Protocol title

Therapeutic efficacy surveillance of malaria treatment and drug resistance monitoring in Gia Lai and Phu Yen provinces of Central Vietnam

Protocol short title

Surveillance of antimalarial drug efficacy and drug resistance in Central Vietnam

Protocol No. VDCP01

Principal Investigator: Dr. Huynh Hong Quang

24 June 2021 - Version No. 2

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3. STATEMENT OF COMPLIANCE

Principal Investigator declaration

I have read the clinical trial protocol and agree that it contains all necessary details for carrying out the study entitled "Therapeutic efficacy surveillance of malaria treatment and drug resistance monitoring in Gia Lai and Phu Yen provinces of Central Vietnam". I will conduct this protocol as outlined herein and will take full responsibility for the study as the Principal Investigator.

I agree to personally conduct or supervise the described study. The study will be conducted in accordance with the following:

- World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (Fortaleza, Brazil 2013);
- Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2) (November 2016);
- Vietnam Ministry of Health Institutional Review Boards in National Biomedical Research (MoH-IRBNBR) that approved the clinical trial protocol.

I agree to inform all participants that the antimalarial drug treatments will be used for surveillance purposes and I will ensure that the requirements relating to informed consent will be obtained in accordance with International Council for Harmonization (ICH) Guidelines for Good Clinical Practice (GCP) section 4.8 and requirements of the MoH-IRBNBR.

I agree to promptly report to the IRB and other ethical agencies associated with the study any changes in the study activities and all unanticipated problems involving risk to subjects. I will not make any changes to the conduct of the study without IRB and Sponsor approval, except when necessary to eliminate apparent immediate harm to subjects.

I will report serious adverse events that occur in the course of the study to the MoH-IRBNBR, and other ethical agencies associated with the study and the sponsor in accordance with ICH guidelines for GCP section 4.11.

I will maintain adequate and accurate records and make those records available in accordance with ICH Guidelines for GCP section 4.11 and requirements of the MoH-IRBNBR.I will ensure that all co-investigators and supporting staff are informed of their responsibilities and duties in the conduct of the study.

I understand that the study may be terminated or enrolment suspended at any time by the Vietnam Ministry of Health or Sponsor, with or without cause, or by myself if it becomes necessary, to protect the welfare of the subjects.

_____ Date: _____

Dr Huynh Hong Quang MD, PhD (Principal Investigator)

4. KEYWORDS, KEY PHRASES, ABBREVIATIONS AND ACRONYMS

Keywords, Key Phrases, Abbreviations and Acronyms	Description
ACPR	Adequate Clinical and Parasitological Response (ACPR) in an antimalarial drug study is a patient who completes the 42 days of follow-up after starting treatment without treatment failure.
ACT	Artemisinin based combination therapy (ACT), a combination of two antimalarial drugs: a rapid acting artemisinin derivative to quickly reduce the parasite load and a slow longer acting drug that assists in preventing the recurrence of infection.
ADF	Australian Defence Force
ADFMIDI	Australian Defence Force Malaria and Infectious Disease Institute
Blood schizonticidal drug	Antimalarial drug that either prevents or kills the development of blood asexual stage malaria parasites.
Clinical efficacy of a drug	Measure of how well a drug treatment succeeds in achieving its aim.
CRF	Case Report Form
DHA-PPQ	Dihydroartemisinin-Piperaquine (DHA-PPQ), an ACT used in Southeast Asia and Africa for the treatment of uncomplicated *Plasmodium falciparum* malaria.
DNA	Deoxyribonucleic acid. A DNA strand consists of the precise order of the four nucleotide bases – adenine, guanine, cytosine and thymine. These bases provide the underlying genetic basis (the genotype) for telling a cell what to do, where to go and what kind of cell to become (the phenotype).
GCPs	Good Clinical Practices (GCPs) is an international ethical and scientific quality standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials. It also serves to protect the rights, integrity and confidentiality of trial subjects.
G6PD deficiency	Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a genetic disorder in which the body doesn't have enough of an enzyme called glucose-6-phosphate dehydrogenase. It is the most common genetic abnormality affecting an estimated 400 million people worldwide. G6PD is important in protecting red blood cells. The lack of G6PD can lead to

	red blood cells breaking down (hemolysis) when the person is exposed to certain drugs such as primaquine or tafenoquine. By breaking down red blood cells there is less oxygen transported around the body causing harm to the normal metabolic functions of the individual.
GMS	Greater Mekong Subregion (GMS) countries are Cambodia, the People's Republic of China, Lao People's Democratic Republic, Myanmar, Thailand and Vietnam.
Genome	A genome is the genetic material of an organism. It consists of DNA. The genome includes both the genes and the noncoding DNA, as well as mitochondrial DNA and chloroplast DNA.
Genotypic	Genetic contribution to the phenotype.
Hb	Hemoglobin (Hb) is a protein in your red blood cells that carries oxygen to your body's organs and tissues and transport carbon dioxide from your organs and tissues back to your lungs.
IMM	Independent Medical Monitor
IMPE-QN	Institute of Malariology, Parasitology, and Entomology located in Quy Nhon, Vietnam
IQR	Interquartile Ranges
IU/g Hb	International units per gram Hb
ICH	International Conference on Harmonization
IMPE-QN	Institute of Malariology, Parasitology, and Entomology located in Quy Nhon, Vietnam
IRB	Institutional Review Board
In vitro drug susceptibility testing	*In vitro* drug susceptibility testing is based on the ability to culture *P. falciparum in vitro* in human red blood cells against varying concentrations of standard antimalarial drugs such as chloroquine, mefloquine, and piperaquine phosphate. Typically, parasites are propagated in white blood cell-free red blood cells at 2-5% hematocrit at 37°C under reduced oxygen (typically 90% nitrogen, 5% oxygen and 5% carbon dioxide) in tissue culture (RPMI 1640) media containing either human serum or plasma for 48 or 72 hours incubation to determine drug sensitivity levels against a battery of common antimalarial drugs. For monitoring *Plasmodium* spp. parasite growth SYBR Green fluorescence staining of parasites is used to compare parasitemia of drug treated and control parasites. After treating various field *P. falciparum* isolates with known antimalarial drugs, their respective 50% inhibitory concentration (IC_{50}) values are

	determined using the fluorescence assay.
In vivo studies (pertaining to antimalarial drug studies)	Studies of antimalarial drugs in animals or humans.
LCMS	Liquid chromatography mass spectrometry
MIPM	Military Institute of Preventive Medicine
MoH	Vietnam Ministry of Health (MoH)
MoH-IRBNBR	Vietnam Ministry of Health Institutional Review Boards in National Biomedical Research.
Pharmacovigilance	Pharmacovigilance also known as drug safety, is the pharmacological science relating to the collection, detection, assessment, monitoring, and discovery of interaction amongst drugs and their effects in humans.
PCR	Polymerase Chain Reaction (PCR) is a method widely used to rapidly make millions to billions of copies of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.
qPCR	Quantitative PCR (real-time) monitors the amplification of the targeted DNA molecule during the PCR, not at its end, as in conventional PCR.
Phenotypic	Observed characteristics
PISCF	Participant Information Sheet and Consent Form
POC	Point-of-Care: A form of testing in which the analysis is performed where healthcare is provided close to or near the patient.
Pyramax®	Pyronaridine-Artesunate is the latest ACT recommended for the treatment of both uncomplicated *Plasmodium falciparum* and *Plasmodium vivax* malaria.
QA	Quality Assurance
QT interval prolongation	QT interval prolongation is a measure of delayed ventricular repolarisation, which means the heart muscles take longer than normal to recharge between beats and thus may trigger dangerous heart rhythms.
Radical cure	Complete elimination of parasites from the body.
RDT	Rapid Diagnostic Test
Relapsing malaria	Recurrence of malaria from dormant (i.e. hypnozoites) liver stages
SAE	Serious Adverse Event
SEA	Southeast Asia

SNP	Single-nucleotide polymorphism (SNP) is a substitution of a single nucleotide at a specific position in the genome that is present in a sufficiently large fraction of the population.
SOP	Standard Operational Procedure
WGS	Whole genome sequencing is the process of determining the complete DNA sequence of an organism's genome at a single time.
WHO	World Health Organization

5. KEY ROLES AND RESPONSIBILITIES

Name	Dr. Huynh Hong Quang (MD, MSc, MPharm, PhD)	**Institution**	IMPE-QN
Position	Vice-Director IMPE-QN; Head of the Department of Clinical and Treatment Research for Tropical Diseases	**Role**	Principal Investigator
Responsibilities	Input into the study design and oversee the conduct of the therapeutic efficacy surveillance studies at Chu R'Cam/la Drech communes (Krong Pa district, Gia Lai province) and Ea Lam/Ea Ly communes (Song Hinh district, Phu Yen province) in Central Vietnam. Perform data analysis and write up of clinical and scientific findings (see curriculum vitae for Dr Quang as supporting documentation of his knowledge and expertise in the conduct and management of therapeutic efficacy studies of antimalarial drugs in Central Vietnam, including the surveillance for drug resistant malaria).		
Contact details	(w): +84 0562 846755	(m): +84 905103496	
	Email: huynhquangimpe@yahoo.com		

Name	Captain Nguyen Van Thanh (MD)	**Institution**	MIPM
Position	Deputy-Head, Department of Malariology, Parasitology and Entomology	**Role**	Co-Investigator
Responsibilities	Input into the study design and assist the Principal Investigator in the conduct of the therapeutic efficacy surveillance studies at Chu R'Cam/l Drech communes (Krong Pa district, Gia Lai province) and Ea Lam/Ea Ly communes (Song Hinh district, Phu Yen province) in Central Vietnam, including the collection of *P. falciparum* isolates for *in vitro* drug susceptibility testing. Oversee the *in vitro* drug susceptibility testing of field *P. falciparum* isolates collected from the studies at MIPM. Perform data analysis and assist in the write up of clinical and scientific findings		

	(see biosketch for Dr Thanh as supporting documentation of his knowledge and expertise in conducting a recent therapeutic efficacy study of Pyramax® in the central highlands of Vietnam, as well as in vitro drug susceptibility studies of field isolates collected from the clinical trial).	
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	Email: mp.mihe@gmail.com	

Name	Senior Colonel Pham Xuan Vinh (MD)	**Institution**	MIPM
Position	Head, Department of Malariology, Parasitology and Entomology	**Role**	Co-Investigator
Responsibilities	Input into the study design. Provide and train MIPM doctors and field technicians to support the conduct of the clinical efficacy surveillance study at Chu R'Cam/la Drech communes (Krong Pa district, Gia Lai province) and Ea Lam/Ea Ly communes (Song Hinh district, Phu Yen province) in Central Vietnam. Provide resources including personnel to perform *in vitro* drug susceptibility testing of field *P. falciparum* isolates at MIPM. Assist with the write up of clinical and scientific findings (see biosketch for Dr Vinh as supporting documentation of his knowledge and expertise in supporting a recent therapeutic efficacy study of Pyramax® in the central highlands of Vietnam).		
Contact details	(w): +84 8177744364	(m): +84 912728272	
	Email: mp.mihe@gmail.com		

Name	Nguyen Thi Minh Trinh (MSc)	**Institution**	IMPE-QN
Position	Head, Molecular Biology Department	**Role**	Co-Investigator
Responsibilities	Input into the study design. Conduct molecular assays of malaria isolates collected from participants in the therapeutic efficacy surveillance study at Chu R'Cam/la Drech and Ea Lam/Ea Ly communes in Central Vietnam. Assist in data analysis and write up of clinical and scientific findings (see biosketch for Mrs Trinh's as supporting documentation of her knowledge and expertise in supporting molecular epidemiological studies for drug resistant malaria in the central highlands of Vietnam).		
Contact details	(w): + 84 0562 3846892	(m): + 84 935228913	
	Email: nguyenminhtrinh1983@gmail.com		

Name	Dr. Ho Thi Thanh Thao	**Institute**	IMPE-QN

Position	Head, Department of Epidemiology	**Role**	Scientific secretary Co-Investigator
Responsibilities	Assist the Principal Investigator in the study design and conduct of the focal case detection surveillance and treatment of asymptomatic malaria at selected sites in Gia Lai and Phu Yen provinces in the Central zone of Vietnam. Perform data analysis and assist in the write up of clinical and scientific findings (see biosketch for Dr Thao as supporting documentation of his knowledge and expertise in conducting a recent malaria case surveillance and management in Central Highlands of Vietnam).		
Contact details	(w): +84 0562 846755	(m): +84 824887016	
	Email: hothanhthaoydh@gmail.com		

Name	Ho Van Hoang (MD, PhD)	**Institution**	IMPE-QN
Position	Director	**Role**	Co-Investigator
Responsibilities	Input into the study design and assist the Principal Investigator in obtaining approval from Ministry of Health of Vietnam for IMPE-QN with support from MIPM to conduct the therapeutic efficacy surveillance study at Chu R'Cam/la Drech and Ea Lam/Ea Ly communes in Central Vietnam. Provide IMPE-QN resources including personnel to conduct the clinical study as well as perform molecular assays for drug resistant malaria at IMPE-QN. Assist with the write up of clinical and scientific findings (see biosketch for Dr Hoang as supporting documentation of his knowledge and expertise in infectious disease in Central Vietnam).		
Contact details	(w): + 84 0256 3846892	(m): + 84 914004629	
	Email: ho_hoang64@yahoo.com		

Name	Senior Colonel Le Van Dong (Ass. Prof, MD, PhD)	**Institution**	MIPM
Position	Director	**Role**	Co-Investigator
Responsibilities	Input into the study design. Obtain approval from the Ministry of National Defence of Vietnam for MIPM to participate with IMPE-QN in the conduct of the clinical efficacy surveillance study at Chu R'Cam/la Drech communes (Krong Pa district, Gia Lai province) and Ea Lam/Ea Ly communes (Song Hinh district, Phu Yen province) in Central Vietnam. Provide MIPM resources including personnel to conduct the clinical study as well as perform *in vitro* drug susceptibility testing of field *P. falciparum* isolates at MIPM. Assist with the write up of clinical and scientific findings (see biosketch for Dr Dong as supporting documentation of his knowledge and expertise in infectious disease in Vietnam).		

Contact details	(w): +84 817744364	(m): +84 989058710
	Email: mp.mihe@gmail.com	

Name	Dr Geoffrey Birrell PhD	**Institution**	ADFMIDI
Position	Senior Analyst (Pharmacology)	**Role**	Project Officer
Responsibilities	Input into the study design. Oversee the measurement of blood concentrations of chloroquine and its active metabolite desethylchloroquine and pyronaridine using LCMS analysis at ADFMIDI. Assist in data analysis and write up of clinical and scientific findings (see biosketch for Dr Birrell as supporting documentation of his knowledge and expertise in antimalarial drug analysis).		
Contact details	(w): +61 7-33324833	(m): +61 458822476	
	Email: geoff.birrell@defence.gov.au		

Name	Dr Marina Chavchich PhD	**Institution**	ADFMIDI
Position	Senior Molecular Parasitologist	**Role**	Project Officer
Responsibilities	Input into the study design and quality assurance of molecular assays conducted at IMPE-QN and *in vitro* drug susceptibility testing at MIPM. Perform whole genome sequencing at ADFMIDI, assist in data analysis and write up of clinical and scientific findings (see biosketch for Dr Chavchich as supporting documentation of her knowledge and expertise in in vitro drug susceptibility testing and molecular analysis of drug resistant markers).		
Contact details	(w): +61 7-33324826	(m): +61 422895573	
	Email: marina.chavchich@defence.gov.au		

Name	Dr Michael Edstein (MSc, PhD)	**Institution**	ADFMIDI
Position	Head, Department of Drug Evaluation	**Role**	Project Officer
Responsibilities	Input into the study design, quality assurance of study documentation and SOPs, assist in data analysis and write up of clinical and scientific findings and overall management of the project for the Australian Defence Organisation (see biosketch for Dr. Edstein as supporting documentation of his knowledge and expertise in malaria		

	chemotherapy).	
Contact details	(w):+61 7-33324930	(m): +61 403321689
	Email: mike.edstein@defence.gov.au	

Independent Medical Monitor (IMM)	Name: Professor Dennis Shanks, MPH, MD Appointment: Director Institution: Australian Defence Force Malaria and Infectious Disease Institute (ADFMIDI) Contact details:(w):+61 7-33324931; (m): +61 418321302 Email: dennis.shanks@defence.gov.au Responsibilities:Provide continuous medical oversight over the study, including the provision of applicable recommendations to principal investigator in monitoring for adverse events and risk mitigation measures (see biosketch for Prof. Shanks as supporting documentation of his knowledge and expertise in malaria chemotherapy).
Institutional Review Board (IRB)	Institute of Malariology, Parasitology and Entomology (IMPE) Quy Nhon (IMPE-QN) Institutional Review Board 611^{B} Nguyen Thai Hoc Street, Quy Nhon City, Binh Dinh, Vietnam Tel: +84-02563846755 E-mail: impe.quynhon@gmail.com Vietnam Ministry of Health Institutional Review Boards in National Biomedical Research (MoH-IRBNBR) 138B Giang Vo Street, Ba Dinh District, Hanoi, Vietnam Tel: +84-243-384-6688 E-mail: iecmoh@gmail.com FWA00022807, IRB00007690

6. PROTOCOL SUMMARY

6.1 STUDY SYNOPSIS

Title	Therapeutic efficacy surveillance of malaria treatment and drug resistance monitoring in Gia Lai and Phu Yen provinces of Central Vietnam
Study Partners	• Vietnam Ministry of Health (MoH) Institute of Malariology, Parasitology, and Entomology Quy Nhon (IMPE-QN) • Vietnam People's Army Military Institute of Preventive Medicine (MIPM) • Australian Defence Force Malaria and Infectious Disease Institute (ADFMIDI)

Sponsor	Australian Defence Organization represented by ADFMIDI and the U.S. Navy (represented by the Naval Medical Research Unit No. TWO – NAMRU-2)
Study description	This is an open-label study in Central Vietnam to monitor the first-line artemisinin based combination therapy, Pyramax® (pyronaridine-artesunate) plus a single dose of primaquine for the treatment of uncomplicated *P. falciparum* malaria and to evaluate chloroquine plus primaquine for the treatment of *P. vivax* malaria. Questionnaire adherence to daily primaquine dosing for the treatment of *P. vivax* when unsupervised by the study team. Drug exposure will be determined by measuring patient's blood drug concentrations after treatment. The prevalence of drug resistant parasites will be determined in blood samples collected from the patients before drug treatment using *in vitro* (phenotypic) drug susceptibility testing and molecular (genotypic) assays with validated molecular markers of drug resistance. The *P. falciparum* and *P. vivax* parasite populations will be genetically characterized. The new point-of-care hemozoin detection device (Gazelle) will be used to measure hemozoin concentration decline in patient's blood samples after starting treatment to determine its feasibility in predicting either drug sensitive or resistant strains of malaria infections.
Aims	**Primary Aims:** • Monitor the therapeutic efficacy of a 3-day course of Pyramax® plus a single dose of primaquine for the treatment of patients with *Plasmodium falciparum* malaria in and near the communes of Chu R'Cam/Ia Drech (Krong Pa district, Gia Lai province) and Ea Lam/Ea Ly (Song Hinh district, Phu Yen province) in Central Vietnam. • Monitor the therapeutic efficacy of a 3-day course of chloroquine plus a 14 day course of primaquine for the treatment of patients with *P. vivax* malaria in and near the communes of Chu R'Cam, Ia Drech, Ea Ly and Ea Lam. **Secondary Aims:** • Assess how well participants adhere to daily primaquine for the treatment of *P. vivax* malaria without supervision by the IMPE-QN/MIPM study team. • Determine blood drug concentrations in patients to ensure adequate drug exposure in patients following treatment. • Characterize the *in vitro* phenotypic drug susceptibility profiles of *P. falciparum* parasites collected from the patients before treatment. • Characterize molecular markers of drug resistance of *P. falciparum* and *P. vivax* in parasites collected from the

	patients before treatment. • Analyze genetic diversity/relatedness, polymorphism, and genetic origin of *P. falciparum* and *P. vivax* parasites. • Evaluate the decline in hemozoin concentration in malaria patients after treatment using the Gazelle (Hemex) device to predict the presence of either drug sensitive or resistant strains of malaria infections. • Investigate microscopically positive and rapid diagnostic test (RDT) negative symptomatic infections for possible histidine-rich protein 2 and 3 (*hrp2/hrp3*) gene deletions.
Inclusion criteria	• People infected with uncomplicated mono-infections of *P. falciparum* and uncomplicated mono-infections of *P. vivax*; • Malaria parasite density of *P. falciparum* (≥ 500 to< 100,000 parasites/µL); • Malaria parasite density of *P. vivax* (≥ 250 parasites/µL); • Children (≥5 years and ≥20 kg to <18 years old) and adults (≥18 to <60 years old) infected with *P. falciparum,* children (≥5 to <18 years old) and adults (≥18 to <60 years old) infected with *P. vivax* malaria; • Gender: Males and females; • Working or residing at the study communes; • Able to provide information and capillary finger prick blood samples; • Written informed consent given to participate in the study by the adult or in case of children up to <18 years old (Assent form for children aged 12 to <18 years old) with parent or guardian permission; • Normal G6PD enzyme activity levels (>70%) of the site median value for G6PD normals for participants to be treated with tafenoquine for the radical cure of *P. vivax* malaria.
Exclusion criteria	• People not infected with malaria infections; • Children (<5 years old and <20 kg)infected with *P. falciparum* and less than 5 years of age infected with *P. vivax* malaria; • Unwilling to provide consent, information, and capillary finger prick blood sample; • Inability to communicate well with the study staff (poor mental development or evidence of psychiatric disorder); • People with *P. vivax* malaria who have G6PD deficient enzyme activity; • Pregnant or lactating females; • Any condition that in the judgment of the IMPE-QN/MIPM doctor would make participation in the study unsafe for the

	potential participant.
Number of participants/records	Up to 120 patients with *P. falciparum* and up to 60 patients with *P. vivax* (collectively from the four field sites)
What organisation has overall responsibility for the project? ADFMIDI	
Anticipated start date	May 2021 (commencement of recruitment)
Anticipated finish date	May 2024 (completion of recruitment, completion of laboratory assays, data analysis and write up of findings for publication in a peer-reviewed scientific journal)
Five keywords/phrases to describe or define the field of research	• *Plasmodium falciparum* and *Plasmodium vivax* malaria • Pyramax® (pyronaridine-artesunate) • Chloroquine plus primaquine • Drug resistant malaria • *In vitro* drug susceptibility testing
Risk level	Negligible risk ☐ Low risk ☐ Greater than low risk ☒

6.2 SCHEDULE ACTIVITIES

The schedule of activities for therapeutic efficacy surveillance of a 3-day course of Pyramax® plus a single dose of primaquine for the treatment of *P. falciparum* and chloroquine plus primaquine for the treatment of *P. vivax* malaria in Central Vietnam are as follows:

1. Pyramax® plus primaquine for treating *P. falciparum* malaria.

2. Chloroquine plus primaquine for treating *P. vivax* malaria.

Schedule of Activities 1:

Pyronaridine-artesunate (Pyramax®) plus primaquine regimen for treating *P. falciparum* malaria

	Day 0 (D0)	Day 1 (D1)	Day 2 (D2)	Day 3 (D3)	Day 7 (D7)	Day 14 (D14)	Day 21 (D21)	Day 28 (D28)	Day 35 (D35)	Day 42 (D42)	Any other day/ unschedule day
PROCEDURES											
Informed Consent	X										
Inclusion/Exclusion Criteria	X										
Clinical assessment of vitals, body systems, physical	X										(X)
Medical History (signs/symptoms)	X										(X)
Pregnancy test (if necessary)	X										
Temperature	X	X	X	X	X	X	X	X	X	X	(X)
Blood films for parasite counts	X	X	X	X	X	X	X	X	X	X	(X)
Blood for Hemozoin level	X	X	X	X	X						
Blood for Molecular analysis	X									X	(X)
Blood for Antimalarial drug level					X					X	(X)
Blood for *In vitro* drug testing (only adults)	X										(X)
Blood for Whole Genome Sequencing (only adults)	X										
Blood for G6PD test	X										
TREATMENT											
Pyramax®	X	X	X								
Adverse Events	X	X	X	X	X	X	X	X	X	X	(X)
Primaquine (1 day course)	X										
Rescue Drug		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)

NOTES: Parentheses denote conditional or optional activities. Rescue treatment could be given on any day, provided that the patient meets the criteria for treatment failure. Any other day are days other than regularly scheduled follow-up days when the patient returns to the health station because of recurrence of symptoms.

Schedule of Activities 2:

Chloroquine plus primaquine regimen for treating *P. vivax malaria* in patients

	Day 0 (D0)	Day 1 (D1)	Day 2 (D2)	Day 3 (D3)	Day 7 (D7)	Day 14 (D14)	Day 21 (D21)	Day 28 (D28)	Day 35 (D35)	Day 42 (D42)	Any other day/ unschedule day
PROCEDURES											
Informed Consent	X										
Inclusion/Exclusion Criteria	X										
Clinical assessment of vitals, body systems, physical	X										(X)
Medical History (signs/symptoms)	X										(X)
Pregnancy test (if necessary)	X										
Temperature	X	X	X	X	X	X	X	X	X	X	(X)
Blood films for parasite counts	X	X	X	X	X	X	X	X	X	X	(X)
Blood for Hemozoin level	X	X	X	X	X						
Blood for Molecular analysis	X									X	(X)
Blood for Antimalarial drug level					X					X	(X)
Blood for *In vitro* drug testing (only adults)	X										(X)
Blood for Whole Genome Sequencing (only adults)	X										
Blood for G6PD test	X										
Morisky Medication Adherence Scale Questionnaire					X	X					
TREATMENT											
Chloroquine	X	X	X								
Primaquine*	X	X	X								
Adverse Events	X	X	X	X	X	X	X	X	X	X	(X)
Rescue Drug		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)

NOTES: Parentheses denote conditional or optional activities. Rescue treatment could be given on any day, provided that the patient meets the criteria for treatment failure. Any other day are days other than regularly scheduled follow-up days when the patient returns to the health station because of recurrence of symptoms.
* Malaria patients with *P. vivax* who will receive a 14 day course of primaquine must have a G6PD enzyme activity >5.87 IU/g Hb. *The administration of primaquine will be observed for the 1st three days of dosing; the other 11 days of dosing will be unsupervised.

6.3 RESOURCES

Resources necessary for the project to be conducted

The clinical study will be carried out by doctors and field technicians from IMPE-QN and MIPM. The study doctors will manage enrolment, obtaining informed consent, screening subjects for malaria infections, blood sample collection before treatment, observed drug administration and follow-up blood sample collections from study patients for monitoring parasite clearance for up to 42 days post-treatment. Staffs from IMPE-QN and MIPM will carry out molecular assays and *in vitro* drug susceptibility testing, respectively, of blood samples collected from participants before treatment. Also, staff from the pharmacology and parasitology sections of the Department of Drug evaluation at ADFMIDI will conduct blood antimalarial drug measurement and whole genome sequencing (WGS), respectively.

The cost to carry out the clinical studies and laboratory testing in Vietnam as well as downstream laboratory testing at ADFMIDI will be covered by the Australian Defence Organisation and NAMRU-2.

7. BACKGROUND

Malaria remains a serious threat for military and civilian personnel in malaria endemic countries across the world and in the absence of a vaccine, the only option available for the treatment and prevention of malaria infections are effective antimalarial medications. Surveillance of antimalarial drug efficacy and drug resistance are critical to inform force health protection and public health policies. The development and spread of multidrug resistant malaria in Southeast Asia (SEA), particularly in the Greater Mekong Sub-Region (GMS) highlights the importance of evaluating more effective drugs and monitoring for drug resistance. For National Malaria Control Programs the monitoring of antimalarial drug resistance is essential to ensure that first-line treatment drugs are effective and adapt drug policy to prevent the development and spread of “super” drug resistant parasites. There is an urgent need for monitoring of the emergence and spread of multidrug resistant malaria parasites.

The spread of multidrug resistant *P. falciparum* out of western Cambodia across the countries of the GMS poses a significant threat to malaria control in SEA (1). First-line ACTs, such as dihydroartemisinin-piperaquine (DHA-PPQ) and artesunate-mefloquine have lost their efficacy in many areas of the GMS, resulting in high rates of treatment failures (1, 2). Furthermore, with vast people movements across the region, there is a high potential for the spread of resistant parasites to other countries of SEA, such as Myanmar and India, as well as to Africa, which would lead to major public health problems should ACTs start failing in Africa, as it has been previously observed after the spread of chloroquine and pyrimethamine resistance (3, 4).

The highly resistant malaria parasites with genetic mutations in the *P. falciparumKelch13* gene and multiple copies of plasmepsins 2/3 genes that are responsible for artemisinin and piperaquine resistance, respectively, have spread from western Cambodia to the southwestern Vietnam province of Binh Phuoc resulting in a drastic increase in DHA-PPQ failure rates (5). Our study (ADHREC Protocol 841-16) and others (5, 6) have shown that these DHA-PPQ resistant parasites are already spreading into the Central Highlands of Vietnam.

There is a growing concern about the spread of these parasites further north into Central Vietnam and possibly into Central Laos. Because of increasing DHA-PPQ resistance in the Central Highlands of Vietnam, the Vietnam MoH has decided to replace DHA-PPQ with the latest ACT, pyronaridine-artesunate (Pyramax®) for the treatment of uncomplicated falciparum malaria in several Central Highland provinces such as Gia Lai and Phu Yen.

In Vietnam, *P. falciparum* also coexists with *P. vivax* and in some areas the prevalence of *P. vivax* sometimes exceeds that of *P. falciparum* with a high frequency of mixed infections. The current recommended treatment for *P. vivax* in Vietnam is chloroquine plus primaquine. There is concern that treatment of *P. vivax* with chloroquine inadvertently provides an additional selective pressure on *P. falciparum,* accelerating development of super-multidrug resistant parasites. Conversely, there is a high chance of exposure of *P. vivax* to piperaquine, that has been used for over a decade as DHA-PPQ for treating falciparum malaria in Central Vietnam, which may affect its susceptibility to chloroquine. Thus, there is a need to continue monitoring the effectiveness of chloroquine plus primaquine in treating vivax malaria in Vietnam.

In monitoring the efficacy of antimalarial treatments and for tracking the emergence of drug resistance better point-of-care (POC) malaria detection tools are urgently needed that will guide treatment changes to avoid the selection and spread of "super" drug resistant parasites and for early detection of resistance. Hemex Health (Portland, Oregon, USA) has developed a POC malaria detection device (called Gazelle) that is based on hemozoin detection. Hemozoin is a malaria pigment, produced in parasites upon hemoglobin digestion during asexual development and can potentially be used as a surrogate marker for measuring parasite decline after treatment. Recently, Molnar et al. (7) demonstrated the feasibility of utilizing hemozoin as surrogate marker for parasite growth to determine the *in vitro* inhibitory concentrations (IC_{50}) of antimalarial drugs as well as differentiating sensitive and resistant *P. falciparum* strains.

Artemisinin-resistant *P. falciparum* parasites carry genetic mutations in the *P. falciparum Kelch13* gene and are associated with delayed parasite clearance and parasite clearance half-life ≥5 hours (8). The measurement of hemozoin concentrations versus time profile after drug treatment is expected to correlate with parasite clearance measured by blood film microscopy and quantitative polymerase chain reaction (qPCR). This needs to be evaluated and validated in a field setting to determine whether hemozoin decline can be used to predict the presence or absence of drug resistant infections. If this can be demonstrated the hemozoin detection device could replace blood film microscopy, be performed at the communal health station as a POC malaria diagnositic tool and possibly identify delay parasite clearance due to drug resistant parasites for early clinical management and early detection of resistance.

Early and accurate diagnosis of malaria infections is crucial for timely and appropriate treatment in preventing severe malaria and death. Rapid diagnostic tests (RDTs) provide rapid malaria diagnosis of malaria infections. The most popular and sensitive RDTs useantigens unique to *P. falciparum*; the histidine-rich protein 2 (PfHRP2) and also the histidine-rich protein 3 (PfHRP3). However, since 2010, parasites lacking PfHRP2 and PfHRP3 have been reported in relatively low malaria transmission areas of South America (9, 10) and Asia (11), and more recently in up to 80% of patients in African countries (12). In Vietnam, *pfhrp3* gene deletions have been identified but as yet not *pfhrp2* gene deletions in falciparum malaria (13).

In this study, we propose to monitor the therapeutic efficacy of Pyramax® for the treatment of patients infected with *P. falciparum* malaria and the efficacy of chloroquine plus primaquine for treatment of *P. vivax* malaria. The patients reside near or in Chu R'Cam/la Drech communes (Krong Pa district, Gia Lai province) and near or in Ea Lam/Ea Ly communes (Song Hinh district, Phu Yen province) in Central Vietnam. To ensure that patients have adequate drug exposure their blood concentrations will be measured. The prevalence of drug resistant malaria at the four communes from participant's blood samples will be determined using *in vitro* (phenotypic) testing and molecular (genotypic) assays. Genetic diversity/relatedness of the patient's parasites will also be investigated.

Non-adherence to daily primaquine dosing for 14 days will markedly compromise the effectiveness of the drug in killing the dormant liver stages (i.e., hypnozoites) for the radical cure of vivax malaria (14). Studies have reported mixed treatment outcomes of supervised versus unsupervised taking of primaquine for 14 days. In a study in Pakistan, primaquine proved equally protective against further episodes of *P. vivax* in supervised and unsupervised groups as compared to placebo (15). However, in a study in Thailand the vivax reappearance rate was significantly lower in the supervised than the unsupervised patients taking of primaquine for 14 days (16,). Poor treatment outcomes will not only lead to an increased risk of relapse but will also contribute to higher health-care and economic costs. To evaluate primaquine adherence by participants given self-administered primaquine an eight-item Morisky Medication Adherence Scale (17) as a structured self-report measure of primaquine-taking behavior should be pursued to inform the National Malaria Control Programs for future treatmemnt strategies for malaria control and elimination.

As part of this study, we also propose to conduct some exploratory studies with blood samples collected from the study patients. The Gazelle (Hemex Health) will be used to measure patient hemozoin concentrations after treatment to determine whether they are infected with either drug sensitive or resistant strains of malaria. Because RDTs play a critical role in informing malaria treatment and surveillance, we will determine whether any study patients are RDT negative using the histidine-rich protein 2 (PfHRP2) and/or the histidine-rich protein 3 (PfHRP3) based RDT but positive based on microscopy for blood film diagnosis.

Overall, the findings of drug resistance from this study will be used by the Vietnam MoH to inform on drug policy and malaria control measures, as well as providing data for designing strategies for malaria elimination.

8. OBJECTIVES AND ENDPOINTS

8.1 Outline the study objectives and endpoints

OBJECTIVES	ENDPOINTS
Primary	
• Monitor the therapeutic efficacy of a 3-day course of Pyramax® plus a single dose of primaquine for the treatment of patients with uncomplicated *Plasmodium falciparum* malaria in and near the	• Day 42 PCR-corrected Adequate Clinical and Parasitological Response (ACPR) for patients treated with Pyramax®. • Day 42 ACPR for patients treated with chloroquine plus tafenoquine.

communes of Chu R'Cam/la Drech (Krong Pa district, Gia Lai province) and Ea Lam/Ea Ly (Song Hinh district, Phu Yen province) in Central Vietnam.	
• Monitor the therapeutic efficacy of a 3-day course of chloroquine plus a 14 day course of primaquine for the treatment of patients with *P. vivax* malaria in and near the communes of Chu R'Cam/la Drech and Ea Lam/Ea Ly.	• Percentage of patients who are free from recurrence at 6 months, defined as *P. vivax* clearance without recurrent parasitemia. • Day 3 parasite positivity proportion. • The median time to clear 50% of parasitemia (PC_{50}) and parasite clearance half-life ($PC_{1/2}$).
Secondary	
• Assess how well participants adhere to daily primaquine for the treatment of *P. vivax* malaria without supervision by the IMPE-QN/MIPM study team.	Participants will be invited to complete an eight-item Morisky Medication Adherence Scale questionnaire on their taking of daily primaquine at days 7 and 14 after starting primaquine treatment with chloroquine.
• Determine blood drug concentrations in patients to ensure adequate drug exposure in patients following treatment.	Blood chloroquine plus desethlychloroquine, and pyronaridine concentrations (ng/mL) collected on day 7 after starting treatment and measured by liquid chromatography mass spectrometry (LCMS).
• Characterize the *in vitro* phenotypic drug susceptibility profiles of *P. falciparum* parasites collected from the patients before treatment.	Fifty percent Inhibitory concentration (IC_{50}, nM) of standard antimalarials such as atovaquone, chloroquine, dihydroartemisinin, lumefantrine, mefloquine, piperaquine, pyronaridine, and tafenoquine against field isolates collected on Day 0 (before treatment) and measured using the SYBR Green I-based fluorescence assay.
• Characterize molecular markers of drug resistance of *P. falciparum* and *P. vivax* in parasites collected from the patients before treatment. • Analyze genetic diversity and origin of parasites by whole genome sequencing (WGS), amplicone sequencing or single-nucleotide polymorphism (SNP) barcode genotyping of *P. falciparum* and *P.*	• Prevalence of polymorphisms in the *Kelch13* (K13) gene, mutations in the Pfcrt gene, increase in gene copy numbers for plasmepsin 2/3 (Pfpm2/3), ExoE415G mutation, and amplification of the multidrug resistance 1 (Pfmdr1) gene for *P. falciparum* molecular markers of artemisinin, chloroquine, piperaquine, and mefloquine resistance, respectively, using blood samples collected from participants on Day 0 (before treatment). • Prevalence of polymorphism in *P. vivax* molecular markers K12, a *P. vivax* homolog of the *PfKelch13* gene, *Pvcrt-o*, *Pvmdr1*, and plasmepsin 4, using blood samples

vivax isolates.	collected from participants on Day 0 (before treatment). • Characterize genetic diversity and origin of *P. falciparum* and *P. vivax* parasites. Identify the origin of the drug-resistant *P. falciparum* parasites using blood samples collected from participants on Day 0 (before treatment).
• Evaluate the decline in hemozoin concentration in malaria patients after treatment using the Gazelle (Hemex) device to predict the presence of either drug sensitive or resistant strains of malaria infections.	• Measurement of hemozoin concentrations on Day 0 (before treatment), at 12 hourly intervals up to Day 3 and then on Day 7 post commencement of treatment. • The median time to clear 50% of hemozoin concentrations (HC_{50}). • Compare hemozoin concentrations decline with parasite density decline by microscopy and qPCR.
• Investigate microscopically positive and rapid diagnostic testing (RDT) negative symptomatic infections for possible histidine-rich protein 2 and 3 (*hrp2/hrp3*) gene deletions.	Presence or absence of hrp2/3 genes will be determined using WGS data analysis using blood samples collected from participants on Day 0 (before treatment).

8.2 Expected study outcomes

The several expected outcomes from the proposed study are as follows:

a. By monitoring the therapeutic efficacy of Pyramax® up-to-date information on the effectiveness (i.e. treatment outcome) of the drugs in treating *P. falciparum* will be obtained in regions of Vietnam with variable susceptibility to ACTs such as DHA-PPQ.

b. The *in vivo* efficacy of chloroquine plus primaquine in treating *P. vivax* malaria will provide blood schizonticidal parasite clearance and patient tolerability data as well as the level of persistence in preventing recurrence of malaria infections.

c. Will be able to determine how well participants infected with *P. vivax* malaria adhere to daily primaquine dosing when drug administration is not supervised by the IMPE-QN/MIPM study team

d. Measurement of blood concentrations of antimalarials will provide drug exposure data to demonstrate that patients have sufficient drug on board to cure their malaria infections.

e. The *in vitro* drug susceptibility and molecular assays will provide phenotypic and genotypic characterization of the level of drug resistance, respectively, at the field sites.

f. The application of WGS is expected to assist in identifying potential new mutations and provide information on genetic origin of drug resistant parasites in Central Vietnam.

g. Genetic diversity/relatedness and origin of *P. falciparum* and *P. vivax* parasites will be characterized to inform and assist the Vietnam MoH in drug resistance management and elimination strategies.

h. The hemozoin concentration versus time profile measured by the Gazelle (Hemex) device will correlate with the parasite clearance curve determined by blood film microscopy and qPCR after commencement of drug treatment and provide early detection of drug resistance.

i. The prevalence of patients who are infected with falciparum parasites lacking PfHRP2 and PfHRP3 will be determined.

9. PROJECT DESIGN

9.1 Study design

The study design is open label treatment of patients with Pyramax® plus a single dose of primaquine for *P. falciparum* malaria in accordance with the guidelines of the Vietnam MoH and chloroquine plus primaquine for the treatment of *P. vivax* malaria.

9.2 Study sites

The study will be conducted at the health stations in four communes in Central Vietnam. Chu R'Cam/la Drech communes are located in Krong Pa district in Gia Lai province and Ea Lam/Ea Ly communes are situated in Song Hinh district in Phu Yen province. Figure 1 shows the location of the communes.

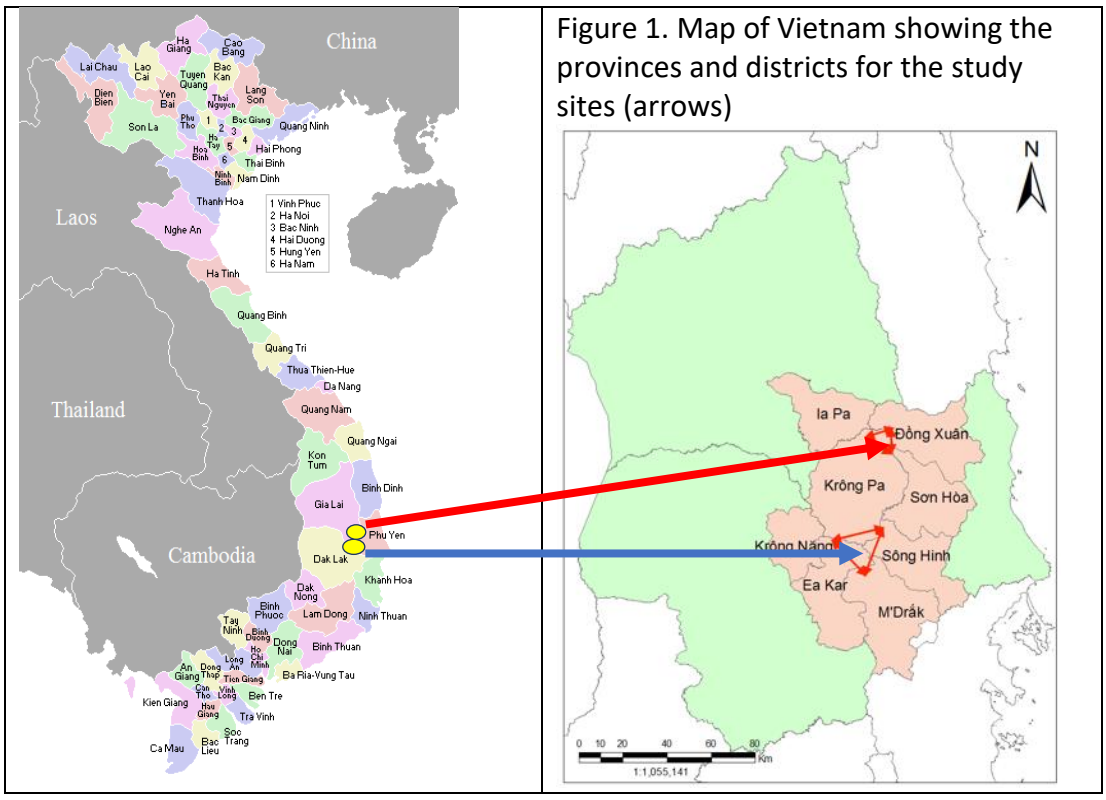

Figure 1. Map of Vietnam showing the provinces and districts for the study sites (arrows)

Gia Lai province is located in the highlands of Vietnam with a population of 2.2 million people in 2019. The province is situated in the western end of Truong Son range, lying in a large plateau with corrugated, relatively flat terrain sloping from the southeast to the northwest, and alternate low plains along main rivers. Phu Yen is a province on Vietnam's south-central coast with a population of 961,152 people in 2019 and is characterized by mountains and beaches like Long Thuy and Vung Ro ports.

Krong Pa district covers an area of 1624 km² with a population of about 86,120 people, with two major ethnic groups (Kinh and Ja Rai). Song Hinh district covers an area of 890 km², with a population of about 58,715 people and has two major ethnic groups (Kinh, E De),with some ethnic groups from Northern part of Vietnam (Thai, Tay, Dao, and Nung). The main source of income for the people living in Krong Pa and Song Hinh districts is agricultural production, including rice farming near their homes or in the forest farms as well as arts, craft and decorative furniture production.

There are 13 and 6 communal health stations in Krong Pa and Song Hinh districts, respectively, belonging to District Health Centers of Krong Pa and Song Hinh, which includes Chu R'Cam/la Drech and Ea Lam/Ea Ly communes. In 2019, the commune population of Chu R'Cam, la Drech, Ea Lam and Ea Ly were 4,556, 3376, 3,179 and 6,719 people, respectively.

There are two distinct seasons each year for Krong Pa and Song Hinh districts: the rainy season lasts from July to the end of December; the dry season begins from January and continues until the end of June.

9.3 Study population and Recruitment

People with malaria infections living near or in Chu R'Cam/la Drech communes (Krong Pa district, Gia Lai province) and near or in Ea Lam/Ea Ly communes (Song Hinh district, Phu Yen province) will be invited to participate in the study. These subjects will be identified by both active and passive case detection using RDTs and blood film microscopy. People with symptoms of malaria such as fever, chills, headaches, tiredness and nausea tend to visit the communal health stations at Chu R'Cam, la Drech, Ea Lam and Ea Ly where they seek diagnosis and treatment, which is provided free by the Vietnam MoH.

The strategy for recruitment consists of the IMPE-QN/MIPM doctor approaching people who have been routinely screened by the health station medical staff and confirmed to be infected with malaria. The IMPE-QN/MIPM doctor will present to the malaria patient the information and documentation for the study. The potential participant will be provided with the Participant Information Sheet and Consent Form (PISCF) to consider participation in the study and return them at a later time if preferred, hence the malaria patient is under no time pressure to make a decision to participate or not. The PISCF is explicit in stating that participation is entirely voluntary and that non-participation will have no effect on the patient receiving free medical care. No record will be kept if the patient decides not to participate in the study. The IMPE-QN/MIPM doctor will be available to answer questions regarding the study as necessary. All study discussion between the IMPE-QN/MIPM doctor and potential participant will be conducted in a private area of the health station, where

conversation is not freely heard by other members of the health station.

Malaria patients, who after reading the PISCF and having any questions satisfactorily answered by the IMPE-QN/MIPM doctor, decide to participate will be asked to provide the signed written consent in the PISCF. The participant will receive a copy of the signed PISCF for their records.

9.4 Selection criteria

The inclusion criteria are as follows:

- People infected with uncomplicated mono-infections of *P. falciparum* and uncomplicated mono-infections of *P. vivax*;
- Malaria parasite density of *P. falciparum* (≥ 500 to < 100,000 parasites/µL);
- Malaria parasite density of *P. vivax* (≥ 250 parasites/µL);
- Children (≥5 years and ≥20 kg to <18 years old) and adults (≥18 to <60 years old) infected with *P. falciparum,* children (≥5 to <18 years old) and adults (≥18 to <60 years old) infected with *P. vivax* malaria;
- Gender: Males and females;
- Working or residing at the study commune;
- Able to provide information and capillary finger prick blood samples;
- Written informed consent given to participate in the study by the adult or in case of children up to <18 years old (Assent form for children aged 12 to <18 years old) with parent or guardian permission;
- Normal G6PD enzyme activity levels (>70%) of the site median value for G6PD normals for participants to be treated with primaquine for the radical cure of *P. vivax* malaria.

The exclusion criteria are as follows:

- People not infected with malaria infections;
- Children (<5 years old and <20 kg)infected with *P. falciparum* and less than 5 years of age infected with *P. vivax* malaria;
- Unwilling to provide consent, information, and capillary finger prick blood sample;
- Inability to communicate well with the study staff (poor mental development or evidence of psychiatric disorder);
- People with *P. vivax* malaria who have G6PD deficient enzyme activity;
- Pregnant or lactating females;
- Any condition that in the judgment of the IMPE-QN/MIPM doctor would make participation in the study unsafe for the potential participant.

9.5 Sample size

This efficacy surveillance study is designed to monitor the effectiveness of Pyramax® for the treatment of *P. falciparum*, which are registered for use in Vietnam, and chloroquine plus primaquine for the radical cure of *P. vivax* malaria. For this purpose a representative number of patients is required to be monitored for parasite clearance and treatment outcome. For conventional therapeutic efficacy drug studies of single-

arm investigations, the World Health Organization (18) has set a minimum of 50 patients is required to detect less than 90% drug efficacy.

Table 1 shows the prevalence of malaria infections from 2018 to 2020 for the four field sites with a noticeable increase in malaria from 2018 to 2019, but a substantial decrease in 2020. In 2020, the malaria season was markedly impacted by three major events that would have reduced malaria transmission in Central Vietnam: 1) The Covid-19 pandemic that restricted the movement of people working in forested areas where malaria is more prevalent, 2) Five consecutive cyclones with torrential rain devastated many areas of Central Vietnam in October and November 2020, with subsequent cold weather affecting the development of mosquito populations and 3) Many factories and companies in Ho Chi Minh city sought out workers from ethnic minor communities for labor employment in industries such as seafood packaging and textile manufacturing, and thus increasing the migration of forest workers from the country regions to the city. Because of these factors, it is too difficult to predict the malaria prevalence rate for 2021 for the four study sites.

Table 1. Malaria prevalence data at the proposed study sites for (2018-2020)

Province	District	Commune	Malaria incidence*		
			2018	2019	2020
Gia Lai	Krong Pa	Chu R'Cam	75 (37 Pf; 38 Pv)	196 (162 Pf; 33 Pv)	44 (39 Pf; 4 Pv, 1 mixed)
		Ia Drech	129 (123 Pf; 6 Pv)	97 (87 Pf; 10 Pv)	33 (31 Pf; 2 Pv)
Phu Yen	Song Hinh	Ea Lam	78 (39Pf; 34 Pv; 5 mix)	66 (33 Pf; 29 Pv; 4 mix)	17 (16 Pf; 1 Pv)
		Ea Ly	68 (34 Pf; 31 Pv; 3 mixed)	105 (55 Pf; 50 Pv)	18 (16 Pf; 2 Pv)

* Data provided by the Vietnam MoH based on blood film positive malaria diagnosis.
Pf -*P. falciparum*; Pv -*P. vivax*

Consequently, we will attempt to recruit up to 120 patients with *P. falciparum* malaria and up to 60 patients with *P. vivax* malaria collectively from the four field sites based on the 2019 malaria prevalence data. With a potential loss to follow-up and withdrawals rate of 15%, we will attempt to collectively recruit a minimum of 100 patients with *P. falciparum* and 50 patients with *P. vivax* malaria from the four sites (with the anticipated population proportion of clinical failures (p) of 15%, confidence (CI) level of 95%, and precision (d) of 10 percentage points (Table 2).

Table 2. The minimum sample size in accordance to treatment failure proportion

Estimated minimum sample size in population(p), CI95%										
d	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
0.05	73	138	196	246	288	323	350	369	380	384
0.10	18	35	**49**	61	72	81	87	92	95	96

9.6 Timeframe for recruitment

Recruitment of people with symptomatic malaria will commence in May 2021 and continue through to May 2022 at the four communes and surrounding areas.

9.7 Ethical approval

Ethical review for this study will be performed by the IMPE-QN IRB and Vietnam MoH-IRBNBR.

10. STUDY METHODS

10.1 Drug treatment and blood collections

People with symptoms (e.g. fever, chills, headache, tiredness and nausea) of malaria who present to the four health communes will be screened for malaria infections using RDTs and blood film microscopy. Those who are diagnosed with malaria will be invited to participate in the study.

At the commune health stations, the IMPE-QN/MIPM doctor will obtain informed consent from the patient with the malaria infection. After obtaining the patient's informed consent, demographic information and vital signs, and his/her answers to health and study questions will be recorded by the IMPE-QN/MIPM doctor.

Adults (patients ≥18 to <60 years old) will be invited to provide a total of 9 mL venous blood sample (before drug treatment): 5 mL for WGS and 4 mL for *in vitro* drug susceptibility testing. Adults who prefer not to provide a venous blood sample will be invited to provide a finger prick capillary blood sample (250 µL)in a Becton Dickinson (BD) microtainer containing EDTA as the anticoagulant for molecular analysis, hemozoin concentration and blood films for microscopy reading (before drug treatment). Children (<18 years old) will only provide a finger prick capillary blood sample (250 µL) in a BD microtainer containing EDTA for molecular analysis, hemozoin concentration and blood film for microscopy (before drug treatment). For the collection of finger prick capillary blood samples, a BD contact activated lancet that is specifically designed to minimize pain of blood collection to the participant will be used.

Drug administration to the participants will be in accordance with the National guidelines of the Vietnam MoH (Decision of 2699/QĐ-BYT dated 26 June 2020) for the treatment of *P. falciparum* malaria. Pyronaridine-artesunate (Pyramax®, Shin Poong Pharmaceutical Co. Ltd, Ansan, South Korea) will be given orally with water once daily for 3 days (days 0 to 2) and dosed according to body weight (Table 3). One tablet of Pyramax® contains 180 mg pyronaridine tetraphosphate plus 60 mg artesunate.

Table 3. Dosage of pyronaridine tetraphosphate-artesunate (Pyramax®)

Body weight	Day 1	Day 2	Day 3
≥ 20 - < 24 kg	1 tablet	1 tablet	1 tablet
≥ 24- < 45 kg	2 tablet	2 tablet	2 tablet

≥ 45 - < 65 kg	3 tablet	3 tablet	3 tablet
≥ 65 kg	4 tablet	4 tablet	4 tablet

Patients infected with *P. vivax* malaria and are G6PD normal will be treated with a standard course of chloroquine plus primaquine. Chloroquine diphosphate (each tablet contains 150 mg chloroquine base from Mekophar Chemical Pharmaceutical, Joint Stock Company, Ho Chi Minh City, Vietnam) will be administered at a dose of 10 mg/kg on days 0 and 1, followed with 5 mg/kg on day 2 (total 25 mg/kg over 3 days). Primaquine dosing will be 0.25 mg/kg daily for 14 days. Primaquine phosphate (each tablet contains 7.5 mg primaquine base) will be obtained from Danapha (Da Nang, Vietnam). Administration of chloroquine and primaquine will be observed and recorded over the first three days of dosing with the following 11 days of primaquine daily dosing unsupervised (Table 4).

The IMPE-QN/MIPM doctor will observe administration of each dose of the ACT, chloroquine and primaquine with the observation recorded in the case report form (CRF). For primaquine dosing the IMPE-QN/MIPM doctor will observe and record the single dose of primaquine (0.5 mg/kg, 30 mg oral adult dose) with the ACT.

All medication will be taken with 100 mL of water. Pyronaridine-artesunate can be given with or without food. Chloroquine and primaquine should be taken with food to minimize gastrointestinal disturbances.

Table 4. Antimalarial drug treatment regimens to be used at the field sites in Gia Lai and Phu Yen provinces

***Plasmodium* spp.**	**Antimalarial drug**	**D0**	**D1**	**D2**
*P. falciparum**	Pyronaridine-Artesunate (Pyramax®)	x	x	x
	Primaquine diphosphate	x		
*P. vivax**	Chloroquine phosphate	x	x	x
	Primaquine diphosphate	x	X	X

* First-line antimalarial drug treatment regimens approved by the Vietnam MoH for 2020. For the treatment of *P. vivax*, the administration of primaquine will be observed for the 1st three days of dosing with chloroquine; the other 11 days of primaquine dosing will be unsupervised.

After starting drug treatment, all patients will be invited to provide a finger prick capillary blood sample on the following days:

- Days 1, 2 and 3 twice daily at about 12 hourly intervals (150 µL for each time pointin a BD microtainer containing EDTA) for the measurement of hemozoin concentrations, qPCR analysis and blood film microscopy to determine parasite clearance.

- Day 7 (250 µL) in a BD microtainer containing EDTA for the measurement of hemozoin concentrations, blood film microscopy, qPCR analysis and measurement of antimalarial drug levels to determine good drug exposure.
- Days 14, 21, 28, and 35 (150 µL for each time point) in a BD microtainer containing EDTA for blood film microscopy and molecular analysis to check for malaria parasites.
- Day 42 (250 µL) in a BD microtainer containing EDTA for blood film microscopy to check for malaria parasites, for molecular analysis of sub-microscopic malaria infections, if present, and measurement of antimalarial drug levels.

If, however, a patient has a recurrent malaria infection after receiving drug treatment he/she will be invited to provide a finger prick capillary blood sample (250 µL) in a BD microtainer containing EDTA on the day of malaria recurrence for the measurement of blood chloroquine and pyronaridine concentrations (2 x 50 µL) and the remaining blood for blood film microscopy to determine *Plasmodium* speciation for subsequent treatment, and for molecular analysis.

Time windows for follow-up visits:

The time-window for the visits on Days 7 to 42 will be plus or minus 2 days. Although it is preferred that patients attend follow-up at the health station, if this is not possible home visits will be arranged by the IMPE-QN/MIPM study team.

10.2 Field and Laboratory Procedures

The following field and laboratory tests will be performed:

(1) Rapid Diagnostic Testing (RDT)

Participants suspected of having malaria will be screened with a RDT [SD Bioline Malaria Ag Pf/Pv HRP2/pLDH for the detection of *P. falciparum* (Pf) and *P. vivax* (Pv), respectively] to quickly check for malaria infection.

(2) Blood film microscopy

Thick and thin blood films (quantity two) will be collected for parasite density counts and species determination, respectively. The blood films will be labelled anonymously (study number and date) and stained with Giemsa.

Counting of the number of asexual parasites will be done against a set number of white blood cells (WBC) (typically 200) with a hand tally counter. Parasite density, expressed as the number of asexual parasites per µL of blood, will be calculated by dividing the number of asexual parasites by the number of WBC counted and then multiplying by an assumed WBC density (typically 8,000 per µL).

Two WHO certified microscopists (Levels 1 or 2) at IMPE-QN will read all the slides independently, and parasite densities will be calculated by averaging the two counts.

In addition, counting of sexual forms (gametocytes) will be performed in symptomatic malaria people to establish prevalence of gametocytes before treatment. Samples which are microscopy-positive but RDT-negative will be

further investigated for potential deletions of *hrp2-hrp3* genes as previously described (19).

(3) G6PD activity deficiency and hemoglobin measurement

The G6PD status of the malaria participants will be determined using a quantitative CareStart™ G6PD Biosensor (Access Bio, USA) that gives a quantitative measurement of total G6PD enzyme activity. Quantification of the participant's G6PD enzyme activity will be normalized against the individual's hemoglobin (Hb) concentration, which will be measured using a quantitative CareStart™ Hb device (Access Bio, USA). For the G6PD deficiency and hemoglobin measurements, about 10 µL of finger prick capillary blood will be required, and G6PD activity values will be calculated in units per gram hemoglobin (IU/g Hb). Patients with normal G6PD enzyme activity levels (>70%) of the site median value for G6PD normal (20) will be invited to participate in this study.

Patients with normal G6PD enzyme activity levels (>70%) of the site median value for G6PD normal (20) will be invited to participate in this study. For this study, we will apply the G6PD activity threshold >5.87 IU/g Hb (21) as approved in 2017 by the Vietnam Ministry of Health (NCT02216123) using the CareStart™ G6PD Biosensor.

(4) **Pregnancy test**

Female patients of child-bearing age (10 to 55 years old) will be asked to take a urine pregnancy test before enrolment in the study, because the ACTs and primaquine are contraindicated during pregnancy. Pregnant and lactating women will not participant in this study. Female participants of child-bearing age who are sexually active will be counselled to use contraception during the study. Birth pills will be offered to the participant by the study doctor at the time informed consent is obtained, with appropriate counselling about the risks of becoming pregnant and exposing the foetus to the study medicine.

(5) ***In vitro* drug susceptibility testing**

All adult participants with *P. falciparum* will be invited to provide a blood sample (4 mL) before commencement of treatment for *in vitro* drug sensitivity testing using the malaria SYBR Green I-based fluorescence assay as described by Johnson *et al.* (22). The standard drugs to be tested will be atovaquone, chloroquine, desethylamodiaquine, dihydroartemisinin, lumefantrine, mefloquine, methylene blue, piperaquine, pyronaridine, and tafenoquine. The experimental antimalarial candidates such as JPC-3210 and a 1,2,4-triazine will also be assessed. Falciparum parasites will be cryopreserved at the field sites with glycerolyte and stored in liquid nitrogen for further evaluation at MIPM and ADFMIDI. The *in vitro* ring-stage survival assay for artemisinin resistance (23) and the piperaquine survival assay for piperaquine resistance (24) will also be performed.

(6) **Confirmation of malaria species and determinations of Day 42 PCR-adjusted ACPR for *P. falciparum* and Day 42 ACPR for *P. vivax* by PCR**

Venous or finger prick capillary blood (50 µL x 2) will be added to Whatman 31 ET Chromatography filter paper from each participant on designated days. Malaria species (*P. falciparum*, *P. vivax*, *P. ovale,* and *P. malariae*) will be detected and identified by a single round of multiplex PCR as described by Padley et al. (25) in samples collected on Day 0, Day 42 as well on the day of malaria recurrence. Recurrent *P. falciparum* parasites will be genotyped using *msp1*, *msp2* and *glurp* markers as previously described (26).

(7) **Characterization of molecular markers of drug resistance in *P. falciparum* field isolates**

For artemisinin resistance, clinically relevant single-nucleotide polymorphism (SNPs) in codons 441-675 of the *Pfkelch13* gene (27) will be characterized using a previously described method (28). For piperaquine resistance, point mutation in the *Exo-E415G* gene and an amplification of the *plasmepsins 2/3* genes will be evaluated as previously described (29, 30). Codons 72-76, 93, 97, 145, 218 and 220 in the *Pfcrt* gene were analyzed by PCR amplification and sequencing of the respective fragments as previously described (31) with modified D2b primer 5'-AACAATAAA GAACATAATCATAC-3' used instead of D2. The copy number of the *P. falciparum* multidrug resistance protein 1 (*Pfmdr1*), associated with lumefantrine and mefloquine resistance (32, 33) will be determined by quantitative PCR assay using SYBR Green chemistry (34).

Limited knowledge is available on molecular drug-resistant markers in *P. vivax.* The prevalence of polymorphism in *P. vivax* molecular markers K12, a *P. vivax* homolog of the *PfKelch13* gene (35, 36), *Pvcrt-o* (37), *Pvmdr1* (38, 39), and copy number variation in *Pvmdr1* (38) and plasmepsin 4 (36) will be determined as these genes may have an association with reduced susceptibility to artemisinin, chloroquine, mefloquine, and piperaquine, respectively.

(8) Whole Genome Sequencing (WGS)

Based on their resistance profile a sub-set of approximately 50 *P. falciparum* field isolates will be selected for WGS to be conducted at ADFMIDI as previously described (40). Parasite genomic DNA will be extracted and sequenced using Illumina MiSeq platform to generate >20x coverage of the *P. falciparum* genome. The sequence assembly of ~150 base pair paired-reads will be guided using *P. falciparum* 3D7 reference genome available from public databases (i.e. PlasmoDB). Bioinformatic analysis of variants will be utilized to identify novel SNPs and copy number variations in the genomes of resistant field isolates relative to the reference 3D7 genome.

(9) Antimalarial drug analysis

As part of monitoring the therapeutic efficacy of the treatment drugs, finger prick capillary blood samples collected at day 7 after the commencement of treatment and at the time of recurrent malaria will be carried out. Blood concentrations of the long acting drugs: chloroquine and pyronaridine concentrations (ng/mL) will be measured by LCMS at ADFMIDI using established methods (41, 42).

(10) Hemozoin-detecting assay for malaria diagnosis

Hemex Health (Oregon, USA) has developed a hemozoin-detecting assay, based on magneto-optical detection of hemozoin crystals from whole blood in a column in a small cartridge. Thirty µL of finger prick blood sample is added to a small cartridge containing diluent (saline water). The blood is lysed by sonication and then light scatter is detected in the presence of a fluctuating magnetic field. The time from finger prick to result is about 1 minute. The assay can distinguish monoinfections of *P. falciparum* from monoinfections of *P. vivax* at above 95% accuracy (crystals are of a different shape and respond differently). The hemozoin assay device will also be tested in malaria patients during routine screening who decide not to participate in the efficacy monitoring of antimalarial drugs.

In terms of sensitivity, the hemozoin assay appears to be able to detect 10-20 parasites/µL for *P. vivax*, which is markedly more sensitive than RDTs with a sensitivity of about 200 parasites/µL for *P. vivax*. However, for *P. falciparum* the sensitivity of the hemozoin assay is less clear but is expected to be at least as sensitive as RDTs (50-100 parasites/µL). Other benefits of the assay is that it detects all *Plasmodium* species, it is not affected by *hrp2-hrp3*deletions, ease of use, digital data, no cold chain requirements, and the portable reader is battery operated with global positioning system, Wifi, and Bluetooth connectivity and affordable at about $USD700 for the reader and $USD0.65 for the disposable cartridge.

(11) Genetic diversification/relatedness

DNA genome parasite sequencing will be performed to determine the diversity of *var* genes associated with malaria pathogenesis and erythrocyte surface antigenic variation as described by Day *et al.* (43).

(12) Rescue treatment

Patients who fail treatment of *P. falciparum* malaria with Pyramax®plus a single dose of primaquine will be re-treated with a 7-day course of daily quinine plus doxycycline and patients who fail treatment of *P. vivax* malaria with chloroquine plus primaquine will be re-treated with a 3-day course of dihydroartemisinin-piperaquine (DHA-PPQ) and daily primaquine for 14 days, as per Vietnam MoH treatment guidelines. The rescue drugs will be administered by the communual health station medical staff who will be responsible for the patient's clinical management.

10.3 Adverse Event monitoring and reporting

It is the responsibility of the IMPE-QN/MIPM doctor to detect and document all adverse events experienced by the patients during the study and that fulfils the definitions and criteria outlined in this protocol in the CRF. Adverse events will be monitored in the participants by the IMPE-QN/MIPM doctor on Day 0 (before drug treatment) and then on days 1, 2, 3, 7, 14, 21, 28, 35 and 42 after the commencement of treatment with observation including severity grading recorded in the CRF.

An adverse event is defined as: "Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product."

An adverse event includes:

1) An exacerbation, or an unexpected increase in frequency or intensity of a pre-existing condition, including intermittent or episodic conditions.
2) Significant or unexpected worsening or exacerbation of the condition/indication under investigation.
3) A suspected drug interaction.
4) An intercurrent illness.
5) Any clinically significant laboratory abnormality.

An adverse event does not include:

1) Anticipated day-to-day fluctuations of any pre-existing conditions, including the disease under study.
2) Signs and symptoms of the disease under study that do not represent a significant worsening or exacerbation.
3) Expected progression of the disease under investigation.

Adverse events will not be prompted but will be reported at the interviews in response to the non-leading question "How do you feel since you took the antimalarial tablets". If a patient responds affirmatively with symptoms, a checklist of expected symptoms will be used and the timing and intensity of the adverse event(s) will be recorded. The intensity and seriousness of the adverse event will be classified as follows:

a. *Assessment of Intensity:* The relative intensity of an adverse event is determined by clinical judgement based on the following guidelines. The maximum intensity encountered during the evaluation period will be recorded as:
    1) Mild: An adverse event, which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
    2) Moderate: An adverse event, which is sufficiently discomforting to interfere with normal everyday activities.
    3) Severe: An adverse event, which prevents normal everyday activities.

b. *Assessment of seriousness:* A serious adverse event (SAE) is one that represents an actual or potentially significant hazard. This includes experiences that are fatal, life threatening, permanently disabling, require hospitalization, and/or result in congenital abnormality, cancer or overdose. All adverse events will be classified according to the following:
    1) Non serious (Adverse Event)
    2) Serious (Serious Adverse Event - SAE). A SAE is an adverse event that:
        - Results in death;
        - Life-threatening;
        - Requires in-patient hospitalisation or prolongation of existing hospitalisation;
        - Results in persistent or significant disability/incapacity;

- Congenital anomaly/birth defect;
- Requires acute medical or surgical care to prevent one of the outcomes listed above;

c. *Action Taken:* Actions taken in response to an adverse event can be classified as follows:
   1) None;
   2) Treatment drug discontinued;
   3) Other action taken (concomitant therapy or other action taken to manage the adverse event).

Causality: Although malaria infection, particularly during the acute phase can cause adverse events such as nausea, abdominal pain, headache and dizziness, which often makes it difficult to distinguish disease effects from drug effects, the IMPE-QN/MIPM doctor will attempt to determine a causal association between the adverse event and the treatment drugs.

Reporting of Serious Adverse Events: The IMPE-QN/MIPM doctor will take immediate action in response to SAEs to ensure the safety and wellbeing of the participant, and will immediately inform the Principal Investigator. The IMPE-QN/MIPM doctor will attempt to identify the cause/s of the SAE. The Principal Investigator will notify the IMPE-QN IRB, MoH-IRBNBR, Independent Medical Monitor (IMM) and ADFMIDI of any SAE within 24 hours of becoming aware of the event. The notification must be in writing by email or fax, and documented on a SAE reporting form. For reporting an SAE and/or an unanticipated problem involving risks in the course of the study, the Principal Investigator must prepare a detailed case history together with the CRF and email the clinical data to the IMPE-QN IRB, MoH-IRBNBR, IMM and ADFMIDI within 72 hours. Additionally, for fatal or life-threatening SAEs whether related or not related to the treatment drugs the IMPE-QN/MIPM doctor must report by telephone to the Principal Investigator as soon as he or she becomes aware of the event.

10.4 Therapeutic efficacy monitoring

Efficacy data will be assessed by means of Kaplan-Meier survival analysis and as *per-protocol* analysis (Table 5). Classification of treatment outcomes will be by the World Health Organization guidelines (44) for Early Treatment Failure, Late Clinical Failure, Late Parasitological Failure and ACPR.

Table 5. Guidelines for *in vivo* analysis of results of therapeutic efficacy studies of antimalarial drugs

End-point for day X (X = 28 or 42)	**PCR-uncorrected results**	
	Cumulative success or failure rate (Kaplan-Meier analysis)	Proportion (per-protocol analysis)
Adequate clinical and parasitological response on day X	Success	Success
Early treatment failure	Failure	Failure
Late clinical failure before day 7	Failure	Failure
Late clinical failure or late parasitological failure on or after day 7	Failure	Failure

Other species infection	Censored day of infection	Excluded from analysis
Lost to follow-up	Censored last day of follow-up according to timetable	Excluded from analysis
Withdrawal and protocol violation	Censored last day of follow-up according to timetable before withdrawal or protocol violation	Excluded from analysis
End-point for day X (X = 28 or 42)	**PCR-corrected results**	
	Cumulative success or failure rate (Kaplan-Meier analysis)	Proportion (per-protocol analysis)
Adequate clinical and parasitological response at day X	Success	Success
Early treatment failure	Failure	Failure
Late clinical failure before day 7	Failure	Failure
Late clinical failure or late parasitological failure on or after day 7		
P. falciparum recrudescence*	Failure	Failure
P. falciparum reinfection*	Censored day of reinfection	Excluded from analysis
Other species mixed with *P. falciparum* recrudescence	Failure	Failure
Other species mixed with *P. falciparum* reinfection	Censored day of reinfection	Excluded from analysis
Other species infection	Censored day of infection	Excluded from analysis
Undetermined or missing PCR	Excluded from analysis	Excluded from analysis
Lost to follow-up	Censored last day of follow-up according to timetable	Excluded from analysis
Withdrawal and protocol violation	Censored last day of follow-up according to timetable before protocol violation or withdrawal	Excluded from analysis

* WHO. *Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations*. Geneva, World Health Organization, 2008 (41)

10.5 Drug treatment side effects profiles

Pyronaridine-artesunate (Pyramax®) is the new first-line ACT that is registered for use by the Vietnam MoH for the treatment of uncomplicated *P. falciparum* malaria. Chloroquine plus primaquine is the first-line drug combination for treating *P. vivax* malaria in Vietnam.

All medicines may cause side effects, but many people have no, or minor side effects. The safety profiles of the ACTs to be used in this study are well understood and their adverse effects are mild compared to the risks of untreated *P. falciparum* and *P. vivax*

malaria. Side effects of antimalarials used for treatment of acute malaria may be difficult to distinguish from the symptoms of malaria. Possible side effects of the treatment regimens to be used in this study are as follows:

Pyronaridine-artesunate (Pyramax®)

The safety of pyronaridine-artesunate for treatment of malaria has been evaluated in clinical trials of more than 4,000 patients. The ACT is generally well-tolerated (45), with the most common adverse events similar to the symptoms of malaria (i.e. dizziness, nausea, vomiting, abdominal discomfort and headache), which are generally mild and transient. Mild to moderate reversible liver enzymes (i.e. alanine aminotransaminase and aspartate aminotransferase) elevation have been observed after treatment in African and Asian patients without clinical manifestations following pyronaridine-artesunate treatment (46). A recent study in Vietnam in 155 patients treated with pyronaridine-artesunate for *P. falciparum* in five provinces reported no clinical drug-related adverse events (47).

Chloroquine diphosphate

The drug is one of the safest antimalarial drugs ever discovered. Serious adverse reaction to chloroquine is rare at the regular dose and it is generally well tolerated when used for treatment (48).The principle limiting adverse effects in practice are the unpleasant taste, which may upset children, and pruritus (itching), which may be severe in dark-skinned patients. Other less common transient side effects include headache, various skin eruptions, blurred vision, and gastrointestinal disturbances, such as nausea, vomiting and diarrhea.

Primaquine diphosphate

The 15-mg adult dose of primaquine daily for 14 days consistently shows good tolerability with methemoglobin levels consistently elevated (typically <5 g%, and >12 g% is rare) (49). Therapeutic doses may cause abdominal pain if administered on an empty stomach. Larger doses can cause nausea and vomiting. The most important adverse effects are hemolytic anaemia in patients with G6PD deficiency.

10.6 Statistical analysis

The primary outcome for treating falciparum and vivax malaria will be the Day 42 PCR-adjusted ACPR and unadjusted-PCR ACPR on Day 42, respectively. The primary outcome will be analyzed using Kaplan-Meier analysis and the proportion of patients with the outcome using a per-protocol analysis and 95% confidence intervals. Patients will be censored if they withdrew from the study, they are lost to follow-up, or PCR results confirms *P. falciparum* reinfection for patients treated for *P. falciparum* or recurrence of *P. vivax* infection for patients treated for *P. vivax* malaria. Values of normally distributed data will be expressed as means with standard deviations and non-normally distributed data as medians with interquartile ranges (IQR). For testing differences in parasite populations and drug concentrations the Mann-Whitney *U* test will be conducted. $P < 0.05$ will be considered significant.

11. RISKS AND BENEFITS

11.1 Risks

The main risks to the participants and researchers associated with the study are:

a. Failure of treatment:
There is a small chance that the treatment drug might fail to cure the patients malaria infections, leaving the participants in danger of severe disease.

b. Side-effects of blood sampling:
Venous blood and finger prick capillary blood sampling carries a risk of mild discomfort and a very small risk of infection and/or bruising. Also, some patients may experience fainting or dizziness when providing blood samples.

c. Privacy risks:
Privacy exist in storage of participant medical information received and generated during the conduct of the study.

d. Researcher risks:
Handling of human clinical samples carries the potential risk of infection with pathogens that may be present in the samples.

11.2 How will the risks be mitigated?

The risk of treatment failure is low but to protect the wellbeing of the patient the IMPE-QN/MIPM study team will check the patient's blood frequently after treatment to make sure that their malaria infection has been cured. However, if the drug does not clear the patient's malaria infection or the malaria comes back the communal health station staff will retreat the patient with another drug combination recommended by the Vietnam MoH.

The risk of infection from venous and finger prick capillary blood collections will be minimized by the use of aseptic technique and disposable sterile lancets. To minimize bruising or dizziness/fainting only trained and experienced IMPE-QN/MIPM doctors and technicians will perform the venipuncture and finger prick blood sample collection.

The participants should be well hydrated and be sitting for the sample collection; those who prefer may lie down. Before collecting the blood sample the participant will be informed of the procedure so they are fully aware of what will be carried out. However, if the participant feels dizzy/faints with pain or at the sight of blood, the IMPE-QN/MIPM doctor or technician will immediately lie the participant down, raise their legs and apply cold sponges to provide relief and will administer first aid and medical assistance if necessary. Before undertaking a venipuncture on a participant, the IMPE-QN/MIPM doctors and technician will plan for safe handling and proper disposal of the needle/lancet to minimize a needle stick injury.

Mitigation methods against privacy risks are (i) Taking of consent to participate in the study in a private place and (ii) Participant information will be handled appropriately, as outlined in the sections "Participant information (Section 12.2)" and "Data management (Section 13.2)".

To reduce the risk of infection to the IMPE-QN/MIPM doctors and technician personal protective equipment will be applied when collecting, handling and processing blood specimens collected from the participants.

11.3 Benefits to participants, wider community and researchers

The main benefits for participants, the wider community and the researchers associated with the study are:

- If their blood film is found to have malaria parasites detected by microscopy they will be treated (free-of-charge) at the commune health station or district health centers. The IMPE-QN/MIPM doctor will check the patient's general wellbeing by collecting health vitals such as pulse rate, respiratory rate and body temperature, as well as their recent medical history of signs/symptoms.
- The IMPE-QN/MIPM doctor will treat the malaria infected participant with first-line treatment drugs for both *P. falciparum* and *P. vivax* malaria in accordance with the Vietnam MoH guidelines. Participants will be followed up for 42 days to ensure that they have been cured of their infection.
- Up-to-date knowledge of the therapeutic efficacy and tolerability of antimalarial treatment drugs is of benefit to the local community in knowing whether the drugs that they are receiving are or are not effective in treating malaria infections in the local area.
- Studying the genetic diversification/relatedness of the malaria parasites will assist in providing important information on the origin of the parasites and whether they have been imported into Vietnam or originated within the country. Such information could also assist in tracking drug resistant parasites.
- By evaluating a potentially new device that measures malaria pigment concentrations it may be able to predict whether patients are infected with either drug sensitive or resistant malaria parasites. If this can be demonstrated the device would provide early detection of resistance and earlier clinical management for patients infected with drug resistant malaria infections.
- By monitoring the therapeutic efficacy of antimalarial drugs in a region of Central Vietnam with changing levels of drug resistance, the researchers will be able to provide up-to-day profiles on drug resistant malaria that will inform on drug policy for the Vietnam MoH and the findings will aid in developing future strategies for malaria control and elimination.

11.4 How do the benefits of the research outweigh the risks associated with the research?

Apart from possible physical discomfort in providing a biological sample and privacy issues associated with accessing and maintaining personal medical information, there appears to be no other particular risks to participants. The benefit to wider community and Vietnam MoH will be a better understanding of the effectiveness of antimalarial drugs in treating malaria in potential regions of evolving and spreading multidrug-resistant malaria parasites.

12. STUDY DOCUMENTATION AND PARTICIPANT INFORMATION

12.1 Study documentation

The Principal Investigator will ensure that all data are collected and recorded correctly in the registers of data and the CRF. Any change or correction to a CRF will be marked with the date and initials of person making the correction. Corrections must be explained and should not obscure the original entry. The CRF for baseline data for each participant is attached as Annex A for people with symptomatic malaria. Participants receiving chloroquine plus primaquine for the treatment of vivax malaria will complete an eight-item Morisky Medication Adherence Scale questionnaire for primaquine user adherence (Annex B). PISCF for adults is attached as Annex C. Participant information and parent/guardian permission form for children is attached as Annex D. Participant information and statement of assent for children aged 12 to <18 years old is attached as Annex E. The SAE report form is attached as Annex F.

For all purposes, procedures and analyses the participant will be de-identified with unique study numbers (code) given and used throughout the study [e.g. ELYSHPY01for the 1st symptomatic malaria patient at the field site in Phu Yen where the letters ELYSHPY refer to Ea Ly (ELY) Commune, Song Hinh (SH) district, Phu Yen (PY) province].

Hard copies of the PISCFs, CRFs and registers of data will be stored in a locked cabinet/ drawer in a locked room at IMPE Quy Nhon. The PISCFs, CRFs and registers of data will also be scanned and transferred electronically to the Principal Investigator's password protected computer(s) at IMPE-QN.

12.2 Participant Information

Clinical and laboratory findings from each participant will be kept with the CRF. The PISCFs and CRFs will be separately stored (hard copies and electronically). During the study, access to the PISCFs and CRFswill be restricted to the Principal Investigator and the study IMPE-QN/MIPM doctors with a need to use these documents. Study members with access to study documents will be counselled on the importance of confidentiality of participant medical information.

The Principal Investigator, co-investigators, project officers, and the IMPE-QN/MIPM study team (doctors, scientists and technicians) have completed GCP and Ethics training. In all electronic records for data analysis, the participant is not to be referenced by name, but only his/her unique study number. This method is designed to protect the privacy of study medical information of the participants. All the PISCFs, CRFs and registers of data will be held at IMPE-QNfor at least 15 years after completion of recruitment of all participants into the study in secured cabinets/drawers with restricted access.

13. SHARING OF BIOLOGICAL SAMPLES AND DATA MANAGEMENT

13.1 Sharing of biological samples

Biological samples can be shared between the study partners (i.e. IMPE-QN, MIPM, and ADFMIDI), particularly if there is scientific merit in conducting further investigations to expand our knowledge of drug resistant malaria in the context of malaria epidemiology.

13.2 Data Management

Storage and access

Hard copy study documents will be held under lock and key cabinet/drawer accessible only by the study IMPE-QN/MIPM doctor at the field sites for up to 6 months or the Principal Investigator at IMPE-QN for 15 years. Scanned copies of hard copy study documents will also be secured by the Principal Investigator on a password protected desktop/laptop and Universal Serial Bus (USB) at IMPE-QN. All study collected data will be entered into a master study database spreadsheet by participant assigned study ID and stored at IMPE-QN in a password protected desktop/laptop and USB stick. The confidentiality of all participants will be protected.

Personal identifiers and assigned study ID will be entered in a separate database only accessible by the Principal Investigator as a secured document in a password protected desktop/laptop and USB stick. This document will be utilized by the Principal Investigator when reporting results.

Use of Biological Samples and Data Confidentiality

Biological samples will be assigned a study code by study investigators for the purpose of testing by study laboratory staff, but will not be linked to any identifying information by laboratory staff. Results will be kept anonymous and combined as cohort data for purposes of publishing in peer reviewed scientific journals and presentations.

The participant's de-identified blood samples (blood films and finger prick and venous blood samplesfor molecular analysis and hemozoin concentration) collected in this study or/and will be kept for up to 15 years from the time of blood collection. If new diagnostic devices and assays are developed for malaria diagnosis or for studying novel genetic and drug resistance markers in malaria parasites, we may re-test the participant's samples with these devices and molecular assays. The PISCF for adults, permission from parents/guardians for children to participate in the study and statement of assent for children (aged 12 to 17 years old) seeks approval for the re-testing of their blood samples with new devices and molecular assays for malaria diagnosis and for studying drug resistance and genetic diversification of malaria parasites. The participants' samples collected under this study will not be used for any other purpose.

Transfer and archiving

De-identified blood samples will be held for up to 15 years after which they will be destroyed by autoclaving.

Data will be stored in a master database spreadsheet only linked to the assigned study code unique for each participant.

The electronic copies of the registers of data can be shared with the other study co-investigators and project officers for data analysis. Hard paper copies of all source data, including PISCFs, CRFs and registers of data will be destroyed via shredding and electronic records will be erased at the end of 15 years of storage, with the exception of the scanned PISCFs which can be retained longer at the discretion of the Principal Investigator or the Director of IMPE-QN. Hard paper copies of all source data will be

13.3 Data ownership, use of results and publication policy

The master database will be shared with the other collaborators, on a mutually agreed basis. Sharing the data with other parties is subject to the approval of the Principal Investigator and ADFMIDI. A report by the Principal Investigator summarizing the findings of this study will be forwarded to all collaborating institutions.

The results from all study sites will be submitted for publication in a peer-reviewed journal. Only those individuals who have made substantial and significant contributions will be co-authors on publications. All the research findings will be disseminated to policy makers and other researchers for an informed decision on drug policy for the treatment of malaria in this region and strategies for malaria elimination.

ADFMIDI will ensure that the study design, selection criteria, name of ethical committees granting approval of the clinical protocol and other aspects of the trial are posted in the publicly accessible database of the Australian New Zealand Clinical Trials Registry before commencement of the study (i.e. before the screening visit).

13.4 Quality assurance

Dr. Michael Edstein (PhD) at ADFMIDI will be responsible for carrying out quality assurance (QA) on the conduct/progress of the study. This covers study procedures associated with recruitment of participants, storage of biospecimens, management and storage of study documentation (e.g. PISCFs, CRFs, SOPs, registers of data), and resourcing and logistics associated with the study. QA monitoring is to be carried out before starting the study and at about 3-4 monthly intervals until completion of the study at the field sites by Dr Edstein or a nominated representative.

Dr. Geoffrey Birrell will QA the antimalarial drug concentration measurements by LCMS as well as drug exposure analysis.

Dr. Marina Chavchich will QA the laboratory procedures performed at MIPM and IMPE-QN. She will continue to train MIPM staff on new *in vitro* survival assays and IMPE-QN staff in new molecular techniques for characterizing drug resistance and genetic relatedness/diversification of malaria parasites.

14. PROTOCOL AMENDMENTS

Version	Date	Description of changes
1.0	15 Feb 2021	The initial VDCP01 protocol had chloroquine plus tafenoquine for the treatment of vivax malaria. Because of the Covid-19 pandemic, tafenoquine cannot be sourced from GlaxoSmithKline for providing the Kozenis tablets from the plant in India.
Version 2	24 Jun 2021	The VDCP01 protocol has been amended with the replacement of chloroquine plus tafenoquine with chloroquine plus primaquine for the treatment of vivax malaria.

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Annex A. CASE REPORT FORM

SENTINEL SITE	
Name of commune health station: Chu R'Cam (CR) ☐ Ia Drech (LD) ☐ Ea Lam (ELA) ☐ Ea Ly (ELY) ☐ tick ☑ only one box	Home location:
District:Krong Pa (KP) ☐ Song Hinh (SH)☐ tick ☑ only one box	Province: Gia Lai (GL)☐ Phu Yen (PY) ☐ tick ☑ only one box

PARTICIPANT INFORMED CONSENT AND ASSENT		
Study number: CRKPGL ___ ___ LDKPGL ___ ___ ELASHPY ___ ___ ELYSHPY ___ ___	Date: ___ ___ / ___ ___ / 20 ___ DD MM YYYY	Consent form signed for adults (≥ 18 yrs old): ☐ No ☐ Yes Parent/guardian permission signed for children (< 18 yrs old): ☐ No ☐ Yes Assent form signed for 12 to < 18 yrs old: ☐ No ☐ Yes

DEMOGRAPHIC DATA

Occupation Categories: ☐ Farmer ☐Teacher ☐ Student ☐ Forest Ranger ☐ Shopkeeper ☐Retired☐ Provincial/District Official ☐Driver ☐ Tradesman ☐Salesperson☐Other

Ethnic group: _____

Age: ___ ___ years Body Weight: ___ ___ ___. ___ kg

Gender (tick ☑ only one box): ☐ Male ☐ Female

INCLUSION CRITERIA FOR VDCP01

1. People infected with uncomplicated mono-infections of *P. falciparum* and uncomplicated mono-infections of *P. vivax*;
2. Malaria parasite density of *P. falciparum* (≥ 500 to < 100,000 parasites/µL);
3. Malaria parasite density of *P. vivax* (≥ 250 parasites/µL);
4. Children (≥5 years and ≥20 kg to <18 years old) and adults (≥18 to <60 years old) infected with *P. falciparum,* children (≥5 to <18 years old) and adults (≥18 to <60 years old) infected with *P. vivax* malaria;
5. Gender: Males and females;
6. Working or residing at the study commune;
7. Able to provide information and capillary finger prick blood samples;

8. Written informed consent given to participate in the study by the adult or in case of children up to <18 years old (Assent form for children aged 12 to <18 years old) with parent or guardian permission;
9. Normal G6PD enzyme activity levels (>70%) of the site median value for G6PD normals for participants to be treated with tafenoquine for the radical cure of *P. vivax* malaria.

EXCLUSION CRITERIA FOR VDCP01

1. People not infected with malaria infections;
2. Children (<5 years old and <20 kg)infected with *P. falciparum* and less than 5 years of age infected with *P. vivax* malaria;
3. Unwilling to provide consent, information, and capillary finger prick blood sample;
4. Inability to communicate well with the study staff (poor mental development or evidence of psychiatric disorder);
5. People with *P. vivax* malaria who have G6PD deficient enzyme activity;
6. Pregnant or lactating females;
7. Any condition that in the judgment of the IMPE-QN/MIPM doctor would make participation in the study unsafe for the potential participant.

Does the patient meet any of the exclusion criteria for VDCP01? ☐ Yes ☐ No

MOVEMENT OF PARTICIPANT OVER THE PAST TWO WEEKS

During the last two weeks did you live in or visit a forest area? ☐ Yes ☐ No

If, "Yes" did you use a bed net for sleeping?
☐ Nightly
☐ A few times a week
☐ Did not sleep under a bed net

Can you show on the map of Krong Pa/Song Hinh districts where you currently live?

GPS coordinates: _____

Can you show on the map of Krong Pa/Song Hinh districts where you worked in the forest or border area over the past two weeks?

GPS coordinates: _____

MALARIA PREVENTION			
Did the participant report the use of the following:	**Yes**	**No**	**Comments**
Bed net use in the commune and if so how frequently did they use (nightly, a few times a week, not often)	☐	☐	☐ Nightly ☐ A few times a week ☐ Not often ☐ Don't know
Bed net is treated	☐	☐	☐ Don't know
Use of insecticide coil	☐	☐	☐ Nightly ☐ A few times a week ☐ Not often ☐ Don't know
Treated fabric or other material to repel mosquitoes	☐	☐	☐ When treated last: ☐ >12 months ago ☐ 12 months ago ☐ 6 months ago ☐ 3 months ago ☐ < 1 month ago ☐ Don't know

Has the participant taken any prior antimalarial medication within the last 28 days or currently?
☐ Yes ☐ No If yes, please specify the name of the antimalarial drug used?

_____

PHYSICAL EXAMINATION	
Pulse rate: ___ ___ ___ /min.	Respiratory rate: ___ ___ ___ /min

BODY TEMPERATURE	
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ☐ Tympanic tick ☑ only one box ___ ___. ___ °C

BLOOD FILMS PARASITE DENSITY *P. falciparum* ☐ *P. vivax* ☐ tick ☑ only one box	
Number of asexual parasites ___ ___ ___ ___ / µL	Number of gametocytes ___ ___ ___ ___ / µL

MEDICAL HISTORY DURING LAST 3 DAYS BEFORE ANTIMALARIAL DRUG TREATMENT						
Signs/Symptoms	**Present**	**Absent**	**Comments/Causality**	**AE Intensity**		
				Mild	**Mod**	**Sev**
Rigors/Chills	☐	☐				
Sweating	☐	☐				
Headache	☐	☐				
Cough	☐	☐				
Nausea	☐	☐				
Abdominal pain	☐	☐				
Vomiting	☐	☐				
Loss of appetite	☐	☐				
Fatigue	☐	☐				
Myalgia	☐	☐				
Jaundice	☐	☐				
Hepatomegaly	☐	☐				
Splenomegaly	☐	☐				
Pruritus	☐	☐				
Other	☐	☐				

AE intensity classifications: Mid – Mild; Mod – Moderate; Sev – Severe

General Comments:

ANTIMALARIAL DRUG ADMINISTRATION			
Name of Drug	tick ☑ only one box	Number of tablets	Time of dosing
Pyramax®	☐	_____	___ ___:___ ___ (HH:MM)
Chloroquine	☐	_____	___ ___:___ ___ (HH:MM)
Primaquine	☐	_____	___ ___:___ ___ (HH:MM)

Drugs are administered with 100 mL of water and food

LABORATORY SPECIMENS	
Biological samples specified below are to be collect at Day 0-0h (just before drug administration)	
1. RDT	☐ Yes ☐ No
2. Finger prick capillary blood (250 µL) in BD microtube with EDTA for blood thick and thin films for microscopy (Qty 2), molecular assays and hemozoin concentration (children<18 years old).	☐ Yes ☐ No
3. Finger prick capillary blood (250 µL) in BD microtube with EDTA for blood thick and thin films for microscopy (Qty 2), molecular assays and hemozoin concentration (adults who decide not to provide venous blood samples).	☐ Yes ☐ No
4. Venous blood sample (4 mL) in a tube with lithium heparin for *in vitro* drug susceptibility testing (only adults ≥18 years old).	☐ Yes ☐ No
5. Venous blood sample (5 mL) in a tube with EDTA for blood thick and thin films for microscopy (Qty 2), hemozoin concentration and whole genome sequencing (only adults ≥18 years old)	☐ Yes ☐ No tick ☑ only one box for each sample
_____ Name of IMPE-QN/MIPM doctor	Time: ___ ___ : ___ ___ (HH:MM)
_____ Signature of IMPE-QN/MIPM doctor	

Study No: _____	Visit date: ___ ___ / ___ ___ / 202 _ DD MM YYYY	Day 0-12h ☐ Day 1-24 h ☐ Day 1-36 h ☐ Day 2-48 h ☐ Day 2-60 h ☐ Day 3-72 h ☐	Day 7 ☐ Day 14 ☐ Day 21 ☐ Day 28 ☐ Day 35 ☐ Day 42 ☐
		tick ☑ only one box	

BODY TEMPERATURE	
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ☐ Tympanic tick ☑ only one box ___ ___· ___ °C

BLOOD FILMS PARASITE DENSITY ***P. falciparum*** ☐, ***P. vivax*** ☐ tick ☑ only one box	
Number of asexual parasites ___ ___ ___ ___ / µL	Number of gametocytes ___ ___ ___ ___ / µL

ANTIMALARIAL DRUG ADMINISTRATION			
Name of drug	tick ☑ only one box	Number of tablets	Time of dosing
Pyramax®	☐	_____	___ ___:___ ___(HH:MM)
Chloroquine	☐	_____	___ ___:___ ___ (HH:MM)
Primaquine	☐	_____	___ ___:___ ___ (HH:MM)

Drugs are administered with 100 mL of water and food

Did the participant vomit after administration of the antimalarial drug?	☐ Yes ☐ No
If the participant vomits within 1 hour of taking the antimalarial drug, he/she will be invited to take the medication again.	
Did the participant vomit the re-administered antimalarial drugs?	☐ Yes ☐ No

Study No: _____	Visit date: ___ ___ / ___ ___ / 202 _ DD MM YYYY	Day 0-12h ☐ Day 1-24 h ☐ Day 1-36 h ☐ Day 2-48 h ☐ Day 2-60 h ☐ Day 3-72 h ☐	Day 7 ☐ Day 14 ☐ Day 21 ☐ Day 28 ☐ Day 35 ☐ Day 42 ☐
		tick ☑ only one box	

ADVERSE EVENT (AE) FOLLOW-UP AFTER STARTING DRUG TREATMENT						
Signs/Symptoms	**Present**	**Absent**	**Comments/Causality**	**AE Intensity**		
				Mild	**Mod**	**Sev**
Rigors/Chills	☐	☐				
Sweating	☐	☐				
Headache	☐	☐				
Cough	☐	☐				
Nausea	☐	☐				
Abdominal pain	☐	☐				
Vomiting	☐	☐				
Loss of appetite	☐	☐				
Fatigue	☐	☐				
Myalgia	☐	☐				
Jaundice	☐	☐				
Hepatomegaly	☐	☐				
Splenomegaly	☐	☐				
Pruritus	☐	☐				
Other	☐	☐				

AE intensity classifications: Mid – Mild; Mod – Moderate; Sev – Severe

General comments:

_____

Study No: _____	Visit date: ___ ___ / ___ ___ / 202 _ DD MM YYYY	Day 0-12h ☐ Day 1-24h ☐ Day 1-36h ☐ Day 2-48h ☐ Day 2-60 h ☐ Day 3-72h ☐	Day 7 ☐ Day 14 ☐ Day 21 ☐ Day 28 ☐ Day 35 ☐ Day 42 ☐
		tick ☑ only one box	

LABORATORY SPECIMENS	
1. Finger prick capillary blood (150 µL) in BD microtube with EDTA for thick and thin blood films (Qty 2), molecular assays and hemozoin concentration for time points: Days 0-12h, 1-24h, 1-36h, 2-48h, 2-60h, 3-72h.	☐ Yes ☐ No
2. Finger prick capillary blood (250 µL) in BD microtube with EDTA for thick and thin blood films (Qty 2), molecular assays, hemozoin concentration and drug concentration for time point D7.	☐ Yes ☐ No
3. Finger prick capillary blood (150 µL) in BD microtube with EDTA for thick and thin blood films (Qty 2) and molecular assays for time points: Days 14, 21, 28 and 35.	☐ Yes ☐ No
4. Finger prick capillary blood (250 µL) in BD microtube with EDTA for thick and thin blood films (Qty 2), molecular assays, and drug concentration for time point D42.	☐ Yes ☐ No tick ☑ only one box for each sample
_____ Name of IMPE-QN/MIPM doctor	Time: ___ ___ : ___ ___ (HH:MM)
_____ Signature of IMPE-QN/MIPM doctor	

Annex B

MORISKY 8-ITEM PRIMAQUINE ADHERENCE QUESTIONNAIRE

Question	**Patient Answer (Yes/No)**	**Score (Yes = 1: No = 0)**
Do you sometime forget to take your primaquine tablets?		
People sometimes miss taking their primaquine tablets for reasons other than forgetting. Thinking over the past two weeks, were there any days when you did not take your primaquine tablets?		
Have you ever cut back or stopped taking your primaquine tablets without telling the IMPE-QN/MIPM study doctor because you felt worse when you took the primaquine tablets?		
When you travel or leave home, do you sometimes forget to bring along your primaquine tablets?		
Did you take all your primaquine tablets yesterday?		
When you feel that your malaria symptoms such as fever, headache, nausea and tiredness are under control, do you sometimes stops taking your primaquine tablets?		
Taking primaquine tablets every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your primaquine dosing plan?		
How often do you have difficulty remembering to take your primaquine tablets? A = 0, B-E = 1 ___ A. Never/Rarely ___ B. Once in a while ___ C. Sometimes ___ D. Usually ___ E. All of the time		
	Total Score	
Scores: >2 = low adherence 1 or 2 = medium adherence 0 = high adherence		

Annex F

SERIOUS ADVERSE EVENT REPORT FORM

Protocol title: Therapeutic efficacy surveillance of malaria treatment and drug resistance monitoring in Gia Lai and Phu Yen provinces of Central Vietnam

Principal investigator: Dr. Huynh Hong Quang, IMPE, Quy Nhon, Vietnam.

Participant ID Number |____|____|____|____|____|____|

To be emailed within 24 hours: ***Email:*** _____

☐ **INITIAL REPORTING FORM** ☐ **FOLLOW-UP REPORTING FORM**

PARTICIPANT'S DEMOGRAPHY			
Gender Male ☐ Female ☐ **Date of Birth** |____|____| |____|____| 20|___|___| (day) (month) (year)			
SAE DESCRIPTION			
Diagnosis	**Start Date** (day)/(month)/(year)	**Elapsed time from last drug administration** if < 24 hours (hour) : (min)	**Stop Date** (day)/(month)/(year)
	|___|___| |___|___| **20**|___|___|	|___|___|:|___|___|	|___|___| |___|___| **20**|___|___|
Symptoms	**Start Date** (day)/(month)/(year)	**Elapsed time from last drug administration**	**Stop Date** (day)/(month)/(year)

	|___|___| |___|___| **20**|___|___|	|___|___|:|___|___|	|___|___| |___|___| **20**|___|___|
	|___|___| |___|___| **20**|___|___|	|___|___|:|___|___|	|___|___| |___|___| **20**|___|___|
	|___|___| |___|___| **20**|___|___|	|___|___|:|___|___|	|___|___| |___|___| **20**|___|___|

NAME OF STUDY MEDICATION: _____

Date(s) of Drug Administration(s) (day)/(month)/(year)

Dose 1 |___|___| |___|___| **20**|___|___| Dose 2 |___|___| |___|___| **20**|___|___| Dose 3 |___|___| |___|___| **20**|___|___|

Dose 4 |___|___| |___|___| **20**|___|___| Dose 5 |___|___| |___|___| **20**|___|___| Dose 6 |___|___| |___|___| **20**|___|___|

Is the SAE related to the study medicine(s)*? ☐ Yes ☐ No

Is the SAE related to the study medicine?** ☐ Yes ☐ No

Details of Last Drug Administration ***(before occurrence of SAE)***

Name(s) of study medicine	**Batch Number(s)**	**Oral Dose Number (1, 2, 3, …)**	**Relationship*** Yes/No (tick one)
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

OUTCOME & SERIOUSNESS

Outcome	**Seriousness** *check all items which apply*

☐ Fatal Date of Death: |___|___| |___|___| **20**|___|___| ☐ Recovered ☐ Recovered with Sequelae ☐ Ongoing **Serious Adverse Event Leading to Termination** ☐ Yes ☐ No	☐ Death ☐ Life threatening ☐ Hospitalization Required inpatient hospitalization *(complete both dates below)* Prolonged inpatient hospitalization *(complete the discharge date only)* Admission Date (day)/(month)/(year): |___|___| |___|___| **20**|___|___| Discharge Date (day)/(month)/(year): |___|___| |___|___| **20**|___|___| ☐ Persistent or significant disability/incapacity ☐ Congenital anomaly/birth defect ☐ Other: Important medical event *specify:*.....

** Yes = at least possibly related, No = no relationship*
*** New event relating to the conduct of the trial or the development of the trial product likely to affect the safety of the subject.*

CONCOMITANT THERAPIES (vaccines/drugs ongoing or taken before onset of symptoms)

Trade Name	Dose Number (1, 2, 3, …)	Start Date dd/mm/yyyy	Stop Date dd/mm/yyyy	Route of Administration	Dosage	Indication	Relationship* Yes/No (tick one)
							☐ Yes ☐ No
							☐ Yes ☐ No
							☐ Yes ☐ No

**** Is the SAE related to concomitant therapies: Yes = at least possibly related, No = no relationship***

DETAILED DESCRIPTION (including complementary investigations)
Narrative Description of the SAE
Treatment Prescribed for the SAE
Relevant ongoing illness / medical history / risk factors *(personal and family)*
Complementary Investigations - Type / Results
REPORTING INFORMATION
SAE Initial Reporting Form sent to the Independent Medical Monitor: **Date:**|____|____| |____|____| **20**|____|____| (day) (month) (year) **SAE Follow-up Reporting Form sent to the Independent Medical Monitor:**

Date: |____|____| |____|____| **20**|____|____|
(day) (month) (year)

Investigator's Name and Address:

Name:
……

Address:……

……

Tel:	**Email:**

Principal Investigator's Signature:

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