDA [14, 23, 28]. Ongoing MDA to eliminate ultralow subclinical malaria is predicated on the assumption that extremely low density infections contribute meaningfully to transmission. It may seem reasonable to assume that *any* infection is potentially transmissible, but several studies (summarized in [24]) have shown that transmissibility falls with declining parasite density.

There is no evidence at present that ultralow subclinical malaria infections contribute significantly to transmission. Skeptics about the value of MDA in Southeast Asia rightly point out that malaria has been eliminated from many regions without detecting or treating submicroscopic infections, much less ultralow density infections. Despite strong advocacy for MDA by prominent malaria thought leaders [30], scaling up of this controversial but potentially promising intervention is stalled in Southeast Asia as NMCPs await clear answers about the

clinical and transmission risks posed by subclinical—and especially ultralow density—malaria infection. This study is designed to provide these clear answers.

Study impact. The results of the longitudinal matched cohort study that this pilot study will lead to will influence national and regional malaria control and elimination policies. Whether or not the results of the cohort study support scaling up MDA, the development of robust, highly sensitive surveillance tools will enhance the ability to map and track malaria, capabilities that become increasingly critical as countries approach elimination. Our results may support "targeted MDA" based on microstratification at the village level, a new concept just now gaining currency.

2.2.2. Dynamics of Asymptomatic or Subclinical Malaria Identified by Ultrasensitive PCR

It is well known that many asymptomatic infections may persist for from weeks to months [31-33]. Persistent chronic cases of malaria infection in the absence of new exposure has been described [34]. A recent paper extensively reviewed the nature and dynamics of asymptomatic malaria and its clinical and transmission impact in different transmission zones [35]. However, most of these studies were conducted in areas of high malaria transmission, and used standard methods such as microscopy or RDTs LoDs far higher than that of usPCR. Studies that used sensitive molecular methods in low transmission regions [36, 37] are limited to cross-sectional surveys, and do not assess the dynamics or chronicity of these low-density infections. The natural dynamics of low density malaria infections in humans is unknown; although it has been suggested that these may be chronic infections, their duration has not been described.

2.2.3. Parasite population structure and migration

There is an urgent push for malaria elimination in the GMS, driven by fear of the spread of ACT-resistant malaria. Information on how parasites are migrating, where they come from and where they end up (i.e. "sources" and "sinks"), could help to guide elimination interventions. Recent studies of *P. falciparum* whole genome sequences from the GMS identified highly structured P. falciparum populations clustering into multiple distinct genetic subpopulations [38, 39], some of which are associated with artemisinin resistance. Some *P. vivax* populations show similar patterns of population structure and diversity [40], while others show *P. vivax* to be more diverse and less structured than *P. falciparum* [41, 42]. Further studies employing genome sequencing and genome-wide genotyping linked to ecological, epidemiological and geospatial information about risk of exposure to malaria parasite and mosquito vector species have the potential to lead to new tools for predicting malaria risk that would aid in elimination efforts.

2.2.4. Serosurveillance: tracking the parasite's footprints

Serological tools can be used to map malaria risk [43] and assess time-dependent malaria exposure [44]. Protein microarrays are used to measure antibody responses to large numbers of malaria proteins, including both polymorphic vaccine antigens [45] and variant surface antigens associated with immune evasion and pathogenesis [46, 47]. These microarrays may prove most useful as surveillance tools to detect recent or remote exposure to specific parasite species, life cycle forms, antigenic variants, and even mosquito vectors. Unlike commercially available protein microarrays [44, 48], which measure seroreactivity to hundreds of proteins, each derived from the reference genome, our diversity-reflecting arrays contains dozens to

hundreds of variants of important polymorphic antigens derived from sequencing many hundreds of field isolates. We hypothesize that breadth of recognition of antigen variants will provide a simple and robust way to estimate malaria exposure history in a population. Moreover, mosquito salivary antigens elicit serological responses in humans [49] that can correlate with exposure [50], and we hypothesize that we will be able to estimate exposure to different mosquito vectors by measuring antibodies against species-specific variants of mosquito proteins. We hope to use this information to target malaria interventions more efficiently based on which human populations are exposed to which vectors, e.g. indoor-biting versus forest-biting species.

2.2.5. Mapping and forecasting malaria risks using geospatial and satellite data

Understanding malaria risk as it varies over time and space is essential for effective targeting of interventions. Changing malaria pattern can be understood using two major transmission pathways, namely human and mosquito. Vector ecology likely dominates in determining individuals' risk of exposure at the local scale, because environmental factors largely control mosquito population dynamics and distribution. However, human mobility plays a more significant role at a regional scale as human flow allows for "import" and "export" of local parasite subpopulations, accelerating the dissemination of drug resistance and other heritable traits. The data generated through this study will be used to develop spatially explicit and temporally dynamic approaches to track, monitor, and forecast malaria risk within Myanmar and along its borders with China and Bangladesh, using satellite and geospatial data to aid malaria control and elimination. Earth-observing satellite systems acquire data characterizing environmental conditions and ecosystem states that are routinely used in habitat and risk modeling applications in ecological studies and management applications. At a regional scale, studies of movement paths or trajectories of populations are starting to improve our understanding of the role of human mobility in spreading infections [51, 52]. We will develop highly dynamic depictions of environmental factors in the study areas, including meteorological metrics, landscape matrix, and land use drivers derived from satellite imagery to model local scale parasite abundance and transmission potential.

2.3. Hypotheses

This preliminary study is largely descriptive. The subsequent matched cohort study will test the hypothesis that individuals harboring persistent chronic subclinical *P. falciparum* and/or *P. vivax* parasitemias are protected from clinical malaria, but represent a source of malaria transmission.

2.4. Choice of study design

This is a short longitudinal preliminary study that aims to describe the dynamics of low-density subclinical malaria to support the final study design of a subsequent matched cohort study. To provide the rationale for the current protocol, both studies are described below; a separate protocol for the matched cohort study will be finalized based in part on results of this preliminary study.

2.4.1. Preliminary study

In this study, a small number of asymptomatic infections detected by ultrasensitive PCR (usPCR) will be followed and tested intensively for three months to measure the temporal dynamics of these infections. A much larger number of uninfected individuals will be followed

for just 2-4 weeks (two visits), providing a comparator group for the infected cohort. The validity of our results in a subsequent matched cohort study depends, in part, on our ability to accurately classify infection status using a single usPCR result. In other words, do we have sufficient confidence in the correct classification of malaria infection positive and negative status using usPCR test at the beginning of the study? While there is no gold standard test more sensitive than usPCR, repeated usPCR testing offers a surrogate. Presumably, in a low transmission setting, someone who is truly uninfected on the first testing should remain negative on multiple consecutive tests, but this remains to be confirmed.

If this initial evaluation confirms that usPCR results are relatively stable - i.e., that most usPCR+ infections remain positive on most or all repeated testing over the course of three months, and that most usPCR- infections remain negative upon re-testing 2-4 weeks later, then we can move forward with the cohort study with confidence that our initial classification of exposure is a meaningful basis for assessing subsequent outcomes. If, however, a single usPCR result is found to be “unstable”, we may need to consider testing at least twice before assigning infection status. Similarly, we may wish to increase the sample size if we determine that the sensitivity and specificity of a single usPCR test (compared to results of multiple testing) is high enough to proceed with single testing at screening and enrollment, but lower than ideal (e.g. sensitivity/specificity 50-75%).

2.4.2. Design of the subsequent matched cohort study (to be submitted for ethical review later as a separate protocol)

To provide the rationale for this preliminary study, the design of the subsequent matched cohort study is described briefly here. The principle design will be a matched cohort study. This choice of study design is based on the knowledge of generally low prevalence of malaria in most of the proposed study sites. Although a standard cohort study would be appropriate in a high transmission setting, it would be difficult, if not impossible, to reach adequate sample size in our study sites with such low prevalence. A retrospective case control design is also unsuitable, since it will not be possible to measure the exposure of interest (prior subclinical malaria) at the time that the primary outcome (RDT+ infection) is detected. Therefore, a matched case-cohort design similar those we have previously done in Mali [53] offers a good alternative to study a rare exposure.

Matching is not typically used in cohort studies because it precludes testing for association between matched variables and outcomes of interest. However, in this situation, independent association between clinical protection and transmission risk needs to be understood. Because subclinical infection is itself likely to signify higher malaria exposure risk, comparing rates of RDT+ infections and transmission capability in unmatched infected and uninfected cohorts would likely show that clinical malaria and transmission capacity are more common in the infected cohort simply because their initial infection signals greater malaria exposure. Therefore, for the subsequent cohort study we will match infected participants (cases) to uninfected controls, to control for covariates known and suspected to be associated with malaria risk. With this design, we will still be able to test for association with matched variables and outcomes of interest within (but not between) infected and control cohorts.

2.5. Potential Risks and Benefits

2.5.1. Potential Risks

Effects of finger sticks and venous blood draws. Risks of bruising, bleeding, infection and fainting can accompany these procedures. Study-related blood collection for malaria diagnostic testing by rapid diagnostic test and for a dried blood spot sample can be accomplished by using finger stick or earlobe stick. Venous blood will only be obtained routinely from participants with RDT+ infections detected either in screening, enrollment, follow-up, or in active or passive surveillance.

DNA sequence information. Samples will be used for genotyping and/or sequencing malaria parasite RNA and/or DNA (nucleic acids). Left-over samples will be archived, with consent from study subjects, for future investigations—potentially including analysis of human nucleic acids or nucleic acids from other infectious organisms—with further appropriate approval. For example, future studies may investigate the presence of other causes of fever in participants with fever but negative malaria tests, or genomic studies of human migration to complement those of parasite migration.

Potential risks to study personnel. The main risks to study personnel are from accidental exposure to blood and body fluid borne infections. SOPs for staff safety are used in clinical and laboratory areas, including sharps management, hazardous waste management, etc. Universal precautions are used for handling all body fluids.

2.5.2. Known Potential Benefits

Participants may not receive any direct benefit from their participation in the study. However, if symptoms of malaria or other illnesses are noted, participants will be referred for prompt evaluation and treatment by the appropriate local health provider. By providing evidence-based strategies for malaria elimination, the information gained from the proposed work is beneficial for the study participants in the future, others living with malaria or in malaria-endemic regions, as well as public health professionals and policymakers in Myanmar and in the GMS, who are leading regional malaria elimination efforts.

3. OBJECTIVES AND OUTCOMES

Primary objective:

Make a preliminary assessment of the dynamics of subclinical malaria detected by ultrasensitive PCR to guide the design of a matched cohort study of subclinical malaria in Myanmar and along its borders with China and Bangladesh

(*The objective of the subsequent matched cohort study, to be reviewed as a separate protocol, will be to compare the incidence of clinical malaria and malaria transmission capacity, in participants with and without subclinical malaria*.)

Secondary and exploratory objectives:

1. Within usPCR+ and usPCR- cohorts, test for association between demographic and other risk factors (e.g. occupation, travel) and incidence of clinical malaria and transmission capacity
2. Collect preliminary data to estimate the history of exposure to malaria parasite and mosquito vector species by serological profiling
3. Collect preliminary data to develop a model system to predict future malaria risks using geospatial and satellite data on environmental conditions, patterns of parasite migration and human mobility, field data on vector abundance and malaria burden over time at study sites

Primary outcomes:

1. Proportion of individuals with initially positive usPCR tests that were followed by 1 or more consecutive positive tests on weekly or biweekly testing
2. Proportion of individuals with initially negative usPCR tests that subsequently had at least one usPCR positive test at a second screening 2-4 weeks later.

Primary endpoints:

Not applicable

Secondary and exploratory outcomes:

Infection outcomes and endpoints

1. Incidence of falciparum malaria infection by RDT
2. Incidence of vivax malaria infection by RDT
3. Incidence of mixed falciparum/vivax malaria infection by RDT
4. Incidence of falciparum malaria infection by usPCR
5. Incidence of vivax malaria infection by usPCR
6. Incidence of mixed falciparum/vivax malaria infection by usPCR

Serology endpoint

Seroreactivity to polymorphic malaria/mosquito proteins

4. STUDY DESIGN

4.1. Overview

- Prospective, multi-site short longitudinal study
- Up to six study sites, each with >2 villages, towns, unions, refugee camps or plantations, or a single military base. Alternative and additional sites may be added to ensure enough infected cases.
- Screening for eligibility (age at least 0.5 year; able & willing to strictly follow study protocol and to provide written informed consent or assent as appropriate)
- Enroll and consent eligible individuals
- RDT-positive: one-time enrollment for data and venous blood collection; Refer to and ensure appropriate treatment by care providing team; No study-related follow up
- RDT-negative: Collect data and DBS samples; Return to research clinic in 2-4 weeks
- PCR-negative participants: One follow up visit approximately 2-4 weeks after enrollment
- PCR-positive participants: Five follow up visits at approximately 4, 6, 8, 10, and 12 weeks (for those with total 12 weeks follow up) or 2, 3, 4, 5 and 6 weeks (for those with total 6 weeks follow up) after enrollment
- Total up to 6000 residents in up to six study site of all ages in villages, refugee camps, military bases, and work sites are to be enrolled
- Finger stick blood sampling with be done for RDT and dried blood spot sampling in all participants at each scheduled and unscheduled visit.
- Venous sampling will be done for participants with RDT+ infections detected at times when study staff trained for venous sampling are present. Blood volumes at any time point are limited to 2 mL for children aged < 3 years, 3 mL for age 3-5 years, and 5 mL for older children and adults.
- Estimated duration of study:18 months

4.2. Description of the Study Design

This is a multi-site short longitudinal study to evaluate the dynamics of subclinical *P. falciparum* and/or *P. vivax* malaria. The study areas are chosen to represent a range of malaria epidemiologies characteristic of the region, and include Townships in Myanmar's Mandalay and Sagaing Regions and Chin and Rakhine States; Unions in the Chittagong Hill Tracts of Bangladesh; and Counties in China's Yunnan Province that border study sites in Shan and Kachin States (Table 1, Figure 3).

A maximum of up to 6,000 participants will be enrolled in the longitudinal study in up to six participating sites. Survey participants will provide consent (and assent, if appropriate) prior to having blood collected for hemoglobin measurement and for dried blood spots used for molecular analyses. A subset of RDT+ participants will also be asked to provide venous blood.

Table 1. Initial study sites

Location	State Region Province or District	Township County or Union*	Estimated population	*P. falciparum* Prevalence by RDT % (range)	*P. falciparum* Prevalence by USqsPCR % (range)	*P. falciparum* API Cases/1000 (range)	*P. vivax* Prevalence by RDT % (range)	*P. vivax* Prevalence by USqsPCR % (range)	*P. vivax* API Cases/1000 (range)
Central Myanmar	Mandalay	Madaya (Singu)	22,000	0.0 (0.0-0.0)	0.5 (0.0-2.0)	1.03† (NA)	0.0 (0.0-0.0)	3.5 (0.0-6.0)	1.03† (NA)
Western Myanmar	Rakhine	Ann (Buthidaung)	2731	2.5 (0.0-8.0)	11.1 (4.0-22.0)	2.09† (0.0-4.59)	0.0 (0.0-0.0)	9.0 (0.0-20.0)	2.09† (0.0-4.59)
Myanmar-India border	Sagaing	Monywa/Tamu (Paletwa)	1,200 troops	--	--	1.05† (0.0-8.38)	--	--	1.05† (0.0-8.38)
China-Myanmar Border	Yunnan	Waingmaw	20,000 refugees	1.0 (1.0-1.0)	0.0 (0.0-0.0)	2.99† (0.0-26.15)	0.0 (0.0-0.0)	6.0 (6.0-6.0)	2.99† (0.0-26.15)
	Shan	Mongmao	15,000	--	--	0.39 (NA)	--	--	2.02 (NA)
	Yunnan	Nabang	1,629	--	--	0.019 (NA)	--	--	0.22 (NA)
	Kachin	Cangyuan	9,000	--	--	0.054 (NA)	--	--	0.22 (NA)
Southeastern Bangladesh	Bandarban	Rajbila	8,619	--	--	3.48 (0.43-14.57)	--	--	0.67 (0.0-2.73)
		Kuhalong	10,361	--	--	2.03 (1.13-7.16)	--	--	0.19 (0.16-2.39)

*Myanmar Townships, Chinese Counties and Bangladeshi Unions are roughly similar administrative units that contain many villages; Chinese counties are much larger in size and population. Alternative sites that may be substituted in the event that too few cases are found at initial sites are shown in parentheses.
†All malaria. API data for some townships is reported only for all malaria (any positive RDT). Based on prevalence data, API for Kachin State site is likely to be mostly *P. vivax*.
RDT, rapid diagnostic test for malaria; usPCR, ultrasensitive polymerase chain reaction; API, annual parasite index (cases per 1000 population at risk/year); NA, not available—range across villages not available for areas only reported by township.

5. STUDY POPULATION

5.1. Selection of the Study Population

Sites broadly representative of the range of malaria epidemiologies seen in the region (Table 1, Figure 3) have been identified based on available data on the incidence of clinical malaria and prevalence of subclinical malaria (measured by both standard testing and by usPCR in surveys we have supported). In a staged fashion, initial screening will be undertaken at these sites, starting with the sites in Myanmar, where laboratories proficient in usPCR have been established.

To account for the possibility that malaria rates have declined since the latest available data were collected, alternative and additional sites may be identified in consultation with our research partners and local NMCP officials. The addition or substitution of additional sites will be reported to the study sponsor and IRBs.

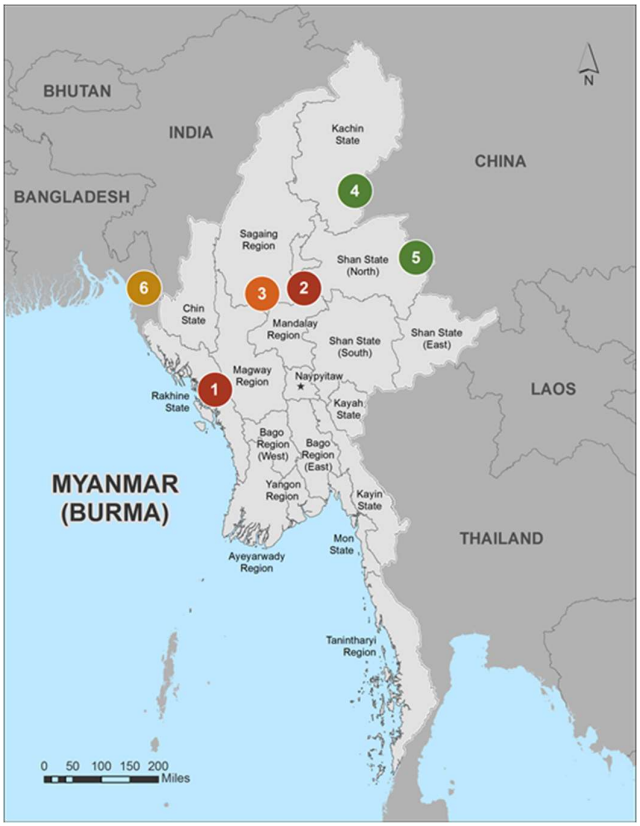


Figure 3. Study Sites and partners. Department of Medical Research: (1) Ann Township, Rakhine State and (2) Madaya Township, Mandalay Region; Defence Services Medical Research Centre: (3) Monywa Township, Sagaing Region; National Institute of Parasitic Diseases, Chinese CDC: (4) Waingmaw Township, Kachin State/Nabang, Yingjiang County, China and (5) Mongmao Township, Shan State/Canguan County, Yunnan Provice, China; icddr,b: Rajbila and Kuhalong Unions, Bandarban District , Bangladesh.

Children will be included because they may be at risk for malaria, as well as to measure age-specific infection pattern which is expected to vary across sites. Adults of all ages—including pregnant women and the elderly—will be included to address the paucity of data on transmission potential in these groups and to detect chronic infections persisting even in the absence of recent exposure.

Because technical capacity for molecular diagnosis required for this study is not yet established at the Bangladesh and China sites, the study will begin at Myanmar sites following review and approval by the Myanmar and U.S.-based IRBs. As laboratory facilities are established in Bangladesh and China, the protocol will be reviewed by IRBs in those countries. Study activities in each country will only be started after review and approval by the IRB(s) in that country.

5.2. Sampling Strategy

The primary aim of the screening process is *not* to obtain an unbiased measurement of malaria prevalence, which is already known to be low in many sites. Rather, it is to identify and enroll in the study the target number of cases of subclinical malaria infection, which are expected to be rare. People and locations suspected or known to harbor malaria will be targeted for screening, based on: RDT and usPCR cross-sectional prevalence data from our ongoing

separately-funded malaria surveillance activities; RDT-based malaria incidence data from NMCPs and our own previous and ongoing research and surveillance activities; village clinic treatment logs; interviews with local health providers and community leaders; and results of initial screening.

As the screening process progresses, household and workplace contacts of RDT+ and usPCR+ cases will be traced to find more infected individuals until enrollment targets are reached. This targeted screening means that the overall sampling framework will be neither random nor fully representative of the larger communities. However, whenever possible, comprehensive or representative sampling will be done to provide unbiased prevalence estimates of *P. falciparum* and *P. vivax* infection for specific locations or populations within the larger study areas (e.g. sampling all individuals in a village).

While sampling strategies will necessarily be locale-specific (e.g. clinic-based, school-based, military-based or household-based), at each location the basis for sampling will be carefully documented so that we can ascertain when unbiased estimates of prevalence can be made for specific locations or populations. Each site has a central location for screening, enrollment and follow-up and satellite locations, e.g. clinics or schools, where study activities will occur.

5.3. Inclusion/Exclusion Criteria

5.3.1. Inclusion criteria for pilot study

Participants must meet all the inclusion criteria to participate in the study: 1) Age 6 months or older at the time of screening; 2) Written informed consent obtained (from the parent/guardian if the subject is less than 18 years old).

5.3.2. Exclusion criteria

Any condition which in the view of the investigator makes participation not in the best interests of the prospective participant. Any condition that would interfere with study participation or pose risks to participants. Those with clinical malaria infection as diagnosed by positive RDT may be invited to return and be re-screened for study eligibility after treatment and resolution of their illness.

6. STUDY PROCEDURES/EVALUATIONS

6.1. Study Procedures

6.1.1. Screening process

People and locations suspected or known to harbor malaria will be targeted for screening, as described above. As screening progresses, household and workplace contacts of RDT+ and usPCR+ cases will be traced to find more infected individuals until enrollment targets are reached. The sample collection schedule is shown in Table 2.

A very small number of individuals who are found to be RDT+ during the screening process will be asked to provide venous blood, dried blood spots, and travel history. Individuals who are found to be RDT+ during screening, or at any time during the study, will be referred promptly to local health providers for ACT treatment, in compliance with WHO recommendations and NMCP policies, which call for treatment of any RDT+ infection.

Table 2a. Study procedures for population with total 12 weeks of follow up

Procedures	Screening	Follow-up visits in weeks after enrollment				
		4	6	8	10	12
Eligibility/ RDT testing	R					
Informed consent/assent	R					
Enrollment	R					
Contact & demographic Information; hemoglobin testing	R					
Travel history	R	R	R	R	R	R
Dried blood spots (RDT- & usPCR+ at enrollment)	R	R	R	R	R	R
Dried blood spots (RDT- & usPCR- at enrollment)	R	R				
Venous blood collection for RDT+	R; Any time during the study					
Treatment referral for RDT+	S; Any time during the study					

RDT, rapid diagnostic test; usPCR, ultrasensitive PCR; R, research-related procedure; S, standard care procedure

Table 2b. Study procedures for population with total 6 weeks of follow up

Procedures	Screening	Follow-up visits in weeks after enrollment				
		2	3	4	5	6
Eligibility/ RDT testing	R					
Informed consent/assent	R					
Enrollment	R					
Contact & demographic Information; hemoglobin testing	R					
Travel history	R	R	R	R	R	R
Dried blood spots (RDT- & usPCR+ at enrollment)	R	R	R	R	R	R
Dried blood spots (RDT- & usPCR- at enrollment)	R	R				
Venous blood collection for RDT+	R; Any time during the study					
Treatment referral for RDT+	S; Any time during the study					

RDT, rapid diagnostic test; usPCR, ultrasensitive PCR; R, research-related procedure; S, standard care procedure

Eligible and consenting individuals will provide dried blood spot (DBS) samples, demographic and travel information, and contact information. DBS will be analyzed using usPCR methods in designated laboratories. Of those tested at the initial screening visit, usPCR- participants will return for just one additional follow up visit at approximately 4 weeks, at which time a second DBS and updated travel information will be collected. Individuals who test usPCR+ at the initial screening will also return approximately 2-4 weeks after the initial screen, and again for four additional visits (weeks ~6, 8, 10, and 12 or weeks ~2, 3, 4, 5 and 6), with DBS and travel history update collected at each visit.

6.1.2. Enrollment in preliminary study

Eligible and consenting individuals will be enrolled in the study. Eligibility criteria are listed in Section 5.3.

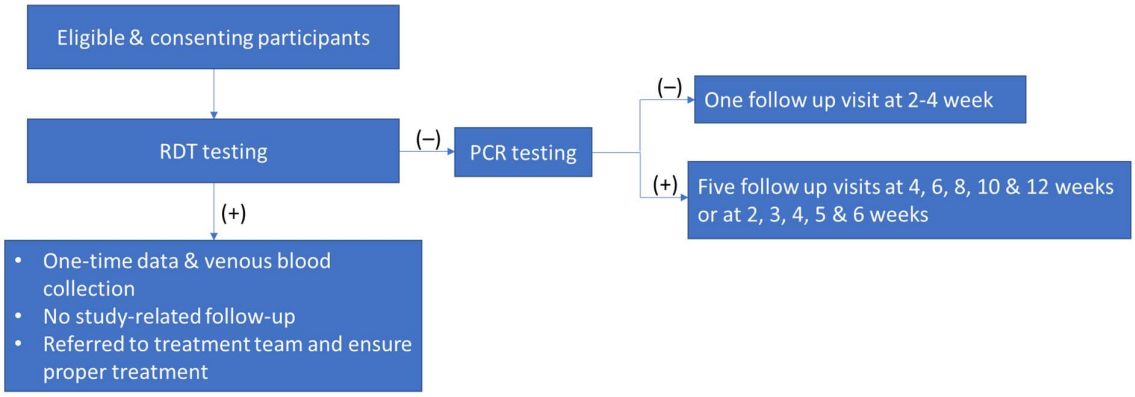
6.1.3. Follow-up

All enrolled participants will follow up 2-4 weeks after enrollment. Those who are found to be usPCR negative will provide one additional DBS at a second visit at 2-4 weeks and discharged from the study. Those who are found to be usPCR positive will provide four additional DBS, two weeks apart (weeks 6, 8, 10, and 12), and discharged from the study at Week 12. For the military population who are likely able to provide a total of only 6 weeks follow up, they will return at 2 weeks after enrollment, and the follow up schedule will be weekly.

At each follow up visit, a brief history (including travel history update) will be taken, and dried blood spots will be collected. Those who miss two consecutive follow-up visits will be

discharged from the study. At any time that participants are found to be RDT+, if study personnel qualified to collect venous blood are present, RDT+ participants will provide venous blood for serology and parasite nucleic acid sequencing. Individuals testing RDT+ at any time during the study will be referred for prompt ACT treatment of malaria by local health providers.

Conceptual framework of the study is shown below:



Anticipated duration of screening and enrollment phase: Screening and enrollment will begin at each site after obtaining ethical approval from all relevant administrative and ethical review committees for that site. Because technical capacity for usPCR is not yet established at the China and Bangladesh sites, the study is expected to begin sooner at the Myanmar sites. Screening will be continued until enrollment targets are reached.

Duration of follow-up for each subject: Up to 4 months (~2-4 weeks initial assessment plus up to 1.5-3 months follow-up).

Total study duration: Up to 1.5 years

Retention: We have achieved retention rates of >95% in longitudinal cohort studies in Madaya (unpublished) and Bandarban [54-58] by maintaining strong relationships with the community, including regular community meetings; contributing to community welfare through training health providers and upgrading facilities; and treating informed consent as an ongoing process for the individual and the community [59, 60]. Retention is expected to be lower at sites with refugees and military troops, requiring additional screening at those sites, as noted above.

Study termination: Participants will end their participation in the study when they miss two or more consecutive follow-up visits. Participants who return after more than two consecutive missed visits will be eligible to be re-screened and re-enrolled as a new participant.

Study visit schedule and primary evaluations: Active surveillance will consist of scheduled visits every 1-2 weeks for procedures shown in Table 2a and 2b. Passive surveillance will be conducted by encouraging participants to see the local health provider when ill. Local providers will collect dried blood spot samples whenever RDTs are done and will notify study staff of positive tests so that venous blood can be collected. In accord with WHO and NMCP policies in all three countries, any positive RDT result, whether testing was done as part of active or passive surveillance, will result in referral for ACT treatment, which will be administered by local providers as routine care and not as part of the study.

6.1.4. Sample Collection

Dried blood spots at all encounters. Our usPCR method preserves both DNA and RNA in 0.3 mL of blood collected in capillary tubes initially [22], and we have successfully adapted this method for DBS on filter paper, achieving nearly the same LoD. At sites and times when our dedicated study personnel are present to collect samples, DBS will be collected on two filter papers from finger-stick samples. When local health providers collect samples in the absence of research staff—e.g. when evaluating fever episodes during unscheduled visits, DBS will be collected, following standard procedures that we have successfully trained local health providers to perform at dozens of remote sites in Myanmar [22], Bangladesh [54] and China [9].

Whole blood during RDT+ malaria episodes. While DBS and no requirement for cold chain is field-friendly and simpler, the ability to sequence parasite genes is impaired by the low parasite density. In addition DNA degrades overtime without cold chain. As DNA quantity from DBS is not sufficient for whole genome sequencing, venous sampling will be done for RDT+ infections detected in screening, follow-up or active surveillance. Blood volumes at any time point are limited to 2 mL for children aged < 3 years, 3 mL for age 3-5 years, and 5 mL for children aged >5 years and adults. We have extensive experience recovering sera and cryopreserved parasites from small blood volumes collected from children [61-65] and have developed and used protocols for preserving RNA from field samples in Southeast Asia [22, 66].

Quality control and assurance. QC/QA procedures will be followed, including routine monitoring and evaluation of study records, using regularly updated study-specific and general standard operating procedures, and providing periodic refresher training in Good Clinical Practices.

6.2. Laboratory Evaluations

6.2.1. Laboratory evaluations/Assays

Dried blood spots (DBS) on filter paper will be used for usPCR assays and other targeted molecular genotyping and serological assays [45-47]. Venous blood samples will be used for serology, genotyping [67, 68], targeted and genome-wide sequencing [69-71], and related analyses.

To the greatest extent possible, laboratory analyses, including molecular assays and probing of microarrays, will be conducted at malaria research laboratories of collaborating institutions in the region (DMR, DSMRC, icddr,b and NIPD), in close collaboration and with technical support from the Duke University malaria team. A subset of samples will be shipped to Duke University malaria and collaborating genomic laboratories for advanced analyses such as whole genome sequencing and assembly [70-72] and for quality assurance procedures.

6.2.2. Specimen Collection, Preparation, Handling and Shipping

6.2.2.1. Instructions for Specimen Preparation, Handling, and Storage

Blood spots on two types of filter paper for study analyses will be collected at the time of blood collection for malaria diagnostic testing. DBS specimens will be collected using standard methods [73].

DBS and venous blood samples will be labeled with a participant ID and demographic information. After collection, blood spot samples will be air dried and stored in zip-lock plastic bags with desiccant at each site in a cool, dry, location.

Venous blood samples obtained from RDT+ participants will be collected into tubes with anticoagulant, frozen, and stored in either liquid nitrogen dry shippers or -20 ºC or -80 ºC freezers. Frozen samples (either blood or extracted DNA) may be shipped to the Duke University in dry ice containers or liquid nitrogen dry shippers.

Future studies of parasite DNA derived from samples may be conducted without restriction. Relevant studies of human RNA or DNA, such as human polymorphisms associated with risk of or protection from malaria, or genomic studies of human migration patterns, may be done on archived samples or nucleic acids only if specific permission for additional such studies is obtained from the relevant IRBs. Study participants will have the right to withdraw their permission for further use of their samples at any time during and after the study.

6.2.2.2. Specimen Shipment

Samples will be shipped periodically to the malaria laboratories at Duke University in Durham NC or other collaborating laboratories for specialized genotyping/probing that cannot be done in the region. Frozen venous blood samples will be sent in liquid nitrogen dry shippers or on dry ice to Duke University. Specimen receipt will be logged into an inventory database.

7. STATISTICAL CONSIDERATIONS

7.1. Study Outcome Measures

Primary objective:

Make a preliminary assessment of the dynamics of subclinical malaria detected by ultrasensitive PCR to guide study design of a subsequent longitudinal matched cohort study.

(*The objective of the subsequent matched cohort study, to be reviewed as a separate protocol, will be to compare the incidence of clinical malaria and malaria transmission capacity, in participants with and without subclinical malaria*.)

Secondary and exploratory objectives:

1. Measure the dynamics of subclinical malaria over time and obtain a preliminary estimate of the chronicity of infection over a short period of time
2. Test for association between demographic and other risk factors (e.g. occupation, travel) and incidence of clinical malaria and transmission capacity
3. Collect preliminary data to estimate the history of exposure to malaria parasite and mosquito vector species by serological profiling
4. Collect preliminary data develop a model system to predict future malaria risks using geospatial and satellite data on environmental conditions, patterns of parasite migration and human mobility, field data on vector abundance and malaria burden over time at study sites

Primary outcomes:

1. Proportion of individuals with initially positive usPCR tests that were followed by 1 or more consecutive positive tests on biweekly testing
2. Proportion of screened individuals with negative usPCR tests that subsequently had usPCR positive tests at a second screening test 4 weeks later

Primary endpoints:

Not applicable

Secondary and exploratory outcomes:

Infection outcomes and endpoints

1. Incidence of falciparum malaria infection by RDT
2. Incidence of vivax malaria infection by RDT
3. Incidence of mixed falciparum/vivax malaria infection by RDT
4. Incidence of falciparum malaria infection by usPCR
5. Incidence of vivax malaria infection by usPCR

6. Incidence of mixed falciparum/vivax malaria infection by usPCR

Serology endpoint

Seroreactivity to polymorphic malaria/mosquito proteins

7.2. Sample Size Considerations

This preliminary study is designed for better understanding of the dynamics and stability of usPCR status. The preliminary data are needed to estimate the required sample size for the subsequent matched cohort study, by assuming a McNemar test at α=0.05, two-sided, on the observed proportions of usPCR+ and usPCR- participants who develop a positive RDT in 5 years of follow-up for all sites combined. The results of this preliminary study as well as data from other recent and ongoing surveillance and studies will allow us to estimate the average annual probability of a positive RDT. We will then be able to be calculate the sample size to obtain adequate statistical power to reject the null hypothesis that the true risks in usPCR+ and usPCR- individuals are equal if the true relative risk of the estimated 5-year probability of a positive RD.

The sample size of this preliminary study (up to 1,000 per site) is planned within logistical and financial constraints. A total of 250 usPCR+ is conservatively estimated. Observing usPCR+ for 6 or 12 weeks (total number of observation = 6) is feasible, and will provide a meaningful preliminary estimate of the stability and dynamics of usPCR+ infections. For every 100 enrollees, we estimate that at least 75% will be usPCR-. Observation of usPCR-individuals (sample size may be as many as 750 per site) for 2-4 weeks (total number of observation = 2) will also be sufficient and meaningful to judge the stability of usPCR- status.

7.3. Participant Enrollment and Follow-Up

Recruitment will begin in the 2018 May-October rainy season and continue with until enrollment targets are reached. The duration of follow-up for each subject will be up to 12 weeks. The total study duration will be up to 1 year. At each site, enrollment will be paced such that at least 100 participants are enrolled in both the rainy and in the dry season, permitting us to assess seasonal variation in prevalence of infection.

7.4. Analysis Plan

Primary analysis will compare the proportion of individuals with initially positive usPCR tests that were followed by 1 or more consecutive positive tests on biweekly testing. Using the screening results, we will also calculate the proportion of individuals with negative usPCR tests that subsequently had usPCR positive tests at the second screening test.

Secondary analyses will include comparisons based on incidence of species-specific RDT+ and usPCR+, and, for serological studies, breadth (% seropositivity) and magnitude (mean fluorescence intensity) of antibody responses on protein and peptide microarrays.

To understand the dynamics of usPCR positivity, we will construct maximum likelihood models using Markov chains to evaluate risk factors that increase or decrease the probability of going from state to state between monthly sampling time points.

8. SUBJECT CONFIDENTIALITY

Subject confidentiality is held strictly in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples, in addition to the demographic information relating to participating subjects. The study protocol, documentation, data and all other information generated will be held in strict confidence. Study records including case record forms and consent forms will be stored in locked cabinets in secure facilities only accessible to authorized investigators, and/or in password-protected devices and computers.

All participants will be assigned a unique study identification (ID) number. This number will be used to label all biologic samples and study-related documents except informed consent/assent forms which will contain the participant's identifier and will be stored in secured space as described below. All data generated from each study sample will be linked to the coded, unique ID and associated study data.

Study data will be stored on password protected computers at the study site under the purview of leadership of the DMR, DSMRC, icddr,b and NIPD, with restricted access to specified study personnel. Genetic/genomic data generated from malaria parasites isolated from study participants may be submitted to publicly available databases, but will not be linked to any identifying information from study participants. Participants will not be identified in any publications resulting from the study.

The data will be used by the PI and study collaborators to assess parasite gene flow and genotype candidate markers of drug resistance as described in this document. The data will be stored in secure, password-protected databases at the malaria laboratories at the Duke University in Durham NC for up to 20 years, after which the data may be destroyed.

8.1. Future Use of Stored Specimens

All study participant will be asked whether we will be permitted to use left-over blood sample for future studies 1) related to malaria; 2) related to any infections or 3) related to any diseases. Studies of microbial RNA or DNA (chiefly malaria, but also other infectious agents that may explain non-malaria fevers) derived from samples may be done without restriction. Studies of human RNA or DNA using archived samples or material will be done only if specific permission for additional such studies is obtained from the relevant IRBs. Study participants will have the right to withdraw their permission for further use of their samples at any time during and after the study. Specimens will be maintained in malaria laboratories at the DMR, DSMRC, NIPD, icddr,b and/or Duke University. Specimens will be labeled with a coded, unique identifier and will not contain identifying information.

9. INFORMED CONSENT PROCESS

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specified procedures are carried out.

Information about the study will be given to prospective participants in both oral and written forms whenever possible. The written consent documents will embody the elements of informed consent as described in the current edition of the Declaration of Helsinki, will adhere to the ICH Harmonized Tripartite Guideline for Good Clinical Practice and 45CFR46 and 21CFR50, and will also comply with applicable Myanmar regulations. The oral consent process will be consistent with 45CFR46.§46.117, 21CFR50.27 and ICH E6 (R1) Section 4.8. Independent witnesses will be used to attest that illiterate potential participants have understood the contents of the informed consent document.

9.1. Informed Consent/Assent Process (in Case of a Minor or Others Unable to Consent for Themselves)

We have published detailed descriptions of the processes we use to obtain community "permission to enter" and individual informed consent [60]. We consider informed consent to be a dynamic, ongoing process, with continuous availability of investigators to answer any questions that arise in the course of the study and to ensure that participants and their parents/guardians understand study procedures. These same principles will be followed in this study. All NMCPs in the GMS routinely conduct multiple annual therapeutic efficacy surveys to monitor efficacy of ACT. As a result, communities throughout the GMS have now become familiar with the informed consent process, including written, signed consent forms, which used for NMCP therapeutic efficacy surveys. The investigators will work with local research staff at each site to assure study information and informed consent procedures meet requirements of the community and the local IRBs.

Since the vast majority of study participants' parents/guardians do not use telephones, fax, or mail, contact information is provided in terms of local physicians who can be visited directly and who can themselves reach the investigators directly or by telephone or fax.

Individuals who are at least 18 years old are considered adults and will provide consent for themselves; children aged up to 12 years will provide verbal assent in addition to written consent provided by their guardians; children aged 13–17 years will provide written assent in addition to written consent provided by their guardians. Guardians need not necessarily be the child's parent(s). Any close relative including aunts, uncles, grandparents, adult siblings etc. who accompany the child to the clinic may serve as the guardian for the sake of providing informed consent. This may include neighbors or village elders in the case of orphans.

10. DATA MANAGEMENT

Source Data, Data Storage, and Access to Data

Hard copy research records generated for this protocol will be stored in locked rooms at project sites. Study data will be entered electronically into a secure, password protected, cloud-based research information portal (RIP) developed, managed, and maintained by the Data Management and Statistics Core. The data warehouse will be developed using MySQL developed by DMB Core staff. The portal will also be built to ensure data security and integrity.

Our primary tool for developing case record forms will be REDCap™ for electronic data capture REDCap™ is a free, secure, web-based application for data capture. Developed by a multi-institutional consortium initiated by Vanderbilt University, REDCap™ provides user-friendly, web-based case report forms, real time data entry and validation (e.g., data types, range checks), audit trails, and the ability to set up a study-related calendar to schedule and track critical study events such as participant visits, reminders, etc. The REDCap consortium of users is comprised of 1,837 institutional partners in 99 countries around the world who use and support REDCap as a research tool [4,5]. REDCap™ will give us the ability to assign different levels of access to members of the research team depending upon their role on the project so that end users only have access to forms and data directly related to their role on the project.

The REDCap™ system provides tools for users to enter data directly through a web-based interface on laptop, Ipad or other tablet device, or enter and save data offline and upload the data onto a web server later via internet or satellite service. In addition, CRFs can be saved as PDFs for printing in order to allow offline data collection. Any data collected on paper in offline mode will require manual entry with double keying to ensure data accuracy. In each field site, data will be entered into a REDCap™ CRF on a mobile phone or a tablet, and saved. Internet service is reliably available at the local base of operations for each field site, located in towns or cities at or near the field site (DMR: Buthidaung and Madaya; DSMRC: Monywa; NIPD: Canyuang and Nabang; icddr,b Bandarban), where data will be uploaded daily to the central database.

Access to the Duke University REDCap™ system requires university-level credentials. Specifically, the tool requires username/password security protection to prevent unauthorized access for entry or editing data. Shibboleth [6] middleware is used for identity management and will be used for federated identity-based authentication and authorization. A list of security level access (full access, edit access, read only access etc.) is documented. REDCap™ complies with the Health Insurance Portability and Accountability Act (HIPAA) regulations and is 21 CFR Part 11 capable through server configuration.

Data will be stored on a secure, cloud-based server. Connections and access to the RIP will be secured with a username and password. Role-based access privileges will be assigned and access will be limited to authorized users. PIs will be granted access to all of the data from their site, while sub-investigators and other project personnel may only have access to data that has been authorized by the study PI. PIs will have final authority for determining who is granted access to data and to which data they will have access.

10.2 Data Standardization

To the extent possible, data elements will be developed following the Clinical Data Acquisition Standards Harmonization (CDASH) developed by Clinical Data Interchange Standards

Consortium (CDISC). Adoption of these standard will facilitate data sharing and harmonization with other ICEMRs.

10.3 Audit Trail System

The RIP and tools embedded within the RIP like REDCap™ will support an automatic electronic audit trail that permanently tracks and logs every access of data, tools, or reports within the RIP. The RIP audit log records the date and time of any access, the RIP element that was accessed (e.g., data table, software tool such as REDCap™) and records all changes to data stored in the RIP. The system will record and store the username of the individual who changes data, the date and time of change, the new and old value of the variable changed and the computer used.

10.4 Data Validation and Query Generation

REDCap™ will apply data validation rules at the time data are entered through the REDCap™ tool. Other programs within the RIP will identify potentially incorrect data through manual and automatic queries. These queries are run after reconciliation procedures, and check for any data discrepancies in the system. These queries are documented on a Data Clarification Form (DCF). The system has an input mask for incorrect data such as out of range/value or an incorrect date. At each in-country partner site (DMR and DSMRC in Myanmar, NIPD/YIPD in China, and icddr,b in Bangladesh) a data clerk documents or flags any unclear data or errors during the data checking process. The data supervisor generates a query for any discrepant data or error and sends it to the field site for resolution. Final quality checks will be done in Durham until regional data teams are fully capable of managing the data validation process. The resolution of queries by the PI or the designee will be documented and dated. After the resolution, the data are updated using the resolved query; the update of this query is then signed. This electronic update of data is tracked by the RIS audit trail system.

10.5 Database Lock (Locked Data)

After the cleaning and validation of data, and when data are ready for statistical analyses, the data manager for each site will lock the database (Locked data base). This lock is documented by the data manager and the Site PI with date/time of locked. Any change after the data lock has occurred must be authorized by the PI responsible for the study.

10.6 Quality Assurance and Quality Control

The data managers and data entry clerks involved in the study data management will be trained beforehand in all aspects of the specific study data management procedures. Standard operating procedures for quality management have been developed and are used to train all personnel; these are kept on file with documentation of training. Data are evaluated for compliance with protocol and accuracy in relation to source documents. Quality control will be also monitored using classical means of the Statistical Process Control (including Control Chart of specific indicators).

10.7 Data Integrity and Data Quality

Data integrity will be verified by several means: 1) Comparison of information in the source documents vs. those that are in the CRF; 2) comparison of data in the database vs. those in the CRF; 3) interviews to search for unacceptable or questionable practices, and review of the

analysis procedures; 4) inspection and independent auditing. Furthermore, access to the RIP and all data tables will be monitored through regular control of the access log.

10.8 Upload and Download Functions

The Research Information Portal will have data upload and download capability via secure file transfer protocol (sFTP). Access to sFTP functionality will be controlled by username/password strategy, and all uploads and downloads will be monitored via our central audit logging tool.

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SUPPLEMENTS/APPENDICES