A clinical study to assess the feasibility of a controlled human *Plasmodium vivax* malaria infection model through experimental sporozoite infection in Thai Adults

Short Study Title: Vivax malaria human infection studies in Thailand

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Funding partner	Wellcome Trust
Medical Monitoring Team Leader	Dr. Lorenz Von Seidlein MD

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigator team, regulatory authorities, and members of the research ethics committees. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Site Principal Investigator, Assist Professor Dr. Podjanee Jittamala **AND** both Co-PIs, Professor Dr. Nicholas Day and Research Professor Dr. Jetsumon Sattabongkot Prachumsri.

Investigator Agreement and Conflict of Interest

We have read this protocol and agree to abide by all provisions set forth therein. We agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice.

We declare no conflict of interest, according to the current version of the Declaration of Helsinki.

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Assist. Professor Podjanee Jittamala		
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AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes

LIST OF ABREVIATIONS:

AE	Adverse event
β-HCG	Beta – Human Chorionic Gonadotropin
BP	Blood Pressure
BT	Body Temperature
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CHMI	Controlled human malaria infection
Cr	Creatinine
CRF	Case Record Form
CRO	Contract Research Organization
CSP	Circumsporozoite
CYP2D6	Enzyme predicting primaquine metabolism (P450 enzyme)
DBP	Duffy Binding Protein
DMFA	Direct Membrane Feeding Assay
DSMB	Data Safety Monitoring Board
FBS	Fasting Blood Sugar

FTM	Faculty of Tropical Medicine
FTMCTU	Clinical Therapeutic Unit (healthy volunteer ward), Faculty of Tropical Medicine
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency virus
HTLV	Human T cell Lymphotrophic virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
JE	Japanese Encephalitis
LDL	Low Density Lipoprotein
LFT	Liver Function Test
MFA	Membrane Feeding Assay
MORU	Mahidol-Oxford Tropical Medicine Research Unit
MVRU	Mahidol Vivax Research Unit
OxTREC	The Oxford Tropical Research Ethics Committee
PCR	Polymerase Chain Reaction
P. falciparum	*Plasmodium falciparum*
PI	Principal Investigator

PQ	Primaquine
PR	Pulse Rate
P. vivax	*Plasmodium vivax*
PvCSP	*P. vivax* circumsporozoite
PSC	Programme Steering Committee
qPCR	Quantitative Polymerase Chain Reaction
RECs	Research Ethics Committees
RR	Respiratory Rate
RUNMC	Radboud University Nijmegen Medical Centre
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
TMDR	Tropical Medicine Diagnostic Reference Laboratory
ULN	Upper Limit of Normal
USMMVP	U.S. Military Malaria Vaccine Program
WHO	World Health Organization
WI	Work Instruction

1. SYNOPSIS

Study Title	A clinical study to assess the feasibility of a controlled human *Plasmodium vivax* malaria infection model through experimental sporozoite infection in Thai Adults
Short Study Title	Vivax malaria human infection studies in Thailand
Study site	Clinical Therapeutic Unit (FTMCTU), Hospital for Tropical Diseases, Mahidol Vivax Research Unit, Medical Entomology Department and TMDR, Faculty of Tropical Medicine, Mahidol University, Bangkok Thailand.
Protocol Number	MAL19002
Study Design	Controlled human infection study. Overview This is a sporozoite-challenge clinical study with the primary objectives of assessing the feasibility and safety of controlled human sporozoite *P. vivax* malaria infection in upto six healthy volunteers, and developing a bank of *P. vivax*-infected blood for use as inocula in future controlled human blood stage *P. vivax* malaria infection studies. Secondary objectives are to assess the growth of and the immune response to *P. vivax* infection and assess the induction of sexual gametocytaemia post-CHMI via the natural route of malaria infection (mosquito bite).
Study Participants	Healthy adult volunteers, aged 20-55 years: malaria naïve and ABO blood group O.
Sample size	Up to 6
Planned Study Period	Approximately 1 year from the time of antimalarial treatment initiation. There will be a 6 months screening process prior to the malaria challenge and approximately 9-16 days pre-patent period.
Objectives	Primary objectives:

	• To assess the feasibility and safety of controlled human *P. vivax* malaria infection in six healthy human volunteers, through experimental sporozoite infection (mosquito bite). • To obtain up to 250 mL of blood from each infected volunteer and freeze down the blood to create inocula for use in future *P. vivax* challenge studies. Secondary objectives: • To assess the immune response to primary *P. vivax* infection delivered by mosquito bite. • To assess gametocytaemia following primary *P. vivax* infection delivered by the mosquito bite.
Study Endpoints	Primary endpoints: • Feasibility and safety of *P. vivax* sporozoite human challenge, as measured by successful infection (development of detectable persistent parasitaemia +/- clinical symptoms) and AE(S) occurrences. • Collection and freezing down of up to 250 mL *P. vivax*-infected blood from each of the 6 volunteers. Secondary endpoint: • Cellular and humoral Immune response to primary *P. vivax* infection. • Gametocytaemia pre-treatment, as measured by qPCR.
Medicinal Product	None
Medical monitor (chair)	Dr. Lorenz Von Seidlein, MD

2. BACKGROUND, OVERVIEW AND RATIONALE

2.1 *Plasmodium vivax* malaria

Plasmodium vivax (*P. vivax*) is one of the five *Plasmodium* species known to cause human malaria. It is geographically the most widespread, and accounts for most of the cases of non-*P. falciparum* malaria. Although generally less severe than falciparum malaria vivax malaria is associated with some mortality (particularly in children) and significant levels of morbidity, a disease burden which has been chronically under-appreciated [1]. *P. vivax* accounts for up to 50% of malaria cases in South and Southeast Asia, and between 71-81% of cases in South America with an estimated global number of between 106 and 313 million clinical cases per year. Following sporozoite inoculation by a mosquito clinical cases are not only due to the resulting primary infection but are also due to relapses from the hypnozoites in the liver, which occur weeks to years after the primary infection [2]. Whereas most of the vivax burden is in Asia and South America, there are between 6 - 15 million cases of vivax malaria in Africa per year [3].

2.2 The impact of Plasmodium vivax infection

Plasmodium vivax infection has been shown to cause significant morbidity and attributable mortality, with reported complications including severe anaemia, acute respiratory distress syndrome, shock [4-6]. A reduction in fatal growth when mothers are infected with *P. vivax*, despite effective antimalarial therapy, has also been shown [7]. The full socio-economic impact of vivax infection is not known, but overall global cost due to lost productivity is estimated at US$ 1.4 to 4.0 billion per year [2]. Those affected by *P. vivax* malaria are typically poor with inadequate access to affordable healthcare and with little financial reserve, perpetuating the cycle of poverty [8].

Control of *P. vivax* is challenging. This is due to multiple factors including relapses, difficulty detecting asymptomatic infection, resistance to antimalarial medications, and a lack of understanding of parasite biology [9]. Recent calls for control and 'eradication' of malaria worldwide [10] have focused attention on this neglected disease and the need for development of an effective *P. vivax* vaccine to be used alongside current control methods. Consequently, the revised Malaria Vaccine Technology Roadmap to 2030 now recognises the importance of *P. vivax* and calls for a vaccine to achieve 75% efficacy over two years – equally weighted with *P. falciparum* in an era of renewed political will to move towards malaria elimination and eradication [11].

Unlike *P. falciparum*, to date there has been relatively little research into this malaria species. One of the limiting factors has been the inability to culture *P. vivax in vitro* over a prolonged period. Recently there has been an increase in vivax vaccine research, with several candidate vaccines being developed and taken

forward to clinical trial [12, 13]. However, none of these vaccines has progressed past a Phase IIa trial, and options for assessing the protective efficacy of new candidates are extremely limited.

2.3 Challenges in *P. vivax* drug and vaccine development

The unique characteristics of *P. vivax* pose some specific challenges in studying its biology and designing strategies to control it [14]. For example, the lack of a method for continuous culture of *P. vivax* blood-stages makes it difficult to perform *in vitro* growth inhibition assays to identify synergistic combinations of blood-stage antigens that can be targeted to inhibit *P. vivax* blood-stage growth with high efficiency. In the absence of a *P. vivax* blood-stage culture system, production of infected mosquitoes for sporozoite- or transmission-stage studies also requires access to *P. vivax* patients in endemic areas. Several groups have recently succeeded in establishing short-term *P. vivax* culture for invasion assays using enriched reticulocytes from cord blood [15], but such methods are still dependent on access to fresh *P. vivax* isolates from malaria patients, limiting the routine use of such assays to endemic regions. In order to study hepatocyte invasion stages of *P. falciparum* as well as *P. vivax*, attempts have been made to establish *in vitro* liver models [16, 17]. In addition, a reliable humanised mouse model for *P. vivax* liver stages is now available [18]. Both approaches will help to identify and test novel drugs and vaccines targeting hepatocyte stages. Studies on expression of parasite proteins in hypnozoites will determine if these latent stages can also be targeted with novel drugs and vaccines.

2.4 Vaccine development

Given that a *P. vivax* vaccine will in most cases be used in low transmission settings, the ideal vaccine would have high efficacy (more than 90%) and block transmission. The development of such a vaccine will likely require a combination of multiple antigens that act synergistically to achieve high efficacy. For example, achieving high rates of blood-stage growth inhibition may require targeting a combination of key blood-stage antigens involved in reticulocyte invasion. A combination of blood-stage antigens with liver-stage antigens may be needed to achieve high efficacy, and inclusion of antigens from sexual- and mosquito-stages will be needed to inhibit transmission. It is therefore necessary to initiate efforts to combine antigens both within and across developmental stages to achieve synergy and attain the goal of developing a highly effective vaccine for *P. vivax* malaria. The purpose of developing and deploying sporozoite- and blood-stage human challenge models is to accelerate and rationalise vivax vaccine development. Conducting Controlled human malaria infection (CHMI) studies in malaria-endemic countries using malaria-exposed volunteers provides a means of testing vaccine candidates in a study population genetically and immunologically similar to the target population for vaccine deployment. *P. vivax* vaccine candidates can be validated and down-selected to ensure that only the most promising candidates proceed to time-consuming and expensive field trials against natural challenge.

Table 1: *P. vivax* vaccine candidates under development: from ref 12.

Vaccine candidate	Development Phase	Lifecycle stage	Antigen	Delivery system
VMP001	Phase I/IIa END	Liver-stage	PvCSP	Rec. protein-AS01B
CSV-S,S	Pre-clinical	Liver-stage	PvCSP	HBsAg fusion-AS01B
PvCSP-LSP	Phase I END	Liver-stage	PvCSP	Synthetic peptides-Montanide ISA 720
ChAd63-PvTRAP/MVA-PvTRAP	Pre-clinical	Liver-stage	PvTRAP	Prime-boost, viral vectors
PvDBPII	Phase I	Blood-stage	PvDBP	Rec. protein-GLA-SE
PvDBPII-DEKnull	Pre-clinical	Blood-stage	PvDBP	Rec. protein
ChAd63-PvDBPII/MVA-PvDBPII	Phase I/IIa	Blood-stage	PvDBP	Prime boost, viral vectors
PvMSP119	Pre-clinical	Blood-stage	PvMSP1	Rec. protein-Montanide ISA720
Pvs25H	Phase Ia END	Transmission-stage	Pvs25	Rec. protein-Alhydrogel; Rec. protein-Montanide ISA 51
Pvs28	Pre-clinical	Transmission-stage	Pvs28	Rec. protein-adjuvant
Pvs25-IMX313	Pre-clinical	Transmission-stage	Pvs25	Rec. protein-adjuvant
AnAPN1	Pre-clinical	Mosquito midgut Ag	AnAPN1	Rec. protein-adjuvant

2.5 Controlled human infection studies

The controlled human infection or the microbial challenge studies of human volunteers is the deliberate infection of human volunteers with micro-organisms under highly controlled conditions. Human challenge models have contributed uniquely to the understanding of the pathogenesis, immune responses and the treatment and prevention of numerous microbial diseases including influenza, cholera, typhoid, hepatitis and malaria [25].

In vaccine and therapeutic drug development there are frequently multiple candidates, and it is often difficult to decide which should be taken forward to expensive clinical field testing. At the proof of concept stage of development controlled human challenge trials can be used to assess the efficacy of a novel therapeutic or the protective efficacy of a candidate vaccine in a small number of volunteers. This alternative research methodology is now more widely used to reduce the cost and time investment to accelerate the development of drugs and vaccines. A review by the UK Academy of Medical Sciences on microbial challenge studies recognised that such studies are desirable for providing proof of concept for prophylactic and therapeutic interventions and can significantly accelerate progress to Phase III studies [25].

2.6 Controlled human malaria infection studies

Controlled Human Malaria Infection (CHMI) can either be undertaken via 1.) Inoculation of sporozoites by mosquito bite or 2.) Direct injection of sporozoites or of plasmodium-infected blood. The inoculation of sporozoites by mosquito bite allows both liver and blood stage infection to develop, while inoculation with parasitized erythrocytes leads to blood stage infection only. After the inoculation, the subject is carefully monitored and the infection is truncated by anti-malarial drug treatment that is initiated promptly according to pre-defined study specific criteria, for instance the onset of microscopic patency and/or detection of a predefined parasitaemia by quantitative polymerase chain reaction (qPCR).

2.7 Controlled human malaria infection with *Plasmodium falciparum*

Plasmodium falciparum (P. falciparum) is a microbe particularly well-suited to "challenge" studies. It has a relatively short asymptomatic period, a well-established diagnostic laboratory test (blood film microscopy), and rapid recovery with no long-term infectious state following appropriate and timely treatment. Studies involving controlled human malaria infection are a powerful tool for investigating malaria vaccine and prophylactic and therapeutic drug efficacy [26]. With an increasing number of candidate malaria vaccines being developed, the number of centres conducting controlled human falciparum malaria infection studies is expanding in developed countries, and they are now also being conducted in endemic areas of Africa [26]. The first well-documented controlled human malaria infection study with laboratory-reared infectious mosquitoes was carried out in 1986 at the US Walter Reed Army Institute of Research (WRAIR), the US Naval Medical Research Institute (NMRI) and the US National Institutes of Health (NIH) [30]. The following year, the efficacies of the first recombinant protein and synthetic peptide *P. falciparum* vaccines were reported for experimentally infected volunteers [31, 32]. Controlled human malaria infection has now become established as a key tool to assess the efficacy of novel malaria vaccines and drugs; a total of 1,343 volunteers were experimentally infected with *P. falciparum* between 1985 and 2009 [33], [26]. As controlled human malaria infection trials are carried out in a controlled environment, they allow detailed evaluation of parasite growth and immunological responses, providing key information for vaccine and drug development [26]. Currently controlled human challenge infections trials are routinely carried out at: the

US Military Malaria Vaccine Program (USMMVP); Maryland, USA; Radboud University Nijmegen Medical Centre (RUNMC), Netherlands; the University Of Oxford, UK; QIMR Berghofer, Brisbane Australia, and, more recently, Seattle Biomed, USA and the KEMRI-Wellcome Programme in Kilifi, Kenya. Other groups in Africa who have done falciparum challenge including IHI in Tanzania and Lamberene, in Gabon.

2.8 Controlled human malaria infection with *Plasmodium vivax*

In contrast to *P. falciparum*, modern controlled human malaria infection studies with *P. vivax* have been conducted in much less frequently, with only a small handful of studies reported over the past decade. This is largely due to the inability to culture *P. vivax* long-term *in vitro*. In only two of the studies published to date has efficacy of immunisation been assessed (Table 2) [34]. Unlike with *P. falciparum*, there is also a risk of clinical relapse weeks to years after sporozoite infection if hypnozoites are not cleared from the liver, as these have the potential to reactivate.

Table 2: Overview of published *Plasmodium vivax* challenge studies.

Trial Site	Number of volunteers	Pre-patent period (days)[a]	Number of infected mosquitoes OR inoculum	Number of volunteers with patent parasitaemia	References
Sporozoite (mosquito-bite) challenge studies					
Cali, Columbia	18	9 – 13	2 - 10	17/18	[19]
Cali, Columbia	17 Duffy positive 5 Duffy negative	9 – 16	2 - 4	17/17 (Duffy positive) 0/5 (Duffy negative)	[20]
Cali, Columbia	7 malaria-naïve 9 semi-immune	11 – 13	2 - 4	16/16	[22]
WRAIR, USA	6 malaria-naïve 6 malaria - naive	9-12 12-14	5 5	6/6 6/6	[57] and unpublished data (NCT00935623)
WRAIR, USA	27 vaccinees	10 – 13 10 – 11	5	33/33	[24]

Trial Site	Number of volunteers	Pre-patent period (days)[a]	Number of infected mosquitoes OR inoculum	Number of volunteers with patent parasitaemia	References
	6 infectivity controls				
Cali, Columbia	12 Duffy positive vaccines 2 Duffy positive controls 5 Duffy negative controls	12 – 13	2 - 4	7/12 vaccinees 2/2 Duffy positive controls 0/5 Duffy negative controls	[23]
Oxford university Oxford, UK (2018)	2 Blood group O Rh negative	10-11	5	2/2	[Personal contact (Draper S. and Minassian A)]
Blood-stage challenge studies (IBSM)					
QIMRB, Australia (ACTRN12612001096842)	2	8-9	13,000 genome equivalents	2/2	[21]
QIMRB, Australia (ACTRN12613001008718)	6	8-9	31,786 (± 11,947) as determined by qPCR (= 15 5 viable *P. vivax* parasites)	6/6	[35]
QIMRB, Australia (ACTRN12614000930684)	2	2/2 positive day 5	Inoculum – 564 PRBC	2/2	[3]Collins *et al* in preparation *

Trial Site	Number of volunteers	Pre-patent period (days)[a]	Number of infected mosquitoes OR inoculum	Number of volunteers with patent parasitaemia	References
QIMRB, Australia (ACTRN12616000174482)	24	21/24 positive day4 3/24 positive day5	Inoculum – 564 PRBC	24/24	[3] Collins *et al* in preparation *
QIMRB, Australia NCT02573857	8	8/8 positive day 4	Inoculum – 564 PRBC	8/8	McCarthy *et al* (submitted 2019)
QIMRB, Australia ACTRN12617001502325	4	All positive day 4	Inoculum – 564 PRBC	4/4	[59]
Oxford, dose finding (inoculation)	6	All positive day 14-16	1:5x2, 1:20x2, whole vialx2	6/6	[Personal contact (Draper S. and Minassian A)]
Oxford, vaccine trial (ChAd63 PvDBP and MVA PvDBP)	8	Data not available	Data not available	8	[Personal contact (Draper S. and Minassian A)]

[a] The pre-patent period refers to the period before malaria diagnosis: by blood film (sporozoite) or qPCR (blood-stage).

Despite a paucity of modern challenge trial experience, there is however an extensive history of deliberate infection with *P. vivax*; most notably in malariotherapy, which was carried out for the treatment of neurosyphilis almost a century ago. The Austrian psychiatrist Julius Wagner-Jauregg later received a Nobel Prize for his work with this treatment [36], and the practice was widely adopted as the only effective treatment available at the time. Malariotherapy provided a wealth of information about *P. vivax* infection, which has been reviewed previously [37]. Deliberate infection with *P. vivax* was also conducted in the USA from the 1940s to 1970s in prisoners involved in the Malaria Research Project at the Illinois State Penitentiary. These studies mainly examined compounds for their potential use as antimalarials [38]. Similar studies were also carried out at the United States Penitentiary, Atlanta. Key discoveries of the biology of *P. vivax* were made during this period including, for example, the association between Duffy negativity and resistance to *P. vivax* infection [39]. Following on from the studies of *P. vivax* infection conducted in Illinois,

controlled human challenge infections were carried out to see if prior exposure to irradiated mosquitoes could confer protection by immunization. Rieckmann *et al*. [40] reported no protection against controlled human challenge infections in three volunteers previously exposed to *P. vivax*-infected irradiated mosquitoes on four occasions at intervals of 2 – 4 weeks (total of <200 mosquitoes).

Three *P. vivax* controlled human challenge infection studies assessing the ability to 'immunise' with X-irradiated sporozoites also took place in Maryland, USA during the 1970s [58]. Following immunisation, challenge infection was initiated by periodic exposure to the bites of non-irradiated infected mosquitoes in three volunteers in separate experiments, and blood films were taken at least daily for all volunteers to assess outcome. These experiments in Maryland demonstrated that controlled human malaria infection studies with both *P. falciparum a*nd *P. vivax* could be successfully carried out, but the studies were very small.

More recent *P. vivax* mosquito-bite controlled human challenge infections trials have taken place in Cali, Colombia and at the Walter Reed Army Institute of Research (WRAIR), Maryland, USA.

The first of the Cali trials involved eighteen healthy volunteers exposed to the bites of 2-10 infected *An. albimanus* mosquitoes [19]. *P. vivax* infection was established in mosquito lots fed on blood from fifteen patients presenting to outpatient clinics at the Immunology Institute in Cali and Buenaventura with *P. vivax* infection. Four mosquito lots had to be discarded due to co-infection with *P. falciparum*, hepatitis B or hepatitis C in the donor blood. Seventeen of the eighteen volunteers developed *P. vivax* malaria, confirmed by blood smear, with a pre-patent period between 9 and 13 days. At diagnosis, volunteers were treated with standard *P. vivax* therapy consisting of chloroquine (600 mg initially, followed by 450 mg 24 and 48 hours later) to clear blood-stage infection and primaquine (PQ) (30 mg/day for 14 days) to achieve radical cure. Levels of parasitaemia ranged from 75-420 parasites/µL; parasites were cleared within 48 hours of starting treatment in all infected volunteers. There were no serious adverse events (SAEs) in this trial, but seven volunteers required fluid therapy due to nausea and vomiting, and five developed blurred vision lasting 2-3 days after treatment initiation. Authors speculated that the volunteer who did not develop malaria had surreptitiously taken anti-malarial medication, but this was never confirmed.

The second *P. vivax* mosquito-bite controlled human challenge infections trial was carried out by the same group in Colombia, aiming to demonstrate the reproducibility of this method of infection using three different *An. albimanus* mosquito lots fed on blood from three *P. vivax*-infected donors [20]. Seventeen individuals whose red blood cells were positive for the Duffy antigen/chemokine receptor (DARC, "Duffy positive") and five Duffy negative controls were enrolled into the study. Volunteers were randomly assigned to three groups (with six Duffy positive individuals in two of the groups and five in the third group), and exposed to the bites of 2-4 infected mosquitoes. The Duffy negative controls were assigned across the three

groups, with two controls in the first two groups and one in the third. All Duffy positive volunteers (and none of the Duffy negative volunteers) developed blood-stage malaria. The pre-patent period ranged from 9-16 days, and was different in the first group, with a median of 14 days, as opposed to a median of 10 days in the second two groups.

A third *P. vivax* mosquito-bite controlled human challenge infections trial was carried out in Cali but this time among both 'semi-immune' (previously-exposed; n=9) and malaria-naïve adult volunteers (n=7) [22]. The pre-patent periods ranged between 11-13 days, with no significant difference between the malaria-naïve and 'semi-immune' volunteers. Symptoms were significantly worse among the malaria-naïve subjects but there were no SAEs. One malaria-naïve volunteer presented three months after treatment with a *P. vivax* infection after visiting an endemic area. However, the investigators were unable to determine whether this episode of malaria was due to reinfection or relapse, as the volunteer had visited the same endemic area that the controlled human malaria infection study strain had come from.

The most recent mosquito-bite controlled human challenge infections trial from the Cali group involved the use of radiation-attenuated sporozoites in Duffy positive and Duffy negative healthy adult volunteers, delivered by mosquito bite. Mosquitoes were infected as described above for the other trials conducted by this group. Sporozoites were attenuated by exposing the mosquitoes to 150 ± 10 cGy of gamma irradiation. Twelve Duffy positive volunteers and five Duffy negative controls completed the immunisation phase with exposure to *P. vivax* infected irradiated *An. albimanus* mosquitoes followed by mosquito-bite controlled human challenge infections. Two Duffy positive controls also completed the trial; these volunteers were exposed to non-irradiated, non-infected mosquitoes. Seven immunisations were carried out for each volunteer on weeks 0, 8, 12, 23, 48, 51 and 56 with a mean of 65 infectious bites for each immunisation. Two weeks after the final immunisation volunteers were treated with chloroquine and primaquine to clear any malaria infections that may have developed during the immunisation phase. Plasma levels of chloroquine and primaquine were checked prior to CHMI to ensure drug clearance. Challenge was carried out at week 64 using 2 - 4 *P. vivax*-infected mosquito bites, and volunteers were monitored daily with blood films from day 6. There were no reported SAEs related to immunisation, although one volunteer developed severe elevation of hepatic transaminases (>10 times the upper limit of normal [x ULN]) with associated abdominal pain and vomiting following mosquito-bite controlled human challenge infections, with no alternative cause found. These symptoms resolved spontaneously. The protective efficacy of the immunisation schedule was 42% (5/12 Duffy positive volunteers protected). In volunteers who developed malaria, the mean pre-patent period until blood film positivity was 12.8 days. Interestingly, all of the volunteers protected in this trial were female [23].

Only one other mosquito-bite controlled human challenge infections trial assessing a *P. vivax* vaccine has been published to date. The VMP001/ AS01B vaccine was tested in healthy malaria-naïve adults at the

WRAIR in the USA [24]. VMP001 is a soluble recombinant protein vaccine [41], encoding the *P. vivax* circumsporozoite protein (PvCSP) administered with the AS01B adjuvant (GlaxoSmithKline). The vaccine was administered to 30 volunteers in three cohorts (10 in each) at doses of 15 μg, 30 μg and 60 μg, given three times at a 4 week interval between the first and second dose; the third dose was given 8 (15 μg cohort), 6 (30 μg cohort) or 4 (60 μg cohort) weeks after the second. Twenty-nine volunteers completed the vaccination phase, with twenty-seven proceeding to mosquito-bite controlled human challenge infections two weeks after final vaccination, along with 6 malaria-naïve controls. The mosquito-bite controlled human challenge infections with 5 *P. vivax*-infected *An. dirus* mosquitoes was undertaken. Laboratory-reared mosquitoes were fed on blood from a *P. vivax*-infected donor in a clinical laboratory in Mae Sot province in Thailand after screening by polymerase chain reaction (PCR) to ensure no co-infection with other Plasmodium species or blood-borne infections. Infected mosquitoes were then transported to WRAIR and maintained in their insectary until mosquito-bite controlled human challenge infections. Volunteers were treated following a diagnosis of vivax malaria by blood smear. The vaccine protective efficacy was 0 %; all volunteers had developed blood film-detectable parasitaemia by day 13. The median pre-patent period for all immunised volunteers was 11.9 days versus 10.7 days for infectivity controls. The challenge was well tolerated with no untoward reactogenicity following mosquito bites. No untoward SAEs were observed following the challenge and treatment phases. Volunteers were treated with standard chloroquine and primaquine therapy with rapid clearance of infection. However, two volunteers went on to have multiple relapses. One volunteer experienced two relapses (at weeks 8 and 18 after challenge), while the other experienced three (at weeks 11, 20 and 48 after challenge) [42]. By study completion the volunteers had been followed up for 5 years, and had not had any further relapses [24]. Exploratory genotyping for the cytochrome P450 (CYP) allele CYP2D6 was undertaken in 25 of the 33 volunteers. The volunteers with relapses were found to have either an intermediate-metaboliser phenotype or poor-metaboliser phenotype. These phenotypes were associated with significantly lower levels of primaquine clearance 24 hours after dosing [42]. Primaquine is metabolised into redox-active metabolites by CYP2D6, and therefore individuals who are unable to metabolise the drug in sufficient quantities appear to be at risk of relapse from *P. vivax.*

Because of these findings, we shall be assessing our healthy Thai volunteers for their ability to metabolise primaquine by genotyping for CYP2D6 at screening, in order to minimise the risk of subsequent relapse of infection. While there is not yet a fully validated correlation between CYP2D6 genotype and failure of primaquine radical cure, it is at present the best predictor of relapse that we have. We shall exclude any volunteer who is not a high metaboliser based on their CYP2D6 genotype. All volunteers will also be screened to ensure they have normal G6PD enzyme activity to prevent haemolytic anaemia during radical cure therapy with primaquine.

The most recent *P. vivax* mosquito-bite controlled human challenge infection trial was in 2 healthy malaria-naïve volunteers carried out by The Malaria Vaccine Group in Oxford University; Oxford, UK in early 2018**.** Laboratory-reared mosquitoes were fed on blood from a *P. vivax*-infected donor in the Mahidol Vivax Research Unit (MVRU), Faculty of Tropical medicine, Bangkok, Thailand after screening of the patient's blood by PCR to ensure no co-infection with other Plasmodium species or blood-borne infections. The infected mosquitoes were then transported to Imperial College, United Kingdom, where the mosquito-bite controlled human challenge infection trial with 5 *P. vivax*-infected *An. dirus* mosquitoes per volunteer was performed in the Imperial College insectary. The subjects were then transferred to the Centre for Clinical Vaccinology and Tropical Medicine, Oxford for clinical follow up. Volunteers were treated following a diagnosis of vivax malaria by blood smear and real time quantitative PCR. The pre-patent period for both participants was around 10-11 days. Both volunteers were blood group O rhesus negative. The blood donation was successfully performed in both subjects and no malaria relapse during first year follow up (Minassian A and Draper S, personal communication) (Table 2).

2.9 Background to first vivax CHMI study in Thailand (covered by current protocol)

To date, most of the challenge models have been successfully performed in non-endemic settings such as in the US, UK and Australia. Up to date the challenge studies in UK in healthy vivax-naïve volunteers have not had any serious adverse event, malaria relapse, and lost follow-up (unpublished data obtained directly from lead investigator). However, the findings in high income settings may not be extrapolated to the target population for future vaccine deployment, which is more heterogeneous both in terms of vivax immunity and genetic background. The best volunteer population to test new vaccines is the eventual target population for vaccine deployment.

As outlined above, the proposed *P. vivax* human challenge in Thailand would allow us to understand the immunological correlates of protection in an endemic setting, thereby informing the development of new vaccine candidates and rapid testing of the protective efficacy of candidate vaccines in the at-risk population in which they will be deployed. Advancing the development of such methods needs renewed emphasis on understanding the biology, pathogenesis and transmission of *P. vivax*. This study aims to assess the safety and feasibility of controlled human *P. vivax* malaria infection in Thailand. It also aims to build on the limited knowledge that exists on parasite growth dynamics, transmission and the human immune response to infection following infection by the natural route of delivery – mosquito bite. **A major objective of the study is to provide a source of *P. vivax* infected blood to use in future vaccine efficacy trials of a sexual and asexual blood- and transmission-stage vaccines – making these studies more feasible in the future**. We will be using *An. dirus* mosquitoes from our in-house sources – reared in Thailand at the MVRU and Entomology Department, Mahidol University, Bangkok. MVRU is the source of the infected mosquitoes used

in previous CHMI study in Oxford described above. We shall be the first Asian centre to perform a controlled human malaria infection trial.

Protocol to prepare *P. vivax* infected mosquitoes was approved by FTM-EC (Protocol # MUTM 2018-017). Patients with *P. vivax* for the infected mosquito preparation will be recruited at the malaria clinics in Thailand by MVRU team.

For MIST 1 study, healthy vivax-naïve volunteers will be recruited at FTMCTU, Bangkok.

2.10 Over view of the whole projects (Position of current study protocol within the overall Thailand vivax Controlled human challenge infection programme)

This section aims to give the overview of all projects in this whole program. This will be a programme comprising multiple studies planning to conduct over the next 5 years in the Faculty of Tropical Medicine, Mahidol University, Thailand as follows:

- Proof of feasibility and safety study and providing banked infected blood inocula for future blood stage human challenge studies.
- Test the protective efficacy of pre-erythrocytic, blood stage and (ultimately) transmission blocking *P. vivax* vaccine candidates in the target population, in both vivax-naïve and semi-immune volunteers.
- Characterise correlates of immune protection in the populations at risk, in vivax-naïve and semi-immune volunteers.
- Test the efficacy of novel drugs under development for anti-hypnozoite (anti-relapse) activity.

To achieve the major aims written above, the programme will compose of 5 or 6 subsequent studies. Each subsequent study will be informed by previous studies in the series (base on the results of previous study) and will be definitely covered by **separate protocols with separate Ethics Committee submissions and approvals**. The 5-year plan of controlled human malaria infection studies are as follows:

- **MIST 1 (*covered by this current protocol*).** Initial mosquito infection (sporozoite) human challenge study (6 malaria-naïve healthy volunteers), MIST 1. This will be a proof of **feasibility and safety** study and providing banked infected blood inocula for future blood stage human challenge studies.
- **MIST 2.** Blood stage inoculum human challenge in volunteers from an endemic area (20 semi-immune volunteers) (using bank blood from MIST 1 to find the proper dose for nest vaccine testing study). This **dose finding study** aims to determine the most suitable inoculum 'dose' for human blood stage challenge in semi-immune subjects for later MIST trials of blood stage vaccine studies. The objective is to determine the most suitable inoculum 'dose' for human blood stage challenge in later CHMI trials of blood stage vaccine candidates.

- **MIST 3**. Mosquito (sporozoite) infection human challenge in volunteers from an endemic area. (20 semi-immune volunteers). This will follow the study procedures of the first study (MIST 1), except that the volunteers will be recruited and screened from a malaria endemic area (Sai Yok, Kanchanaburi Province). The challenge (MIST 3) will take place in FTMCTU in Bangkok. These volunteers will be variably immune to malaria. The volunteers will receive full radical cure treatment regimen before challenge to clear any low level parasitaemia and hypnozoites from the liver at base line and then will be inoculated by infected mosquito bite. The radical cure will be given following the detection of parasitaemia. The immunological and parasitological data obtained will help define correlates of protection, and inform the design of future challenge studies for vaccine efficacy assessment in semi- immune volunteers.
- **MIST 4**. Blood stage inoculum human challenge study to assess protective efficacy of a blood stage vaccine (50 volunteers, semi-immune). Study procedures will follow those of MIST 2 (dose selection study) above. This study (MIST 4) will be used to assess DBP-based or other blood stage vaccine. Volunteers will be randomized to vaccination or control groups, and inoculated using the **blood banked from MIST 1** above in a **dose determined by the results of MIST 2**.
- **MIST 5.** Mosquito (sporozoite) challenge studies to assess pre-erythrocytic vaccines (50 volunteers, semi-immune). These studies (MIST 5) will be used initially to test a CS-based or other pre-erythrocytic vaccine. Study procedures will follow those in MIST 3, and radical cure will be given. Volunteers will be randomised to vaccination or control groups.
- **MIST 6 and 7** will be blood stage inoculum challenge or mosquito challenge studies to test further upcoming *P. vivax* vaccine candidates. The study procedures will follow MIST 4 or MIST 5 above, depending on type of vaccine to be tested.

For the avoidance of doubt, this current protocol only covers MIST 1 ONLY, and all ethical submissions involving this protocol ONLY relate to this **first study** (MIST1) with the specified objectives described in the objectives section of this study in the following section.

This *P. vivax* human challenge programme is a highly collaborative project involving the Faculty of Tropical Medicine (FTM), Mahidol Oxford Research Unit (MORU), Mahidol University, the Wellcome Kenya and Thailand Asia and Africa Programmes, in liaison with all the vaccine developers expected to have vaccine candidates ready for testing in the next five coming years. Multiple departments within FTM are involved, including the world leading Mahidol Vivax Research Unit (MVRU), Department of Entomology, Molecular Tropical Medicine and Genetics, The Tropical Medicine Diagnostic Reference Laboratory (TMDR), and the FTM Clinical Therapeutics Unit.

The programme will be led, coordinated and capacity-built by an executive trial management team consisting of the programme co-PIs, site PIs, project/programme manager, and Controlled Human Malaria

Infection study experts. A Programme Steering Committee (PSC) led by an independent chair and the Dean of Faculty of Tropical Medicine will oversee the research.

3. OBJECTIVES

This protocol will cover only the initial mosquito infection human challenge study in up to 6 healthy malaria naïve volunteers (MIST 1). This will be a 1) **Proof of feasibility study**, 2) **Providing banked *P. vivax* infected blood inocula for future blood stage human challenge studies**, **and 3) Providing data on the development of host immunity**.

The trial has the following objectives:

3.1 Primary objectives:

- To assess the feasibility and safety of controlled human *P. vivax* malaria infection in six healthy human volunteers, through experimental sporozoite infection (mosquito bite).
- To obtain up to 250 mL of blood from each infected volunteer and freeze down the blood to create inocula for use in future *P. vivax* challenge studies.

3.2 Secondary objectives:

- To assess the immune response to primary *P. vivax* infection delivered by mosquito bite.
- To assess gametocytaemia following primary *P. vivax* infection delivered by the mosquito bite.

4. STUDY END POINTS

4.1 Primary endpoints:

- Feasibility and safety of *P. vivax* sporozoite human challenge, as measured by successful infection (development of detectable persistent parasitaemia +/- clinical symptoms) and AE(S) occurrences.
- Collection and freezing down of up to 250 mL *P. vivax*-infected blood from each of the 6 volunteers.

4.2 Secondary endpoints:

- Cellular and humoral Immune response to primary *P. vivax* infection.
- Gametocytaemia pre-treatment, as measured by qPCR.

5. STUDY DESIGN

5.1 Summary of Trial Design

This is a *Plasmodium vivax* mosquito-bite controlled human challenge infection trial in healthy malaria-naïve Thai adults.

6. PARTICIPANT IDENTIFICATION AND RECRUITMENT

6.1 Trial Participants

Up to six healthy, malaria-naïve Thai adults aged between 20 and 55 years with at least an undergraduate degree will be recruited at the FTMCTU at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

6.2 Inclusion criteria

The volunteer must meet all of the following criteria to be eligible for the study:

- Healthy adult aged 20 to 55 years with weight more than 50 kg.
- Blood group O.
- Red blood cells positive for the Duffy antigen/chemokine receptor (DARC).
- Normal CYP2D6 genotype.
- Normal blood levels of Glucose-6-phosphate dehydrogenase (G6PDH) **by the WHO definition.**
- COVID-19 vaccination at least two doses of COVID-19 vaccine(s) approved by WHO.
- Agree to practice continuous effective contraception for the duration of study period until 3 months post-challenge.
- Agreement to refrain from blood donation during the course of the study and for 1 year after the end of their involvement in the study.
- Willing to take a curative antimalarial regimen following challenge.
- Willing to be admitted in the Hospital for Tropical Diseases for blood donation and clinical monitoring, until antimalarial treatment is completed and their symptoms are settling.
- Willing to reside in Bangkok for the duration of the study, until all antimalarial treatment has been completed.
- Reachable (24/7) by mobile phone during the period between challenge CHMI and completion of all antimalarial treatment.
- Able to read and write **in Thai** and able to answer **ALL** questions on the informed consent questionnaire correctly.
- Provided written informed consent to participate in the trial.
- Educational level: has at least an undergraduate degree.

6.3 Exclusion criteria

The volunteer **MUST NOT** enter the study if any of the following apply:

- History of clinical malaria.
- Positive malaria PCR **OR** malaria film **OR** malaria serology
- History of severe allergy to mosquito bite
- Presence of any medical condition (either physical or psychological) which in the judgment of the investigator would place the participant at undue risk or interfere with the results of the study (e.g. serious underlying cardiac, renal, hepatic or neurological disease; severe malnutrition; congenital defects or febrile condition)
- Presence of chronic disease or chronically use of medication.
- Plan to travel outside of Bangkok within the period of challenge until 3 months after.
- Use of systemic antibiotics with known antimalarial activity in the 30 days before challenge (e.g. trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones and azithromycin).
- Use of immunoglobulins or blood products (e.g. blood transfusion) at any time in the 1 year preceding enrolment.
- Venipuncture unlikely to allow blood donation according to the protocol as determined by the investigator.
- Receipt of an investigational product or any vaccine in the 30 days preceding enrolment (D0), or planned receipt during the study period.
- Prior receipt of an investigational vaccine likely to impact on interpretation of the trial data or the *P. vivax* parasite as assessed by the Investigator.
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection, asplenia, history of splenectomy, recurrent, severe infections, and chronic infection.
- Immunosuppressant medication within the past 6 months preceding enrolment (D0) (inhaled and topical steroids are allowed).
- History of allergic disease or reactions likely to be exacerbated by malaria infection.
- Female participant who is pregnant, lactating or planning pregnancy during the course of the study.
- Contraindications to the use of antimalarial treatment (e.g. chloroquine or primaquine or atovaquone / proguanil, DHA piperaquine). **
- Use of medications known to have a potentially clinically significant interaction with antimalarial drug that will be used in this study (chloroquine or primaquine or atovaquone / proguanil, DHA/ piperaquine).
- Use of medications known to cause prolongation of the QT interval as state in the section of prohibited drugs that may have effect on prolongation of the QT interval.

- Known existing positive family history in both 1st AND 2nd degree relatives < 50 years old for cardiac disease.
- Family history of congenital QT prolongation or sudden death.
- Any clinical condition known to prolong the QT interval.
- History of cardiac arrhythmia, including clinically relevant bradycardia.
- Screening ECG demonstrates a QTc interval ≥ 450 ms
- Suspected or known or history of alcohol abuse
- Suspected or known or history of drug abuse.
- Concurrently participating in another clinical study, at any time during the study period.
- Haemoglobin < 13 g/dL in male, < 12g/dL in female (Thai Red Cross).
- Mean corpuscular volume (MCV) < 80 fL/cell
- Finding on safety laboratory values as defined below:
    - AST > 40 U/L for male, and > 32 U/L for female (upper normal range), or
    - ALT > 41 U/L for male, and > 33 U/L for female (upper normal range), or
    - Total Bilirubin > 1.2 mg/dL, (upper normal range), or
    - Creatinine (Cr) > 1.17 mg/dL for male, and > 0.95 mg/dL for female (upper normal range), or
    - Abnormalities corrected calcium and magnesium blood levels
    - Fasting blood sugar (FBS) > 100 mg/dL, or
    - Cholesterol LDL (low density lipoprotein) > 160 mg/dL or Triglyceride > 200 mg/dL
- Thalassaemia disease or haemoglobinopathies.
- Positive for a blood borne or vector borne infectious disease (HIVI-II, HBV, HCV, Dengue, Zika, Chikungunya, Filariasis, JE, and malaria antigen, Anti HTLVI and Anti-HTLVII antibody, Syphilis test (TPHA)
- Positive for COVID-19 testing as diagnosed by RT-PCR

  ** Link of the lists of medications with QTc prolongation:
  https://crediblemeds.org/pdftemp/pdf/CombinedList.pdf

7. STUDY PROCEDURES

This is a sporozoite-challenge clinical study with the **primary objectives** of assessing the **feasibility and safety** of controlled human sporozoite *P. vivax* malaria infection in six healthy volunteers, and **developing a bank of *P. vivax*-infected blood for use as inocula in future** controlled human blood stage *P. vivax* malaria infection studies. Secondary objectives are to assess the growth of and the immune response to *P. vivax* infection, and assess the induction of sexual gametocytaemia post-CHMI via the natural route of malaria infection (mosquito bite).

Up to six healthy, malaria-naïve adults aged between 20 and 55 years with weight more than 50 kg will be recruited at the FTMCTU in the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok. Volunteers will be recruited and receive mosquito-bite sporozoite challenge in two batches. The first batch will consist of **2** volunteers, and the second batch of **4** volunteers. A *P. vivax* mosquito-bite controlled human challenge infection (CHMI) by sporozoites will be delivered to the first batch of volunteers at the insectarium unit, Department of Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok. The procedures will be performed strictly following the approved work instruction (WI) and clinical monitoring will be performed during the post-challenge period at the FTMCTU as an inpatient until the antimalarial medications (chloroquine) are completed. Chloroquine and primaquine will be prescribed according to current Thai national guideline. The second batch of volunteers will receive mosquito bite CHMI after the careful evaluation of the safety profiles / outcomes of the first 2 volunteers (at 28 days after treatment initiation as the minimum time interval).

During admission, the volunteers will have blood taken at regular intervals post-challenge to assess parasitemia, immune response following *P. vivax* infection, and they will be monitored closely until they meet the criteria for blood donation and treatment. Up to 250 mL of blood will be taken prior to treatment from the **successfully** infected volunteers. The volunteers will then be treated with a standard course of oral chloroquine and a 2-week course of directed observed therapy (DOT) of oral primaquine for radical cure of *P. vivax* hypnozoites.

Volunteers will be followed up for 1 year after antimalarial treatment initiation. They will be asked to come back to the outpatient clinic on D 28, D 90, 180, and 1 year after treatment initiation. During this phase, the volunteers will be contacted fortnightly by email/ phone call / other social communications e.g message (SMS), WhatsApp, Line, to ensure they remain well and asymptomatic.

Alternative medications including dihydroartemisinin + piperaquine, atovaquone + proguanil and intravenous artesunate may be prescribed if indicated as per the treatment algorithm in Appendix B.

7.1 Recruitment of volunteers

Volunteers will be screened and recruited at the FTMCTU, Faculty of Tropical Medicine, Mahidol University following Recruitment Process for Healthy Volunteer SOP.

In brief, the volunteers may be recruited by use of an advertisement **approved by the ethics committee** and distributed or posted in the following places:

- On a MIST website operated by the study group
- In public places with the agreement of the owner / proprietor.
- Via presentations (e.g. presentations at lectures or invited seminars).

We may contact individuals who have participated in previous clinical trials from FTMCTU's databases. These volunteers will have expressed an interest in receiving information about all future studies in FTMCTU for which they may be eligible.

7.2 Informed consent

The informed consent process: The investigator will explain the purpose of the study with the help of and consistent with the Participant Information Sheet (PIS) prior to any study related procedures being undertaken. The volunteer must personally sign and date the latest approved version of the informed consent form (ICF) before any study specific procedures are performed.

The information sheet and informed consent form will be explained to the volunteers detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. The aims of the study and all procedures to be carried out will be explained. **It will be emphasised that there is no direct benefit from participating the trial**.

- It will be clearly stated that participation is entirely voluntary and that refusing to participate will not involve any penalty or affect the volunteers' right to receive standard medical care.
- It will also be emphasized that if they do consent to participate and are enrolled, that they are free to withdraw from the study at any time, for any reason, without any penalty or prejudice to future care, and with no obligation to give the reason for withdrawal.

The consent information will emphasize that blood samples (from blood donation) will be taken and stored indefinitely for use in other, ethically approved research. The future use of samples may result in the development of a commercially viable product to which the volunteers may not have any rights. The volunteer will have the opportunity to question the investigator, or other independent parties to decide whether or not they will participate in the study.

If they do decide to participate, volunteers will be asked to **complete a questionnaire testing their understanding of the trial before signing the consent.** This helps to ensure that individuals understand the trial sufficiently to give informed consent. After the volunteer answers all questions in the questionnaire correctly, they will be asked to sign and date two copies of the consent form, one for them to take away and keep, and one to be stored in the investigator's site file and retained at the study site. These forms will also be signed and dated by the investigator. Volunteers who fail to answer all questions correctly on their first attempt will be allowed to re-take the questionnaire following further discussion with the investigator. Only the subject who able to subsequently answer all questions in the questionnaire correctly will be asked to give the consent and will be screened for the trial. Only 2 attempts will be permitted in total.

Hospital consent for HIV test: This will be obtained prior to counselling and HIV test using the standard HIV consent form of Hospital for Tropical Diseases. Pre and post counselling for HIV screening and reporting will

be conducted with the support of physician from the Hospital for Tropical Diseases. In case a volunteer is found to be HIV positive, follow up measures including but not limited to providing counselling and treatment according to standard hospital procedure will be arranged through a physician from the Hospital for Tropical Diseases.

7.3 Screening and eligibility assessment

The screening will aim to recruit up to 6 healthy volunteers and at least 2 back-up healthy volunteers using the same screening procedures.

The screening and eligibility assessment will be performed in the FTMCTU, Hospital for Tropical Diseases, Mahidol University following hospital registration as an outpatient. This will ensure that volunteers will receive prompt medical care as patients if required. Registration through the hospital system can also help prevent the volunteer from participating in other clinical trials simultaneously. This issue will be discussed and emphasized with the volunteer prior to screening and also through the study period.

Screening procedures are outlined in the schedule of procedures in Table 6.

7.3.1 Screening visit 1

Informed consent will be obtained prior to performing any study activities, including screening tests. Once informed consent is given, a screening number will be assigned in sequential order. Screening numbers will be issued consecutively (SCR-MIST1-001, SCR-MIST1-002…and so on). The volunteer will be advised to fast at least 8-12 hours prior to the screening process. All volunteers will have their medical history taken, a physical examination, urinalysis, electrocardiogram (ECG), ABO and rhesus system blood grouping, haemoglobin, testing for G6PD genotyping, G6PD enzyme level, a complete blood count (CBC) and blood disease, biochemistry tests, Duffy antigen/chemokine receptor (DARC), **blood-borne diseases including** Hepatitis B profile (HBsAg, anti-HBc, anti-HBs), anti-HCV, HIV antibodies, anti-HTLV-I and anti-HTLV-II, and syphilis test (TPHA) and **vector-borne diseases** including Dengue, Zika, Chikungunya, JE, and malaria diagnosis, a filariasis RDT. The serum pregnancy test (for females), CYP2D6 genotyping, and malaria serology (exposure to malaria) will be done during this stage.

If an abnormal finding at screening is deemed to be clinically significant, the volunteer will be informed and appropriate medical care arranged with the permission of the volunteer.

Exclusion of the volunteer from enrolling in the trial or withdrawal of a volunteer from the trial will be at the discretion of the Investigator.

The maximum time between the screen **visit 1 and the malaria challenge day is 180 days**

Additional screening for safety reassurance will be performed on day -7 and day -1 (7 and 1 days before the mosquito bite) as described in the following sections.

7.3.2 Screening visit 2: 7 days before challenge (D-7)

The volunteers will attend for a repeat blood test 7 days before challenge (D-7) for HIV I-II, HBV, HCV, Dengue, Zika, Chikungunya, JE, malaria diagnosis, serology for anti HTLV-I and anti HTLV-II antibody, syphilis test (TPHA), filariasis, malaria serology (exposure to malaria), urinalysis, and a urine pregnancy test (for females) according to Table 6.1 to ensure no new acquisition of infection prior to challenge. Nasopharyngeal swab will be collected for testing of COVID-19 by RT-PCR.

Other screening activities will be undertaken according to Table 6.

This is important to ensure they have not acquired any new infections listed in the exclusion criteria since their first screening as their blood donation will be used in the future to administer malaria challenges to healthy volunteers.

These tests for vector borne and blood borne infections are in line with the standard guidelines for blood donor selection;

- The guidelines of the Joint UK Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee (https://www.transfusionguidelines.org/transfusion-handbook/3-providing-safe-blood/3-2 tests-on-blood-donation)
- Guidelines on Assessing Donor Suitability for Blood Donation of World Health Organization 2012; https://www.who.int/bloodsafety/publications/guide_selection_assessing_suitability.pdf)
  The guidelines of National Blood Center, Thai Red Cross;
  (https://english.redcross.or.th/content/page/949)
- Expert advices

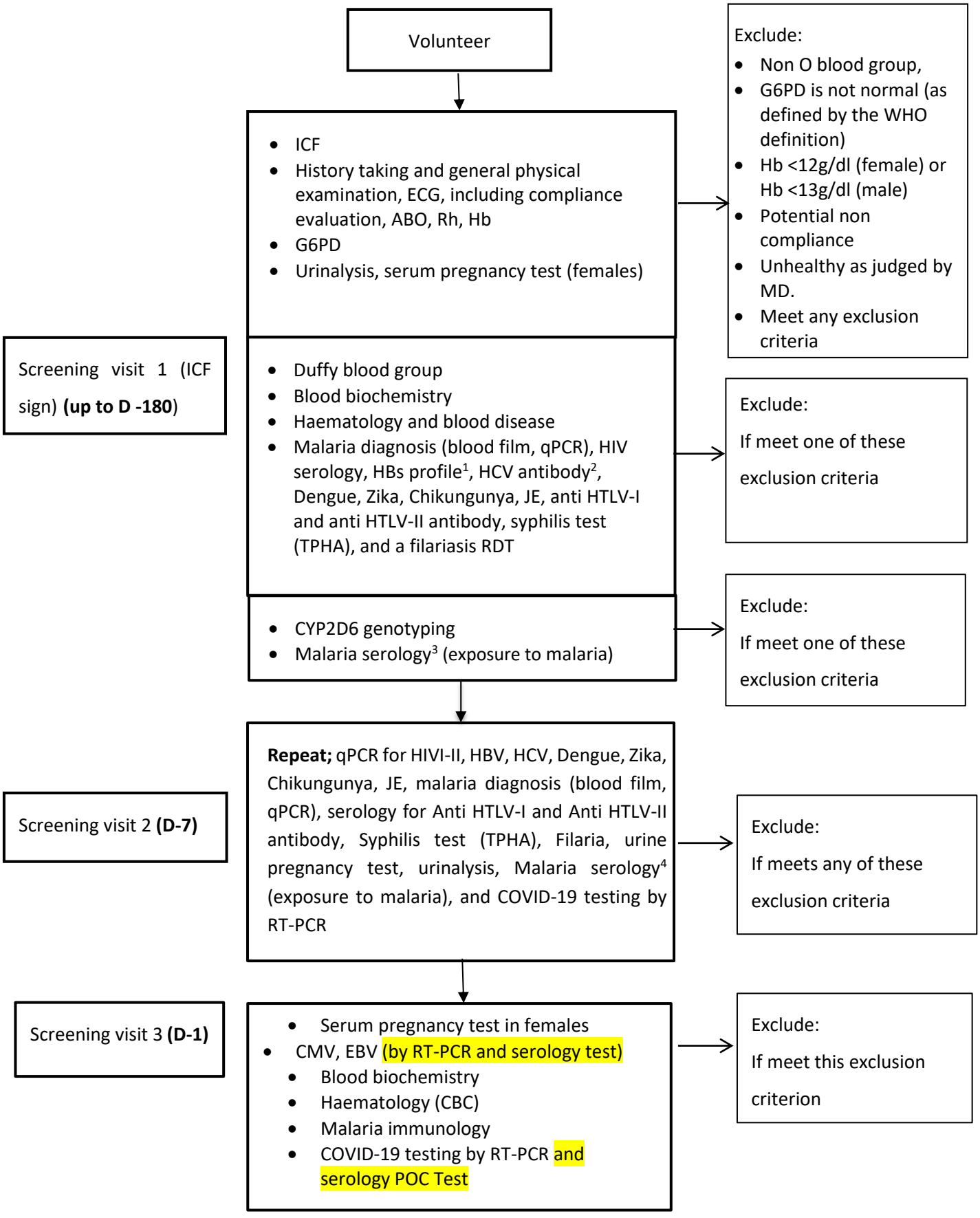
The maximum time between **the screening visit 2 and the challenge day is 14 days**.

7.3.3 Screening visit 3/ a day before the challenge day (1 day before challenge, D-1)

Blood will be taken for haematology (CBC), biochemistry testing, Epstein Barr virus (EBV), cytomegalovirus (CMV), malaria immunology, and serum pregnancy test for females, in accordance with Table 6.1 and the subject screening work flow shown in **Figure 1.** In addition, blood collection will be performed to test for CMV IgM/IgG antibody, EBV VCA (IgM)/IgG antibody, and anti EBNA and EA complex antibody. COVID-19 serology Point-of-Care (POC) test will be done to look at participants' immunity from the current/previous COVID-19 infection (asymptomatic) and/or prior immunization. Nasopharyngeal swab will also be collected for COVID-19 testing by RT-PCR.

Other screening activities will be undertaken according to Table 6.

Figure 1. Screening Work Flow



[1] *HBsAg or isolate HBc antibody*

[2] *HCV antibody*

[3] *AlphaScreen assay will be used to determine the quantitation of malaria antigens-specific antibodies (up to 300 P. falciparum and 300 P. vivax antigens). A seropositivity cut-off will be set as half the lowest non-negative value from of the assayed samples. #a positive malaria history refers to a volunteer who previously had malaria infection which enables to reactive to more than 10% malaria antigens in the library.*

[4] *Multiplex Bead Based Immunoassay will be performed to determine the magnitude of antibodies against recent exposure of 5-8 blood-stage malaria antigens. A seropositivity cut-off will be calculated by the average of median fluorescence intensity (MFI) plus 2 standard deviation of negative control.*

7.4 Interventions in volunteers

This section describes the clinical procedures for evaluating study volunteers and management **during** and **after** challenge.

7.4.1 Inpatient phase until end of antimalarial treatment:

This section involves the baseline assessment, enrolment, challenge by mosquito bite (mosquito bite), blood donation, and antimalarial treatment.

7.4.1.1 Admission day (baseline assessment) (D-1)

The volunteers will be admitted to Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University as inpatients following hospital registration to ensure the maximum safety practice. The registration can help detect if the volunteer has participated in any other clinical trial during the study period. Any new medical issues or symptoms that may have arisen will be again assessed. The venipuncture for serum pregnancy, CMV, EBV, CBC, malaria immunology and blood biochemistry will be performed according to Table 6.1. The history taking and physical examination, COVID-19 testing, and other study activities will be undertaken according to Table 6. All inclusion and exclusion criteria will be checked.

Full contact details for each volunteer will again be confirmed and documented, including home and work addresses, mobile phone number, home and work landline telephone numbers where available and next-of-kin address and telephone numbers. Volunteers must also provide the investigators with the name and 24-hour telephone number of a close friend, relative or housemate who will be kept informed of their whereabouts for the duration of the study.

7.4.1.2 Challenge: infected mosquito bites on day 0 (D0)

All inclusion and exclusion criteria will be checked again to ensure eligibility criteria have been met. Study activities will be undertaken according to Table 7. Any new medical issues or symptoms that have arisen will be assessed. Results of vector borne and blood borne infectious diseases (of D-7) and pregnancy test (of D-1, if female) must be available and reviewed prior to challenge. If all inclusion criteria are fulfilled and none

of the exclusion criteria apply, then the volunteer will be enrolled into the full study (proceeding to the challenge phase). Enrolment number will be assigned to each volunteer in sequential number of the format Study Code, Site Number, Sequential Number e.g. MIST1-TH01-001, MIST1-TH01-002…… MIST1-TH01-006. The back-up volunteers must also be available during the period of the malaria challenge (during the mosquito bites), in case one of the volunteers in the batch withdraws at the last minute or any unexpected event occurs. This means that confirmation of whether or not a back-up volunteer will be needed for the challenge will not be made until the challenge (the whole mosquito biting process) has been successfully completed.

Challenge will be administered according to the SOP for sporozoite challenge by mosquito bites at the designated insectarium unit, Department of Entomology, Faculty of Tropical Medicine, Mahidol University. Following successful completion volunteers will return to FTMCTU for clinical management. The procedures are described in section 8. Other study activities will be undertaken according to Table 7. Summary of the mosquito biting process as detailed in Appendix A.

1. Prepare infected mosquitoes

2. Briefing of volunteers before the malaria challenge.

3. Perform malaria challenge process in a secure room in the insectary.

   3.1 Clean volunteer forearm following to the mosquito biting work instruction.

   3.2 Bite each volunteer by five infectious mosquitoes (by placing his or her forearm over the pot containing mosquitoes) for 5-10 minutes.

4. Return volunteer to Hospital for Tropical Diseases for admission until disease management is completed

7.4.1.3 Day 1 to Day 4 post challenge

The liver-stage of malaria infection is asymptomatic and lasts approximately one week. Even so the volunteers will be daily reviewed in the morning on days D1 to D4 post-challenge by using the **daily well-being checklist** form. The once daily general physical examination will be done. Blood will be collected on Day 2 for assessing the immune response and CBC and Day 3 for liver function tests.

In case volunteers have fever but malaria blood film, malaria qPCR, and testing for vector-borne diseases are both negative, COVID-19 testing will be performed.

7.4.1.4 Days 5 post challenge to Day of blood donation (D5 – $D_{Bl\ donation}$)

Volunteers will be clinically monitored **twice daily** from D5 (in the morning and evening) by clinical research team. The team will consist of the investigators who are physicians (with experience in acute medicine and infectious diseases and familiarity assessing patients with malaria) and nurses.

- Well-being checklist questioning, physical examination will be performed. Venipuncture will be performed according to Table 6.1.
- In the clinical well-being checklist form, the volunteers will be questioned whether they have experienced any malaria-related symptoms (such as fever, chills, sweating, malaise). These symptoms will be listed as solicited AEs.
- The severity of symptoms will be carefully assessed.
- The qPCR and blood film for malaria and gametocyte qPCR will be monitored twice daily from D5 until blood donation. Then once daily until the volunteer has two consecutive negative blood film readings and on the day 7 of primaquine treatment ($D_{pq\ 7}$) and day 14 of primaquine treatment ($D_{pq\ 14}$).
- If volunteer present with a clinical feature(s) of COVID-19 at the discretion of the trial physician, and malaria blood film and malaria qPCR are both negative, COVID-19 testing will be performed.
- Blood for assessing the immune response on day 5 and day of blood donation.
- Blood collection will be performed to test for CMV IgM/IgG antibody, EBV VCA (IgM)/IgG antibody, and anti EBNA and EA complex antibody at 2-4 weeks after the inoculation.
- COVID-19 serology Point-of-Care (POC) test will also be assessed at 14 days after the inoculation to look at participant's immunity.
- Urine pregnancy test before chloroquine treatment.

Other activities and the details of blood taken during the study period are given in Table 6.1 and Table 7.

At a time-point that will depend on the level of parasitaemia and/or the degree of the volunteers' symptoms (as per algorithm, Table 3), we will take up to 250 mL of blood from them within 24 hours of the clinical OR laboratory criteria being met (as per Table 3). The blood donation will be performed following the Blood Donation SOP then processed and frozen down following the parasite banking SOP for future use in the future subsequence blood-stage controlled human challenge trials (MIST2 - MIST7). The study team will make sure that volunteer tolerate the blood donation procedure and that they are provided with adequate symptom relief.

Blood donation will be done only in volunteers with a haemoglobin higher than 10 g/dL and who are clinically stable.

The ranges of haemoglobin level for a blood donation are describe as follow;

- Hb > 11 g/dL: blood donation 250 mL
- Hb > 10 g/dL to 11 g/dL: blood donation 200 mL
- Hb ≤ 10 g/dL: no blood donation

Table 3. Blood donation criteria

Blood Donation Criteria	• Febrile illness and any parasitaemia, and volunteer considered clinically fit for blood donation **OR** • Parasitaemia > 50,000 parasite /mL by microscopy without signs and symptoms, and volunteer considered clinically fit for blood donation **Blood donation must be complete within 24 hour of these criteria being meet.**

7.4.1.5 Antimalarial treatment

A study physician will then immediately prescribe antimalarial treatment, chloroquine and a 14-day course of primaquine, 30 mg once daily using the direct observed therapy (DOTs) to ensure 100% drug compliance. Any side effect from the antimalarials will be rapidly dealt within a controlled environment. They will continue to have blood films and qPCR once daily until clinically recovered AND two consecutive negative of malaria blood films (completion of the chloroquine treatment course). In case blood donation occurred before day 14, blood collection will also be performed to test for CMV IgM/IgG antibody, EBV VCA (IgM)/IgG antibody, and anti EBNA and EA complex antibody at 2-4 weeks after the inoculation. In addition, COVID-19 serology Point-of-Care (POC) test will also be assessed at 14 days after the inoculation to look at participant's immunity.

The volunteers will be then discharged from the hospital. Before discharge from the hospital, COVID-19 testing will be carried out to confirm that subjects did not acquire COVID-19 during their inpatient stay. After discharge, they will attend FTMCTU daily for directly observed treatments (DOTs) until they have completed the remaining dosages of 14-day course of the primaquine treatment (D_{endPQ}). Diary card will be given to the volunteers on the discharge day and will be reviewed on every subsequent visit.

If any volunteer reaches day 21 post-challenge without reaching the treatment criteria, they shall be started on 3-day course of antimalarial (chloroquine) **and a 14-day course of primaquine, 30 mg once daily.**

If any contraindications to chloroquine are identified, an alternative antimalarial dihydroartemisinin + piperaquine or Malarone (atovaquone + proguanil) will be prescribed. If a volunteer is unable to tolerate an oral antimalarial, the appropriate parenteral antimalarial therapy will be prescribed.

If a volunteer withdraws/is withdrawn from the study after challenge but before reaching the criteria for malaria treatment, then a **complete, appropriate, curative course of antimalarial therapy must be completed.** The importance of this will be emphasized to volunteers at screening.

Table 4*:* Antimalarial treatment criteria.

Malaria treatment Start Criteria	• Blood donation completed **OR** • Symptoms highly suggestive of malaria infection and judged by investigator as requiring antimalarial treatment, in the absence of a positive blood smear (other causes of symptoms should be considered and if appropriate treated) (Appendix B. Treatment algorithm) **OR** • Day 21 reached and not develop symptom and blood parasitemia according to the blood donation criteria

Table 5: Chloroquine and Primaquine treatment dose

Weight	Day 1		Day 2		Day 3		Day 4-14
	CQ Tab	PQ mg	CQ Tab	PQ mg	CQ Tab	PQ mg	PQ mg
25-50 kg	4	10	1	10	1	10	10
Above 50 kg	4	30	4	30	2	30	30

- *National Malaria Treatment Guideline 2015 (Thailand), CQ 150 mg base/tablet*

7.4.1.6 Supportive treatments and medications

The malaria symptoms (e.g. fever, nausea, vomiting, and dehydration) will be relieved accordingly. Paracetamol (500 mg orally up to four times a day) and dimenhydrinate (50 mg orally) will be prescribed according to clinically needed. Intravenous fluid supplementation may be given following physician decision.

All medications used in the trial will be recorded in medical record.

If the volunteers meet the criteria for severe malaria (by WHO definition) or fail to improve within 48 hours of starting anti-malarial therapy, malaria experts in the Faculty of Tropical Medicine will be consulted. The volunteers will be managed according to Thai National Guideline as well as the Hospital for Tropical Diseases' standing order/ procedures/ guideline for severe malaria.

The algorithm to be used for *P. vivax* malaria treatment is attached in the protocol as Appendix B. The Investigators are able to treat any volunteer for malaria regardless of the blood film microscopy or qPCR result if they are clinically concerned or a volunteer wishes to withdraw from the study.

7.4.2 Follow up assessment phase (after completion of antimalarial treatment)

After discharge from the hospital and completion of 14 days of primaquine daily DOT ($D_{end\ PQ}$), and if the volunteer remains asymptomatic or has mild, self-resolving symptoms, the follow-up assessment will be performed at FTMCTU as an out-patient on day 28 (D_{Rx28}), day 90 (D_{Rx90}), day 90 (D_{Rx90}), and 1 year ($D_{Rx\ 1yr}$) post-treatment initiation.

On the primaquine end date ($D_{end\ PQ}$) the volunteers will receive full counselling on the remaining possibility of relapse and the signs and symptoms that they have to be able to observe early and report in the diary card and seek medical advice. A diary card to self-document any abnormal symptoms will be given to them, and the contact channel with the study team will be again emphasized.

All volunteers will be followed up according to the schedule in Table 7.

Follow up visit after complete the radical cure are detailed as following.

7.4.2.1 Day 28 post antimalarial treatment initiation visit ($D_{Rx\ 28}$)

The volunteers will be asked to visit FTMCTU as an outpatient using the hospital registration system. Diary cards will be reviewed and collected, a history taken using the clinical well-being check list, a physical examination will be performed and AEs assessed. Venipuncture will also be performed to detect malaria parasites by blood film and qPCR, malaria gametocyte qPCR, malaria immune response, CBC, and biochemistry according to Table 6.1. Other study activities will be undertaken according to Table 7.

7.4.2.2 Between Day 28 and Day 90 post antimalarial treatment initiation (D_{Rx29} - D_{Rx89})

The volunteers will be contacted fortnightly (biweekly) by email / phone call / other social communications e.g. message (SMS), WhatsApp, Line, to ensure they remain well and asymptomatic. They will not be limited to contact only with the study team, but the volunteer will have to understand that they must stay in Bangkok until day 90 after challenge and always immediately contact the study team as soon as they observe any abnormal symptoms or would like to contact the team for any other reason.

7.4.2.3 Day 90 post antimalarial treatment initiation ($D_{Rx\ 90}$)

The volunteers will be asked to visit FTMCTU as an outpatient using the hospital registration system. History taken using the clinical well-being check list, physical examination will be performed and AEs will be assessed. Diary cards will be reviewed and collected. According to Table 6.1, venipuncture will be performed to detect malaria antigen by blood film and qPCR, malaria gametocyte qPCR, malaria immune response, CBC, biochemistry, and blood collection for future cross matching* with the volunteers who will receive the blood inoculum in the future MIST studies. Other study activities will be undertaken according to Table 7.

** Blood sample for cross matching may be collected any time ≧ 90 days after antimalarial treatment initiation (time of blood donation)*

7.4.2.4 Between Day 90 and Day 1 year post antimalarial treatment initiation ($D_{Rx\ 91}$ to $D_{Rx\ 364}$)

The volunteers will be contacted fortnightly by email/ phone call / other social communications e.g message (SMS), WhatsApp, Line to ensure they remain well and asymptomatic.

7.4.2.5 Day 180 post antimalarial treatment initiation ($D_{Rx\ 180}$)

The volunteers will be asked to visit FTMCTU as an outpatient using the hospital registration system. According to Table 6.1, venipuncture will be performed to test for the serologic markers of hepatitis B virus (HBsAg, Anti-HBc, Anti-HBs), hepatitis C virus (Anti-HCV), HTLV I and HTLV II (anti HTLV I and anti HTLV II antibody), HIV (HIV antibodies), and syphilis (TPHA).

*** Blood sample may be collected any time ≥ 180 days after antimalarial treatment initiation (time of blood donation)*

7.4.2.6 1 year post antimalarial treatment initiation

The volunteers will be asked to visit FTMCTU as an outpatient. Venipuncture will be performed to detect malaria antigen by blood film and qPCR, malaria gametocyte qPCR, malaria immune response, and CBC according to Table 6.1. Other study activity will be undertaken according to Table 7.

Figure 2. Study diagram

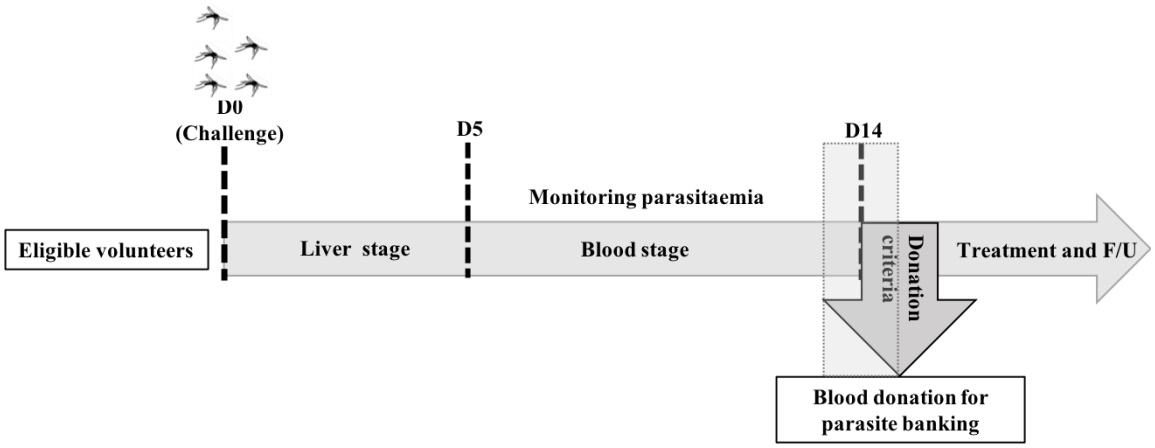


Table 6: Schedule of clinical reviews for volunteers pre-challenge (Timeline/Window are in relation to the day of challenge).

	Screening phase		
Event	Screening 1	Screening 2	Screening 3
Attendance number	1	2	3
Timeline (days)	Up to D-180	D-7	D-1
Window (days - days)	(D-180) - (D-7)	(D-14) - (D-7)	D-1
Inclusion / Exclusion criteria	X	X	X
Informed Consent Questionnaire	X		
Informed consent	X		
Medical History	X	X	X
Physical Examination	X	X	X
Body weight and height[f]	X		X
Urinalysis	X	X	
Electrocardiogram	X		
Vital signs [a]	X	X	
Filariasis RDT	X	X	
G6PD	X		
Haemoglobin	X		
Blood typing ABO, Rhesus	X		
Duffy antigen	X		

	Screening phase		
Event	Screening 1	Screening 2	Screening 3
Attendance number	1	2	3
Timeline (days)	Up to D-180	D-7	D-1
Window (days - days)	(D-180) - (D-7)	(D-14) - (D-7)	D-1
CBC and haematological diseases (e.g. haemoglobinopathy, thalassaemia)	X		
Blood biochemistry	X [b]		X [c]
Infectious diseases (HIVI-II, HBV, HCV, Dengue, Zika, Chikungunya, JE, malaria diagnosis, Anti HTLV I and Anti HTLV II antibody, Syphilis test (TPHA)	X [d]	X [e]	
β-HCG testing (women only)	X (Serum pregnancy test)	X (Urine pregnancy test)	X (Serum pregnancy test)
Epstein-Barr virus (EBV), Cytomegalovirus (CMV) (by PCR and serology testing)			X
Haematology (CBC)			X
CYP2D6 genotype	X		

	Screening phase		
Event	Screening 1	Screening 2	Screening 3
Attendance number	1	2	3
Timeline (days)	Up to D-180	D-7	D-1
Window (days - days)	(D-180) - (D-7)	(D-14) - (D-7)	D-1
Malaria serology	X	X	
Malaria Immunology			X
COVID-19 testing (nasopharyngeal swab [RT-PCR])		X (RT-PCR)	X (RT-PCR)
COVID-19 testing (nasopharyngeal swab (serology POC test)			X (POC test)

[a] Vital signs include blood pressure, pulse, respiratory rate and temperature.

[b] Biochemistry including electrolytes (sodium, potassium, calcium, magnesium, chloride, bicarbonate), blood urea nitrogen (BUN), creatinine (Cr), liver function tests (LFTs), fasting blood sugar (FBS), and lipid profile (cholesterol, triglyceride, HDL, LDL)

[c] Biochemistry including electrolytes (sodium, potassium, calcium, magnesium, chloride, bicarbonate), blood urea nitrogen (BUN), creatinine (Cr), and liver function tests (LFTs)

[d] HIV, HBV, and HCV will be tested for antibodies. HBV serological profile includes HBsAg, anti-HBc, and anti-HBs.

[e] HIVI-II, HBV, and HCV will be tested by qPCR.

[f] Height will be measured only at Screening 1

Table 6.1: Blood collection during study period

Phase	Clot blood		EDTA blood		NaF		CPD		Total blood volume (ml)	Total accumulation blood volume (ml)
	Volume	Test	Volume	Test	Volume	Test	Volume	Test		
Screening 1	7	Blood typing	13.5	Duffy antigen	2	Fasting blood sugar			22.5	22.5
		Blood biochemistry		Thalassemia						
		Serum HCG		CBC						
		HIV Ab		G6PD						
		HBs profile, anti-HCV		CYP2D6						
		Syphillis (TPHA)		HTLVI-II						
				Filaria						
				Malaria						
				malaria exposure (serology)						
				JE						
				DEN						
				CHIK						
				Zika						
Screening 2	1	Syphillis (TPHA)	13.5	malaria					14.5	37
				Malaria exposure (serology						
				JE						
				DEN						
				CHIK						

Phase	Clot blood		EDTA blood		NaF		CPD		Total blood volume (ml)	Total accumulation blood volume (ml)
	Volume	Test	Volume	Test	Volume	Test	Volume	Test		
				Zika						
				Filaria						
				HCV Ag						
				HBV Ag						
				HTLVI-II						
				HIV Ag						
Screening 3	9	Blood biochemistry	16	malaria immunology					25	62
		Serum HCG		CBC						
		CMB, EBV serology		CMV						
				EBV						
Admission D0-D4	3	Liver function test	12	Malaria immunology					15	77
				CBC						
Admission D5-blood donation	7	Liver function test	64	Malaria blood film, qPCR, gametocyte qPCR, hemoglobin			Up to 250	Blood donation	321	398
		CMB, EBV serology		Immunology						
				CBC						

Phase	Clot blood		EDTA blood		NaF		CPD		Total blood volume (ml)	Total accumulation blood volume (ml)
	Volume	Test	Volume	Test	Volume	Test	Volume	Test		
Treatment phase	3	Liver function test	22	Malaria blood film, qPCR, gametocyte qPCR, hemoglobin					25	423
				Malaria immunology						
				CBC						
$D_{Rx\ 28}$	5	Blood biochemistry	14	CBC					19	442
				Malaria blood film, qPCR, gametocyte qPCR						
				Malaria immunology						
$D_{Rx\ 90}$	5	Blood biochemistry	14	CBC					69	511
				Malaria blood film, qPCR, gametocyte qPCR						
				Malaria immunology						
			50	Blood collection for cross matching*						

Phase	Clot blood		EDTA blood		NaF		CPD		Total blood volume (ml)	Total accumulation blood volume (ml)
	Volume	Test	Volume	Test	Volume	Test	Volume	Test		
$D_{Rx\ 180}$**	4	Syphillis HIV HBs profile anti-HCV	1	HTLVI-II					5	516
$D_{Rx\ 1\ Yr}$			14	Malaria blood film, qPCR, gametocyte qPCR CBC Malaria immunology					14	530

** Blood sample for cross matching may be collected any time ≥ 90 days after antimalarial treatment initiation (time of blood donation)*

*** Blood sample may be collected any time ≥ 180 days after antimalarial treatment initiation (time of blood donation)*

Table 7: Schedule of Clinic Attendances Post-challenge

Event	Admission Phase				OPD visit for PQ DOT	Follow up Phase					
Timeline	D0	D1-D4	D5 - $D_{BI\ donation}$	$D_{Start\ CQ/PQ}$ - $D_{d/c}$	$D_{1st\ OPD}$ - $D_{End\ PQ}$	D_{Rx28}	Between D_{Rx28} & D_{Rx90}	D_{Rx90}	$D_{Rx91\ to\ 1\ yr.}$	D_{Rx180}	$D_{Rx1\ yr.}$
Window (days)	0	0	0		0	±9		±14		±14	±14
Inclusion / Exclusion criteria	X										
Medical History (well-being check list)[a]	X	X (Once daily)	X (Twice daily)	X (Once daily)	X	X		X			X
Body weight	X		X (Blood donation)			X		X			X
Physical examination [a]	X	X	X	X	X	X		X			X
Urine pregnancy test (women only)			X (before CQ Tx)								
Vital signs [a]	X	X	X	X	X	X		X			X
Local & Systemic Events (AE/SAE) [b]	X	X	X	X	X	X		X			X

Event	Admission Phase				OPD visit for PQ DOT	Follow up Phase					
Timeline	D0	D1-D4	D5 - $D_{BI\ donation}$	$D_{Start\ CQ/PQ}$ - $D_{d/c}$	$D_{1st\ OPD}$ - $D_{End\ PQ}$	D_{Rx28}	Between D_{Rx28} & D_{Rx90}	D_{Rx90}	$D_{Rx91\ to\ 1\ yr.}$	D_{Rx180}	$D_{Rx1\ yr.}$
Window (days)	0	0	0		0	±9		±14		±14	±14
Mosquito bite malaria sporozoite challenge	X										
Treatment for Malaria [c]				X	X						
Diary card					X	X		X			
Malaria Blood Film and qPCR and Gametocyte PCR			X (Twice daily)	X (Once daily)	X (Once daily on $D_{PQ\ 7}$ and $D_{PQ\ 14}$)	X		X			X
Haemoglobin			X	X	X ($D_{End\ PQ}$)						
Haematology (CBC)		X (D2)	X (D5, $D_{BI\ donation}$)		X (D_{Rx7})	X		X			X
Biochemistry [d]						X		X			
Liver function Tests		X	X		X						

Event	Admission Phase				OPD visit for PQ DOT	Follow up Phase					
Timeline	D0	D1-D4	D5 - $D_{BI\ donation}$	$D_{Start\ CQ/PQ}$ - $D_{d/c}$	$D_{1st\ OPD}$ - $D_{End\ PQ}$	D_{Rx28}	Between D_{Rx28} & D_{Rx90}	D_{Rx90}	$D_{Rx91\ to\ 1\ yr.}$	D_{Rx180}	$D_{Rx1\ yr.}$
Window (days)	0	0	0		0	±9		±14		±14	±14
		(D3)	(Present with parasitaemia)		($D_{End\ PQ}$)						
Malaria Immunology		X (D2)	X (D5, $D_{BI\ donation}$)		X (D_{Rx7})	X		X			X
Blood donation			X								
Contact volunteers fortnightly							X		X		
COVID-19 testing (RT-PCR by nasopharyngeal swab)		X [e]	X [f]	X ($D_{d/c}$)							
COVID-19 testing (serology POC test)			X [h]								
Blood collection for cross matching								x			
Infectious diseases (HBV, HCV, anti HTLV I and anti HTLV II antibody, HIV, and Syphilis test (TPHA)[g]										X	

Event	Admission Phase				OPD visit for PQ DOT	Follow up Phase					
Timeline	D0	D1-D4	D5 - $D_{BI\ donation}$	$D_{Start\ CQ/PQ}$ - $D_{d/c}$	$D_{1st\ OPD}$ - $D_{End\ PQ}$	D_{Rx28}	Between D_{Rx28} & D_{Rx90}	D_{Rx90}	$D_{Rx91\ to\ 1\ yr.}$	D_{Rx180}	$D_{Rx1\ yr.}$
Window (days)	0	0	0		0	±9		±14		±14	±14
EBV and CMV serology			X[i]								

[a] Vital signs include blood pressure, pulse, respiratory rate and body temperature.

[b] AE/SAEs will be collected until 1 year.

[c] This treatment period could occur on any day between D6 and D21, blood draws will continue as per the Table for the relevant study day, up until the time-point of blood donation or Treatment. The treatments will be done on D21 if no criteria meet.

[d] Biochemistry including electrolytes (sodium, potassium, chloride, bicarbonate), blood urea nitrogen (BUN), creatinine (Cr), liver function tests (LFTs)

[e] In case volunteers have fever but malaria blood film, qPCR, and testing for vector-borne diseases are negative, COVID-19 RT-PCR will be tested.

[f] In case volunteers have fever but malaria blood film and qPCR are both negative, COVID-19 RT-PCR will be tested.

[g] HBV, HCV, and HIV will be tested for antibodies. HBV serological profile includes HBsAg, anti-HBc, and anti-HBs

h COVID-19 serology POC test will be done at 14 days after the inoculation.

i EBV and CMV serology will be done at 2-4 weeks after the inoculation. EBV and CMV including EBV VCA (IgM)/IgG antibody, anti EBNA and EA complex antibody, and CMV IgM/IgG antibody

7.5 Details of study clinical procedures/laboratory tests

Procedures will be performed at the time points indicated in the schedule of procedures (Tables 6-7). Additional procedures or laboratory tests may be performed, at the discretion of the investigators if clinically necessary. All clinical and laboratory systems will be inspected by a Contract Research Organization (CRO) appointed by the Wellcome Trust (first inspection in March 2019, second inspection prior to study initiation), and study procedures monitored by the MORU Clinical Trials Support Group.

7.5.1 Clinical procedures

7.5.1.1 Body Weight and Height

Body Weight and Height will be measured at the time points indicated in the schedule of procedures (Tables 6 & 7).

7.5.1.2 Vital signs

Pulse rate (PR), blood pressure (BP), respiratory rate (RR) and body temperature (BT) will be measured at the time points indicated in the schedule of procedures (Tables 6 & 7).

7.5.1.3 Urinalysis

Urine will be tested for the presence of clinically significant proteinuria, glucosuria or haematuria (out of normal range) at the screening visit.

7.5.1.4 Electrocardiogram

An electrocardiogram will be performed at the screening visit.

7.5.2 Laboratory procedures

The Tropical Medicine Diagnostic Reference Laboratory (TMDR) will be the central reception point for all blood samples collected from FTMCTU, and will follow a sample management system designed by the MORU Sample Management Centre. TMDR will aliquot diagnostic and research samples and be responsible for transfer to designated laboratories for testing and retrieve the result generated from the following laboratories

- **Diagnostic Laboratory Unit, Hospital for Tropical Disease, Faculty of Tropical Medicine, Mahidol University**

    1. Haematology: complete blood count (CBC) and blood grouping (A/B/O, Rhesus status)
    2. Biochemistry: consisting of electrolytes, blood urea nitrogen (BUN), creatinine (Cr), liver function tests (LFTs), fasting blood sugar (FBS) and lipid profile (cholesterol, triglyceride, HDL, LDL).
    3. Serum beta-human chorionic gonadotrophin (serum β-HCG)
    4. Diagnostic serology; HBs profile (HBsAg, anti-HBc, anti-HBs), HCV antibodies, and HIV antibodies.
    5. Urinalysis
    6. Urine pregnancy test

7. CMV, EBV serology

- **Tropical Medicine Diagnostic Reference Laboratory (TMDR)**
  1. Hemoglobin
  2. Serology for HTLV I, HTLV II
  3. Dengue PCR
  4. Filariasis by rapid antigen test

- **Malaria Laboratory, Mahidol-Oxford Tropical Medicine Research Unit**
  1. Malaria microscopic examination

- **Microbiology and Immunology Department, Faculty of Tropical Medicine, Mahidol University**
  1. qPCR for Chikungunya
  2. qPCR for Zika virus

- **Molecular Tropical Medicine & Genetics Department, Faculty of Tropical Medicine, Mahidol University**
  1. qPCR for Malaria

- **ATGenes**
  1. G6PD genotyping
  2. Duffy antigen
  3. Haematological diseases: haemoglobinopathy analysis, CBC, haemoglobin electrophoresis and molecular studies for alpha and beta thalassaemia trait
  4. CYP2D6 genotype testing and reporting of expected metaboliser phenotype.

- **Virology Department, Ramathibodi Hospital, Mahidol University**
  1. qPCR for HIV1 virus, HIV 2 virus
  2. Hepatitis B PCR
  3. qPCR for hepatitis C virus
  4. qPCR for Japanese encephalitis virus.
  5. Cytomegalovirus
  6. Epstein-Barr virus

- **Serology Department, Ramathibodi Hospital, Mahidol University**
  1. Syphilis serology

- **Division of Hematology and Oncology, Siriraj Hospital**
  1. G6PD enzyme level

- **Mahidol Vivax Research Unit (MVRU), Faculty of Tropical Medicine, Mahidol University**
    1. Screening for background exposure to malaria (screening 2): Multiplex Bead Based Immunoassay will be performed to determine the magnitude of antibodies against recent exposure of 5-8 blood-stage *P. vivax* antigens. A seropositivity cut-off will be calculated by the average of median fluorescence intensity (MFI) plus 2 standard deviations of negative control.
    2. Gametocyte qPCR
    3. Immunology: Immunological responses to *P. vivax* infection.
    4. Cryopreservation of *P. vivax* infected blood (parasite banking)

Samples may also be sent to collaborating laboratories within and outside the Thailand for immunomonitoring and/or harmonisation of key immunological assays. There are two collaborating laboratories;

1) Ehime University, Matsuyama, Japan, and 2) KEMRI, Kenya

The AlphaScreen assay will be used to quantify malaria antigens-specific antibodies (up to 300 *P. falciparum* antigens and 300 *P. vivax* antigens). A seropositive cut-off will be set as half the lowest non-negative value of the assayed samples. Positive malaria history refers to a volunteer who previously had a malaria infection leading to antibodies in their serum which reactive to more than 10% of the malaria antigens in the library. This may include human DNA and RNA analysis.

With the volunteers' informed consent, any leftover cells and serum [**not including up to 250 mL blood inoculum**] will be stored for 10 years in the FTM/MORU Sample Management System for future immunological and genetic analysis of malaria-specific responses.

7.5.3 Blood donation, processing and storage

Two hundred fifty cubic centimeters (cc) of blood will be taken with a CPD whole blood collection kit (Imuflex whole blood filter PL saving with, Terumo). The blood will be leukocyte depleted using the inline leukodepletion filter that is a component of the blood collection pack. The blood will be transported back to the facility at Hospital for Tropical Diseases where it will be processed and frozen according to laboratory procedures in the spirit of Current Good Manufacturing Practice (cGMP). Immediately on arrival, the red cells will be separated from the plasma by centrifugation, and the blood then cryopreserved using a method described elsewhere [21]. Briefly, a ratio of 2 volumes of GMP quality Glycerolyte 57 to 1 volume of infected cell pellet will be used. The first 20% of the Glycerolyte 57 volume will be added dropwise with gentle agitation, and the suspension will be incubated for 5 minutes at room temperature before the remaining Glycerolyte 57 is added. The RBC-Glycerolyte mixture will be stored as 1.5 ml aliquots in 2 ml cryovial tube then frozen under controlled conditions at a temperature of -80°C for overnight. Aliquots are transferred and stored in a dedicated liquid nitrogen tank in a secure environment. This freezing process will be performed according to the blood donation and sample processing work instruction (WI) and Laboratory Standard Operating Procedure (SOPs) under controlled conditions in the laboratory, similar to the procedures used to freeze and

thaw the blood being used for CHMI trials in Oxford [54]. Aliquots of blood will be tested for bacterial contamination with a validated blood culture technique, mycoplasma, endotoxin as well as sterility and other key QC parameters according to a defined testing plan. CMV, EBV, Blood-borne (HIV, HBV, HCV, HTLV I-II, Syphilis) and vector-borne (DEN, CHIK, JE, and Zika) infectious diseases will be tested to ensure no new acquired infection during the admission phase. Genetic analysis of the parasite will be performed for in depth characterization of the banked parasite. There is good evidence that P. vivax withstands the freezing and thawing process and can subsequently be successfully used in short-term culture and invasion assays [55].

7.6 Definition of start and end of trial

The start of the trial is defined as the date of the first screening visit for first volunteer. The end of trial is the date of the last follow-up (1 year) of the last volunteer.

7.7 WITHDRAWAL OF VOLUNTEERS

In accordance with the principles of the current revision of the Declaration of Helsinki (updated 2013) and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. In addition the volunteer may withdraw/be withdrawn from further study procedures at any time in the interests of the volunteer's health and well-being, or for any of the following reasons:

- Administrative decision by the Investigator.
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- Volunteer non-compliance with study requirements.
- An AE which requires discontinuation of the study involvement or which results in inability to continue to comply with study procedures.
- Current COVID-19 infection during a participant's admission in the hospital

The medical monitors may recommend withdrawal of volunteers. The reason for withdrawal from further study procedures will be recorded in the case record form (CRF). If a volunteer withdraws after having completed a course of antimalarials, as much long-term safety data collection as possible, including procedures, such as safety bloods, will continue to be collected, with the agreement of the volunteer. For all AEs, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

If a volunteer withdraws from the study after challenge but before reaching the criterion for malaria diagnosis, a complete, appropriate, curative course of antimalarial therapy must be completed by standard chloroquine treatment and a 2-week course of 30 mg/day of primaquine. The importance of this will be emphasized to volunteers at screening. If a volunteer refuses to take antimalarial therapy after malaria diagnosis, a rapid assessment of mental state and capacity will be undertaken, with the involvement of psychiatric and infectious

diseases specialists. If necessary and if in accordance with the law in Thailand the volunteer may be detained for appropriate medical management.

If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored unless the volunteer specifically requests otherwise. Similarly, all data collected up to the point of withdrawal will be stored, unless they specifically request for it to be destroyed. Volunteers are free to request that their blood samples be destroyed anytime during or after the study. However, up to 250 mL of blood that they donate will not be destroyed after they have donated it, as it will be impossible to repeat this from a "back-up" volunteer half way through the study. However, the sample will be "delinked" so that there can be no tracing of it back to the volunteer who donated it. This shall all be made clear in the consent form.

In all cases of volunteer withdrawal, excepting those of complete consent withdrawal, long-term safety data collection will continue if volunteers have already undergone challenge process. Withdrawn volunteers before blood donation will be replaced.

8. Description and administration of the study agents (which is the *P. vivax* infected mosquitoes)

In this study, the study agent that we apply / administer to the volunteer is not a drug (since it is not drug trial). It is "*P. vivax* infected-mosquitoes" and the dosage given is "5 infected mosquitoes per one volunteer".

This section composes of 2 parts; 1) How to prepare the **study agent** (infected mosquitoes) (See section 9.1.) and 2) How to **administer** the **study agent** to the volunteers (how to perform mosquito biting) (See section 9.2.)

8.1 Preparing the study agent: Infected mosquito preparation stage

The process to producing the *"P. vivax* infected mosquito" are a) Source case identification (*P. vivax* patient(s)), b) mosquito preparation, and c) membrane feeding:

8.1.1 Source case identification (P. vivax *patient(s)):*

P. vivax patients are recruited from a clinic or hospital in endemic areas in Thailand where FTM has a study site. The molecular analysis is performed by the FTM laboratory in Bangkok to exclude mixed malaria species infection. Testing of donor blood for **blood-borne** and **endemic mosquito-borne (vector borne)** infections in the source patient as per the following details;

- Screening for **blood-borne infection:** HIV-1 and -2, HTLV I-II infection, Hepatitis B and C (HBV and HCV), and syphilis
- Screening for **vector-borne** infections endemic in Thailand that could potentially be transmitted from the patient via the *Anopheles* mosquito: Dengue virus, Zika virus, Chikungunya virus, Japanese B encephalitis virus and Filariasis

- Screening for **mixed malaria infections**: molecular speciation for *P. vivax, P. falciparum*, *P. ovalae* , *P. malariae* , and *P knowlesi*

8.1.2 Mosquito preparation and membrane feeding:

The Mahidol Vivax Research Unit (MVRU) and the Department of Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, will provide the *Anopheles* mosquitoes for the challenge. They are reared in the laboratory and fed on rigorously screened human blood (purchased from the Red Cross) to maintain the colony and induce egg-laying. The prepared mosquitoes are infected via a membrane feeder (membrane feeding assay (MFA)) containing infected blood from a *P. vivax* patient (as explained in #1). Up to 3,000 mosquitoes are used per patient's blood feed.

Prior to the mosquitoes biting volunteers, **the presence of *P. vivax* infection** is checked:

a) **7-9 days post blood-feeding by counting oocysts visualised on dissection of the midgut;** and

b) **14 days post blood-feeding by grading the number of sporozoites visualised on dissection of salivary gland.**

The mosquitoes are only used for challenge if the **infection rate is >75% and grading of sporozoite $\geq$ +3.** (This mosquito preparation protocol was already approved by FTMEC (No# MUTM 2018-017).

8.2 Administering the study agent to the volunteers:

- Mosquito challenge of volunteers at the insectarium Unit, Department of Entomology

Once the mosquitoes have been fed with *P. vivax* infected blood (by membrane feeding assay (MFA)) they need to be used in a sporozoite challenge (biting the study subject) between 14-21 days later to ensure **maintained infectivity** (it usually takes 14 days for the sporozoites to reach the salivary glands).

The challenge steps: Challenge (mosquito biting) of volunteers by using these infected mosquitoes will be carried out in the insectarium unit within the Faculty of Tropical Medicine, Mahidol University, Bangkok.

The volunteer(s) will be hospitalized a night before the mosquito biting day (D0) to ensure their safety / illegibility and to avoid the traffic burden.

Mosquitoes are transported directly from MVRU in a temperature-controlled box at 26 +/- 2^{o}C and immediately transferred to a secure room within the insectary of the Entomology Department. Each pot of mosquitoes is kept in a strong transparent plastic storage box. All manipulation of mosquitoes is performed within a secure room. The challenge is performed in a part of the secure insectary that is isolated by appropriate and approved mechanisms.

Prior to challenge, all volunteers are briefed. The arms will be cleaned according to the mosquito biting work instruction. Use by the volunteer of lotion or perfume is forbidden on the day of mosquito challenge.

For challenging, 5 mosquitoes will be aspirated into a small box. Each volunteer will be exposed to the bites of five infectious mosquitoes by placing their forearms over the mosquito cup for 5-10 minutes at a time.

Engorged mosquito(es), as indicated by the presence of a blood meal in the abdomen), is/are individually taken out of the pot to be dissected and assessed for sporozoite load (grading the load of sporozoite by using graded 0 to +4), ONLY a gland rating of +2 or more, representing 10 or more observed sporozoites, **qualifies as being infectious**). If, by this method the volunteer is found to have been inoculated by less than five infected mosquitoes, further mosquitoes are allowed to feed on the volunteer until a total of 5 appropriately infected mosquitoes have fed. The bite-challenge procedure continues until the volunteer has been bitten by 5 infectious mosquitoes (Appendix A). This mosquito biting process will be performed strictly following the Mosquito Bite Malaria Sporozoite Challenge work instruction.

The rationale for 5 infectious mosquito bites is based on the extensive previous experience with *P. falciparum*, where sporozoites inoculated by <5 mosquitoes have led to an irregular infection in malaria-naive human volunteers. However, this does not appear to be the case for *P. vivax:* 100% of the 45 volunteers (controls and vaccines) bitten by five *P. vivax*-infected mosquitoes in the WRAIR US study developed patent parasitaemia, and also of the 53 non-vaccinated volunteers (either malaria-naïve or semi-immune) bitten by between 2 and 4 infected mosquitoes in the Cali studies, 52 developed patent parasitaemia (about 100-1,000 sporozoites are inoculated per bite). However, to ensure reliable infection in our small study of just 6 volunteers, we have chosen to proceed with 5 successful infectious mosquito bites.

9. SAFETY

9.1 Safety experience from previous CHMI studies

Following a collaborative consensus process involving investigators from the USMMVP, Sanaria, University of Maryland, University of Oxford, RUNMC, the Seattle Biomedical Research Institute and the KEMRI-Wellcome Kilifi Research Programme, a consensus document; "Standardization of Design and Conduct of *P. falciparum* Sporozoite Challenge Trials" was developed, and provides a comprehensive guide to the appropriate conduct of controlled human malaria infection studies [33]. Although there remain minor differences between centres in follow-up procedures in controlled human malaria infection trial conduct, there is consensus on the following key points.

- All volunteers should have a medical assessment no longer than 48 hours before challenge, including an interim medical history, directed physical examination, pregnancy test for female volunteers.

- Follow-up visits should be scheduled at least once daily, but may increase in frequency to twice daily, starting at day 5-7 post-sporozoite challenge. At all visits volunteers should be questioned about the occurrence of adverse events (AEs) and use of medication.
- Grading and reporting of adverse events should be performed using international and local guidelines. It should be noted that the occurrence of a low frequency of grade 3 severe adverse events, of short duration, and with no long-term sequelae, is not unexpected in control human challenge studies. A minority of those challenged are known to experience grade 3 systemic adverse events and this fact should be included in the informed consent form.
- Vital signs should be recorded at least once daily and at any subsequent visits for medical attention. Directed physical examination should be performed when necessary.
- It is critical that every volunteer must receive every dose of antimalarial therapy. In some settings fully directly observed treatment will be essential. Where directly observed treatment is not used, investigators must follow volunteers closely to ensure compliance with the treatment regimen.
- After challenge, all volunteers should be followed until they have completely finished antimalarial treatment.
- Volunteers should be evaluated at least two weeks after finishing treatment.
- A local safety monitor and an independent safety monitoring committee should be established to act as independent experts in evaluating adverse events. The safety monitor or monitoring committee may advise the investigators on initiating anti-malarial treatment for a specific volunteer or volunteer group. While safety monitoring committees are not a requirement for Phase I trials, they should be considered a requirement for challenge trials which have an efficacy component and which have major potential safety concerns.

Volunteer safety is of paramount importance, and we will follow these guidelines. The following measures are in place to safeguard volunteer safety:

- Volunteers will only be enrolled in the study if investigator judges this is appropriate.
- Volunteers' understanding of the trial information will be tested **by means of a questionnaire** at screening. This provides further confidence that fully informed consent has been obtained.
- Before challenge, full contact details for each volunteer will be documented, including home address and mobile telephone numbers. Mobile telephone numbers will be verified prior to challenge to ensure the volunteers are easily contactable. Home and work landline telephone numbers where available and next-of-kin address and telephone numbers will also be documented. Volunteers must also provide the Investigators with the name and 24 hour telephone number of a close friend (s), relative (s) or housemate (s) who live nearby and who will be kept informed of their whereabouts for the duration of the study.

- The volunteers will be hospitalized throughout the challenge processes until clinically recovered **AND** the completion of the antimalarial course (chloroquine) **AND** until two consecutive negative blood films. The 14 days of primaquine for radical cure will be performed under direct observed therapy (DOT).
- Volunteers will be able to contact a medically qualified member of the study team 24 hours a day throughout the study period.
- Volunteers will be required to contact the study team and their GP if they feel feverish or unwell in the 1 years following the challenge.
- The 5 years insurance for malaria infection will be provided.

9.2 What do we expect to discover during the challenge?

This part of the protocol aims to clearly review the events related to experience from the previous control human challenge studies in order to foresee the possible event(s) after the challenge that we may have to prepare to encounter within this study (almost all of the data comes from *P. falciparum* controlled human challenge studies).

• Nearly all unvaccinated volunteers in challenge studies develop symptoms of clinical malaria infection within 2 weeks following the challenge (means nearly all success in the induce the infection by this intervention)

• Approximately 1/5 of volunteers in *P. falciparum* CHMI studies temporarily develop symptoms graded as severe (symptoms that prevent daily activities, most of them were fever and dehydration), but severe or life-threatening malaria has never occurred [43]. Vivax malaria is less dangerous than falciparum malaria, though severity of initial symptoms may be similar.

• Routine laboratory checks generally show a moderate decrease in leukocyte and platelet numbers during infection, with no change in haemoglobin concentration [44].

• Bleeding or thrombogenic complications have never been described [43, 44].

• Abnormalities of liver enzymes have been observed, but these abnormalities have rarely resulted in clinical manifestations (just one volunteer with raised ALT associated with abdominal pain and vomiting in the recent RCT from Cali [23]) and they resolved after a few days.

As of 2011, human malaria challenge infections have been conducted with *P. falciparum* in over 1,300 volunteers [43-45]. Recently, safety concerns were raised in a young volunteer who suffered a cardiac event shortly after treatment for diagnosed malaria. This was diagnosed as probable myopericarditis, although ischaemia could not be ruled out. Although a definite relationship between the cardiac event, which resolved fully and rapidly, and the experimental malaria infection was not established [46] (**and this was also seen with *P. falciparum*, not *P. vivax***), it has been generally agreed that volunteers with an increased risk of cardiac

disease should be excluded from such trials [33]. A further case of myopericarditis has since been identified in a recent challenge study, also at the Nijmegen, Netherlands, centre, but in this case the individual was also diagnosed with an intercurrent rhinovirus infection so that the relation to malaria infection is again uncertain. There was a brief episode of clinical chest pain and the volunteer made a full recovery [47].

9.3 Risks

9.3.1 Phlebotomy

The maximum volume of blood drawn over the study period should not compromise these otherwise healthy volunteers. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venipuncture, which will not be documented as AEs if they occur.

Due to the 250 mL blood donation, each volunteers will have a total (maximum) of 530 mL taken over the study period (~3 months from admission phase to 1 year). This is approximately in the range of the amount of a regular blood donation (350-500 mL) every 3 months. In addition, a recent multicentre study by NHS Blood and Transplant (in Oxford and Cambridge) compared outcomes in 50,000 regular blood donors who were randomised to different intervals between blood donations (as regularly as giving 470 mL every 8 weeks in one group). They found no differences in tolerance/ outcomes over a 3-year follow-up between the different groups (verbal communication, data as yet unpublished). Donating 816 mL over 4 months amounts to 124 mL less than that donated by the 8-week interval group in the NHSBT study, so we are confident that this volume should not compromise our volunteers in any way. However, we do acknowledge that these volunteers will not be true "healthy volunteers" at the time of blood donation as they will be infected with malaria and may be symptomatic from this. For this reason, they shall be closely monitored in a controlled environment at the time of donation, with access to symptomatic relief, including fluid therapy, if required. Reassuringly, a similar amount of blood has been taken from research volunteers with *P. vivax* infection in 3 previous studies, and this has resulted in no untoward adverse events over and above those attributable to the *P. vivax* infection itself [21, 49, Minassian A and Draper S, personal communication]. The same or larger amount (500 mL) has also been taken from patients with *P. falciparum* malaria [50, 51].

Participants' blood samples will be collected by well - trained medical personnel with aseptic technique to prevent infection or complications.

9.3.2 Blood donation (up to 250 mL)

Close clinical monitoring to ensure that volunteer tolerates the blood donation. This procedure will be carried out during the working hour. If the volunteer's haemoglobin falls below 10 g/dL with clinical stable regardless of their level of parasitaemia, then the blood donation shall be withheld and they shall be treated with antimalarials immediately.

9.3.3 Mosquito bites

Mosquito bites may cause local inflammatory reactions with redness, itching, swelling, scaling and/or tenderness. Topical anti-histamine cream for use twice daily for 3 days post-mosquito bite will be dispensed to the volunteers on the day of challenge. Serious allergic reactions including anaphylaxis have not been seen in challenge studies to date, but may occur and for this reason volunteers will be inoculated in an area where Advanced Life Support trained physicians and a defibrillator are immediately available. The volunteers will be screen and excluded if suspected as severe allergy to the insect bite or mosquito bite. Data regarding itching and use of antihistamine cream will be collected on diary cards but, as this is an expected part of the challenge procedure, it will not be classed as an adverse event in the immediate post-challenge period.

9.3.4 *Plasmodium vivax* infection

Base on previous studies, volunteers are likely to develop symptomatic malaria infection following challenge [48]. Volunteers will be followed up closely post-challenge and only enrolled in the study if they are deemed reliable and capable of complying with the intensive follow-up schedule.

As described above, the volunteers will be hospitalized in the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University for close clinical monitoring, treatment (antimalarials) and symptomatic relief (fluids, analgesia and/or anti-emesis) until parasite becomes negative. The treatments algorithm as well as the hospital guideline will be fully reviewed and strictly followed. This study initiation will be officially acknowledge to the hospital to ensure all the facility are in place. The expert of the Faculty of Tropical Medicine will be officially acknowledged prior to the study. The medical monitors will be reachable for the whole study processes.

A very small proportion of volunteers in previous *P. vivax* blood-stage and sporozoite-stage challenge studies have temporarily required intravenous fluid therapy for nausea and vomiting prior to treatment [23, 49].

It is relevant to note that safety and parasitological data from 128 malaria-naïve volunteers participating in challenge studies in Oxford and the Netherlands were analysed and compared to a report from the US Military Malaria Vaccine Program. The authors found that cohorts with a longer prepatent period or a higher peak parasitaemia did not consistently show a higher frequency of adverse events [52]. However, these data were on *P. falciparum* challenge, and there is a lot less experience with *P. vivax* challenge.

9.3.5 Risk of relapse of malaria

Infection with *P. vivax* malaria carries with it a significant risk of relapsing disease if sub optimally treated. This can occur because parasites (hypnozoites) can stay dormant in the liver and then reactivate into the blood causing clinical disease weeks, months or even years later. This risk is minimised and mitigated by:

a.) giving volunteers a 14-day course of primaquine treatment after / together with their initial 3-days of schizonticidal antimalarials, as this drug specifically eradicates any remaining dormant parasites from the liver.

b.) screening volunteers for their ability to metabolise primaquine efficiently, by checking their blood for CYP2D6 genotypes. Only volunteers who have genotypes associated with “high-metaboliser” status will be enrolled into the study.

c.) following up volunteers after treatment by email and / or telephone every two weeks for a year in order to capture any symptoms suggestive of relapse and any medical attention sought. They shall also be encouraged to inform their local doctor and the study investigators as soon as possible should any symptoms occur. If they do relapse they shall be referred to hospital for treatment immediately. The study group will provide treatment for 5 years of *P. vivax* malaria relapse.

9.3.6 COVID-19

The risk mitigation steps will be taken for the face to face research during COVID-19 pandemic which follow the local guidance (Hospital for Tropical Diseases).

9.3.7 Contingency plan if subject going missing post-challenge

The volunteers will be inpatients for entirety of the challenge phase, from the day before mosquito challenge until the completion of schizonticidal antimalarial treatment. They will then receive daily directly observed primaquine treatment for 14 days. It is unlikely that a volunteer will become absent during hospitalization (before schizonticidal treatment is completed). The chance of a subject missing radical treatment with primaquine daily DOT should be very low following the counseling carried out during the screening process. If the volunteer does fail to attend for a scheduled clinical visit after discharge from the hospital (during the primaquine DOT phase), and if the volunteer is un-contactable by telephone or instant messaging, the following stakeholders will be informed:

- All Investigators.
- The volunteer’s nominated contact and next of kin.
- The trial sponsor.
- The medical monitor group.
- The ethical committee(s).
- The local police department.

Active efforts will be made to locate the volunteer while all parties will aim to preserve the volunteer’s confidentiality. Volunteers will be informed of this during screening.

9.4 Safety detection, assessment, documentation and reporting

The investigators and designated site staff is/are responsible for the detection, assessment, documentation and reporting of events meeting the criteria and definition of an AE or SAE as provided in this protocol. Each

volunteer will be instructed to contact the investigator immediately should the volunteer manifest any signs or symptoms they perceive.

9.4.1 Definitions

9.4.1.1 Adverse Event (AE):

An AE is any untoward medical occurrences in a volunteer, which may occur during or after administration of a study intervention (in this case, vivax parasite challenge) and does not necessarily, have a causal relationship with the intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention. Any events occurring between screening and D-1 will be considered as baseline/, pre-existing conditions. This information will be recorded in the medical records.

9.4.1.2 Serious Adverse Event (SAE):

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention.

- Death (i.e. results in death from any cause at any time).
- Life-threatening event (i.e. the volunteer was, in the view of the Investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Transfer of inpatient care to the intensive care unit, if the Investigators assess that a higher level of intervention and intense monitoring is required to manage symptoms following controlled human malaria infection, blood donation, or drugs (over and above what can be provided by the Investigators in research bay). Hospitalization (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a SAE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Relapse of malaria would be classified as an SAE.
- Congenital anomaly or birth defect.

9.4.2 Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events:

In absence of diagnosis, clinically abnormal laboratory findings (e.g. clinical chemistry, haematology, and urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

In addition, COVID-19 infection in volunteers acquired during in-patient stay challenge will be considered an SAE and COVID-19 treatment will be provided to the volunteers free of charge. If volunteers have to stay in the hospital for SAE, admission compensation at 2,000 THB per night will be provided to the volunteers.

9.4.2.1 Causality assessment

The investigator is obligated to assess the relationship between study procedure and/or antimalarial medications and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study procedure and antimalarial medications will be considered and investigated. The relationship of the adverse event with the study procedures will be categorized as unrelated, unlikely to be related, possibly related, probably related or definitely related (Table 8). An intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to the study intervention. The delegated clinician will use clinical judgment to determine the relationship.

Table 8: Guidelines for assessing the relationship of study intervention to an AE

0	Unrelated	No temporal relationship to study intervention ***and*** Alternate aetiology (clinical state, environmental or other interventions);***and*** Does not follow known pattern of response to CHMI, blood donation or drug.

1	Unlikely to be related	Unlikely temporal relationship to study intervention ***and*** Alternate etiology likely (clinical state, environmental or other interventions) ***and*** Does not follow known typical or plausible pattern of response to challenge, blood donation or drug.
2	Possibly related	Reasonable temporal relationship to study intervention; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with previous challenge, blood donation or similar drug.
3	Probably related	Reasonable temporal relationship to study intervention; and Event not readily produced by clinical state, environment, or other interventions or Known pattern of response seen with previous challenge, blood donation or drug.
4	Definitely related	Reasonable temporal relationship to study intervention; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with previous challenge, blood donation or drug.

9.4.2.2 Assessment of intensity

Each adverse event will be graded by the investigator and designated study staff according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

In the rare case that an adverse event is not graded in the CTCAE, then that event should be graded as follows:

Grade 1: Mild AE

Grade 2: Moderate AE

Grade 3: Severe AE

Grade 4: Life-threatening or disabling AE

Grade 5: Death related to AE

All AE/SAE will be collected throughout the first 3 month after challenge or until a satisfactory resolution occurs. Malaria relapse will be collected throughout the entire 1 year period. All AEs that result in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the volunteer consents to this). In cases where the volunteer has to do AE self-detection and self- assessment, the simple grading for the self-assessment on the diary card will be used (Table 9)

The simple grading for use for the self- assessment for severity as in Table 9

Table 9: Severity grading criteria for the self-assessment for AEs.

GRADE 0	None
GRADE 1	Mild: Transient or mild discomfort (<48hours);no medical intervention/therapy required
GRADE 2	Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	Severe: Marked limitation in activity, some assistance usually required; may require medical intervention/therapy

9.4.2.3 Assessment of outcomes

The investigator will assess the outcome of all AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

During the inpatient phase, the adverse events (including all SAEs) will be assessed by the investigator and the delegated study staff.

While the volunteer is followed as an outpatient, a diary card will be provided, and the volunteer will be instructed how to self-assess the cause and severity of AEs. This self-assessment symptom diary will be checked, discussed, collected and recorded at each clinic visit.

9.4.3 Reporting procedures

For SAEs

In order to comply with current regulations on serious adverse event reporting to Ethics and regulatory authorities (if applicable), the event will be documented accurately and notification deadlines respected.

SAEs will be reported to the medical monitor group immediately (within 24 hours) of the Investigators' being aware of their occurrence.

SAEs will also be reported to ethics committees, the Data and Safety Monitoring Board (DSMB), and the regulatory authority (if applicable), in accordance with reporting requirements and according to required timelines.

9.5 Events or outcomes not qualifying as adverse events or serious adverse events

9.5.1 Pregnancy

Volunteers are informed that for the safety of volunteer and their child, pregnancy is prohibited until 3 months after the challenge. Female study volunteers are asked to use appropriate contraceptive methods to prevent pregnancy while they participate in the study for 3 months after the mosquito challenge.

Appropriate contraceptive methods include:

- Established use of oral, injected or implanted hormonal contraceptives

- Intrauterine Device or Intrauterine System

- Barrier methods (condoms or diaphragm with additional spermicide)

- Male sterilisation and female sterilisation (with appropriate post-vasectomy documentation of absence of sperm in the ejaculate)

- True abstinence, when this is in line with the preferred and usual lifestyle of the volunteer. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Female volunteers will be tested for pregnancy immediately prior to challenge and during follow up as indicated in the schedule of procedures (Table 6).

Should a volunteer become pregnant during the trial, she will be treated with antimalarials immediately and will be withdrawn from the study. We will not routinely perform venepuncture on such volunteers, other than blood films to check that the parasitaemia has been cleared by the antimalarial treatment. With the volunteer's permission she shall be followed up until pregnancy outcome. The management of any volunteer found to be pregnant at any time after challenge up to the point of malaria treatment will be discussed with the on-call infectious diseases consultants at Faculty of Tropical Medicine and the Mahidol-Oxford Tropical Medicine Research Unit, including advice on antimalarial drug choice.

Should a volunteer become pregnant after receiving antimalarial treatment (but prior to the end of the study), they shall be discontinued from the study as soon as we have confirmed that their parasitaemia has cleared. The volunteer will still be followed up until 1 year after challenge.

During pregnancy the following should always be considered as an SAE:

- Spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation)
- Ectopic and molar pregnancy
- Stillbirth (intrauterine death of fetus after 22 weeks of gestation).
- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).

- Any congenital anomaly or birth defect identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the fetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

However, pregnancy occurring in participants within 3 months of challenge will be considered a SAE.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the study procedures will be reported. While the investigator is not obligated to actively seek this information from former study volunteers, he/she may learn of a pregnancy through spontaneous reporting.

9.6 Safety monitoring

9.6.1 Monitoring

Monitoring will be performed using established Monitoring plan. Independent monitoring will be performed by Clinical Trials Supporting Group (CTSG), MORU. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol and Good Clinical Practice (GCP). The investigators will provide direct access to all trial related source data/documents and reports for the purpose of auditing by the Sponsor and inspection by local and regulatory authorities.

9.6.2 Medical Monitor Group

A Medical Monitor group, representing the Sponsor, will be appointed for oversight of safety in this clinical study. The Medical Monitor group will be responsible for safety assessments. The monitor will review the study prior to initiation and will be available to advise the Investigators on study-related medical issues and to act as a representative for the welfare of the subjects. The medical monitor does not have direct involvement in the conduct of the study. All serious adverse events will be reported to the medical monitor within 24 hours of becoming aware of the event. The medical monitor group is responsible for the review of the safety data and communicate with the PI and/or the DSMB, as appropriate.

The medical monitor group will be chaired by Dr. Lorenz Von Seidlein M.D., and the members of the medical monitor group are

1. Dr. Elizabeth Ashley, MD
2. Dr. Rupam Tripura, MD

9.6.3 Data and Safety Monitoring Board

DSMB will be independent of the Clinical Trials and Research Governance, Oxford University (Sponsor) and MIST programme team. The DSMB will review the study prior to initiation; review the interim safety data

reports; and review all SAEs according to DSMB charter. The Board may convene additional reviews if deemed necessary, on review of the safety data, as sent, periodically by the medical monitor. All SAEs will be reported by the site principal investigator to the DSMB at the same time as they are submitted to the ethics committees. The PI(s) will notify the Board and obtain a recommendation concerning continuation, modification, or termination of the study. The site principal investigator will submit written DSMB summary reports with recommendations to the ethics committees(s). The roles and responsibilities of the DSMB will be formalised in a charter agreed with the members of the DSMB.

10. STATISTICS

The safety of the CHMI will be assessed by descriptive analysis of the frequency, incidence and nature of adverse events and serious adverse events arising during the study. Since this is a feasibility study conducted in six volunteers, only a brief statistical analysis plan is applicable.

Sample size calculation

The number of volunteers undergoing malaria challenge in this CHMI study will be up to 6, deemed sufficient to meet the primary objective of **assessing the safety and feasibility** of controlled human *P. vivax* malaria infection in Thailand. It is the first study in Asia, and if successful, larger similar studies will be performed per the study package described in the background, overview and rationale of the study section (section 2).

11. DATA, DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Source documents are original documents, data, and records from which volunteers' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarized into the CRF), clinical and office charts, registration logbook, laboratory and pharmacy records, diaries, radiographs, referral notes and correspondence. All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the volunteer will be referred to by the study volunteer number/code, not by name. Direct access to all trial related source data/documents will be granted to authorized representatives from the sponsor, Research Ethics Committees (RECs), and the regulatory authorities to permit trial-related monitoring, audits and inspections.

12. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol.

12.1 Audit & Inspection

The Quality Assurance will perform system based internal audits to check that trials are being conducted, data recorded, analysed and accurately reported according to study protocols, departmental SOPs and in compliance with ICH GCP. The audit schedule includes laboratory activities. The internal audits will supplement the monitoring process and will review processes not covered by the monitor.

The Sponsor, trial sites, ethical committee(s), and authorised individuals may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the regulatory authority to ensure compliance with protocol and national regulations. The sponsor will assist in any inspections.

13. ETHICS

Ethical approval will be sought prior to commencing the study through the relevant Research Ethics Committees. Indemnity for the trial will be provided by the University of Oxford. SAEs will be reported to the medical monitor group, DSMB, and ethics committees. GCP certificate will be obtained by all staff/investigators prior to commencing the studies.

The Investigators will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki 2013. The trial will adhere to the ICH GCP.

The protocol, informed consent form, participant information sheet, and other written participant information/materials and Advertisement will be submitted to appropriate Research Ethics Committees (RECs), and regulatory authorities (if applicable) for written approval. The Principal Investigator (PI) will submit and, where necessary, obtain approval from the above parties for all amendments to the original approved documents. The Investigator will notify deviations from the protocol or SAEs occurring at the site to REC(s) of these if necessary in accordance with procedures. The principal investigator shall submit a report once a year throughout the study, or on request, to the ethic committees. In addition, an End of Study notification and final report will be submitted to the ethic committees.

13.1 Participant confidentiality

All data used for analysis will be de-identified; personal identifying information such as names and telephone numbers will not be used for analysis. Files containing identifiable information will be stored separate from other study data, in secure locations. Only the Sponsor's representative, Investigators, the clinical monitor, the ethical committee(s) and the regulatory authorities will have access to these records. Photographs may be taken to be shown to other professional staff, used for educational purposes, or included in a scientific publication. Photographs will not include the volunteer's face and if required the volunteer's written informed consent will be sought before photographs are taken. The photographs will be stored as confidential records, as above.

Volunteers' data and results from blood analyses stored in our database may be shared with other researchers to use in the future. However, the other researchers will not be given any information that could identify the subject.

13.2 Benefit

The expected benefit is to provide information about the feasibility and safety of controlled human *P. vivax* malaria infection studies for future testing of pre-erythrocytic candidate vaccines, about the growth of and the immune response to *P. vivax* infection, and to create a bank of *P. vivax*-infected blood for future testing of candidate blood stage vaccines. All of these will benefit patients with malaria infections in the future, and potentially lead to development of a vaccine to prevent infections altogether. However there are no direct benefits to the participants taking part in this study.

13.3 Data handling and record keeping

The investigators will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH E6 (R2) GCP and regulatory and institutional requirements for safety and protection of confidentiality of volunteers. The principal Investigators are responsible for data management and for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. The study staff will enter the data into the volunteers' CRFs, which will be in a paper and/or electronic format. This includes safety data, laboratory data (both clinical and immunological) and outcome data. Data will be managed and stored in MACRO® database, a GCP-compliant electronic data capture system. A study data management plan will outline detailed procedures for data capture, storage, curation and preservation.

The study protocol, documentation, data and all other information generated will be held in strict confidence. Only authorized, trained study staff will have access to study records. The investigators will permit authorized representatives of the sponsor, ethical committee(s), regulatory authorities (if applicable), authorized representative of sponsor, and the monitors to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. No information concerning the study or the data will be released to unauthorised third parties, without prior written approval of the Sponsor.

Medical's record will be kept at study site for approximately 15 years after study completion. Data will be fully anonymised and stored indefinitely in the secure Database.

14. INSURANCE

The University of Oxford has a specialist insurance policy in place - Newline Underwriting Management Ltd, at Lloyd's of London – which would operate in the event of any volunteer suffering harm as a result of their involvement in the research.

15. PUBLICATION POLICY

All Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authorship will be determined in accordance with the International Committee of Medical Journal Editors (ICMJE) guidelines and other contributors will be acknowledged.

16. COMPENSATION

Volunteers will be compensated for their time and for the inconvenience caused by procedures as below. This amount of compensation is calculated based on the cost of living in Bangkok for time and traveling. According to the consensus recommended by the controlled human challenge study group meeting in 2015, the compensation specifically on the risk was also advised (Bambery B, Selgelid M, Weijer C, et al., Ethical criteria for human challenge studies in infectious disease. Public Health Ethics. 2016. 9; 92-103.)

Table 10*:* Estimated compensation amounts.

Activity	**Compensation (THB)**	**Number of visits**	**Total**
1. Screening visit 1 (D -180)	2,000	1	2,000
2. Screening visit 2 on D-7	1,500	1	1,500
3. Admission per night	2,000	Around x 18 nights	36,000 (estimated)
4. Inconvenience of blood donation:	5,000	1	5,000
5. Primaquine DOT per visit (out-patient)	1,000	11	11,000
6. D_{Rx28}	1,500	1	1,500
7. D_{Rx90}	1,500	1	1,500
8. D_{Rx180}	1,500	1	1,500
9. 1 yr.	1,500	1	1,500

Time in Trial (approx.)	Maximum no. of visits	Maximum volume of blood taken[1] (mL)	Total compensation amount (THB)
1 year and 6 months	18	530	61,500

Remark:

1. Maximum volume of blood taken is 530 ml (estimated blood donation is D12-D14). In case volunteer does not reach the treatment criteria, microscopy and malaria qPCR and Gametocyte qPCR will be performed twice daily until D21, so the maximum volume of blood taken will be 260 mL.

In case volunteers has to come for extra visit(s), they will be compensated for their time and for the inconvenience for 1,000 THB per day.

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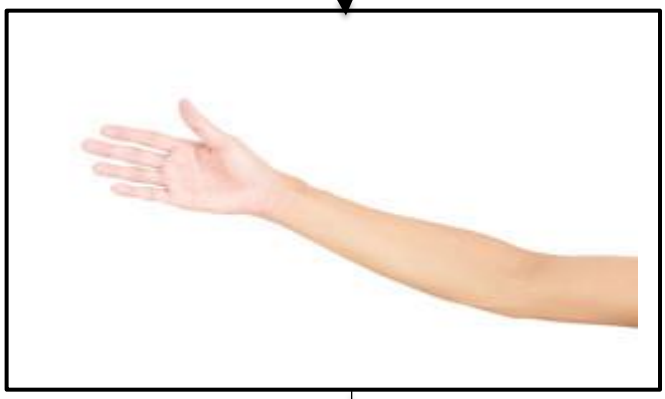
18. Appendix A: MOSQUITO BITING PROCESSES DIAGRAM

Infected mosquitoes

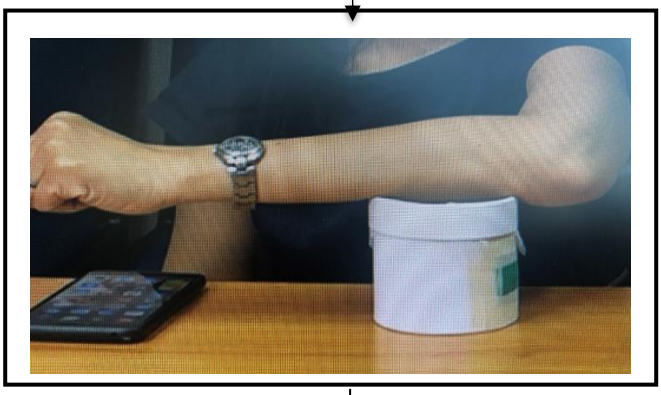
Subject will be briefed before the challenge.

The challenge process will be performed in the secure room in the insectary.

Forearm will be cleaned.

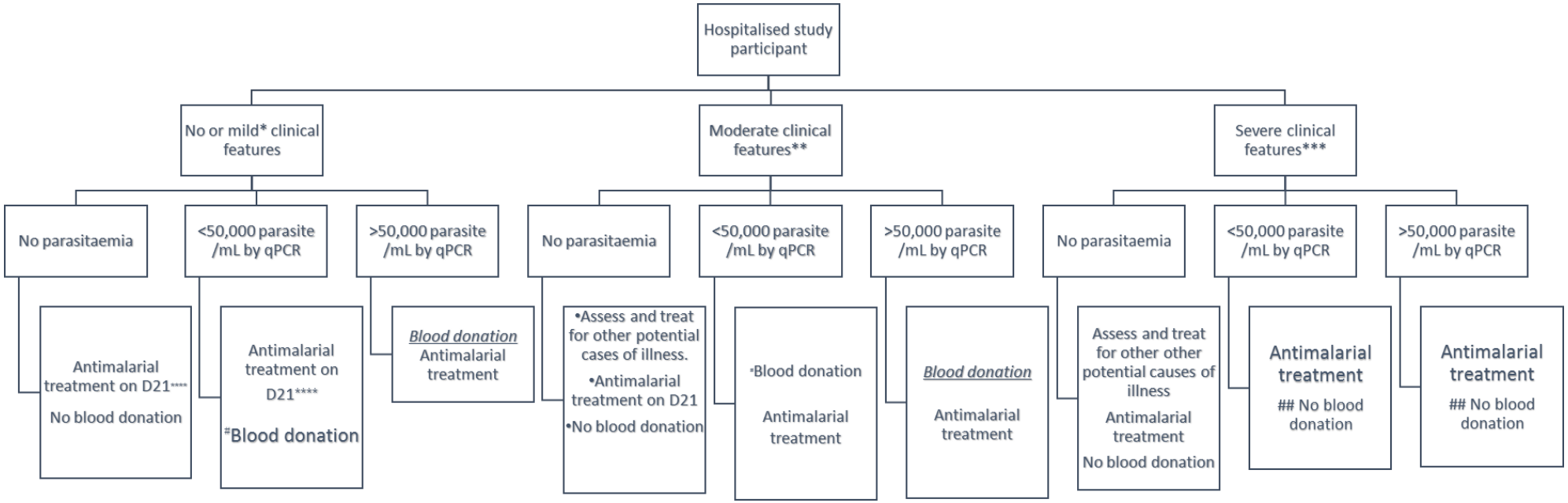


Subject will be bitten by five infectious mosquitoes per volunteer (by placing their forearms over the pot containing mosquitoes) for 5-10 minutes.



Subject will be hospitalized at Bangkok Hospital for Tropical Disease until disease management is completed.

19. APPENDIX B: TREATMENT ALGORITHM



*Mild clinical features, any one of the following and no moderate or severe features described below:

- Intermittent fever (< 38 °C, in 4-6 hour)
- Nausea ≤2 vomiting episodes in 24 hours
- Discomfort
- Mild dehydration

**Moderate clinical features, any one of the following and no severe features described below:

- Sustained high fever (> 38 °C, in 4 to 6 hour)
- Severe vomiting (≥ 3 times in 24 hours),
- Moderate dehydration

***Severe clinical features, any one of the following:

- Severe prostration
- Unstable vital signs (BP less than 80/50 mmHg, resting pulse > 120, weak pulse, RR > 25 BPM)
- Alteration of consciousness

- Severe dehydration
- Acute dyspnea
- Oliguria in previous 24 hours
- Clinical deteri

****Antimalarial treatments will be prescribed either on D21 or earlier following the physician judgements based on clinical and laboratory data.

[#]Blood donation will be done according to physician judgement based on parasitaemia.