PART I: COVER PAGE

Title	An open label study to assess the efficacy, safety and tolerability of pyronaridine-artesunate in the treatment of malaria infection caused by single or mixed species of *Plasmodium falciparum, P. vivax,* or *P. malariae* in Vietnam.
Short title	Efficacy, safety and tolerability of pyronaridine-artesunate in treating uncomplicated malaria in Vietnam.
Protocol number	VNPA-01
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Co-investigator	LTCOL Michael Edstein (MSc, PhD), Australian Defence Force Malaria and Infectious Disease Institute (ADFMIDI), Brisbane, Australia.
Project officer	LCDR Kristina St.Clair (DO, MTM&H), US Naval Medical Research Centre-Asia (NMRC-A), Singapore.
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Proposed Start and End Dates	Start and End Dates, including data analysis and publication of results in a peer-reviewed scientific journal: From May 2018 to May 2021.
Sponsor	US NMRC-A

STUDY SYNOPSIS

Abstract	Reports by WHO of reduced susceptibility of dihydroartemisinin-piperaquine for the treatment of *Plasmodium falciparum* in six provinces of Vietnam severely threatens the artemisinin combination therapy (ACT) for first-line treatment of falciparum malaria in Vietnam. To inform the Vietnam Ministry of Health drug policy, it is crucial to have information on other potentially more effective ACTs such as pyronaridine-artesunate, which is presently under investigation in areas with proven artemisinin resistance in Western Cambodia. Pyronaridine-artesunate is a newer ACT and was recently approved by the European Medicine Agency for use in adults and children >20 kg, particularly in countries with documented artemisinin resistance. In addition to treating uncomplicated *P. falciparum* malaria, pyronaridine-artesunate is recommended for the treatment of *P. vivax* malaria or mixed infections of *P. falciparum* and *P. vivax*. We propose to carry out an open-label, single-arm, clinical trial to assess the efficacy, safety and tolerability of pyronaridine-artesunate for the treatment of uncomplicated mono-infections of falciparum, vivax and malariae malaria, or mixed infections of the *Plasmodium* species in a province of Vietnam, with reduced susceptibility to artemisinins.
Study Design	Observational study in an area with reduced susceptibility to artemisinin**s** of the efficacy and tolerability of pyronaridine-artesunate over 42 days for the treatment of mono-infections of *P. falciparum, P. vivax* and *P. malariae* or mixed infections of these *Plasmodium* species.
Participants	Patients with acute uncomplicated mono-infections of *P. falciparum, P. vivax* and *P. malariae* malaria or mixed infections of the *Plasmodium* species.
Drug Treatment	Participants with mono-infections of *P. falciparum, P. vivax* and *P. malariae* or mixed infections of these *Plasmodium* species will be treated orally with pyronaridine-artesunate (Pyramax®) given daily for 3 days. Participants with mono-infections of *P. vivax* or mixed infections of *P. vivax* will also receive a standard 14-day course of primaquine treatment.
Study Sites	Dak Drong Commune in Cu Jut District and Thuan An Commune in Dak Mil District, both in Dak Nong Province with the communes having WHO confirmed reduce susceptibility to artemisinins.
Sample Size	55 patients each for both the *P. falciparum* and *P. vivax* mono-infection arms. With the additional inclusions of 10 patients with *P. malariae* and/or mixed *Plasmodium* species, the overall sample size goal is 120 patients.
Inclusion Criteria	• Adults and children ≥ 20 kg • Symptomatic of malaria infection (i.e. history of fever within 24 hours and/or presence of fever >37.5°C for axillary temperature or >38.0°C for tympanic temperature. • Microscopic confirmation of asexual (i.e. blood) stages of mono-infections of *P. falciparum*, *P. vivax* and *P. malariae* or mixed infections of the *Plasmodium* species. • Parasitemia between 250/µL and < 100 000/µL. • Glucose-6-Phosphate Dehydrogenase (G6PD) normal patients with mono-infection of *P. vivax* or mixed infections of *P. vivax* will be treated with primaquine to kill liver hypnozoites. • Ability to take oral medication. Written informed consent given to participate in the trial by the patient or in case of children up to 17

	years old (assent for children aged 10 to 17 years old) with adult or guardian permission.
Exclusion Criteria	• Pregnancy or lactation (urine test for β HCG to be performed on any woman of child bearing age 10 to 55 years old). • Hematocrit <20%. • Parasitemia <250/µL or > 100 000/µL. • Signs or symptoms indicative of severe/cerebral malaria (WHO, 2014). • Liver function test (AST/ALT levels) more than 2.5 times the upper limit of normal (ULN) range. • Total bilirubin > 2 ULN. • Use of an antimalarial drug in the preceding 4 weeks. • History of splenectomy, heavy alcohol use or injecting drugs of abuse. • Any other condition, which in the judgment of the study physician would make participation in the study unsafe for the potential study patient.
Primary Objective	To assess the therapeutic efficacy of pyronaridine-artesunate for the treatment of uncomplicated mono-infections of *P. falciparum* and *P. vivax* in an area of reduced susceptibility to artemisinins.
Secondary Objectives	Main secondary objectives: • To assess the therapeutic efficacy of pyronaridine-artesunate for the treatment of *P. malariae* malaria or mixed infections of the *Plasmodium* species. • To assess the safety and tolerability of pyronaridine-artesunate. • Genotypic assessment of *P. falciparum* resistance. • Determine drug exposure by measuring patient's plasma artesunate/dihydroartemisinin and blood pyronaridine concentrations. • *In vitro* drug susceptibility of patient's falciparum parasites against standard antimalarial drugs
Primary Endpoints	To assess Days 28 and 42 Polymerase Chain Reaction (PCR) corrected Adequate Clinical and Parasitologic Response (ACPR) for the treatment of mono-infections of *P. falciparum*, *P. vivax* and *P. malariae* or mixed infections of these *Plasmodium* species.
Secondary Endpoints	Main secondary endpoint: • To assess the safety and tolerability of pyronaridine-artesunate in patients. • Polymorphism of molecular markers of *P. falciparum* resistance. Other secondary endpoints • The numbers of patients with a positive malaria slide 72 hours after treatment initiation. • Fever clearance time (i.e. the time taken for axillary temperature to fall below 37.5˚C or tympanic temperature to fall below 38.0˚C and remain there for at least 24 hours). • Kaplan Meier analysis over 28 and 42 days for recrudescences and reinfections. Hazard ratio of recurrent malaria parasites of falciparum and vivax malaria. • PCR uncorrected and corrected ACPR at Days 28 and 42 following treatment.

	• Gametocyte carriage rates and gametocyte clearance times. • Documented adverse events (AEs) and serious adverse events (SAEs) and relationships to study drugs. • Patient's plasma artesunate/dihydroartemisinin and blood pyronaridine concentrations. • *In vitro* drug susceptibility testing of falciparum parasites collected from study participants against standard antimalarial drugs

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LIST OF ABBREVIATIONS

ACPR	Adequate Clinical and Parasitological Response
ACT	Artemisinin Combination Therapy
ADFMIDI	Australian Defence Force Malaria and Infectious Disease Institute
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CITI	Collaborative Institutional Training Initiative
CRF	Case Report Form
DDVAHREC	Departments of Defence and Veterans' Affairs Human Research Ethics Committee
EMA	European Medicine Agency
ETF	Early Treatment Failure
GCP	Good Clinical Practice
G6PD	Glucose-6-Phosphate Dehydrogenase
HCG	Human Chorionic Gonadotropin
ICD	Information Consent Document
ICH	International Conference on Harmonization
ITT	Intention-To-Treat
IMPE	Institute of Malariology, Parasitology and Entomology
IRB	Institutional Review Board
LC-MS	Liquid Chromatography-Mass Spectrometry
LCF	Late Clinical Failure
LFU	Lost to Follow-Up
LPF	Late Parasitological Failure
MIPM	Military Institute of Preventive Medicine
MoH	Ministry of Health
NAMRU-2	Naval Medical Research Unit-2
NMRC-A	Naval Medical Research Center-Asia
PCR	Polymerase Chain Reaction
PP	Per Protocol
QA	Quality Assurance
RBC	Red Blood Cells
RDT	Rapid Diagnostic Test
SAE	Serious Adverse Event
ULN	Upper Limit of Normal
US	United States
WBC	White Blood Cells
WHO	World Health Organization
WTH	Withdraw

PART II. RECORD OF CHANGES TO PROTOCOL AS REQUESTED BY OTHER IRBS

PART III: SIGNATURES

Signatures below acknowledge that this research proposal has been reviewed and that the investigator agrees with the proposal as submitted.

_____

Nguyen Ngoc San — Date
MIPM Principal Investigator

_____

Huynh Hong Quang — Date
IMPE Co-investigator

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Nguyen Duc Manh — Date
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Michael D. Edstein — Date
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Kristina St.Clair — Date
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Kimberly Edgel Date
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Jean de Dieu Bizimana Date
Vysnova Partners Co-investigator

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Ho Huu Tho Date
Quality Monitor

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Nguyen Xuan Thanh Date
Research Monitor

PART IV:

A. SCIENTIFIC BACKGROUND AND OBJECTIVES

1. Background

Fixed-dose artemisinin based combination therapies (ACT) are still the cornerstone of malaria treatment globally. The artemisinins are very rapid acting antimalarial drugs and are combined with a slower but longer acting drug to prevent the original malaria infections from returning (i.e. recrudescence). Artemisinin reduced susceptibility in *Plasmodium falciparum* malaria (i.e. malignant malaria parasite) has emerged in six provinces (Binh Phuoc, Gia Lai, Dak Nong, Quang Nam, Khanh Hoa and Ninh Thuan) of Vietnam (World Health Organization - WHO, 2016) with prolonged parasite clearance times (i.e. the time it takes for the parasite to be removed from the patient's blood) following treatment with artemisinin compounds (Hien et al., 2012; Thriemer et al., 2014) and development of resistance to the long acting partner drug, piperaquine (Thriemer et al., 2014). However, a recent study in Binh Phuoc province has revealed dihydroartemisinin-piperaquine, the country's first-line ACT is starting to fail, with 26% treatment failures (Thanh et al, 2017). This worrisome situation reflects the emergence of artemisinin resistance that appeared about 10 years ago in western Cambodia (Denis et al., 2006) followed by high treatment failure rates of both artesunate-mefloquine (Rogers et al., 2009) and dihydroartemisinin-piperaquine (Saunders et al., 2014; Spring et al., 2015). Thus, there is an urgent need to combat and confine ACT resistance to prevent resistant parasites spreading throughout Southeast Asia, including Vietnam.

The indicator of choice for identifying suspected artemisinin resistance in *P. falciparum* is the proportion of patients who are parasitemic (i.e. parasites in blood) on Day 3 (WHO, 2016) after starting ACT treatment. A threshold of 10% as a Day 3 positivity rate has been useful in identifying malarious areas for more definitive clinical and laboratory studies of artemisinin resistance (Woodrow and White, 2016). Slow parasite clearance in malaria patients have provided the opportunity to carry out genotype–phenotype association studies that have revealed mutations in the *P. falciparum* Kelch-13 'propeller' region, as a molecular marker for artemisinin resistance (Ariey et al., 2014).

Until more effective non-ACTs are developed, WHO continues to recommend ACTs for frontline treatment of uncomplicated *P. falciparum* malaria, and also for uncomplicated *P. vivax* (WHO, 2015). The most recently developed ACT is pyronaridine-artesunate. Each drug has powerful blood stage action and the combination of the two is expected to show additional drug action in treating malaria infections. The action of artesunate is to rapidly kill the malaria parasites with the drug being cleared quickly due to its short elimination half-life in human blood. Pyronaridine is also effective in the short-term but has a lengthy blood half-life (14-16 days - Jittamala et al., 2015) thus providing a sustained blood action effect. As with all ACTs the aim of the fixed dose combination of pyronaridine-artesunate in the treatment of uncomplicated malaria is to provide a rapid reduction in parasitemia with a three-day regimen, thereby improving compliance and reducing the risk of recrudescence through the slower blood elimination of pyronaridine.

Clinical trials called Phase II/Phase III studies with pyronaridine-artesunate have been carried out in Africa and Asia. These studies compared the efficacy and tolerability of pyronaridine-artesunate and other ACTs (artemether-lumefantrine and artesunate-mefloquine) in adults and children with *P. falciparum* malaria and chloroquine for the treatment of benign relapsing *P. vivax* malaria. Overall, in two Phase II and four Phase III studies, a total of 2,815 patients were

treated with pyronaridine-artesunate, including 1,929 adults and older children (≥ 12 years) and 886 younger children (< 12 years) (Tshefu et al., 2010; Poravuth et al., 2011; Kayentao et al., 2012; Rueangweerayut et al., 2012; reviewed by Duparc et al., 2013). yPronaridine-artesunate was non-inferior (i.e. new treatment no worse than an established treatment) to artesunate-mefloquine or artemether-lumefantrine with a day-28 Polymerase Chain Reaction (PCR) corrected Adequate Clinical and Parasitologic Response (ACPR) >98% in the per-protocol population for the treatment of *P. falciparum* malaria (reviewed by Duparc et al., 2013). For the treatment of *P. vivax* malaria, pyronaridine-artesunate was non-inferior to chloroquine in treating patients from Cambodia, India, Indonesia and Thailand (Poravuth et al., 2011), and the ACT was well tolerated without untoward events and cleared parasitemia within three days when Indonesian patients were treated with pyronaridine-artesunate combined with primaquine (Nelwan et al., 2015).

These Phase III studies revealed pyronaridine-artesunate and the comparator drugs were well-tolerated with the only safety finding was a mild transient increase in liver enzymes (reviewed by Duparc et al., 2013). Re-treatment of pyronaridine-artesunate did not increase safety risk based on laboratory values, reported adverse event frequencies, or electrocardiograph findings (Sagara et al., 2016). More recent studies of pyronaridine-artesunate carried out in African and Southeast Asian countries including Vietnam also revealed the ACT to be well-tolerated (Nelwan et al., 2015; Leang et al., 2016). No drug related serious adverse events were reported in patients given pyronaridine-artesunate, including 163 malaria patients from Vietnam (Rueangweerayut et al., 2012).

In Southeast-Asia, the epicenter of multidrug-resistant *P. falciparum* for many years has been western Cambodia. Pyronaridine-artesunate has been studied at several field sites in western Cambodia with proven artemisinin resistance (Rueangweerayut et al., 2012; Leang et al., 2016). The efficacy cure rates range from 82% and 90% at Day 42 of follow-up, which is at and below the WHO recommended cut-off of 90% (WHO, 2009) when an alternative ACT needs to be sought. Most patients (95.9%) in the Leang et al. (2016) study conducted in the resistance zone of Cambodia harbored *P. falciparum* Kelch-13 C580Y mutant parasites. The poor efficacy response to pyronaridine-artesunate may have been due the high level of artemisinin resistance at the field sites, and low patient immunity of the Cambodian patients. No doubt these factors also contribute to the lower cure rates of other ACTs studied in Cambodia over the past 8 years such as artesunate-mefloquine and dihydroartemisinin-piperaquine with efficacies of 81% (Rogers et al., 2009) and 46% (Spring et al., 2015), respectively.

Although the efficacy findings of pyronaridine-artesunate in western Cambodia were disappointing, notwithstanding the small patient numbers, the ACT could still be an alternative option for the treatment of uncomplicated falciparum malaria in areas with a lower level of artemisinin resistance. In 2012, the European Medicine Agency (EMA) Article 58 adopted a positive scientific opinion for pyronaridine-artesunate (Pyramax®) tablets 180 mg/60 mg for the treatment of acute, uncomplicated malaria infection caused by *P. falciparum* and *P. vivax* in adults and children weighing 20 kg or more, albeit in areas of low transmission with evidence of artemisinin resistance as a single treatment course. No data are reported with pyronaridine-artesunate in the treatment of malaria due to *P. malariae* or *P. ovale*. In 2015, these recommendations were expanded by EMA with new labelling that allowed for repeated treatments and removed the geographic restrictions for tablets as well as extending to a specific paediatric formulation for 5 kg and above (www.mmv.org, 2015; Sagara et al., 2016).

Although pyronaridine-artesunate is registered for malaria therapy in Vietnam its current use is limited to investigational purposes only by the Vietnam Ministry of Health (MoH). Because reduced susceptibility to artemisinins is spreading in Vietnam it would be very useful to know whether pyronaridine-artesunate would be effective in treating malaria patients in areas of Vietnam with known artemisinin resistance. *In vitro* susceptibility data has shown pyronaridine to be markedly more active than piperaquine against both *P. falciparum* and *P. vivax* malaria, particularly at ring stages (Price et al., 2010). Thus, the long acting pyronaridine may be a better partner drug both in killing artemisinin resistant ring stages of *P. falciparum* and preventing recrudescences than piperaquine.

In this study, we propose to assess the effectiveness, safety and tolerability of pyronaridine-artesunate in treating *P. falciparum, P. vivax* and *P. malariae* malaria or mixed infections of these *Plasmodium* species in central Vietnam with known artemisinin resistance. Participants with mono-infections of *P. vivax* or mixed infections of *P. vivax* will also receive a standard 14-day treatment course of primaquine to kill the liver dormant stage hypnozoites.

2. Study Objectives

a. Primary objective:

To assess the therapeutic efficacy of pyronaridine-artesunate for the treatment of uncomplicated mono-infections of *P. falciparum* and *P. vivax* malaria in an area of reduced susceptibility to artemisinins.

b. Secondary objectives:

- To assess the therapeutic efficacy of pyronaridine-artesunate for the treatment of uncomplicated mono-infections of *P. malariae* malaria or mixed infections of the *Plasmodium* species.
- To assess the safety and tolerability of pyronaridine-artesunate for the treatment of uncomplicated malaria.
- Genotypic assessment of *P. falciparum* resistance.
- Determine drug exposure by measuring patient's plasma artesunate/dihydroartemisinin and blood pyronaridine concentrations.
- *In vitro* drug susceptibility of patient's falciparum parasites against standard antimalarial drugs.

B. STUDY METHODS

1. Study Procedures and Rationale

a. Justification for the Use of Human Subjects

The objective of the study is to measure the efficacy, safety and tolerability of the new ACT, pyronaridine-artesunate for the treatment of mono-infections of *P. falciparum. P. vivax* and *P. malariae* and mixed infections of the *Plasmodium* species. Although it is possible to conduct *in vitro* drug resistance assays, such procedures cannot accurately determine the clinical cure rate of drugs, particularly in settings in which there is some degree of acquired immunity to malaria. Therefore, information about clinical cure rates are needed to make decisions about antimalarial drug policy that can only be obtained from human studies.

b. Study Design

In two WHO sponsored therapeutic efficacy studies of dihydroartemisinin-piperaquine conducted at Dak Drong, Cu Knia, and Nam Dong Communes in Cu Jut District, Dak Nong Province in 2012 and 2014 the percentage of participants with Day 3 parasitemia after starting treatment were 29% and 27%, respectively, suggestive of reduced susceptibility to the first-

line treatment dihydroartemisinin-piperaquine (Quang HH, 2016). Mutations (C580Y and I543T) in the *P. falciparum* Kelch-13 gene have also been identified in Day 3 positive patients treated with dihydroartemisinin-piperaquine in the Cu Jut district of Dak Nong Province (Quang HH, 2016). Although dihydroartemisinin-piperaquine was highly efficacious (100% ACPR) in curing infections at the three communes, it is highly likely that ACT failures will occur in the not too distant future.

We propose to conduct a prospective, non-randomized, single-arm, open-labelled clinical trial of pyronaridine-artesunate at Thuan An Commune (Dak Mil District) and Dak Drong Commune (Cu Jut District). Both districts neighbour on Cambodia. We will also attempt to recruit patients from other nearby communes and districts. As with Cu Jut District we suspect falciparum parasite populations from Dak Mil District to have reduced susceptibility to artemisinins. It would be of scientific importance to carry out an observational trial of pyronaridine-artesunate in this area of Vietnam of reduced susceptibility to artemisinins before conducting a randomized comparative study of pyronaridine-artesunate versus dihydroartemisinin-piperaquine. Recruitment will be challenging as the numbers of malaria cases being reported in various malaria regions of Vietnam, including Dak Nong Province has markedly declined in 2015 and 2016. For this reason patient recruitment will be planned over 2 years.

c. Study Endpoints

The primary endpoints of the study are:

- Days 28 and 42 PCR corrected ACPR to assess therapeutic efficacy of pyronaridine-artesunate for the treatment of mono-infections of *P. falciparum*, *P. vivax* and *P. malariae* or mixed infections of these *Plasmodium* species in an area of known reduced susceptibility to artemisinins.
- New infection as evidenced by PCR-genotyping will be considered as treatment success.

Main secondary endpoints:

- To assess the safety and tolerability of pyronaridine-artesunate.
- Polymorphism of validated molecular markers of *P falciparum* resistance.

Other secondary endpoints:

- The numbers of patients with a positive malaria slide 72 hours after treatment initiation.
- Fever clearance time (i.e. the time taken for axillary temperature to fall below 37.5˚C or tympanic temperature to fall below 38.0˚C and remain there for at least 24 hours).
- Kaplan Meier analysis over 28 days and 42 days for recrudescences and reinfections. Hazard ratio of recurrent malaria parasites of falciparum and vivax malaria.
- PCR uncorrected and corrected ACPR at Days 28 and 42 following treatment.
- Gametocyte carriage rates and gametocyte clearance times.
- Documented AEs and SAEs and relationships to study drugs.
- Patient's plasma artesunate/dihydroartemisinin and blood pyronaridine concentrations.
- *In vitro* drug susceptibility of patient's falciparum parasites to standard antimalarial drugs.

d. Study Sites, Study Population, and Start and End Dates

The pyronaridine-artesunate study will be conducted at the health stations at Dak Drong and Thuan An Communes. The resident civilian population at Thuan An Commune has about 11,506 people, with 10 hamlets and Dak Drong Commune has about 14,450 people, with 19 hamlets. The people are from various ethnic minority groups with the Nùng group representing 46.3% of the population. Peak malaria transmission occurs from April to June and September to January, with the prevalence of *P. falciparum* and *P. vivax* being similar, with some *P.*

malariae. The time to complete recruitment, data analysis and publication of results in a peer-reviewed scientific journal will be over 3 years from May2018 to May 2021. Patients will be recruited from the surrounding study sites by active case detection by pre-screening febrile patients by malaria microscopy and rapid diagnostic test (RDT) before assessing their eligibility for study inclusion.

e. Sample Size

We propose a sample size of 55 patients for the *P. falciparum* mono-infection arm. Allowing for a 10% lost to follow-up and withdrawal, 50 patients will still fulfil the minimum sample of 50 patients that is required for a study to be representative as a WHO (2009) *P. falciparum* therapeutic efficacy study, with sufficient numbers to cover for patients who are likely to be lost during follow-up, withdrawal, and PCR-corrected reinfections (Stephniewska et al., 2006). With regards to the *P. vivax* mono-infection arm, since the baseline rates of vivax and falciparum malaria in this region are essentially equal, the same sample size goal of 55 patients will be pursued. With the additional inclusions of *P. malariae* and/or mixed infections of the three *Plasmodium* species of about 10 patients ACPR at Days 28 and 42 following treatment, the overall sample size goal is 120 patients.

f. Volunteer Recruitment and Enrolment

In areas of low endemicity, such as Vietnam where immunity is low, severe malaria infections can occur in all age groups. Because of this it is most important to evaluate antimalarial drugs in Vietnam in all age groups, including children. For this study, the efficacy of pyronaridine-artesunate will be assessed in both children weighing ≥20 kg and adults. At the study site, children weighing 20 kg are usually 7-9 years old.

A study doctor will ask adult and child patients entering the health station and who have a qualifying blood film examination for malaria if they would like to participate in this study. The study doctor will explain to the patient the study objectives and what is expected of them if they volunteer to participate in the trial. The study doctor will present the informed consent document to the potential participant or the parent/guardian of eligible minors. Consent will be obtained from either adults (≥18 years old) or permission from the parent or guardian on behalf of children under the age of 18. Assent will be obtained from all children greater than 10 years of age and older, and their assent will be accompanied by the permission of a parent or guardian. A witness, who is literate will ensure the accurate reading of the information and consent forms to participant's who are illiterate. The witness can be a parent/guardian, friend, relative or Commune leader/representative. A nurse from the Commune Health Station will also witness the accurate reading of the information and consent forms to participant's and will assist the study doctor in explaining health and medical questions associated with the study.

The study doctor will evaluate the detailed inclusion/exclusion criteria listed below to determine participant eligibility. Eligible women of childbearing age (10 to 55 years old) will be asked to provide a urine sample for pregnancy testing and will be considered eligible only if the test is negative. Eligible, consenting subjects will be enrolled; ineligible persons will be referred for routine therapy at the health station.

g. Inclusion Criteria:

- Adults and children ≥ 20 kg.
- Symptomatic of malaria infection (i.e. history of fever within 24 hours and/or presence of fever >37.5°C for axillary temperature and >38.0°C for tympanic temperature).
- Microscopic confirmation of asexual (i.e. blood) stages of *P. falciparum*, *P. vivax* or *P. malariae,* or mixed infections of the *Plasmodium* species.

- Glucose-6-phosphate dehydrogenase (G6PD) normal patients with mono-infection of *P. vivax* or mixed infections of *P. vivax* will be treated with primaquine to kill liver hypnozoites.
- Parasitemia between 250/µL and 100,000/µL.
- Ability to take oral medication.
- Written informed consent given to participate in the trial by the patient or in case of children up to 17 years old (assent for children aged 10 to 17 years old) with adult or guardian permission.

h. Exclusion Criteria:

- Pregnancy or lactation (urine test for β HCG to be performed on any woman of child bearing age 10 to 55 years old).
- Hematocrit <20%.
- Parasitemia <250/µL or > 100 000/µL.
- Signs or symptoms indicative of severe/cerebral malaria (WHO, 2014).
- Liver function test (alanine aminotransferase – ALT and aspartate aminotransferase - AST levels) more than 2.5 times the upper limit of normal (ULN) range.
- Total bilirubin > 2 ULN.
- Use of an antimalarial drug in the preceding 4 weeks.
- History of splenectomy, heavy alcohol use or injecting drugs of abuse.
- Any other condition, which in the judgment of the study physician would make participation in the study unsafe for the potential study patient.

i. Enrolment Procedure

All subjects enrolled in the study will be given a unique code. A case record form (CRF) will be completed with each patient documenting symptoms prior to health station attendance, concomitant illness and drug history. Height, weight, vital signs and physical examination findings will be also recorded.

j. Antimalarial Treatment - Drugs and Dosages

- G6PD normal participants with mono-infections of *P. vivax* or mixed infections of *P. vivax* will in addition to receiving the 3-day course of pyronaridine-artesuante be administered primaquine (0.25 mg/kg body weight daily for 14 days) as per Vietnam MoH national treatment guidelines. Primaquine treatment will commence on Day 0.

- Pyronaridine-artesunate tablet contains 60 mg artesunate + 180 mg pyronaridine. Dosing will be according to body weight (Table 1) with the target dosage range for pyronaridine–artesunate of 7.2-2.4 mg per kg of body weight to 13.8-4.6 mg per kg.

Table 1: Pyronaridine-artesunate dosage by body weight

Body Weight (kg)	Daily dose (mg)		Number of tablets daily for 3 days
	Pyronaridine	Artesunate	
≥20 - < 24 kg	180	60	1 tablet
≥24 - <45 kg	360	120	2 tablets
≥45 - < 65 kg	540	180	3 tablets
≥ 65 kg	720	240	4 tablets

Pyronaridine-artesunate will be taken orally with water (100 mL), once daily for 3 days. Each dose will be administered under supervision in the health station or if not possible

by a home visit to the patient's home. Patients will remain at the health station or at home for observation for one hour after each dose. If he/she vomits within 30 minutes after drug administration on the first dosing, the dose will be repeated in full. If the patient vomits their second administration, they will be withdrawn from the study and referred to a local doctor for possible parenteral therapy based on the national malaria treatment guidelines. The investigator will observe consumption of all medication, and will document drug administration in the case report form (CRF).

- **Rescue Treatment**

The following parasitemia findings will prompt administration of the rescue therapy as per WHO guidelines (2009):

1) Asexual parasitemia on Day 2 > count on day 0 (first day of treatment).
2) Asexual parasitemia on Day 3 with axillary temperature ≥37.5ºC.
3) Asexual parasitemia on Day 3 ≥ 25% of count on day 0.
4) Asexual parasitemia recurs between Days 4 and 42.

If a patient is unable to tolerate the trial medication he/she should discontinue the treatment and alternative antimalarial drug should be initiated. In this case, the reason for discontinuation should be recorded in the CRF as "Adverse Event" and be withdrawn from the study. The patient will receive parenteral therapy with artesunate as per national treatment guidelines. Any patient who develops signs of severe or complicated malaria will be hospitalized and will receive parenteral therapy of artesunate (i.e. first dose of 2.4 mg/kg followed by 2.4 mg/kg at 12 and 24 hours, and then 2.4 mg/kg daily until the patient becomes conscious or can swallow alternative medication). When the patient is able to swallow he/she will be given treatment with oral dihydroartemisinin-piperaquine as per Vietnam MoH national treatment guidelines (2016).

Recurrent infections with *P. falciparum* will be treated with a 3-day course of dihydroartemisinin-piperaquine according to the Vietnam MoH national treatment guidelines (2016) and the patients will be followed up by the health station staff. Recurrent *P. malariae* infections will be treated with chloroquine (25 mg/kg over 3 days) according to the Vietnam MoH national treatment guidelines (2016) and the patients will be followed up by the health station staff.

Pyronaridine-artesunate is expected to clear the blood stages of *P. vivax*, but if an episode of *P. vivax* malaria occurs during the 42 day follow-up period, the participant will be treated with chloroquine (25 mg/kg over 3 days) and primaquine (0.25 mg/kg daily for 14 days) according to the Vietnam MoH national treatment guidelines (2016) and the patients will be followed up by the health station staff. Before receiving primaquine the G6PD status of the participant must be determined.

- **Pregnancy Treatment**

In case of pregnancy, the antimalarial treatment shall be the one recommended by the Vietnam MoH. The patient will not be included in the study. Female study patients will be encouraged to communicate to the Principal Investigator and manufacturer Shin Poong Pharmaceutical Company if they become pregnant within a period of two months after the start of the treatment. The evolution of the pregnancy will be monitored by the study doctors with visits at 3, 6, 9 months and after the delivery. If the study doctor is not present the outcome of the pregnancy will be determined by the health station medical doctor and reported to the Principal Investigator. Generally,

follow-up will be no longer than 4 weeks after the estimated delivery date. Information on the drugs taken during the pregnancy as well as AEs/SAEs and the health status of the newborn will be collected; thereafter the newborn will be followed at 6 and 14 weeks. Any premature termination of pregnancy will also be reported. While pregnancy itself is not considered an adverse event, any complication of pregnancy or elective termination for medical reasons must be recorded as an adverse event or a serious adverse event and reported to Shin Poong Pharmaceutical Company. A spontaneous abortion is always considered a serious adverse event and will be reported as such.

- **Adjunctive Therapy**

Adjunctive therapy, including analgesics, anti-pyretics, or rehydration, will be given at the discretion of the study doctor to provide adequate supportive care except for antibiotics with antimalarial activity (e.g. doxycycline, azithromycin). If these are required, the patients will be kept in the study and for antibiotics will be noted as a protocol deviation. Antimalarials for recurrent infections will be prescribed as described above. Haematinics and anti-helminthics may be prescribed after Day 7 if indicated. Any medication, other than the study medication taken during the study period will be recorded in the CRF. For fever control, tepid sponging and oral acetaminophen (0.5 to 1 gm every 4 hours) will be used for axillary temperature ≥37.5ºC or tympanic temperature ≥38.0^{o}C.

- **Primaquine Therapy**

For the treatment of hypnozoites of *P. vivax* or mixed infections of vivax malaria, patients will be treated with a standard course of primaquine. Participants who are G6PD normal will be given primaquine as per the Vietnam MoH national treatment guidelines of 0.25 mg/kg body weight daily for 14 days. G6PD deficiency will be determined for each patient using the Carestart G6PD Rapid Diagnostic Test (RDT) (CSG; Access Bio, New Jersey, USA) (Espino et al., 2016).

Primaquine treatment will commence on Day 0 with pyronaridine-artesunate treatment. Primaquine administration will be observed over the first 3 days of treatment by the study team but the remaining 11 days of primaquine treatment will be unsupervised. The participant will be responsible for taking their remaining medication as they would normally do if not participating in the present study. It has been reported by Leslie et al. (2004) that supervised or unsupervised primaquine treatment showed a similar degree of protection against relapsing vivax malaria. For G6PD deficient participants primaquine will not be administered. For these participants the blood schizontocidal action and tolerability of pyronaridine-artesunate alone will be assessed. The slowly eliminated pyronaridine (half-life of 14-16 days - Jittamala et al., 2015) is expected to prevent relapses up to 5-7 weeks following treatment with the ACT (WHO, 2015).

k. Source of Study Drugs and Storage

Pyronaridine-artesunate (Pyramax®) will be obtained from National Institute of Malariology Parasitology and Entomology which will be sponsored by Shin Poong Pharmaceutical Company with a certificate of analysis for the batch/lot number of the obtained Pyramax® tablets provided to the Vietnam IRB MoH before commencement of the study. Artesunate (IV), Arterakine (dihydroartemisinin-piperaquine), chloroquine, and primaquine will be sourced from a reliable supplier recommended by the Vietnam MoH. All drugs will be stored in a secure temperature-controlled (<30°C) area, with access limited to the investigators. All movements of study medication will be recorded. Both individual patient and overall drug

accountability records will be kept up to date by the study staff. Pyramax tablets will be destroyed by incineration at the end of the study after the Principal Investigator has completed a close-out audit of drugs used.

l. Monitoring and Follow-Up

After commencement of treatment blood smears and temperatures will be collected twice daily (i.e. about 12 hours apart) until the patient's blood smears are negative on two consecutive collections and his/her axillary temperature remains <37.5ºC/tympanic temperature remains <38ºC for more than 24 hours. The follow-up period for participants on pyronaridine-artesunate will be 42 days. Participants will be asked to re-visit the health station on Days 7, 14, 21, 28, 35 and 42, plus any other day that he/she feels sick, in order to monitor clinical recovery/recurrence of malaria symptoms. If this is not possible the study team will try to visit the subject at their home. In the event that a subject fails to re-visit the health station on schedule, a member of the study team will attempt to locate the subject and encourage he/she to visit the health station for the scheduled follow-up. Any time a subject is referred to the study doctor for a reason other than routine follow-up, the doctor-subject contact will be recorded.

m. Discontinuation / Withdrawal of Participants from the Study

Each participant has the right to withdraw from the study at any time. In addition, the investigator may withdraw a participant if he or she considers it necessary for any reason including:

- Development of severe malaria.
- Repeated vomiting within 30 minutes after administration of study drug after the second day of dosing.
- A determination by the on-site doctor that the subject risks his/her health by further participation.
- Development of a serious disease that requires referral to hospitalized treatment to be reported as an SAE.
- An adverse event or disease progression which requires discontinuation of the study medication or results in inability to continue to comply with study procedures.
- Significant non-compliance with treatment regimen or study requirements such as the inability to locate a subject.
- Ingestion of any antimalarial by subject outside protocol parameters.
- Allergic reaction to study drug.

If an individual is prematurely discontinued from study treatment for any reason, the investigator must make every effort to perform the following evaluations: physical examination and vital signs assessment, hematocrit level, parasite count and AE assessment. The reason for withdrawal will be recorded in the CRF. If the subject is withdrawn due to an adverse event, the investigator will arrange for follow-up visits until the adverse event has resolved or stabilised.

n. Adverse Event Monitoring and Reporting

Shin Poong Pharmaceutical Company have a responsibility to European Medicines Agency (EMA) in terms of the oversight of Pyramax pharmacovigilance.

It is the responsibility of the study doctor to detect and document all adverse events which occur during the study and which fulfil the definitions and criteria outlined in this protocol 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 unfavourable 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 is one that represents an actual or potentially significant hazard. This includes experiences that are fatal, life threatening, permanently disabling, require hospitalisation, 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 AE that:
    - results in death
    - is life-threatening
    - requires inpatient hospitalisation or prolongation of existing hospitalisation
    - results in persistent or significant disability/incapacity
    - is a congenital anomaly/birth defect
    - requires acute medical or surgical care to prevent one of the outcomes listed above

- ALT or AST >3xULN and total bilirubin .2xULN (Hy's Law)

c. *Action Taken:* Actions taken in response to an adverse event can be classified as follows:
   1) None
   2) Study 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 study doctor will attempt to determine a causal association between the adverse event and the study drug.

Reporting of Adverse Events of Special Interest: An adverse event of special interest (AESI) is an adverse event for which ongoing monitoring is appropriate within the context of the study. These events necessitate complementary examinations in order to characterize and understand them. AE's is in this study can be related to hepatotoxicity.

The study team is to take particular notice of symptoms/signs suggestive of clinical and biological signs of possible hepatotoxicity such as:

1. ALT or AST >3xULN with the appearance of worsening of fatigue, nausea, vomiting, fever, rash, or eosinophilia.
2. AST or ALT increase >5xULN.

In all the cases of hepatotoxicity, confirmed by the Principal Investigator and Research Monitor, the Principal Investigator is to notify the Institutional Review Boards (IRB) that approved the study and Shin Poong Pharmaceutical Pharmacovigilance within 72 hours of the occurrence.

Reporting of Adverse Events: In the unlikely event of a death or a related adverse event which is rated as serious, the Principal Investigator will immediately notify the Research Monitor and no further patients will be admitted to the study until the details of the adverse event have been evaluated by the Research Monitor. If a SAE and/or an unanticipated problem involving risks to subjects or others occur in the course of the study, the Principal Investigator must prepare a detailed case history and together with the CRF, Fax the clinical data within 24 hours or earlier to the Research Monitor and Shin Poong Pharmaceutical Pharmacovigilance. The Principal Investigator will also notify the IRB that approved the study within 72 hours of the occurrence.

o. Laboratory procedures

1) Blood Smear Microscopy

Thick and thin blood films for parasite counts should be obtained and examined at screening on Day 0 to confirm adherence to the inclusion and exclusion criteria. Thick and thin blood films will be also examined on Days 1, 2, 3, 7, 14, 21, 28, 35 and 42 or on any other day if the patient returns spontaneously and parasitological reassessment is required. Specimens will be labelled anonymously (study number, time, date).

A fresh Giemsa stain dilution will be prepared at least once a day and possibly more often, depending on the number of slides to be processed. Giemsa-stained thick and thin blood films will be read by a qualified microscopist at a magnification of 1000× to identify the parasite species and to determine the parasite density.

Two thick and two thin blood slides per patient will be obtained. One slide will then be stained rapidly (10% Giemsa for 15 min) for initial screening, while the others will be retained. If the patient is subsequently enrolled, the second slide will be stained more carefully (4% Giemsa for 45 min), and slower staining will also be used for all slides obtained at follow-up visits. The study number of the patient, the time and the date of follow-up will be recorded on the frosted edge of the slide with a permanent black pen or pencil. The thick blood smear for initial screening will be used to count the numbers of asexual parasites and white blood cells in a limited number of microscopic fields.

The second blood smear will be used to calculate the parasite density, by counting the number of asexual parasites in a set number of white blood cells (WBC) (typically 200) with a hand tally counter. Once a field has been started, it must be counted to completion; the final number of WBC will therefore rarely be exactly 200. If more than 500 parasites have been counted before 200 WBC have been reached, the count will be stopped after the reading of the last field has been completed. 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).

The same technique will be used to establish the parasite count on each subsequent blood film. When the number of asexual parasites is less than 100 per 200 WBC in follow-up smears, counting will be done against at least 500 WBC (i.e. to completion of the field in which the 500th white blood cell is counted). A blood slide will be considered negative when examination of 1,000 WBC reveals no asexual parasites.

The presence of gametocytes on an enrolment or follow-up slide will be counted against 500 (WBC). In addition, 100 fields of the second thick film will be examined to detect mixed infections; in case of any doubt, the thin film will be examined for confirmation.

Two qualified microscopists, preferably WHO certified Level 1 or 2, will read all the slides independently, and parasite densities will be calculated by averaging the two counts. Blood films with discordant results (differences between the two microscopist in species diagnosis, in parasite density of >50% or in the presence of parasites) will be re-examined by a third, independent microscopist, and parasite density will be calculated by averaging the two closest counts.

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 study team.

In addition to microscopy, patient's blood will be screened at admission (Day 0) with the RDT [i.e., SD Bioline Malaria Ag *Pf*/*Pf*/*Pv* (HRP2/pLDH; Product Code 05FK120)] from Standard Diagnostics Inc. to provide a cross-check against microscopy and to assist in detecting mixed infections.

2) Basic hematology and blood biochemistry

Blood sample (3 mL) for hematology and biochemistry assessments will be collected from each participant before treatment (Day 0), Day 7 and Day 28. The following hematology tests will be performed:

- Hemoglobin
- Hematocrit

- Platelets
- White Cell Count
- Granulocytes
- Lymphocyte and Monocyte Count
- Red Blood Cell Count

The following biochemical assessments will be done:

- Urea
- Total Bilirubin
- Aspartate Aminotransferase
- Alanine Aminotransferase
- Gamma Glutamyl Transferase
- Alkaline Phosphatase
- Creatinine

3) Genotyping of Malaria Parasites

In order to differentiate a recrudescence (same parasite strain) from a newly acquired infection (different parasite strain) of *P. falciparum*, a genotype analysis will be conducted. This is based on the extensive genetic diversity among the malaria parasite genes *msp1*, *msp2* and *glurp* (Padley et al., 2003; Imwong et al., 2009; WHO, 2008). The genotypic profiles of pre- and post-parasite strains will be compared. The copy number of the *P. falciparum* mdr1 gene for mefloquine resistance will be measured as well as sequencing of the Kelch-13 gene for artemisinin resistance. For the Kelch-13 gene, polymorphisms associated with codon changes (C580Y, Y493H, R539T, I543T, N458Y, R561H) identified by the WHO report (April 2017) on artemisinin resistance will be evaluated using a previously described method (Tun et al., 2015). Recently, two molecular markers for piperaquine resistance have been identified by Amato and colleagues (2016), an exonuclease gene polymorphism on chromosome 13 (*exo-E415G*) and an amplification in *plasmepsins 2* and *3* will be also be evaluated.

For *P. vivax* malaria, three microsatellite markers (Pv3.27, MS16 and *msp1*F3) will be used to genotype the admission parasitemia (Day 0) as described by Koepfi et al. (2009) and compared with any recurrent infections of *P. vivax* over the 42 day follow-up period. If the recurrent *P. vivax* malaria is genotypically different to the Day 0 parasites, then the treatment outcome will be considered successful.

The most popular and sensitive RDTs use an antigen unique to *P. falciparum*; the histidine-rich protein 2 (PfHRP2) and also the histidine-rich protein 3 (PfHRP3). Because RDTs play a critical role in informing malaria treatment and surveillance, we will also determine whether any participant is RDT negative for the histidine-rich protein 2 (PfHRP2) and/or the histidine-rich protein 3 (PfHRP3) but blood smear positive. In Vietnam, *pfhrp3* gene deletions have been identified but as yet not *pfhrp2* gene deletions in falciparum malaria (Cunningham, 2017). The detection of *pfhrp2* and *pfhrp3* gene deletions in *P. falciparum* will be performed as previously described (Gamboa et al., 2010; Cheng et al., 2014).

In order to minimize discomfort to the patient due to repeated finger pricks, four drops of blood will be collected on Whatman 31 ET Chromatography filter paper during screening or enrolment, Day 1 after starting treatment and each time blood smears are required according to the protocol on and after Day 7. Specimens will be labelled anonymously (study number, day of follow-up, date), kept in individual plastic bags with

desiccant pouches and protected from light, humidity and extreme temperature until analysed. The plastic bags containing blood spots on filter paper will stored in a refrigerator with great care taken to protect samples from moisture. The PCR techniques will be performed be performed at MIPM or ADFMIDI. Paired filter papers will be used for parasite DNA extraction and genotyping.

The patient's de-identified blood spots will be kept for up to 10 years from the time of blood collection and if new genetic markers for parasite antimalarial drug resistance are identified we may re-test the patient's samples for these markers. The participant information consent document seeks approval from the patient for the re-testing of their filter paper blood spots/parasite DNA for future new genetic markers of antimalarial drug resistance. The patient's samples collected under this protocol will not be used for genetic analysis of other diseases.

4) Antimalarial Drug Analysis

Patients will be invited to provide a blood sample (0.5 mL) 1 hour after the last dose on Day 2 (i.e. 3 days after starting treatment) to determine baseline plasma concentrations of artesunate and dihydroartemisinin (the major active metabolite of artesunate) and blood concentrations of pyronaridine on Day 7 after commencement of treatment. The time to peak concentration of dihydroartemisinin is about 1 hour after artesunate administration and between 2 and 8 hours post-dose for pyronaridine (European Medicines Agency, 2014). Previously, Day 7 blood concentrations of partner antimalarials such as lumefantrine (WWARN, 2015) and piperaquine (Price et al., 2007) administered as ACTs have provided valuable information as a predictor of drug exposure and efficacy. In this study, Day 7 pyronaridine concentrations will also be measured to determine whether the sample point could act as a measure of exposure and treatment outcome.

The blood samples for artesunate/dihydroartemisinin analysis will be collected by venipuncture using potassium oxalate/sodium fluoride as the anticoagulant and plasma separated by centrifugation. Blood samples for pyronaridine measurement will be collected in EDTA tubes. Plasma and blood samples will be stored in liquid nitrogen in the field and transported to MIPM and ADFMIDI on dry ice. Plasma artesunate/dihydroartemisinin and blood pyronaridine concentrations will be measured by liquid chromatography mass spectrometry (LC-MS) using the method described by Hodel et al. (2009).

5) *In Vitro* Drug Susceptibility Testing

All 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 (MSF) assay as described by Johnson et al. (2007) or the [^{3}H]-hypoxanthine growth inhibition assay (Desjardins et al., 1979) for falciparum malaria. The drugs to be tested will be chloroquine, desethylamodiaquine, piperaquine, pyronaridine, mefloquine, lumefantrine, methylene blue and dihydroartemisinin. Falciparum parasites will be cryopreserved with glycerolyte and stored in liquid nitrogen for further evaluation at MIPM and ADFMIDI. The *in vitro* ring-stage survival assay (RSA) for artemisinin resistance (Witkowski et al., 2013) and the piperaquine survival assay (PSA) for piperaquine resistance (Duru et al., 2015) will also be performed.

6) 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 pyronaridine-artesunate is

contraindicated during pregnancy. They will also be asked to take a urine pregnancy test on Day 28 and Day 42 after starting the pyronaridine-artesunate treatment course or on early withdrawal from the 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.

p. Detailed Description of Study Phases and Visits

Visit 1: Day 0: Screening and Enrollment of Patients

- Collect from potential patients a small sample of finger prick blood to produce two thick and two thin smears to confirm *P. falciparum, P. vivax, P. malariae* or mixed infections;
- Briefing of the potential patient for enrollment;
- Provide patient information and obtain informed consent/ascent;
- Re-check inclusion and exclusion criteria;
- Vital signs including body temperature and medical history;
- Conduct physical examination;
- For the treatment of patients with *P. vivax* or mixed infections of *P. vivax* a G6PD test will be performed;
- Collect blood for hematology and biochemical measurement;
- Urine HCG pregnancy test for all female subjects 10 to 55 years of age;
- Officially enroll patients, initiate CRF (Screening and treatment Day 0);
- Collect four blood spots on Whatman 31 ET Chromatography filter paper for PCR analysis, and hematocrit;
- For adults with mono-infection of *P. falciparum* collect 4 mL of venous blood for *in vitro* drug susceptibility testing;
- Administer first dose (Day 0) of the 3 day regimen of pyronaridine-artesunate according to the patient's body weight. G6PD normal patients with mono-infections of *P. vivax* or mixed infections of *P. vivax* will also receive a dose of primaquine according to the patient's body weight. All drug treatments will take place at either the health station or at the patient's home with observed treatment by the study team.
- All subjects will remain at the health station or at their home for at least an hour following the administration of the medication;
- At Enrollment the patients will be informed by the Study Doctor of the following:
  1. To either re-visit the health station daily for another 2 days or be treated daily for another 2 days at the patient's home for supervised treatment and until they are blood smear negative for malaria for 24 hours and without fever for 24 hours.
  2. To revisit the health station on Days 7, 14, 21, 28, 35 and 42 after commencement of pyronaridine-artesunate treatment for monitoring of parasitemia and physical assessment.

Visits 2-3: Day 1, 2: Continuation of Drug Treatment

- Administer, review and update CRF (treatment Day 1, Day 2);
- Review of medical history and assess vital signs (temperature will be collected twice daily at intervals of about 12 hours apart until afrebile for 24 hours);
- Obtain finger prick blood for blood smears (two thick and two thin) at about 12

hour intervals apart until blood smear negative for malaria on three consecutive bleeds;

- Conduct physical examination and adverse events assessment;
- Continue to administer pyronaridine-artesunate on Day 1 and Day 2 according to the patient's body weight until completion;
- Continue to administer primaquine to G6PD normal patients with mono-infections of *P. vivax* or mixed infections of *P. vivax* on Day 1 and Day 2 according to the patient's body weight;
- All subjects will remain at the health station for at least an hour following the administration of the treatment course;
- Collect blood sample (0.25 mL) from the vein of patients 1 hour after the last dose of pyronaridine-artesunate on Day 2 (3 days after starting treatment) for the measurement of plasma artesunate/dihydroartemisinin concentrations by LC-MS;
- If the subject is found to have parasitemia suggestive of early treatment failure or late clinical failure during the visits, an additional blood sample (0.5 mL) from the vein will be collected for blood smears, PCR and LC-MS analysis. Rescue therapy will then be administered;
- The rescue therapy will consist of a 3 day course of dihydroartemisinin-piperaquine according to national treatment guidelines;
- Any subject who experiences a second episode of parasitemia will be withdrawn from the study and will not be required to continue any follow-up visits after completion of the rescue therapy. Once the rescue therapy has been initiated, the subject will be referred to the health station for any follow-up procedures based on national treatment guidelines. These subjects' data will be included in the final data set but censored up to the day of second episode of parasitemia;
- If a subject develops severe malaria, the subject will be withdrawn from the study, the study medication will be discontinued, and the subject will be referred to the district or referral hospital for intravenous artesunate therapy;
- In the unlikely events of a death or any SAE which is related as serious, the investigator will immediately notify the Research Monitor and Shin Poong Pharmaceutical Pharmacovigilance and no further patients will be admitted to the study until the details of the SAE have been evaluated by the Research Monitor in conjunction with Shin Poong Pharmaceutical Company;
- If a SAE and/or an unanticipated problem involving risks to subjects or others occur in the course of the study, the Principal Investigator must prepare a detailed case history and together with the CRF, fax the clinical data within 24 hours or earlier to the Research Monitor and Shin Poong Pharmaceutical Pharmacovigilance. The Principal Investigator will also notify each IRB that approved the study within 72 hours of its occurrence.

Visits 4: Day 3: Parasitemia check

- Administer, review and update CRF (parasitemia check);
- Review of medical history and assess vital signs (temperature will be collected twice daily at intervals of about 12 hours apart until afebrile for 24 hours);
- Obtain finger prick blood for blood smears (two thick and two thin) at about 12 hour intervals apart until blood smear negative for malaria on three consecutive bleeds;
- The sample on Day 3 will be taken as close as possible to 72 h after the initial blood smear (i.e. just before commencement of treatment with pyronaridine-artesunate);
- Conduct physical examination and adverse events assessment;

Visits 5-10: Days 7, 14, 21, 28, 35 and 42(± 2 days): Post Treatment Follow-up Visits, including Unscheduled Visits

- Administer CRF (follow-up Days 7, 14, 21, 28, 35 and 42 for the treatment of *P. falciparum, P. vivax, P. malariae* or mixed infections*;*
- Review of medical history and assess vital signs;
- Conduct physical examination and adverse events assessment;
- Collect finger prick blood for blood smears (two thick and two thin) and two blood spots on Whatman 31 ET Chromatography filter paper for PCR analysis;
- Collect blood for hematology and biochemical measurement on Day 7 and Day 28 only;
- Collect blood sample (0.25 mL) from the vein of patients on Day 7 after starting pyronaridine-artesunate treatment for the measurement of blood pyronaridine concentrations by LC-MS;
- If the subject is found to have a late parasitological failure during the visits, an additional blood sample (0.25 mL) from the vein will be collected for blood (two thick and two thin) smears, PCR and LC-MS analysis. Rescue therapy will then be administered;
- Female patients of child-bearing age (10 to 55 years old) will undergo a urine pregnancy test on Day 28 and Day 42;
- The rescue therapy will consist of a 3 day course of dihydroartemisinin-piperaquine according to national treatment guidelines;
- Any subject who experiences a second episode of parasitemia will be withdrawn from the study and will not be required to continue any follow-up visits after completion of the rescue therapy. Once the rescue therapy has been initiated, the subject will be referred to the health station for any follow-up procedures based on national treatment guidelines. These subjects' data will be included in the final data set but censored up to the day of second episode of parasitemia;
- If a subject develops severe malaria, the subject will be withdrawn from the study, the study medication will be discontinued, and the subject will be referred to the district or referral hospital for intravenous artesunate therapy;
- In the unlikely events of a death or an adverse event which is related as serious, the investigator will immediately notify the Research Monitor and no further patients will be admitted to the study until the details of the adverse event have been evaluated by the Research Monitor;
- If a SAE and/or an unanticipated problem involving risks to subjects or others occur in the course of the study, the Principal Investigator must prepare a detailed case history and together with the CRF, fax the clinical data within 24 hours or earlier to the Research Monitor and Shin Poong Pharmaceutical Pharmacovigilance. The Principal Investigator will also notify each IRB that approved the study within 72 hours of its occurrence.

2. Data Handling and Analysis

a. Data and Material Management

The Principal Investigator will ensure that the study protocol is strictly adhered to and that all data are collected and recorded correctly in the CRF. Any change or correction to a CRF will be dated and explained and should not obscure the original entry. The Principal Investigator will be responsible for the safe keeping of CRFs and the completed patient identification code list in a secure location (i.e. locked cabinet). The Principal Investigator will complete the CRF for inputting into a computerized database. A checklist for blood sample storage for blood

smears, filter paper blood spots and cryopreserved blood will be maintained to track the collection of material.

b. Therapeutic Efficacy

The primary endpoint will be PCR-adjusted Days 28 and 42 cure rates for patients treated with pyronaridine-artesunate. Secondary endpoints will be parasite and fever clearance times and the occurrence of adverse events. Efficacy data will be assessed by means of Kaplan-Meier survival analysis and a *per-protocol* analysis, with patients reinfected during the follow-up period considered as cured. Classification of treatment outcomes will be by WHO guidelines (WHO, 2009) as follows:

1) Early treatment failure (ETF)
   - danger signs or severe malaria on Day 1, 2 or 3 in the presence of parasitemia.
   - parasitemia on Day 2 higher than on Day 0, irrespective of axillary or tympanic temperature.
   - parasitemia on Day 3 with axillary temperature ≥37.5ºC or tympanic temperature ≥38.0ºC.
   - parasitemia on Day 3 ≥ 25% of count on Day 0.

2) Late clinical failure (LCF)
   - danger signs or severe malaria in the presence of parasitemia on any day between Days 4 and 42 for mono-infections of *P. falciparum, P. vivax* and *P. malariae* or mixed infections of these *Plasmodium* species in patients who did not previously meet any of the criteria of ETF.
   - presence of parasitemia on any day between Days 4 and 42 for mono-infections of *P. falciparum, P. vivax* and *P. malariae* or mixed infections of these *Plasmodium* species with axillary temperature ≥37.5ºC or tympanic temperature ≥38.0ºC (or history of fever) in patients who did not previously meet any of the criteria of ETF.

3) Late parasitological failure (LPF)
   - presence of parasitemia on any day between Days 7 and 42 for mono-infections of *P. falciparum, P. vivax* and *P. malariae* or mixed infections of these *Plasmodium* species with axillary temperature <37.5ºC or tympanic temperature <38.0ºC in patients who did not previously meet any of the criteria of ETF or LCF.

4) Adequate clinical and parasitological response (ACPR)
   - absence of parasitemia on Days 28 and 42 for mono-infections of *P. falciparum, P. vivax* and *P. malariae* or mixed infections of these *Plasmodium* species after treatment, irrespective of axillary or tympanic temperature, in patients who did not previously meet any of the criteria of ETF, LCF or LPF.

c. Statistical Analysis

The primary analysis will be on an '*intention-to-treat'* (ITT) and '*per protocol'* (PP) basis, including all patients enrolled into the study. Kaplan-Meier analysis will be used to estimate the primary efficacy endpoints that is the proportion of patients with uncomplicated mono-infections of falciparum, vivax and malariae malaria as well as mixed *Plasmodium* infections who achieved a PCR-adjusted ACPR at Days 28 and 42. Hazard ratio of recurrent malaria parasites of falciparum and vivax malaria will be calculated. The proportion of patients with parasite or fever clearance at days 1, 2, and 3 were compared by use of exact 95% confidence intervals (CIs).

C. ORGANIZATION OF RESEARCH EFFORT AND RESPONSIBILITIES

1. Duties and Responsibilities of Investigators, Quality Monitor and Research Monitor

- Dr. Nguyen Ngoc San (MIPM), the Principal Investigator, will input into the study design, be responsibility for the day-to-day conduct of the study, will assist in data analysis and the write up of the study. The Principal Investigator will ensure that the study doctors and technicians are appropriately qualified to conduct the therapeutic efficacy study to Good Clinical Practice (GCP) standards and that appropriate study Standard Operating Procedures and Registers of Data are available to the study team. If a SAE and/or an unanticipated problem involving risks to subjects or others occur in the course of the study, the Principal Investigator must prepare a detailed case history and inform the Research Monitor and Shin Poong Pharmaceutical Pharmacovigilance within 24 hours or earlier of the occurrence. The Principal Investigator will also notify each IRB that approved the study within 72 hours of its occurrence. Progress SAEs, unanticipated events, protocol deviations, security of documentation, and progress and final reports will also be provided by the Principal Investigator to the approving IRBs in a timely manner as specified by these agencies.

  Dr. Nguyen Ngoc San (MIPM) will be responsible for the conduct of the study at Thuan An and Dak Drong Communes, and other nearby communes and districts with the support of Dr. Huynh Hong Quang, who is a senior infectious disease doctor employed by the Vietnam MoH.

- Dr. Huynh Hong Quang (IMPE) will input into the study design, provide direct support to the Principal Investigator, participate in the conduct of the study, assist in data analysis and the write up of the study.

- Dr. Nguyen Duc Manh (MIPM) will input into the study design, assist with selecting the field site, provide resource allocation, review clinical documentation of participants, and assist in writing up of the study.

- Dr. Michael Edstein (ADFMIDI) will advise on the study design, coordinate the laboratory tests, analysis of the data, assist in writing up of the study and will provide quality assurance on the conduct of the study. Drs San and Edstein will coordinate the laboratory analysis for *in vitro* drug susceptibility testing of field isolates, PCR analysis of filter paper blood spots for *Plasmodium* species and genotyping for drug resistance, and antimalarial drugs analysis by LC/MS.

- Dr. Kristina St. Clair (NMRC-A) and Dr. Kimberly Edgel (NAMRU-2) will be responsible for adherence to all relevant Navy regulations for the conduct of human subject research. Drs. St Clair and Edgel will also assist in the design, implementation, analysis of data, write up of the study and will provide quality assurance on the conduct of the study.

- Dr. Jean de Dieu Bizimana (Vysnova Partners) will advise on statistical analysis, study design and calculation of sample size to match with study objectives and assist with the presentation and write up of the study.

- Dr. Ho Huu Tho (Vietnam Military Medical University) the quality monitor will advise on coordination of study activities at the field site within Vietnam, will perform quality monitoring and reporting of protocol procedures, and assist with the presentation and write up of the study.

- Dr. Nguyen Xuan Thanh **(**Thu Cuc International Hospital) will serve as the research monitor. He will ensure compliance to the medical ethics guidelines described above and will act as the point-of-contact for subjects with ethical or medical concerns about their participation in the study.

2. Qualification of Principal Investigators and Research Monitor

The Principal Investigator, Dr. Nguyen Ngoc San is a senior medical doctor with PhD in malaria chemotherapy with knowledge and experience in therapeutic efficacy studies and drug resistance in the central highlands of Vietnam. The Research Monitor, Dr. Nguyen Xuan Thanh has medical and PhD degrees and over 20 years of experience in infectious diseases, including malaria.

3. Multicenter Organizational Plan for IRB Review and Approval

The Principal Investigator will ensure that this study is conducted in compliance with the current revision of the Declaration of Helsinki (Seoul 2008) and that it will follow the principles of the ICH Guidelines for GCP 1996. Ethical review will be performed by the IRBs of the Vietnam MoH and DDVAHREC (formerly the Australian Defence Human Research Ethics Committee). US Navy review will be conducted in accordance with guidelines established for extramural human research. The ethics team may visit the study sites at any time during the course of the study to ensure that all research process and procedures are completely adhered to that stated in the submitted protocol. The study may begin once final approvals by the IRBs of Vietnam MoH, DDVAHREC and NMRC-A, and NMRC-A Commanding Officer have been granted.

4. Technology Transfer/Capacity Building/Sample Sharing Plan

The conduct of this protocol will strengthen the capacity of Vietnamese doctors and scientists at MIPM and IMPE to perform clinical trials under good clinical practice. Vietnamese scientists and technical staff at MIPM will obtain up-to-date training in PCR analysis. The use of these assays will, ultimately facilitate monitoring the spread of drug resistant strains of *P. falciparum* in Vietnam. It is anticipated that biosamples will be sent to ADFMIDI for confirmation of parasitemia, *in vitro* drug susceptibility testing, PCR analysis and antimalarial drug analysis.

5. Registration of clinical trial

The clinical trial will be registered on the website: http:// www.clinicaltrials.gov or www.anzctr.org.au

6. Quality Assurance

Dr. Ho Huu Tho (MD, DSc), Dr Michael Edstein (MSc, PhD), and Dr. Kimberly Edgel (PhD) will be responsible for carrying out quality assurance on the conduct of the clinical trial. This covers recruitment of patients, drug administration, follow-up of patients, study documentation (e.g. CRFs and standing operational procedures), training, resourcing and logistics associated with the study. Dr. Edgel will provide progress reports to the financial sponsor (the US Department of Defense) of the study.

D. RISKS, BENEFITS AND ALTERNATIVE TREATMENT FOR SUBJECTS

1. Risks

There are four main risks in the study; the risk of treatment failure due to drug resistance, the risk of known side effects of the antimalarial drugs to be used in this study, risks associated with venipuncture and finger prick blood sampling and the risk of being pregnant.

a. Treatment Failure

There is a small, but very real risk of treatment failure. There has been recent evidence of reduced susceptibility of artesunate in Dak Nong Province in central Vietnam (WHO, 2017) and 26% treatment failures in patients treated with dihydroartemisinin-piperaquine in Binh Phuoc province (Thanh et al., 2017).

b. Side Effects of Antimalarial Drugs

All medicines may cause side effects, but many people have no, or minor, side effects. The safety profiles of the ACTs and artesunate are well understood and their adverse effects are mild compared to the risks of untreated *P. falciparum* malaria. Similarly, the adverse event profile of the rescue therapy, dihydroartemisinin-piperaquine, for falciparum is well understood as are chloroquine and primaquine for recurrent *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 are as follows:

1) Pyronaridine-artesunate

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, 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. The most commonly reported (>1/100 to <1/10) adverse event were headache, eosinophilia, neutropenia, anaemia, increased platelet count, vomiting, abdominal pain, bradycardia, transaminase increases and hypoglycaemia (EMA, 2014).

In a Cochrane database systematic review (Bukirwa et al., 2014) of three clinical trials (Tshefu et al., 2010; Kayentao et al., 2012, Rueangweerayut et al., 2012) comparing the safety of artesunate-pyronaridine and a comparator ACT (artemether-lumefantrine or artesunate-mefloquine) the mean haemoglobin level fell in both groups during the first seven days after starting treatment with the decline slightly larger with artesunate-pyronaridine compared to the comparator ACT but by day 28 mean haemoglobin levels returned to normal or higher values than baseline in the treatment groups. A similar pattern was observed with platelet and white cell counts in the trial by Rueangweerayut et al. (2012). Overall, the differences in these haematological indices between the treatment groups were small and unlikely to be of clinical significance.

Mild to moderate and reversible liver enzyme elevation (i.e., ALT and AST) have been observed after treatment in few African and Asian patients without clinical manifestations following pyronaridine-artesunate treatment. In four clinical trials involving 3,523 adults and children the analysis of liver function tests showed biochemical elevation were four times more frequent with artesunate-pyronaridine than with other comparator ACTs (Bukirwa et al., 2014). In these studies no patients developed signs or symptoms of liver disease and there were no significant differences for other liver enzymes, including bilirubin. Furthermore, the elevation of the transferases was similar following the first and second administrations of the

3-day regimen of pyronaridine-artesunate, with no marked differences between 60-day and 90-day redosing (Morris et al., 2014).

2) Dihydroartemisinin-piperaquine

Reported side effects include nausea, vomiting, abdominal discomfort, headache, dizziness, and anorexia, which are generally mild and transient, and similar to the symptoms of malaria.

3) Chloroquine

The drug is generally well-tolerated. 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 side effects include headache, various skin eruptions and gastrointestinal disturbances, such as nausea, vomiting and diarrhoea.

4) Primaquine

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 haemolytic anaemia in patients with G6PD deficiency.

c. Risk of Phlebotomy and Finger Stick

The primary risks of phlebotomy include local discomfort, occasional bleeding or bruising of the skin at the site of needle puncture, and rarely haematoma or infection. The risk of infection will be minimized by the use of aseptic technique and disposable, sterile needles and lancets.

d. Special Risks to Pregnant or Potentially Pregnant Women Volunteers

Female subjects aged 10 years and older must have a negative urine pregnancy test before enrolling in the study. Pregnant patients will not be enrolled in the study.

2. Benefits

There are four main benefits for participating in this study; falciparum malaria is a fatal disease and needs to be treated quickly, patients will receive close monitoring of treatment response, food and milk will be provided for each visit, and the study will cover the cost for drug related side effects:

a. Malaria is a Life-Threatening Disease

Malaria is a disease that needs to be treated promptly. All patients will benefit from receiving efficacious treatment at no cost. They will receive prompt rescue therapy, if clinically indicated**.** In addition, they may receive some satisfaction from contributing to efforts to control malaria in Vietnam.

b. Careful Monitoring of Treatment Response

Participants in the study will obtain more extensive post-treatment monitoring to detect treatment failure than they would routinely. The study team will keep one doctor on site 24 hours a day, 7 days a week for the duration of the study. This will greatly reduce the risk of morbidity or mortality resulting from treatment failure. The antimalarials are well tolerated and the known side effects are well understood. Individuals who have contraindications for or known hypersensitivity to any of the antimalarials in the study will be excluded from the study.

c. Compensation for Participating in the Study

Study patients or their parent/guardian (in the case of children) will be reimbursed the cost of local transport to attend follow-up visits. During each visit the patient will receive food and milk. The study will pay for treatment for drug-related SAEs determined by the study doctor in consultation with the Principal Investigator, and the Research Monitor or other research-related injuries. In case of treatment failure or development of serious side effects (including severe malaria), the cost of treatments and other medical care will be paid by the sponsors of the study unless the participant withdraws willingly from the study and goes to a hospital or other health station for independent treatment.

3. Alternatives to Study Participation

The participation of the patients in this study is entirely voluntary. Whether the patients choose to participate or not, all the services they receive at the study site will continue and nothing will change. If any patient chooses not to participate in this project he/she will be referred to the routine treatment service provided in this health facility and the treatment follows the national treatment guideline. The project will not cover any cost for those who decide not to participate in the study.

E. DESCRIPTION OF THE SYSTEM FOR MAINTENANCE OF RECORDS

1. Study Data

Study data will be maintained in the form of CRFs stored in a locked filing cabinet in a locked room at MIPM. Laboratory results will be stored on password protected computers at MIPM, IMPE, NMRC-A and ADFMIDI.

2. Regulatory File

The original research protocol, consent forms, and related documents for protection of human research volunteers will be stored at MIPM. These documents will be stored in locked cabinets in locked offices.

3. Individual Medical Records

Clinical and laboratory findings from each subject will be kept in individual folders of CRFs. The participant's name from the ICD and their study number from the CRF will be listed both electronically and as a hard-copy and secured by the Principal Investigator and Research Monitor. This represents the only link established between the subject and the study code number. During the study, the CRFs are held in a secured cabinet with access restricted to research team members with a need to use the CRFs. Team members with access to CRFs are counselled on the importance of confidentiality of subject medical records. All investigators involved in this study have completed GCP training such as Collaborative Institutional Training Initiative **(**CITI). In all electronic records for data analysis, the subject is not referenced by name, but only study subject number. This method is designed to protect the privacy of study subject medical information. All CRFs will be held at MIPM for 15 years after completion of the study in secured cabinets with restricted access.

4. Data Ownership, Use of Results and Publication Policy

A final copy of the database containing all the data will be held at MIPM. The database can 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 sponsor. A report summarizing the findings of this trial will be forwarded to all collaborating institutions. The results from all study sites will be published in a peer-reviewed journal. Any data published in

the peer-reviewed medical literature will protect the identity of the subjects. Only those 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.

5. Confidentiality and Sample Storage

The study team will ensure that the participants' anonymity is maintained. Participant's biospecimens will be re-identifiable in case there is a need to identify the participant such as an unexpected SAE. All study documents (i.e. CRFs, ICDs, patients' registers) will be stored in a locked filing cabinet at the study site and then transported to the MIPM, where they will be stored in a locked office and will only be accessed by members of the study team on a needs basis or officials with responsibility for study oversight.

6. Sample Sharing and Storage

Samples collected will be used for the purpose of this study as stated in the protocol and properly stored before shipment to MIPM and ADFMIDI. Samples will be used only for the purposes of the study described in this protocol. Any proposed plans to use samples other than for those investigations detailed in this protocol will be submitted to the relevant ethics committees prior to any testing.

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APPENDIX A. CASE SCREENING FORM

Screening number: **S** ___ ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Screening (Day 0)**

SENTINEL SITE		
Name of the health station:	Locality:	District:
Address:	Province:	

DEMOGRAPHIC DATA

Occupation: _____

Age: ___ ___ years Height: ___ ___. ___ cm Weight: ___ ___. ___ kg

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

PRE-TREATMENT TEMPERATURE

☐ Axillary ___ ___. ___ ° C

☐ Tympanic Temperature

History of fever within previous 24 hours ☐ Yes ☐ No _____

_____

THICK AND THIN BLOOD SMEARS

Are malaria parasites present? ☐ Yes ☐ No

Which species? *P. falciparum* ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* ☐ Mixed species ☐

Rapid Diagnostic Test: Positive for *P. falciparum* only ☐ Yes ☐ No

Rapid Diagnostic Test: Positive for *P. vivax* only ☐ Yes ☐ No

Note: RDT is used to confirm blood smear findings. However, because RDTs are not 100% accurate at low parasitemias, the blood smear result is accepted as the gold standard for malaria diagnosis. If RDT negative but blood smear positive the result for blood smear parasitemia will be used to continue processing the subject for study recruitment.

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species		
Was species other than *P. falciparum*, *P. vivax*, *P. malariae* or mixed infection of *Pf*, *Pv* and *Pm* detected upon review of slides? If yes, the patient is to be excluded. ☐ No ☐ Yes	Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
	Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
	Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

PARASITE DENSITY

For *P. falciparum* **only** (250-100,000/µL): >8/200 WBC and <20/1000 RBC
☐ Yes ☐ No* **(*NOT ELIGIBLE IF OUTSIDE THE PARASITEMIA RANGE)**

For *P. vivax* **only** (250-100,000/µL): >8/200 WBC and <20/1000 RBC
☐ Yes ☐ No* **(*NOT ELIGIBLE IF OUTSIDE THE PARASITEMIA RANGE)**

For *P. malariae* **only** (250-100,000/µL): >8/200 WBC and <20/1000 RBC
☐ Yes ☐ No* **(*NOT ELIGIBLE IF OUTSIDE THE PARASITEMIA RANGE)**

For *P. falciparum* plus *P. vivax* **only** or *P. falciparum* plus *P. malariae* **only** or *P. vivax* and *P. malariae* **only** (250-100,000/µL): >8/200 WBC and <20/1000 RBC
☐ Yes ☐ No* **(*NOT ELIGIBLE IF BOTH *PLASMODIUM* SPECIES ARE OUTSIDE THE PARASITEMIA RANGE)**

INCLUSION CRITERIA FOR VNPA STUDY

- Adults and children ≥ 20 kg.
- Symptomatic of malaria infection (i.e. history of fever within 24 hours and/or presence of fever >37.5°C for axillary temperature and >38.0°C for tympanic temperature).
- Microscopic confirmation of asexual stages of *P. falciparum*, *P. vivax*, *P. malariae* or mixed infections of *Plasmodium* species.
- Parasitemia between 250/µL and 100,000/µL.
- G6PD normal patients with mono-infection of *P. vivax* or mixed infections of *P. vivax* will be treated with primaquine to kill liver hypnozoites.
- Ability to take oral medication.
- Written informed consent given to participate in the trial by the patient or in case of children up to 17 years old (assent for children aged 10 to 17 years old) with adult or guardian permission.

Does the patient meet all of the inclusion criteria **FOR VNPA STUDY**? ☐ Yes ☐ No*

***NOT ELIGIBLE**

If no, please specify reason of exclusion:_____

_____

EXCLUSION CRITERIA FOR VNPA STUDY

- Pregnancy or lactation (urine test for β HCG to be performed on any woman of child bearing age 10 to 55 years old).
- Hematocrit <20%.
- Parasitemia <250/µL or > 100 000/µL.
- Signs or symptoms indicative of severe/cerebral malaria (WHO, 2014).
- Liver function test (AST/ALT levels) more than 2.5 times the upper limit of normal (ULN) range.
- Total bilirubin > 2 ULN.
- Use of an antimalarial drug in the preceding 4 weeks.
- History of splenectomy, heavy alcohol use or injecting drugs of abuse.
- Any other condition, which in the judgment of the study physician would make participation in the study unsafe for the potential study patient.

Does the patient meet any of the exclusion criteria **FOR VNPA STUDY**? ☐ Yes* ☐ No

***NOT ELIGIBLE**

If yes, please specify reason of exclusion:_____

_____

URINE PREGNANCY TEST (This section applies only to female patients.)

Result of pregnancy test: ☐ Negative ☐ Positive*

***NOT ELIGIBLE**

FOR ELIGIBLE PATIENTS TO BE IN THE VNPA STUDY

Screening number **S** ___ ___ ___

will be given

Subject number: VNPA ___ ____

APPENDIX B. CASE REPORT FORM

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**(Day 0)** **Before treatment**

SENTINEL SITE		
Name of the health station:	Locality:	District:
Address:	Province:	

DEMOGRAPHIC DATA
Occupation: _____
Age: ___ ___ years Height: ___ ___. ___ cm Weight: ___ ___. ___ kg
Gender (please tick ☑ only one box): ☐ Male ☐ Female

PRE-TREATMENT TEMPERATURE
Time: ___ ___ : ___ ___ (HH:MM)
☐ Axillary ☐ Tympanic Temperature ___ ___. ___ ° C

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**(Day 0)** **Before treatment**

PARASITE DENSITY
For *P. falciparum* **only** (250-100,000/µL): >8/200 WBC and <20/1000 RBC ☐ Yes ☐ No* **(*NOT ELIGIBLE IF OUTSIDE THE PARASITEMIA RANGE)** For *P. vivax* **only** (250-100,000/µL): >8/200 WBC and <20/1000 RBC ☐ Yes ☐ No* **(*NOT ELIGIBLE IF OUTSIDE THE PARASITEMIA RANGE)** For *P. malariae* **only** (250-100,000/µL): >8/200 WBC and <20/1000 RBC ☐ Yes ☐ No* **(*NOT ELIGIBLE IF OUTSIDE THE PARASITEMIA RANGE)** For *P. falciparum* plus *P. vivax* **only** or *P. falciparum* plus *P. malariae* **only** or *P. vivax* and *P. malariae* **only** (250-100,000/µL): >8/200 WBC and <20/1000 RBC ☐ Yes ☐ No* **(*NOT ELIGIBLE IF BOTH *PLASMODIUM* SPECIES ARE OUTSIDE THE PARASITEMIA RANGE)**

HEMATOCRIT
Hematocrit: ___ ___ %

PATIENT INFORMED CONSENT AND ASSENT		
Subject Number: **VN** ___ ___ ___	Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	Consent Form signed: ☐ No ☐ Yes Assent Form signed: ☐ No ☐ Yes

DEMOGRAPHIC DATA
Possible place of infection (where did you go in the last 2 weeks?):_____
How many days have you been sick? ___ ___ days History of drug allergy: ☐ No ☐ Yes, what drug? _____

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**(Day 0)** **Before treatment**

PHYSICAL EXAMINATION
Time of measurement: ___ ___ : ___ ___ (HH:MM)
Systolic blood pressure / Diastolic blood pressure Blood Pressure: ___ ___ ___ / ___ ___ ___ mmHg
Pulse Rate: ___ ___ ___ /min. Respiratory Rate: ___ ___ ___ /min

	Please tick ☑ the applicable box			
Body System	**Normal**	**Abnormal**	**Not examined**	**Findings (if abnormal)**
General Appearance	☐	☐	☐	
Head and Eyes	☐	☐	☐	
Ears, Nose and Throat	☐	☐	☐	
Chest and Lungs	☐	☐	☐	
Cardiovascular	☐	☐	☐	
Abdomen	☐	☐	☐	
Neurological	☐	☐	☐	
Lymphatic (excluding head and neck)	☐	☐	☐	
Musculo-skeleton	☐	☐	☐	
Other, specify:	☐	☐	☐	

Study Doctor:

_____ _____

Name Signature

Subject number: **VNPA ___ ___**	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**(Day 0)** **Before treatment**

Medical History						
Signs/Symptoms	**Present**	**Absent**	**Comments**	**AE Intensity**		
				Mild	**Mod**	**Sev**
Rigors/Chills	☐	☐				
Sweating	☐	☐				
Headache	☐	☐				
Cough	☐	☐				
Nausea	☐	☐				
Abdominal Pain	☐	☐				
Vomiting	☐	☐				
Loss of appetite	☐	☐				
Fatigue	☐	☐				
Myalgia	☐	☐				
Jaundice	☐	☐				
Hepatomegaly	☐	☐				
Splenomegaly	☐	☐				
Pruritus	☐	☐				
Other (list in comments)	☐	☐				

Study Doctor:

_____ _____

Name Signature

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**(Day 0)** **Before treatment**

LABORATORY SPECIMENS	
1. Thick and thin blood smears x 2 2. Whatman 31 ET Chromatography filter paper blood spots x 4 3. Hematocrit 4. Blood (3 mL) for hematology and biochemical tests	☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No
Laboratory Technician's initials: ___ ___	Time: ___ ___ : ___ ___ (HH:MM)

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 0)**

STUDY MEDICATION ADMINISTRATION				
Name of the antimalarial drug	Time of dosing (HH:MM)	Number of tablets	Did the patient vomit?	Time of vomiting (HH:MM)
Pyronaridine-Artesunate	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___
Primaquine (only for mono-infections of *P. vivax* or mixed infections of *P. vivax*)	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___

ADJUNCTIVE MEDICATION				
Name of <u>other</u> medication	Time of dosing (HH:MM)	Number of tablets	Did the patient vomit?	Time of vomiting (HH:MM)
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 0)**

TEMPERATURE	
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ___ ___. ___ ° C ☐ Tympanic Temperature

LABORATORY SPECIMENS (Morning/Afternoon-about 12 hours apart)	
1. Thick and thin blood smears x 2	☐ Yes ☐ No Time: ___ ___ : ___ ___ (HH:MM)

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 1)**

CLINICAL STATUS		
Presence of signs of severe or complicated malaria?	☐ Yes	☐ No

TEMPERATURE		
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ☐ Tympanic Temperature	___ ___. ___ ° C

LABORATORY SPECIMENS (Morning/Afternoon-about 12 hours apart)		
1. Thick and thin blood smears x 2 2. Whatman 31 ET Chromatography filter paper blood spots x 4	☐ Yes ☐ No ☐ Yes ☐ No Time: ___ ___ : ___ ___ (HH:MM)	

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment** **(Day 1)**

Medical History						
Signs/Symptoms	**Present**	**Absent**	**Comments**	**AE Intensity**		
				Mild	**Mod**	**Sev**
Rigors/Chills	☐	☐				
Sweating	☐	☐				
Headache	☐	☐				
Cough	☐	☐				
Nausea	☐	☐				
Abdominal Pain	☐	☐				
Vomiting	☐	☐				
Loss of appetite	☐	☐				
Fatigue	☐	☐				
Myalgia	☐	☐				
Jaundice	☐	☐				
Hepatomegaly	☐	☐				
Splenomegaly	☐	☐				
Pruritus	☐	☐				
Other	☐	☐				

Study Doctor:

_____ _____

Name Signature

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 1)**

ADVERSE EVENTS				
Is it a serious adverse event? ☐ Yes ☐ No If yes, contact Dr Huynh Hong Quang (Tel: 0905103496) Dr. Nguyen Ngoc San (Tel: 0989821168)				
Description of Adverse Events	Onset Date & Time (DD/MM/YYYY)	Ongoing (Yes/No)	Resolution Date & Time (DD/MM/YYYY)	Any medication given
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	

STUDY MEDICATION ADMINISTRATION				
Name of the antimalarial drugs	Time of dosing (HH:MM)	Number of tablets	Did the patient vomit?	Time of vomiting (HH:MM)
Pyronaridine-Artesunate	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___
Primaquine (only for mono-infections of *P. vivax* or mixed infections of *P. vivax*)	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 1)**

ADJUNCTIVE MEDICATION				
Name of <u>other</u> medication	Time of dosing (HH:MM)	Number of tablets	Did the patient vomit?	Time of vomiting (HH:MM)
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___

TEMPERATURE	
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ☐ Tympanic Temperature ___ ___. ___ ° C

LABORATORY SPECIMENS (Morning/Afternoon-about 12 hours apart)	
1. Thick and thin blood smears x 2	☐ Yes ☐ No Time: ___ ___ : ___ ___ (HH:MM)

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA ___ ___**	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 1)**

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 2)**

CLINICAL STATUS		
Presence of signs of severe or complicated malaria?	☐ Yes	☐ No

TEMPERATURE		
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ☐ Tympanic Temperature	___ ___. ___ ° C

LABORATORY SPECIMENS (Morning/Afternoon-about 12 hours apart)	
1. Thick and thin blood smears x 2	☐ Yes ☐ No Time: ___ ___ : ___ ___ (HH:MM)

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 2)**

Medical History						
Signs/Symptoms	**Present**	**Absent**	**Comments**	**AE Intensity**		
				Mild	**Mod**	**Sev**
Rigors/Chills	☐	☐				
Sweating	☐	☐				
Headache	☐	☐				
Cough	☐	☐				
Nausea	☐	☐				
Abdominal Pain	☐	☐				
Vomiting	☐	☐				
Loss of appetite	☐	☐				
Fatigue	☐	☐				
Myalgia	☐	☐				
Jaundice	☐	☐				
Hepatomegaly	☐	☐				
Splenomegaly	☐	☐				
Pruritus	☐	☐				
Other	☐	☐				

Study Doctor:

_____ _____

Name Signature

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 2)**

ADVERSE EVENTS				
Is it a serious adverse event? ☐ Yes ☐ No If yes, contact Dr Huynh Hong Quang (Tel: 0905103496) Dr. Nguyen Ngoc San (Tel: 0989821168)				
Description of Adverse Events	Onset Date & Time (DD/MM/YYYY)	Ongoing (Yes/No)	Resolution Date & Time (DD/MM/YYYY)	Any medication given
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	

STUDY MEDICATION ADMINISTRATION				
Name of the antimalarial drugs	Time of dosing (HH:MM)	Number of tablets	Did the patient vomit?	Time of vomiting (HH:MM)
Pyronaridine-Artesunate	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___
Primaquine (only for mono-infections of *P. vivax* or mixed infections of *P. vivax*)	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___

Subject number: **VNPA ___ ___**	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 2)**

ADJUNCTIVE MEDICATION				
Name of <u>other</u> medication	Time of dosing (HH:MM)	Number of tablets	Did the patient vomit?	Time of vomiting (HH:MM)
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___

TEMPERATURE	
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ☐ Tympanic Temperature ___ ___. ___ ° C

LABORATORY SPECIMENS (Morning/Afternoon-about 12 hours apart)	
1. Thick and thin blood smears x 2 2. LC-MS (Day 2 – patient's plasma artesunate/dihydroartemisinin concentrations at 1 hour post dose – 0.25 mL of blood to be collected)	☐ Yes ☐ No ☐ Yes ☐ No Time: ___ ___ : ___ ___ (HH:MM)

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA ___ ___**	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 2)**

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 3)**

CLINICAL STATUS		
Presence of signs of severe or complicated malaria?	☐ Yes	☐ No

TEMPERATURE		
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ☐ Tympanic Temperature	___ ___. ___ ° C

LABORATORY SPECIMENS (Morning/Afternoon-about 12 hours apart)	
1. Thick and thin blood smears x 2	☐ Yes ☐ No Time: ___ ___ : ___ ___ (HH:MM)

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 3)**

Medical History						
Signs/Symptoms	**Present**	**Absent**	**Comments**	**AE Intensity**		
				Mild	**Mod**	**Sev**
Rigors/Chills	☐	☐				
Sweating	☐	☐				
Headache	☐	☐				
Cough	☐	☐				
Nausea	☐	☐				
Abdominal Pain	☐	☐				
Vomiting	☐	☐				
Loss of appetite	☐	☐				
Fatigue	☐	☐				
Myalgia	☐	☐				
Jaundice	☐	☐				
Hepatomegaly	☐	☐				
Splenomegaly	☐	☐				
Pruritus	☐	☐				
Other	☐	☐				

Study Doctor:

_____ _____

Name Signature

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 3)**

ADVERSE EVENTS				
Is it a serious adverse event? ☐ Yes ☐ No If yes, contact Dr Huynh Hong Quang (Tel: 0905103496) Dr. Nguyen Ngoc San (Tel:0989821168)				
Description of Adverse Events	Onset Date & Time (DD/MM/YYYY)	Ongoing (Yes/No)	Resolution Date & Time (DD/MM/YYYY)	Any medication given
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	

ADJUNCTIVE MEDICATION				
Name of <u>other</u> medication	Time of dosing (HH:MM)	Number of tablets	Did the patient vomit?	Time of vomiting (HH:MM)
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Treatment (Day 3)**

TEMPERATURE	
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ☐ Tympanic Temperature ___ ___. ___ ° C

LABORATORY SPECIMENS (Morning/Afternoon-about 12 hours apart)	
1. Thick and thin blood smears x 2	☐ Yes ☐ No Time: ___ ___ : ___ ___ (HH:MM)

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Follow-up (Day 7, 14, 21, 28, 35 and 42)** Circle one

CLINICAL STATUS		
Presence of signs of severe or complicated malaria?	☐ Yes	☐ No

TEMPERATURE		
Time: ___ ___ : ___ ___ (HH:MM)	☐ Axillary ☐ Tympanic Temperature	___ ___. ___ ° C

LABORATORY SPECIMENS		
1. Thick and thin blood smears x 2	☐ Yes	☐ No
2. Whatman 31 ET Chromatography filter paper blood spots x 4	☐ Yes	☐ No
3. LC-MS (Day 7 – patient's blood pyronaridine concentration – 0.5 mL of blood to be collected)	☐ Yes	☐ No
4. Blood (3 mL) for hematology and biochemistry tests for Day 7 and Day 28	☐ Yes	☐ No
	Time: ___ ___ : ___ ___ (HH:MM)	

Blood smears for quantitative parasite counts and qualitative gametocyte counts for *P. falciparum, P. vivax, P. malariae* or mixed *Plasmodium* species	
Number of asexual *P. falciparum* parasites ___ ___ ___ ___ / µL	*P. falciparum* gametocytes? ☐ No ☐ Yes
Number of asexual *P. vivax* parasites ___ ___ ___ ___ / µL	*P. vivax* gametocytes? ☐ No ☐ Yes
Number of asexual *P. malariae* parasites ___ ___ ___ ___ / µL	*P. malariae* gametocytes? ☐ No ☐ Yes

Subject number: **VNPA** ___ ___	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Follow-up (Day 7, 14, 21, 28, 35 and 42)** Circle one

Medical History						
Signs/Symptoms	**Present**	**Absent**	**Comments**	**AE Intensity**		
				Mild	**Mod**	**Sev**
Rigors/Chills	☐	☐				
Sweating	☐	☐				
Headache	☐	☐				
Cough	☐	☐				
Nausea	☐	☐				
Abdominal Pain	☐	☐				
Vomiting	☐	☐				
Loss of appetite	☐	☐				
Fatigue	☐	☐				
Myalgia	☐	☐				
Jaundice	☐	☐				
Hepatomegaly	☐	☐				
Splenomegaly	☐	☐				
Pruritus	☐	☐				
Other	☐	☐				

Study Doctor:

_____ _____

Name Signature

Subject number: **VNPA ___ ___**	Visit Date: ___ ___ / ___ ___ / 201 ___ DD MM YYYY	**Follow-up (Day 7, 14, 21, 28, 35 and 42)** Circle one

ADVERSE EVENTS				
Is it a serious adverse event? ☐ Yes ☐ No If yes, contact Dr Huynh Hong Quang (Tel: 0905103496) Dr. Nguyen Ngoc San (Tel: 0989821168)				
Description of Adverse Events	Onset Date & Time (DD/MM/YYYY)	Ongoing (Yes/No)	Resolution Date & Time (DD/MM/YYYY)	Any medication given
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	
	___ ___/___ ___/201__ Time: __ __:__ __ HH : MM	☐ Yes ☐ No	___ ___/___ ___ /201__ Time: __ __:__ __ HH : MM	

ADJUNCTIVE MEDICATION				
Name of <u>other</u> medication	Time of dosing (HH:MM)	Number of tablets	Did the patient vomit?	Time of vomiting (HH:MM)
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___
	___ ___:___ ___	___ ___	☐ Yes ☐ No	___ ___:___ ___

Subject number: **VNPA** ___ ___

Outcome : ☐ ACPR ☐ ETF ☐ LCF ☐ LPF ☐ LFU ☐ WTH
Date of Outcome: ___ ___ / ___ ___ / 201 ___ DD MM YYYY
PCR Results: ☐ Reinfection ☐ Recrudescence ☐ Undetermined ☐ Not available
After PCR correction: ☐ ACPR ☐ ETF ☐ LCF ☐ LPF
Reason for lost to follow-up (LFU):
Reason for withdrawal (WTH):
Other comments:

APPENDIX C.

ADULT INFORMATION AND CONSENT FORM

PURPOSE OF THE STUDY

You will be one of 120 people invited to take part in this malaria research treatment study at your commune. This study is being carried out by the Vietnam People's Army, the Ministry of Health, the Australian Defence Force and the United States Navy. Malaria is a disease caused by a parasite transmitted by mosquitoes that can cause fever, muscle aches, cough, headache, nausea, vomiting, abdominal cramping, diarrhoea, seizures, weakness, unconsciousness, and death. Malaria can kill you without effective treatment. Presently, in Vietnam our best antimalarial drugs are starting to fail and because of this it is important that we evaluate new drugs. The purpose of this study is to learn how effective a new drug called pyronaridine-artesunate is in treating malaria at your commune. We also would like to know how well tolerated is the study drug. Knowing how well the study drug works will help us plan malaria control programs in Vietnam.

Please take time to read this information carefully, ask any questions that you may have, so that you understand what will happen to you during the study. Then consider if you want to take part. If you need more time to read this information, tell the health station staff. You will be given a copy of this form to take home with you. Your taking part in this study is completely voluntary. You can decide not to be in the study without any reason. If you do not want to be in the study, you will still receive free treatment for your malaria. If you agree to be in the study, you may withdraw at any time without loss of benefits to which you have a right to receive.

YOUR INVOLVEMENT IN THE STUDY AND RIGHTS

You will need to take pyronaridine-artesunate daily for 3 days under close observation by the medical staff. If you are infected with vivax malaria, you will also receive a standard 14 day treatment course of primaquine to kill parasites in your liver. We expect the drugs to clear your malaria infection but we will need to follow your response to the treatment for up to 42 days to make sure the malaria does not come back. You will take the study drug here at the health station or at home under supervision of study staff. After each dose you will be monitored for at least an hour to make sure you do not vomit the medication. During the first three days of the study we need you to take the study medication under supervision of our staff who will also check your health to determine how well the medication is working to cure your malaria infection. Water will be given with the drug.

The drug to be tested in this study is not recommended during early pregnancy. Women of childbearing age (10 to 55 years old) will be asked to provide a urine sample for pregnancy testing. Only if the test is negative can you be in the study. If you are pregnant, we will still provide you with treatment for malaria, but you cannot be in the study. The pregnancy test results will be kept private and provided to you in person by the study doctor.

Before starting treatment, we will obtain a finger prick blood sample (a few drops) from you for rapid diagnostic testing for malaria and to produce blood films to determine the number of parasites in your blood. The blood will also be added to filter paper to be used to determine whether the parasites are unique and drug resistant based on laboratory assays. We will keep your de-identified blood spots for up to 10 years from the time of blood collection and if new genetic markers for parasite antimalarial drug resistance are identified we may re-test your samples for these markers.

We will also use a needle to collect a small sample of blood (a teaspoon full about 3 mL) from your arm to determine that your blood chemistries are good. Blood (about 3 mL) will also be collected at Day 7 and Day 28 to determine that the treatment drug does not adversely affect your blood chemistries. If you have falciparum malaria we will invite you to give another blood sample (about 4 mL) to determine how well the parasites in your body are killed by standard antimalarial drugs grown in special containers in the laboratory. This will tell us the level of parasite resistance in your community.

After starting drug treatment we will continue to collect a few drops of blood from your finger to determine how well the treatment is working. This will be done about every 12 hours for the next three days, one week from today, and again every week for 6 weeks. Your body temperature will also be measured. We will give you a simple physical examination on each day that you receive the drug and will ask you "How do you feel after taking your last tablets?" to determine how the drug is affecting your health. If you feel sick at any time during the next 6 weeks, please come back so that we can check you for malaria. You will also be invited to provide a small sample of blood (1/16 of a teaspoonful about 0.25 mL) from your arm 1 hour after the last dose of the study drug and on Day 7 after starting drug treatment to determine that you have good drug levels in your body. If you experience an early treatment failure we will invite you to provide a blood sample (0.5 mL) to assist us in determining why the drug did not work.

WHAT YOUR BLOOD WILL BE USED FOR
Your blood sample will be used only for the purpose of this study as stated above.

RISKS AND DISCOMFORTS
1) Failure of Treatment. There is a small chance that the study drug might fail to cure your malaria, leaving you in danger of severe disease. For this reason we will check your blood frequently after treatment to make sure that your malaria has been cured. If we find that the drug does not clear your malaria infection or the malaria comes back we will retreat you with another drug combination recommended by the Ministry of Health.

2) Side effects from the drugs. As with any drug, a small number of people may have side effects that cannot be predicted. The most common side effects of the drugs that you may receive in this study are dizziness, muscle pains, fever, vomiting, headache, chills diarrhea, skin rash, abdominal pain, fatigue, loss of appetite, blurred vision, and ringing in the ears. These side effects are usually mild, should not affect your daily activities and generally go away quickly once treatment is finished. You must always come quickly to see a study doctor if you have any illness or injury. A medical doctor is available 24 hours a day, seven days a week to see you during the study. If you have a medical problem due to your involvement in the study, we will help you get medical care for it.

3) Risks and discomforts from blood sampling. Taking blood from the finger or arm causes a brief sharp pain. The experienced technician will clean the tip of your finger and collect a few drops of blood by pricking your finger with a clean lancet. There is a very low risk of infection where the needle is stuck in the finger or arm. Bruising may occur after collecting blood from your arm but usually disappears in a few days. However, the study team knows how to reduce these risks and they will help you if a problem occurs. If you become dizzy with pain or at the sight of blood, please tell the technician, so that the technician can lie you down.

4) Risk of early withdrawal before completing treatment and/or before the 42 day follow-up period is completed. You are free to withdraw from the study at any time. You may also be

withdrawn by the investigators. Possible reasons for withdrawal by the investigators include a serious side effect or failure by yourself to carry out the requirements of the study. If you select to withdraw before completing treatment you run the serious risk of developing life threatening malaria. So it is very important that you complete your treatment. If you withdraw before the Day42 of follow-up is completed you run the risk that the malaria may come back. So it is important that the follow-up is completed to make sure that you are cured.

BENEFITS

We will give you the highest quality medicine and we will make sure that you receive the full correct dose for your body weight. All care and medications given to you in this study are free. If you need special treatment for your malaria infection or for any other sickness or injury that occurs while you are in this study we will quickly provide first aid. If the doctors think it is necessary we will transport you to another health station or hospital for additional care and treatment. Results from this study will help the Ministry of Health select better antimalarial treatments for your community in the future.

PROTECTION OF PERSONAL DETAILS

All investigators and the Research Monitors will make sure that your safety and personal details are kept private during and after the study. Your name will not appear on your blood samples, blood smears and filter paper blood spots. Only the doctor and technicians at the health station will know who are in the study. The consent form and information obtained from you will be stored at the Military Institute of Preventive Medicine in Hanoi, in a locked filing cabinet. After completing the study, we will sit down with you and tell you about what we learnt. Afterwards, we will be telling more people what we found. Your name will not appear on any reports or presentations.

COMPENSATION

You will receive no money or other gifts for being in the study. However, if you live far away from the health station that the study team is unable to pick you up, we will give you enough money to pay for your transportation to and from the health station to your home. Also, we will cover any additional medical costs that may be due to you for being in the study. You will be provided with water for drug administration at the health station and you will receive food for your weekly return to the health station during the follow-up period. In case of treatment failure or development of serious side effects (including severe malaria), the cost of treatments and other medical care will be paid by the sponsors of the study unless you withdraw willingly from the study and go to a hospital or other health station for independent treatment.

WHAT ARE THE ALTERNATIVES

If you do not take part in this study you can choose to receive your malaria treatment by going to the local health station. There the people are treated with either dihydroartemisinin-piperaquine or chloroquine depending on the type of malaria, which is recommended by the Ministry of Health. Your doctor will tell you how you may receive the standard malaria treatment without being in the study.

QUESTIONS

If you have any questions, complaints or worries about being in the study, you can call or visit a doctor at the commune health station to discuss them. Also, you may contact Dr. Nguyen Ngoc San (Tel. 0989821168), Dr Huynh Hong Quang (Tel: 0905103496), or Dr Nguyen Xuan Thanh (Tel. 0989099352) to have your questions or worries answered. If you have questions about the study and your rights as a study subject, you may contact the Institutional Review

Board at the Ministry of Health, Hanoi (Tel. 04-62732249). If you do not have phone access, the study doctor at the health station can help you contact them.

Signature: After you fully understand what will take place with you in this study, you will need to sign your name or provide a thumb print on the consent form. By signing you have informed us that you have received a copy of this consent form and understand that signing this form proves your willingness to be in this study.

STATEMENT OF CONSENT FOR ADULTS (AGE ≥18 YEARS)

Protocol Title: Efficacy and tolerability of pyronaridine-artesunate in treating uncomplicated malaria in Vietnam.

Principal Investigator: Dr. Nguyen Ngoc San, Military Institute of Preventive Medicine, Hanoi), Vietnam.

Your signature on this Consent Form shows that you have had this study explained to you and you have had a chance to ask questions. You agree to voluntarily be in this study. The information collected from you will be kept private and used for the purpose of this study. Your study results will be made available to you freely upon your request and any published reports of this study will not include your personal details. You understand that you are not bound to be in this study and that you are free to withdraw from the study at any time with no loss of benefits or rights to your future health care.

You will be given a copy of the signed information/consent sheet for your records. Should you have any worries about how the study is being carried out, please come forward and contact the investigators in person.

Volunteer's Name (Print)		___ ___ / ___ ___ / _____ DD MM YYYY
Volunteer's Signature	(THUMB PRINT)	Time: ___ ___ : ___ ___ (HH:MM)

Witness signature: (If the patient is illiterate, a literate witness must sign. The witness can be a friend, relative or Commune leader/representative. If possible, this person should be selected by you and should have no connection with the study team).

I have witnessed the accurate reading of the consent form to the patient, who has had the opportunity to ask questions. I confirm that the patient has given consent freely.

Print name of witness:	
Signature of witness:	
Date:	___ ___ / ___ ___ / _____ DD MM YYYY

Commune Health Station nurse's signature:

I have witnessed the accurate reading of the consent form to the patient, who has had the opportunity to ask questions. I confirm that the patient has given consent freely.

Print name of nurse:	
Signature of nurse:	
Date:	___ ___ / ___ ___ / _____ DD MM YYYY

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the patient, who has had the opportunity to ask questions. I confirm that the patient has given consent freely.

Name of Doctor taking Consent (Print)		___ ___ / ___ ___ / _____ DD MM YYYY
Signature of Doctor taking Consent		

APPENDIX D.

PARENT/GUARDIAN PERMISSION FORM FOR CHILDREN TO BE IN THE STUDY

PURPOSE OF THE STUDY

I understand that my child has been invited to take part in a malaria research study at my commune because he/she has malaria. This study is being carried out by the Vietnam People's Army, the Ministry of Health, the Australian Defence Force and the United States Navy. Malaria is a disease caused by a parasite transmitted by mosquitoes that can cause fever, muscle aches, cough, headache, nausea, vomiting, abdominal cramping, diarrhea, seizures, weakness, unconsciousness, and death. Malaria can kill your child without effective treatment. Presently, in Vietnam our best antimalarial drugs are starting to fail and because of this it is important that we evaluate new drugs. Your child will be one of 120 people invited to take part in this 6 weeks study. The purpose of this study is to learn how effective a new drug called pyronaridine-artesunate is in treating malaria at your commune. We also would like to know how well tolerated is the study drug. Knowing how well the study drug works will help us plan malaria control programs in Vietnam.

As the child's parent or guardian you have been asked to take your time to read this information carefully and to ask any questions, so that you can fully understand what will happen to your child during the study. Your child does not have to take part in this antimalarial drug study, it is voluntary. By deciding not to have your child take part in the study, he/she will still receive the standard treatment for malaria and future health care. You also understand that you can change your mind and discontinue your child's involvement in the study at any time without loss of benefits to which your child has a right to receive.

YOUR CHILD'S INVOLVEMENT IN THE STUDY AND RIGHTS

Your child will take pyronaridine-artesunate daily for 3 days under close observation by the medical staff. If your child is infected with vivax malaria, he/she will also receive a standard 14 day treatment course of primaquine to kill parasites in their liver. We expect the drugs to clear your child's malaria infection but we will need to follow your child's response to the treatment for 42 days to make sure the malaria does not come back. Your child will take the medication here at the health station or at home under the supervision of study staff. After each dose your child will be monitored for at least an hour to make sure he/she does not vomit the drug. During the first three days of the study we need your child to take the study medication under supervision of our staff who will also check his/her health to determine how well the medication is working to cure your child's malaria infection. Water will be given with the medication.

The drug to be tested in this study is not recommended during early pregnancy. Women of childbearing age (10 to 55 years old) will be asked to provide a urine sample for pregnancy testing before enrolment, on Day 28, Day 42 and/or study withdraw. Only if the test is negative can your female child be in the study. If she is pregnant, we will still provide her with treatment for malaria, but she cannot be in the study. The pregnancy test results will be kept private and will be provided by the study doctor to your child and you.

Before starting treatment, we will obtain a finger prick blood sample (a few drops) from your child which will be used for rapid diagnostic testing for malaria and to produce blood films to determine the number of parasites in your child's blood. The blood will also be added to filter paper to determine whether the parasites are unique and drug resistant based on laboratory assays. We will keep your child's de-identified blood spots for up to 10 years from the time of

blood collection and if new genetic markers for parasite antimalarial drug resistance are identified we may re-test your child's samples for these markers.

We will also use a needle to collect a small sample of blood (a teaspoon full about 3 mL) from your child's arm to determine that his/her blood chemistries are good. Blood (about 3 mL) will also be collected at Day 7 and Day 28 to determine that the treatment drug does not adversely affect your child's blood chemistries. If your child has falciparum malaria we will invite him/her to give another blood sample (about 4 mL) to determine how well the parasites in your child's body are killed by standard antimalarial drugs grown in special containers in the laboratory. This will tell us the level of parasite resistance in your community.

After starting treatment, we will continue to collect a few drops of blood from your child's finger to determine how well the treatment is working. This will be done about every 12 hours for the next three days, one week from today, and again every week for 6 weeks. Your child's body temperature will also be measured. We will give your child a simple physical examination on each day that he/she receives medication and we will ask your child "How does he/she feels since taking the last tablets?" to determine how the drug is affecting your child's health. If your child feels sick at any time during the next 6 weeks, please come back so that we can check for malaria. Your child will also be invited to provide a small sample of blood (1/16 of a teaspoonful about 0.25 mL) from his/her arm 1 hour after the last dose of the study drug and on Day 7 after starting drug treatment to determine that he/she has good drug levels in their body. If your child experiences an early treatment failure we will invite him/her to provide a blood sample (0.5 mL) to assist us in determining why the drug did not work.

RISKS AND DISCOMFORTS

1) Failure of Treatment. There is a small chance that the study drug might fail to cure your child's malaria, leaving he/she in danger of severe disease. For this reason we will check your child's blood frequently after treatment to make sure that his/her malaria has been cured. If we find that the drug does not clear your child's malaria infection or the malaria comes back we will retreat the child with another drug combination recommended by the Ministry of Health.

2) Side effects from the drugs. As with any drug, a small number of people may have side effects that cannot be predicted. The most common side effects of the drugs that your child may experience are dizziness, muscle pains, fever, vomiting, headache, chills diarrhea, skin rash, abdominal pain, fatigue, loss of appetite, blurred vision, and ringing in the ears. These side effects are usually mild, should not affect his/her daily activities and they generally go away quickly once treatment has finished. Your child must always come quickly to see a study doctor if he/she has any illness or injury. A medical doctor is available 24 hours a day, seven days a week to see your child during the study. If your child has a medical problem due to his/her involvement in the study, we will help your child get medical care for it.

3) Risks and discomforts from blood sampling. Taking blood from the finger or arm causes a brief sharp pain. The experienced technician will clean the tip of your child's finger and collect a few drops of blood by pricking his/her finger with a clean lancet. There is a very low risk of infection where the needle is stuck in the finger or arm. Bruising may occur after collecting blood from your child's arm but usually disappears in a few days. However, the study team knows how to reduce these risks and they will help your child if a problem occurs. If your child becomes dizzy with pain or at the sight of blood, please tell the technician, so that the technician can lie him/her down.

4) Risk of early withdrawal before completing treatment and/or before the 42 day follow-up period is completed. Your child can withdraw from the study at any time. He/she may also be withdrawn by the investigators. Possible reasons for withdrawal by the investigators include a serious side effect or failure by your child to carry out the requirements of the study. If your child selects to withdraw before completing treatment, he/she will run the serious risk of developing life threatening malaria. So it is very important that your child completes his/her treatment. If your child withdraws before Day 42 of follow-up is completed, he/she will run the risk that the malaria may come back. So it is important that the follow-up is completed to make sure that your child is cured.

BENEFITS

We will give your child the highest quality medicine and we will make sure that he/she receives the full correct dose for his/her body weight. All care and medications given to your child in this study are free. If your child needs special treatment for his/her malaria infection or for any other sickness or injury that occurs while he/she is in this study we will quickly provide first aid. If the doctors think it is necessary we will transport your child to another health station or hospital for additional care and treatment. Results from this study will help the Ministry of Health select better antimalarial treatments for your community in the future.

PROTECTION OF PERSONAL DETAILS

All investigators and the Research Monitor will make sure your child's safety and personal details are kept private during and after the study. Your child's name will not appear on blood smears, blood samples and filter paper blood spots. Only the doctor and technicians at the health station will know your child's personal details. The parent/guardian permission form and assent form and information obtained from you on behalf of your child will be stored at the Military Institute of Preventive Medicine in Hanoi, in locked filing cabinet. After completing the study, we will sit down with your child and tell him/her about what we learnt. Afterwards, we will be telling more people about the study and what we found. Your child's name will not appear on any reports or presentations.

COMPENSATION

Your child will receive no money or other gifts for taking part in the study. However, if your child lives far away from the health station and the study team is unable to pick your child up, we will give you enough money to pay for the transportation of your child to and from the health station to his/her home. Also, we will cover any additional medical costs that may be due to your child for being in the study. Your child will be provided with water for drug administration at the health station and he/she will receive food at the weekly return to the health station during the follow-up period. In case of treatment failure or development of serious side effects (including severe malaria), the cost of treatments and other medical care will be paid by the sponsors of the study unless your child withdraws willingly from the study and goes to a hospital or other health station for independent treatment.

WHAT ARE THE ALTERNATIVES

If your child does not take part in this study, he/she can choose to receive malaria treatment by going to the local health station. There the people are treated with either dihydroartemisinin-piperaquine or chloroquine depending on the type of malaria, which is recommended by the Ministry of Health. Your doctor will tell you how your child may receive the standard antimalarial treatment without being in the study.

QUESTIONS

If you have any questions, complaints or worries about your child being in the study, you can call or visit a doctor at Commune health station to discuss them. Also, you may contact Dr.

Nguyen Ngoc San (Tel. 0989821168), Dr Huynh Hong Quang (Tel: 0905103496) or Dr Nguyen Xuan Thanh (Tel. 0989099352) to have your questions or worries answered. If you have questions about the study and your child's rights as a study subject, you may contact the Chairperson of the Institutional Review Board at the Ministry of Health, Hanoi (Tel. 04-62732249). If you do not have phone access, the study doctor at the health station can help you contact them.

Signature: After you fully understand what will take place with your child being in this study, you will need to sign your name or provide a thumb print on the parental/guardian permission form, with your child's name. By signing you have informed us that you have received a copy of this parental/guardian permission form and understand that signing this form proves your willingness to have your child in this study.

PARENT/GUARDIAN PERMISSION FOR CHILDREN (AGE UP TO 17 YEARS)

Protocol Title: Efficacy and tolerability of pyronaridine-artesunate in treating uncomplicated malaria in Vietnam.

Principal Investigator: Dr. Nguyen Ngoc San, Military Institute of Preventive Medicine, Hanoi), Vietnam.

Your signature on this Permission Form shows that you have had this study explained to you and you have had a chance to ask questions. You are willing to have your child be in this study. The information collected from your child will be kept private and used for the purpose of this study. Your child's study results will be made available to you freely upon your request and any published reports of this study will not have your child's personal details. You understand that your child is not bound to be in this study and that you are free to withdraw your child from the study at any time with no loss of benefits or rights to his/her future health care. You will be given a copy of the signed information/permission form. Should you have any worries about how the study is being carried out, please come forward and contact the investigators in person.

For girls aged 10 to 17 years old, your permission is given for the child to provide a urine sample for a pregnancy test before enrolment, on Day 28, Day 42 and/or study withdraw. If she is pregnant, she cannot be in the study. The pregnancy test results will be kept private and be provided to you and your child by the study doctor.

Child Name (Print)

Child's Parent/Guardian Name (Print)		___ ___ / ___ ___ / _____ DD MM YYYY
Child's Parent/Guardian Signature	(THUMB PRINT)	Time: ___ ___ : ___ ___ (HH:MM)

Witness signature: (A witness signature is required only if the child's parent/guardian is illiterate. In this case, a literate witness must sign. The witness can be a friend, relative or Commune leader/representative. If possible, this person should be selected by the child's parent/guardian and should have no connection with the study team.)

I have witnessed the accurate reading of the parental/guardian permission form to the parent/guardian, who has had the opportunity to ask questions. I confirm that the child's parent/guardian has given permission freely for their child to take part in the study.

Print name of witness:	
Signature of witness:	
Date	___ ___ / ___ ___ / _____ DD MM YYYY

Commune Health Station nurse's signature:

I have witnessed the accurate reading of the parental/guardian permission form to the parent/guardian, who has had the opportunity to ask questions. I confirm that the child's parent/guardian has given permission freely for their child to be in the study.

Print name of nurse:	
Signature of nurse:	
Date:	___ ___ / ___ ___ / _____ DD MM YYYY

Investigator's signature:

I have accurately read or witnessed the accurate reading of the parental/guardian permission form to the parent/guardian, who has had the opportunity to ask questions. I confirm that the parent/guardian has given permission freely for their child to be in the study.

		___ ___ / ___ ___ / _____ DD MM YYYY
Name of Doctor taking parental/guardian permission (Print)		
Signature of Doctor taking parental/guardian permission		

APPENDIX E.

STATEMENT OF ASSENT FOR CHILDREN (AGE 10 to 17 YEARS)

Protocol Title: Efficacy and tolerability of pyronaridine-artesunate in treating uncomplicated malaria in Vietnam.

Principal Investigator: Dr. Nguyen Ngoc San, Military Institute of Preventive Medicine, Hanoi), Vietnam.

This informed assent form is for children aged 10–17 years who have been invited to be in a research study to test how good the new antimalarial drug, pyronaridine-artesunate is in treating malaria. We also would like to know how well tolerated is the drug.

This informed assent form has two parts:

I. Information sheet (to share information about the study with you)

II. Certificate of assent (for signatures or thumbprint if you agree to take part)

You will be given a copy of the full informed assent form.

Part I. Information sheet

I am an investigator of the study team for the treatment of malaria. You will be one of 120 people (adults and children >20 kg) invited to take part in this malaria research treatment study at your commune. This study is being carried out by the Vietnam People's Army, the Ministry of Health, the Australian Defence Force and the United States Navy. Malaria is a disease caused by a parasite transmitted by mosquitoes that can cause fever, muscle aches, cough, headache, nausea, vomiting, abdominal cramping, diarrhea, seizures, weakness, unconsciousness, and death. Malaria can kill you without effective treatment. Presently, in Vietnam our best antimalarial drugs are starting to fail and because of this it is important that we evaluate new drugs. The purpose of this study is to learn how effective a new drug called pyronaridine-artesunate is in treating malaria at your commune. We also would like to know how well tolerated is the study drug. Knowing how well the study drug works will help us plan malaria control programs in Vietnam. We do expect the drug treatment to clear your malaria but we need to follow you for 42 days to make sure that the malaria does not come back.

I am going to give you information and invite you to be in this study. You can choose whether you want to be in the study. We have discussed this study with your parent or guardian, and they know that we are also asking you for your agreement. If you decide to be in the study, your parent or guardian also have to agree. If you do not wish to be in the study, you do not have to do so, even if your parent has agreed. It is your choice. If you decide not to be in the study, nothing will change; this is still your health station and you will receive the standard malaria treatment from the health station. Even if you say 'Yes' now, you can change your mind later and it will still be okay. You may discuss anything on this form with your parents or friends or anyone else you feel comfortable talking to. There may be some words you do not understand or things that you want me to explain more because you are interested or concerned. Please ask me to stop at any time, and I will take time to explain.

Investigator: I have checked with the child, and he/she understands that being in the study is voluntary. _____ (initials)

Antimalarial treatment

Tablets of pyronaridine-artesunate will be given daily for 3 days with water. If you are infected with vivax malaria, you will also receive a standard 14 day treatment course of primaquine to kill parasites in your liver.

Drug treatment plan and blood collections

During the first week of the study, you will have to take the medication under supervision of the study staff that will observe you for 1 hour and make sure you are well. The study will take place over 42 days.

Before starting treatment, we will obtain a finger prick blood sample (a few drops) from you for rapid diagnostic testing for malaria and to produce blood films to determine the number of parasites in your blood. The blood will also be added to filter paper to be used to determine whether the parasites are unique and drug resistant based on laboratory assays. We will keep your de-identified blood spots for up to 10 years from the time of blood collection and if new genetic markers for parasite antimalarial drug resistance are identified we may re-test your samples for these markers.

We will also use a needle to collect a small sample of blood (a teaspoon full about 3 mL) from your arm to determine that your blood chemistries are good. Blood (about 3 mL) will also be collected at Day 7 and Day 28 to determine that the treatment drug does not adversely affect your blood chemistries. If you have falciparum malaria we will invite you to give another blood sample (about 4 mL) to determine how well the parasites in your body are killed by standard antimalarial drugs grown in special containers in the laboratory. This will tell us the level of parasite resistance in your community.

After starting treatment, we will continue to collect a few drops of blood from your finger to determine how well the treatment is working. This will be done every 12 hours for the next three days, one week from today, and again every week for 6 weeks. We will measure your body temperature and carry out a simple physical test on each day that you receive medication so as to determine how the medication is affecting your health. If you feel sick at any time during the next 6 weeks, please come back to the health station so we can recheck for malaria. You will also be invited to provide a small sample of blood (1/16 of a teaspoonful, 0.25 mL) from your arm 1 hour after the last tablet of the study drug and on Day 7 after starting drug treatment to determine that you have good drug levels in your body. Although we expect the medicine to work very well in curing your malaria, if the malaria does not go away quickly after starting treatment we will ask you to provide a blood sample (0.5 mL) to help us understand why the medication is not working as it should. We will then give you another medication that we know will kill your malaria.

Taking blood from your finger or arm will cause a brief sharp pain. However, the person collecting your blood is very good at blood collections and will reduce any pain to you as much as possible. Bruising may occur on your arm but usually disappears in a few days.

You are not allowed to use any other medication during the follow-up period unless permission is obtained from the study doctor. Girls aged 10 years and older will have a urine pregnancy test and if found to be pregnant they will not take part in the study. The pregnancy test results will be kept private and will be provided by the study doctor to you and your parent or guardian.

Investigator: I have checked with the child, and he/she understands what is being asked of them to do in the study. _____ (initials)

Side effect from medicines
The medicine can have some side effects such as dizziness, nausea, stomach pain and vomiting; however, for most people they are not common. We will follow you closely and keep track of any side effects, if they arise, or any other problems. If anything unusual happens to you, we need to know, and you should feel free to call us any time with your concerns or questions. If you get sick or have concerns or questions between visits to the health station, you should let me or the staff nurse know as soon as you can. We have also given your parents information about what to do if you are hurt or get sick during the study.

Investigator: I have checked with the child, and he/she understands the risks and discomforts. _____ (initials)

Compensation
You will receive no money or other gifts for taking part in the study. If, however, you decide to be in this study, any illnesses related to malaria or to the medicine will be treated at no cost to you or your parents. Because you might live quite far from the health station, we will give your parent or guardian enough money to pay for the trip here and back.

Investigator: I have checked with the child, and he/she understands the benefits. _____ (initials)

Protection of personal details
We will not tell other people that you are in this study and we will not share information about you with anyone who does not work in the study. Information about you that will be collected from the study will be hidden away, and no one but the study team will be able to see it. Any information about you will have a number on it, not your name. Only the study team will know what your number is and we will lock that information up.

When we have finished the study, I will sit down with you and your parent or guardian and tell you about what we learnt. Afterwards, we will be telling more people about the study and what we found.

You can ask me questions now or later. You can ask the health station nurse questions. I have written a number and address where you can reach the study team or, if you are nearby, you can come and see us. If you want to talk to someone else whom you know, like your teacher, doctor or auntie, that is okay too.

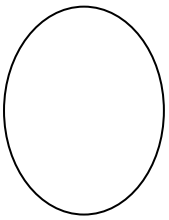
Investigator: I have checked with the child, and he/she understands how their personal details will be protected. _____ (initials)

Part II: Certificate of Assent

I have been invited to be in a study of a new antimalarial drug. If I am a girl aged 10 to 17 years old I assent to provide a urine sample for a pregnancy test and if the test is positive I cannot be in the study. I understand that I will receive pyronaridine-artesunate for the treatment of malaria. I have been informed that the risks are small and may include pain at my arm or finger. I am aware that there may be no benefit to myself personally and that I will not be given a gift (example money) other than travel cost to and from the health station. I have been provided with the name of an investigator who can be contacted easily with the telephone number that I was given.

- I know that I can choose to be in the study or not to be in the study. I know that I can stop whenever I want.

- I have read this information (or had the information read to me), and I understand it.
- I have had my questions answered and know that I can ask questions later if I have them.
- I understand that any changes to this study will be discussed with me.
- I agree to take part in the study.

Child's Name (Print)		___ ___ / ___ ___ / _____ DD MM YYYY Child's Date of Birth
Child's Signature	(THUMB PRINT)	Time: ___ ___ : ___ ___ (HH:MM)

Witness signature: (A witness signature and the child's thumbprint are required only if the child is illiterate. In this case, a literate witness must sign. The witness can be a friend, relative or Commune leader/representative. If possible, this person should be selected by the child's parent/guardian and should have no connection with the study team.)

I have witnessed the accurate reading of the assent form to the patient, who has had the opportunity to ask questions. I confirm that the patient has given assent freely to be in the study.

Print name of witness:	
Signature of witness:	
Date:	___ ___ / ___ ___ / _____ DD MM YYYY

Commune Health Station nurse's signature:

I have witnessed the accurate reading of the assent form to the patient, who has had the opportunity to ask questions. I confirm that the patient has given assent freely to be in the study.

Print name of nurse:	
Signature of nurse:	
Date:	___ ___ / ___ ___ / _____ DD MM YYYY

Investigator's signature:

I have accurately read or witnessed the accurate reading of the assent form to the patient, who has had the opportunity to ask questions. I confirm that the patient has given assent freely to be in the study.

		___ ___ / ___ ___ / _____ DD MM YYYY
Name of Doctor taking Assent (Print)		
Signature of Doctor taking Assent		

APPENDIX F.

STATEMENT OF CONSENT FOR A PREGNANCY TEST FOR ADULTS (AGE ≥18 YEARS)

Protocol Title: Efficacy and tolerability of pyronaridine-artesunate in treating uncomplicated malaria in Vietnam.

Principal Investigator: Dr. Nguyen Ngoc San, Military Institute of Preventive Medicine, Hanoi), Vietnam.

I have been invited to be in a study on a new medicine to treat malaria. I have been asked to supply a sample of urine before enrolment, on Day 28, Day 42 and/or study withdraw, which will only be used for pregnancy testing. I understand that the results of the tests will be kept private and that the study doctor will report the results to me. I understand that I must avoid becoming pregnant during the study because the medicine I will be taking could be dangerous for my child. I have discussed the different methods of birth control with my doctor, and I have been offered condoms. I understand that if the test is positive, I cannot take part in this study.

Volunteer's signature:

I accept to be tested. _____ (volunteer's initials) or

I do not want to be tested, and I have not signed the consent form below. _____ (volunteer's initials).

Volunteer's Name (Print)		___ ___ / ___ ___ / _____ DD MM YYYY
Volunteer's Signature	(THUMB PRINT)	Time: ___ ___ : ___ ___ (HH:MM)

Witness signature: (If the patient is illiterate, a literate witness must sign. The witness can be a friend, relative or Commune leader/representative. If possible, this person should be selected by the patient and should have no connection with the study team).

I have witnessed the accurate reading of the consent form to the patient, who has had the opportunity to ask questions. I confirm that the patient has given consent freely for a pregnancy test.

Print name of witness:	
Signature of witness:	
Date:	___ ___ / ___ ___ / _____ DD MM YYYY

Commune Health Station nurse's signature:

I have witnessed the accurate reading of the consent form to the patient, who has had the opportunity to ask questions. I confirm that the patient has given consent freely for a pregnancy test.

Print name of nurse:	
Signature of nurse:	
Date:	___ ___ / ___ ___ / _____ DD MM YYYY

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the patient, who has had the opportunity to ask questions. I confirm that the patient has given consent freely for a pregnancy test.

Name of Doctor taking Consent (Print)		___ ___ / ___ ___ / _____ DD MM YYYY
Signature of Doctor taking Consent		

APPENDIX G.

Schedule of Activities

Pyronaridine-artesunate (Pyramax) regimen for mono-infections of *P. falciparum* and *P. malariae* or mixed infections of falciparum malaria

	Day 0	Day 1	Day 2	Days 3 - 6	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Any other day
PROCEDURES											
Inclusion / Exclusion Criteria	X										
Informed Consent	X										
Clinical assessment of vitals, body systems, physical examination	X									X	(X)
Medical History (signs/symptoms)		X	X	X	X	X	X	X	X	X	X
Temperature	X	X	X	X	X	X	X	X	X	X	(X)
Pregnancy test (if necessary)	X							X		X	X
Blood smears for parasite counts	X	X	X	X	X	X	X	X	X	X	(X)
Blood for:											
hematology and biochemical tests	X				X			X			(X)
filter paper for molecular analysis	X	X			X	X	X	X	X	X	(X)
hematocrit	X										
antimalarial drug level			X		X						(X)
in vitro drug testing	X										
TREATMENT											
Pyramax	X	X	X								
Primaquine			XX								
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.

Schedule of Activities

Pyronaridine-artesunate (Pyramax) regimen for *P. vivax* or mixed infections of vivax malaria.

	Day 0	Day 1	Day 2	Day 3 - 6	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Any other day
PROCEDURES											
Inclusion / Exclusion Criteria	X										
Informed Consent	X										
Clinical assessment of vitals, body systems, physical examination	X									X	(X)
Medical History (signs/symptoms)		X	X	X	X	X	X	X	X	X	X
Temperature	X	X	X	X	X	X	X	X	X	X	(X)
Pregnancy test (if necessary)	X							X		X	X
Blood smears for parasite counts	X	X	X	X	X	X	X	X	X	X	(X)
Blood for:											
hematology and biochemical tests	X				X			X			(X)
filter paper for molecular analysis	X	X			X	X	X	X	X	X	(X)
hematocrit	X										
antimalarial drug level			X		X						(X)
G6PD test	X										
TREATMENT											
Pyramax	X	X	X								
Adverse Events	X	X	X	X	X	X	X	X	X	X	X
Primaquine (14 day course)	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.