COVER SHEET FOR THERAPEUTIC EFFICACY TEST PROTOCOL

Title	Monitoring and evaluation of the efficacy and safety of Artesunate-Mefloquine (ASMQ) and Artesunate-Pyronaridine (ASP) for the treatment of uncomplicated Plasmodium *falciparum* and *Plasmodium vivax* malaria in Cambodia
Study sites	Site 1: Trapeang Cho Health Center, Kampong Speu district, Kampong Speu province ACT tested: Artesunate-Mefloquine (ASMQ) Site 2: Veunsai Health Center, Veunsai district, Rattanakiri province ACT tested: Artesunate-Pyronaridine (Pyramax) Site 3: Chambak Health Center, Phnom Sruoch district, Kampong Speu province ACT tested: Artesunate-Mefloquine (ASMQ) Site 4: Cheu Tom Health Center, Krakor district, Pursat province ACT tested: Artesunate-Pyronaridine (Pyramax) Site 5: Keo Seima Health Center, Saen Monourom district, Mondulkiri ACT tested: Artesunate-Mefloquine (ASMQ)
Protocol submission date	10/03/2020
Protocol number	Cambodia 1
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Study dates	From June to December 2018
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SYNOPSIS

Title: Artesunate-Mefloquine (ASMQ) and Artesunate-Pyronaridine (ASP) for the treatment of uncomplicated Plasmodium falciparum and Plasmodium vivax malaria.

Purpose: To assess the efficacy of the current first line treatment policy; To assess the efficacy of a new antimalarial drug to support updating of the national policy.

Study period: June to December 2020

Study Sites:

1- ASMQ: Trapeang Cho, Kampong Speu district, Kampong Speu province; Chambak, Phnom Srouch district, Kampong Speu; Keo Seima, Saen Monourom, Mondulkiri.

2- ASP: Veunsai, Veunsai district, Rattanakiri; Cheu Tom, Krakor district, Pursat

Study Design: This surveillance study is a one arm prospective study

Patient population by treatment regimen:

Artesunate-Mefloquine (ASMQ)

Febrile patients aged between 18 years and 60 years, with confirmed uncomplicated *P. falciparum and P. vivax* infections.

Artesunate-Pyronaridine (ASP)

Febrile patients aged between 2 years and 60 years, with confirmed uncomplicated *P. falciparum and P. vivax* infections.

Sample Size: 600 (120 in one site: 60 P. falciparum cases and 60 patients P. vivax cases)

Treatment(s) and follow-up: artesunate-mefloquine (ASMQ) will follow a dosing chart in Annex 3; one tablet of Pyronaridine-Artesunate contains 60mg artesunate+ 180mg pyronaridine; dosing is given based on the weight band (Annex 3). Primaquine will be administered as a single 15-mg adult dose (0.25 mg base/kg).

Clinical and parasitological parameters will be monitored over a 42-day follow-up period to evaluate drug efficacy.

Primary endpoints: The proportion of patients with early treatment failure, late clinical failure, late parasitological failure or an adequate clinical and parasitological response as indicators of efficacy. Recrudescence will be distinguished from re-infection by polymerase chain reaction (PCR) analysis.

Secondary endpoints: The frequency and nature of adverse events

Exploratory endpoints:

- to assess the in vitro susceptibility of *P. falciparum* isolates to dihydroarteminisinin, piperaquine, artesunate and mefloquine at D0 and Day of recrudescence;
- to determine the polymorphism of molecular markers of *Plasmodium falciparum* and Kelch gene

1. BACKGROUND

The current first line treatment of both P. *falciparum* and *vivax* malaria infections in Cambodia is artesunate-mefloquine (ASMQ) fixed dose and Quinine and Tetracycline; the patients receive the treatment at all facilities with free of charge.

Emerging resistance to artemisinins and its partner drugs severely threaten the treatment of falciparum malaria in Cambodia. The ACT resistance has growlingly spread to other regions in Cambodia in the last five years to other regions of the country— in the North, East, and South of Cambodia; it may undo all the efforts in malaria elimination and potentially spreads to the regional and global levels. DHA-PIP became less effective after introduced as the first line drug in 2008; the efficacy level of DHA-PIP had gradually decreased to as low as 37.5% in Siem Reap and 60% in Stung Treng in 2014. The recommendation from the drug policy meeting suggested there is an urgent need to change DHA-PIP, the first-line treatment between 2007 and 2014, to artesunate-mefloquine (ASMQ).

Sensitized with the previous observation of efficacy of artesunate-mefloquine enduring about 3 or 4 years, the national program in collaboration with WHO is to closely monitor the efficacy and safety of ASMQ even though its efficacious level is high. At the same time, there is a need to explore the alternative ACT before the current ACT becomes less effective. Pyronaridine-artesunate could be an alternative option for the treatment of uncomplicated falciparum malaria in western Cambodia. On 16 February 2012, the European Medicine Agency (according to Article 58) adopted a positive scientific opinion for pyronaridine-artesunate (Pyramax®). Pyronaridine-artesunate was pre-qualified by WHO in adults and children >20 kg in countries with documented artemisinin resistance including Cambodia, where the drug was tested in 2005, 2008 and 2104.

Pyronaridine-artesunate was first used as a fixed combination in 2005 in the context of the Phase II development; it is however not yet commercially available in Cambodia. The TES result in 2017 indicated that ASMQ remained effective in the west part of the country and the potential alternative ACT, artesunate-pyronaridine (ASP), also proved to be highly effective in the north and in the east of the country.

It is necessary to closely monitor the efficacy and safety of ASMQ in the east and the south of and re-evaluate the efficacy of ASP for the treatment of both species (P. f and P. v malaria) in the west of Cambodia in 2020; the results of this study will be used to assist the Ministry of

Health of Cambodia in assessing the current national treatment guidelines and to update the policy if necessary.

2. OBJECTIVES

The general objective of this study is to assess the efficacy and safety of artesunate-mefloquine (ASMQ) and artesunate-pyronaridine (ASP) with a single 15-mg adult dose (0.25 mg base/kg) for the treatment of uncomplicated *P. falciparum* and *P. vivax* malaria infection.

The primary objectives are:

- **to measure the clinical and parasitological efficacy of ASMQ and ASP for the treatment of uncomplicated *P. falciparum* and *P. vivax* in patients who meet the inclusion criteria, suffering from *P. falciparum* and *P. vivax* by determining the proportion with early treatment failure, late clinical failure, late parasitological failure or an adequate clinical and parasitological response as indicators of efficacy;**
- to differentiate recrudescence from new infection by polymerase chain reaction (PCR) analysis for *P. falciparum* malaria;

The secondary objectives are:

- to evaluate the incidence of adverse events; and
- to formulate recommendations and to enable the Ministry of Health to make informed decisions about whether the current national antimalarial treatment guidelines should be updated.

The optional exploratory objectives are:

- to assess the in vitro susceptibility of P. falciparum isolates to name of the antimalarial drug(s);
- to determine the polymorphism of molecular markers for name of the antimalarial drug(s) resistance; and
- to determine the blood concentration of name of the antimalarial drug(s).

3. INVESTIGATIONAL PLAN

3.1 Study design

This surveillance study is a one-arm prospective evaluation of clinical and parasitological responses to directly observed treatment for uncomplicated malaria[1,2]. People with uncomplicated *P. falciparum* who meet the study inclusion criteria will be enrolled, treated on site with ASMQ and ASP, and monitored for 42 days for *P. falciparum* and 28 days for *P. vivax*. The follow-up will consist of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. On the basis of the results of these assessments, the patients will be classified as having therapeutic failure (early or late) or an adequate response.

[1] WHO. *Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria*. Geneva, World Health Organization, 2003 (WHO/RBM/HTM/2003.50) (http://www.who.int/malaria/resistance).

[2] WHO. *Method for surveillance of antimalarial drug efficacy*. Geneva, World Health Organization, 2009 (http://www.who.int/malaria/resistance).

The proportion of patients experiencing therapeutic failure during the follow-up period will be used to estimate the efficacy of the study drug(s). PCR analysis will be used to distinguish between a true recrudescence due to treatment failure and episodes of reinfection (only P. f patients).

3.2 Study site

Kampong Speu

Oral Health Centre in Oral district, Kampong Speu province is located around 100km far from Capital city, Phnom Penh. The estimated population is 76,000 living in 42 villages and in three operational districts: Kampong Speu, Kong Pisey and Odong. Malaria transmission occurs from May to December with a peak between July and September. Most people are a farmer; the others are hunters, forest workers making charcoal or collecting firewood. Anopheles Dirus is predominant and not common for An. Minimus and An. Maculatus.

Mondulkiri

Mondulkiri border the provinces of Kratié to the west, Stung Treng to the northwest, Ratanakiri to the north and the country of Vietnam to the east and south, it is the most sparsely populated province in the country despite being the largest in land area. The capital is the town of Senmonorom. The population lives off the land, planting rice, fruit trees, and a variety of vegetables. Others grow strawberries, coffee, rubber, and cashew nuts. Kaoh Nheaek is a district located in Mondulkiri Province, in Cambodia. According to the 1998 census of Cambodia, it had a population of 8,919. This year the study will conduce at Kaoh Nheake

Pursat

Located in the west of the country, Pursat is 188km far from Phnom Penh and from Pursat town to Veal Veng is 125km. The total population in Pursat province is 449,599 in 501 villages, 6 districts and 49 communes. The most people in the area are farmer. People go to their farms “called as Chamka” which is far from villages, especially, in the rainy season to harvest season of vegetables and fruits.

3.3 Study population

The study population will consist of patients with uncomplicated P. falciparum or P. vivax malaria attending the study health clinic who are aged between 2-60 years for ASP and 18-60 years for ASMQ. All adult patients who are above 18 years, age of majority in this country, will sign an informed consent form for participation. Parents or guardians will give informed consent on behalf of children who have not reached the age of majority. Children aged from 12 years and age of majority will be required to consent for participation by signing an informed assent form.

The criteria of the age range included in this surveillance relies on the pattern of malaria infection and their potential involvement in the surveillance study. Those patients under 2 and over 65 are excluded because they may need additional health service support at the higher level while the surveillance study is based at the health centers.

3.4 Timing and duration of study

The study will be conducted during the malaria transmission season, from June to December,

2018.

3.5 Inclusion criteria

- For those receiving ASP: age between 2 and 60 years
- For those receiving ASMQ: age between 18 and 60 years;
- mono-infection with *P. falciparum* or *P. vivax* by microscopy; *P. falciparum* or *P. vivax* parasitaemia of 500-100,000/µl asexual forms;
- presence of axillary ≥ 37.5 °C or history of fever during the past 24 h;
- ability to swallow oral [by mouth] medication;
- ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule; and
- informed consent from the patient or from a parent or guardian in the case of children aged less than 12 years;
- informed assent from any minor participant aged more than 12 years and less than 60 years; and
- consent for pregnancy testing from female of child-bearing potential and from their parent or guardian if under 18 years.

3.6 Exclusion criteria

- presence of general danger signs in children aged under 5 years or signs of severe falciparum malaria according to the definitions of WHO (Appendix 1);
- Age less than 2 years old and over 60 for ASP, under 18 and over 60 for ASMQ;
- mixed or mono-infection with another *Plasmodium* species detected by microscopy;
- presence of severe malnutrition (defined as a child whose growth standard is below –3 z-score, has symmetrical oedema involving at least the feet or has a mid-upper arm circumference < 115 mm);
- presence of febrile conditions due to diseases other than malaria (e.g. measles, acute lower respiratory tract infection, severe diarrhea with dehydration) or other known underlying chronic or severe diseases (e.g. cardiac, renal and hepatic diseases, HIV/AIDS);
- regular medication, which may interfere with antimalarial pharmacokinetics;
- history of hypersensitivity reactions or contraindications to any of the medicine(s) being tested or used as alternative treatment(s);
- a positive pregnancy test or breastfeeding;
- unable to or unwilling to take pregnancy test or to use contraception for women of child-bearing age (defined as age > 12 years and sexually active).

3.7 Loss to follow-up

Loss to follow-up occurs when, despite all reasonable efforts, an enrolled patient does not attend the scheduled visits and cannot be found. No treatment outcome will be assigned to these patients. Every effort must be made to schedule a follow-up visit for patients who fail to

return to the study site, especially during but also after administration of the study drug. These patients will be classified as lost to follow-up and censored or excluded from the analysis. Patients who are lost to follow-up, but who subsequently return to the study site before day 42 for *P. falciparum* patients or before day 28 for *P. vivax* will not be turned away and will be encouraged to return for check-up visits. The principal investigator will decide whether the patient is to be definitely classified as lost to follow-up on the basis of his or her history or is to be maintained for the analysis.

3.8 Patient discontinuation or protocol violation

Study patients who meet any of the following criteria will be classified as withdrawn.

- withdrawal of consent. A patient may withdraw consent at any time, without prejudice for further follow-up or treatment at the study site.
- failure to complete treatment, due to:
  - persistent vomiting of the treatment. A patient who vomits the study medication twice will be withdrawn from the study and given rescue treatment.
  - failure to attend the scheduled visits during the first 3 days; or
  - serious adverse events necessitating termination of treatment before the full course is completed. A patient can be discontinued from the study if the principal investigator decides so due to an adverse event of adequate nature or intensity. In this case, information on the adverse event and symptomatic treatment given must be recorded on a case report form. If the adverse event is serious, the principal investigator must notify the sponsor or its designee immediately and follow the reporting procedures described in section 5.3.
- enrolment violation:
  - severe malaria on day 0; or
  - erroneous inclusion of a patient who does not meet the inclusion criteria.
- voluntary protocol violation: self- or third-party administration of antimalarial drug (or antibiotics with antimalarial activity) (Appendix 2);
- involuntary protocol violation:
  - occurrence during follow-up of concomitant disease that would interfere with a clear classification of the treatment outcome;
  - detection of mono-infection with another malaria species during follow-up; or
  - misclassification of a patient due to a laboratory error (parasitaemia), leading to administration of rescue treatment.

Patients who are withdrawn will nevertheless be followed up until recovery or the end of follow-up, if possible; however, no treatment outcome will be assigned to these patients, and they will be censored or excluded from the analysis. The reasons for discontinuation or protocol violation will be recorded on the case report form.

Pregnancy detected during the course of follow-up does not constitute a reason for withdrawal but the event must be recorded and managed as described in section 5.3.

4. TREATMENT

4.1 Antimalarial treatment

ASMQ (Artesunate + Mefloquine co formulated).
The adult dose tablet contains 100mg Artesunate plus 220mg Mefloquine and the paediatric tablet contain 55mg Artesunate plus 55 mg Mefloquine. (Refer to Annex table for more detailed dosage).

ASP (Artesunate + Pyronaridine/ Pyramax): Adults and children ≥ 20 kg (Refer to Annex table for more detailed dosage).

All doses of medicine will be administered under the supervision of a qualified member of the staff designated by the principal investigator. The study patients will be observed for 30 min after medicine administration for adverse reactions or vomiting. Any patient who vomits during this observation period will be re-treated with the same dose of medicine and observed for an additional 30 min. If the patient vomits again, he or she will be withdrawn and offered rescue therapy. The patients are recommended to stay at the health facility until they complete the treatment course for malaria infection.

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4.2 Concomitant treatment and medication that should not be used

Fever over 38°C can be treated with paracetamol or acetaminophen. Parents or guardians will be instructed in the use of tepid sponging for children under 5 years of age.

Prior treatment with antimalarial drugs will not be considered an exclusion criterion; however, during follow-up, if infections other than malaria require the administration of medicines with antimalarial activity, the patient will be withdrawn from the study. Patients given tetracycline as an eye ointment will not be excluded (Appendix 2). Patients will be withdrawn from the study in the case of self-medication or if an antimalarial drug or an antibiotic with antimalarial activity is administered by a third party.

Adverse events requiring treatment can be treated according to local practice to the best available local practice. If there is a clinical indication for any additional medication during the course of the study, including medication given to treat an adverse event related to the study medicine, the name of the medicine, the dosage and the date and time of administration must be recorded on the case report form.

The use of herbal remedies during the study should be avoided, and participants should be encouraged to return to the study site for treatment if they feel unwell. If any herbal remedies

are taken during the study, this should be captured on the case report form, under 'study medication administration'.

4.3 Rescue treatment

If the patient vomits twice the treatment he/she will receive parenteral therapy based on national treatment guideline. If a patient is unable to tolerate the trial medication he/she should discontinue the treatment and alternative anti-malaria medication should be initiated. In this case, the reason for discontinuation should be recorded in the case record form (CRF) as "Adverse experience" and be withdrawn from the study.

Any patient with sign of severe malaria, will be hospitalized and receive parenteral therapy with artemether or quinine 7 days (IV) + tetracycline 7 days as well as relevant supportive treatments. If a patient meets one of the criteria for therapeutic failure, he/she will receive artemether at D1 3.2mg/kg (max 160mg) intramuscularly, D2 1.6 mg/kg (max 80mg) IM, from D3 to D5 1.6 mg/kg (max 80mg) per day IM and D6 plus mefloquine 25mg/kg spreading over 3 days (max 1250mg) according to the current national recommendations.

5. EVALUATION CRITERIA

The study end-point is the classification assigned to a patient. Valid study end-points include: treatment failure, completion of the follow-up period without treatment failure (adequate clinical and parasitological response), loss to follow-up, withdrawal from study, and protocol violation. At all times, the well-being of the patient will take priority over his or her continuation in the study.

5.1 Efficacy and safety evaluation

5.1.1 Classification of treatment outcomes

Treatment outcomes will be classified on the basis of an assessment of the parasitological and clinical outcome of antimalarial treatment according to the latest WHO guidelines.[3] Thus, all patients will be classified as having early treatment failure, late clinical failure, late parasitological failure or an adequate clinical and parasitological response, as defined in Appendix 4.

As parasitological cure is the goal of antimalarial therapy, all study patients who show treatment failure will be given rescue treatment. Follow-up will continue until recovery. The results from these patients do not need to be recorded systematically for the purpose of the surveillance study.

5.1.2 Safety end-points

The incidence of any adverse event will be documented. All patients will be asked routinely about previous symptoms and about symptoms that have emerged since the previous follow-up visit. When clinically indicated, patients will be evaluated and treated appropriately. All adverse events will be recorded on the case report form. Serious adverse events (see definitions in 5.3) must be reported to the sponsor.

[3] WHO. *Susceptibility of* Plasmodium falciparum *to antimalarial drugs. Report on global monitoring 1996–2004*. Geneva, World Health Organization, 2005 (WHO/HTM/MAL/2005.110) (http://www.who.int/malaria/resistance).

5.2 Clinical evaluation

All patients will be evaluated clinically as described below.

5.2.1 Physical examination

A standard physical examination will be performed at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, 28, 35 and 42. A complete medical history including prior and concomitant medication, demographic information and contact details will be taken at baseline.

5.2.2 Body weight

Body weight will be recorded on day 0 to the nearest kilogram on a Salter scale or on a hanging scale for young children. The scales will be properly calibrated. Patients should not wear excessive clothing while being weighed as this can overestimate their true weight. All young children should only wear undergarments while being weighed. The screening weight will be used to satisfy the inclusion or exclusion for nutrition status as well as to calculate the dose (number of tablets) to be administered. When assessing weight-for-height, children over 24 months of age should have their heights measured while standing. For simplicity, however, infants and children under 87 cm can be measured lying down (or supine) and those above 87 cm standing.

The reliability of the scales will be verified before the study begins and checked at regular intervals.

The circumference of the left mid-upper arm will be measured, at the mid-point between the elbow and the shoulder, and will be recorded to the nearest 0.2 cm. Oedema will be assessed by thumb pressure for 3 s on the dorsal surface of both feet.

5.2.3 Body temperature

Axillary temperature will be measured at baseline (day 0 before dosing) and on days 1, 2, 3, 7, 14, 21, 28, 35 and 42. Temperature will be measured with a thermometer that has a precision of 0.1 °C. Temperature will also be measured as clinically indicated. If the result is < 36.0 °C, the measurement will be repeated. The same route should be used throughout the study.

The quality of the temperature-taking technique and the thermometers should be assessed regularly. Thermometers should be tested in a water-bath of known temperature before the study begins and at regular intervals thereafter.

5.2.4 Microscopic blood examination

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 blood films will be also examined every 8 hours on days 1, 2, 3 and once on days 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 labeled anonymously (screening number or study number, day of follow-up, date). If this is a study to investigate parasitological response to a monotherapy with artemisinin-related compounds the number of parasite counts must be specified, e.g.: 2 thick blood films should be taken 12-hourly after screening for the 3 days or until parasite count is negative.

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 examined at a magnification of 1000 × to identify the parasite species and to determine the parasite density.

Three blood slides per patient will be obtained: two thick blood smears and one thin blood smear. One slide will then be stained rapidly (10% Giemsa for 10–15 min) for initial screening, while the others will be retained. If the patient is subsequently enrolled, the second slide will be stained more carefully (e.g. 2.5–3% Giemsa for 45–60 min), and slower staining will also be used for all slides obtained at follow-up visits. The study number of the patient, the date and the day of follow-up will be recorded either on the frosted edge of the slide or on the glass with a permanent glass pen.

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 adequate parasitaemia for enrolment is at least one parasite for every 16 white blood cells, corresponding to approximately 500 asexual parasites per microlitre for low-to-moderate transmission areas.

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 (typically 200) with a hand tally counter. Once a field has been started, it must be counted to completion; the final number of white blood cells will therefore rarely be exactly 200. If more than 500 parasites have been counted before 200 white blood cells 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 white blood cells counted and then multiplying by an assumed white blood cell density (typically 6000 per µl).

$$\text{Parasite density (per µl)} = \frac{\text{number of parasites counted} \times 6000}{\text{Number of leukocytes counted}}$$

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 white blood cells in follow-up smears, counting will be done against at least 500 white blood cells (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 1000 white blood cells or 100 fields containing at least 10 white blood cells per field reveals no asexual parasites. The presence of gametocytes on an enrolment or follow-up slide will be noted, but this information will not contribute to basic evaluation.

In addition, 100 fields of the second thick film at day 0 will be examined to exclude mixed infections; in case of any doubt, the thin film will be examined for confirmation. If examination of the thin film is not conclusive, the patient will be excluded from the analysis after complete treatment and follow-up.

Two qualified microscopists will read all the slides independently, and parasite densities will be calculated by averaging the two counts. Blood smears with discordant results (differences between the two microscopists 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.

5.2.5 Genotyping of malaria parasites

In order to differentiate a recrudescence (same parasite strain) from a newly acquired infection (different parasite strain), a genotype analysis will be conducted. This is based on the extensive genetic diversity among the malaria parasite genes msp1, msp2 and glurp 4 The genotypic profiles of pre- and post-parasite strains are compared. The copy number of P. *falciparum mdr1* gene will also be measured as well as sequencing of Kelch gene.

In order to minimize discomfort to the patient due to repeated finger pricks, two to three drops of blood will be collected on filter paper Whatman 3MM during screening or enrolment and each time blood smears are required according to the protocol on and after day 7. Specimens will be labeled 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 analyzed. When such room temperature conditions are not possible, for example in extremely humid environments where air-conditioning is not available, storage in a refrigerator or freezer will be considered, but great care will be taken to protect samples from frost and moisture. The PCR technique used will be performed by laboratory of Institute Pasteur in Cambodia. Paired filter papers will be used for parasite DNA extraction and genotyping. Unused filter papers will be kept in safe locations until data are eventually validated or for further research needs. All filter papers will be destroyed immediately after the PCR analyses have been completed. The sponsor will provide instructions to the principal investigator regarding shipment or destruction procedures of biological specimen collected during the study.

5.2.6 Pregnancy test

Female patients of child-bearing age, defined as those who menstruate and who are sexually active, will be asked to take a urine pregnancy test before enrolment in the study, because ASMQ and ASP is contraindicated during the first trimester. They will also be asked to take a urine pregnancy test on day 42 or on early withdrawal from the study.

Female participants of child-bearing age, defined as those who menstruate and sexually active should use barrier contraceptive devices for the duration of the study. They are highly recommended to use condom or the practice of sexual abstinence during the study period as to prevent the risks of becoming pregnant and exposing the fetus to the study medicines.

5.2.7 Haematological assessment

Haematocrit will be determined at local laboratory at D0 using the capillary tube centrifuged in the machine.

5.2.8 *In vitro* susceptibility of *P. falciparum*

A blood sample (5 ml) for *in vitro* drug testing will be collected at day 0 and day of failure to evaluate the *in vitro* susceptibity of *P. falciparum* parasites to mefloquine, artesunate, piperaquine, chloroquine, quinine and dihydroartemisinin. Specimens will be labeled anonymously (study number, day of follow-up, date). The *in vitro* drug sensitivity of *P.*

[4] WHO. *Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations.* Geneva, World Health Organization, 2008 (http://www.who.int/malaria/resistance).

falciparum isolates will be assessed using a classical isotopic microtest. In addition, artemisinin susceptibility will be evaluated using the RSA (Ring-stage Survival Assay) a new in vitro assay described in Witkowski et al (AAC 2013). Briefly, blood samples will be rapidly transported to the laboratory after collection. Thin smears will be performed and only those containing pure *P. falciparum* with parasitaemia greater than 0.1% will be tested immediately or after culture adaptation during one or 2 weeks. In the classical isotopic microtest, parasites will be cultivated 48h in presence of 3H hypoxantine and different concentration of antimalarial drugs. The parasite maturation will be assessed by measuring the incorporation of the radioactive product. The Malaria Molecular Epidemiology Unit at Institut Pasteur in Cambodia will perform these tests. Results will be expressed as IC_{50} (corresponds to the drug concentration which inhibits the parasite growth by 50%).

5.2.9 Molecular markers for antimalarial drug resistance

Three drops of blood will be collected on a filter paper (3M Whatman filter) at day 0 and day of failure to define putative markers related to antimalarial drugs resistance (pfmdr1, pfcrt, , cytb, pfmdr1 copy number, etc…), parasite genetic diversity and *Plasmodium* species. *Plasmodium* species will be determined using real time PCR (Chou et al, Malaria Journal 2012), genetic diversity using Nested PCR/gel migration (Leang et al, AAC 2012), bar coding (Hoyer et al, PlosOne 2012) and whole genome sequencing. Putative markers related to antimalarial drugs resistance will be analyzed by PCR/sequencing approach or by a multiplex real-time PCR assay (Leang et al, AAC 2012).Malaria Molecular Epidemiology laboratory at Institut Pasteur in Cambodia will be performing these tests. 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 analyzed. In order to identify whether Piperaquine is resistant blood spots at day 7 will be additionally collected with a special type of filter paper from every patients from all the study sites.

5.2.10 Anti-malarial drugs blood concentration

By using the 100 ml capillary pipette, 100 ml capillary blood samples will be collected on day 7 for determining the blood concentration of piperaquine. The collected blood will be transferred on to the special filter paper (Watman ETCH31). Specimen will be labeled as follows: study number, day of follow up and date. Blood level will measured by using HPLC at Institute Pasteur in Cambodia.

5.3 Safety assessment

Safety will be assessed by recording the nature and incidence of adverse events and serious adverse events. Adverse events will be assessed by direct questioning. An adverse event is defined as any unfavorable, unintended sign, symptom, syndrome or disease that develops or worsens with the use of a medicinal product, regardless of whether it is related to the medicinal product. All adverse events must be recorded on the case report form.

A serious adverse event is defined as any untoward medical occurrence that at any dose:

- results in death, is life threatening;
- requires hospitalization or prolongation of hospitalization;
- results in a persistent or significant disability or incapacity; or

- is a congenital anomaly or birth defect.

'Life-threatening' means that the person was at immediate risk for death; it does not refer to a adverse event that might have caused death if it were more severe. 'Persistent or significant disability or incapacity' means that a person's ability to carry out normal life functions is substantially disrupted.

All serious adverse events occurring during the study must be recorded and reported by the principal investigator to the sponsor, regardless of whether the principal investigator considers the events to be related to the investigated medicine.

The investigator will collect information on any women who become pregnant while participating in this study and will record the information on the appropriate form. The pregnant woman will also be followed to determine the outcome of the pregnancy. Generally, follow-up will be no longer than 6–8 weeks after the estimated delivery date. Any premature termination of pregnancy will be reported. While pregnancy itself is not considered an adverse event or a serious adverse event, any complication of pregnancy or elective termination for medical reasons will be recorded as an adverse event or a serious adverse event. A spontaneous abortion is always considered a serious adverse event and will be reported as such.

6. STUDY ASSESSMENT

6.1 Screening and enrolment

All patients who meet the basic enrolment criteria (age, fever or history of fever if appropriate, symptoms of malaria, absence of danger signs in children in relation to malaria—child unable to drink or breastfeed, vomiting everything, recent history of convulsions, lethargic or unconscious state, unable to sit or stand, difficulty in breathing—, absence of signs of severe malaria, absence of severe malnutrition, pregnancy) during screening will be assigned a consecutive number and evaluated in greater depth by clinical staff. In children, care will be taken to detect early signs of febrile diseases other than malaria, as their presence will necessitate exclusion from the evaluation. The most frequent confounding condition is a lower respiratory tract infection: cough or difficult breathing, together with fast breathing, is an indicator for exclusion. Fast breathing is defined as a respiratory frequency > 50/min in infants under 12 months of age and > 40/min in children aged 12–59 months. Other relatively common febrile conditions are otitis media, tonsillitis, measles and abscesses. Patients with these conditions will not be enrolled but should be treated for both malaria (if they have parasitaemia) and the other infection, as appropriate.

The screening record form (Appendix 5) will be used to record the general information and the clinical observations on each patient being screened. If the patient meets the clinical criteria, he or she will be examined for parasitaemia. Once the patient meets all the enrolment criteria, he or she (if adults are included) or a parent or guardian in case of children will be asked for consent to participate in the study. Children from 12 years of age will also need to provide their assent to participate.

6.2 Follow-up

Patients who meet all the enrolment criteria will be given a personal identification number and will receive treatment only after the study has been fully explained to them and they have willingly provided informed consent. Any person who decides not to participate in the study will be examined, treated and followed-up by the health facility staff according to the standard of care established by the Ministry of Health.

The basic follow-up schedule is summarized in Appendix 6. A case report form (Appendix 7) and a serious adverse event report form (Appendix 8) will be used to record the general information and clinical observations on each patient enrolled into the study. The appointment schedule will be clearly explained, and a follow-up card with a personal identification number will be provided. House visits will be made in the event patient cannot make it to the clinic. If this is the case provide detail about the visiting staff and the study procedures that will be done at home. The health staff will send to the patient's residence and provides a transportation support for bringing the patient to the study site where he or she will receive the physical examination and laboratory test if necessary.

The day a patient is enrolled and receives the first dose of medicine is designated 'day 0'. All antimalarial treatment will be given by a study team member under supervision. Enrolled patients will be observed for at least 30 min after treatment to ensure that they do not vomit the medicine. If vomiting occurs within 30 min of treatment, the full treatment dose will be repeated. Ancillary treatment, such as antipyretics, will be provided if necessary to patients by the study team and documented on the case report form. Patients with persistent vomiting (i.e. necessitating more than a single repeat dose) will be excluded from the study and immediately referred to the health facility staff for appropriate management.

Thereafter, patients are required to undergo regular clinical reassessment. Blood films for parasite counts will be made on days 2, 3 and 7 and then weekly for the remainder of the follow-up period, i.e. on days 14, 21, 28, 35 and 42. Patients will be advised to return on any day during the follow-up period if symptoms return and not to wait for the next scheduled visit day. In particular, parents or guardians should be instructed to bring children to the centre at any time if they show any sign of danger (unable to drink or breastfeed, vomiting everything, presenting with convulsions, lethargic or unconscious, unable to sit or stand, presenting with difficult breathing), if they are still sick or if there is any cause for worry. Clinical reassessment will be sufficiently thorough to ensure patient safety and will include assessment not only for potential treatment failure but also for potential adverse reactions to the medicine. Additionally, blood films will be obtained whenever parasitological reassessment is requested by the clinical staff.

Because many medicines have to be given over several days, the initial visits are critical not only for assessing efficacy but also for ensuring patient safety; defaulters at this stage will not have received a complete course of treatment and may be at risk for clinical deterioration. All reasonable efforts will be made to find defaulters to ensure complete treatment. Similarly, the ultimate success of the study rests on minimizing loss to follow-up. While patients are encouraged to return on their own for scheduled follow-up visits, it is essential that provisions be made ahead of time for locating patients at home if they do not attend as requested. This requires obtaining detailed directions to the home during enrolment, and study team members familiar with the community will be responsible for home visits and means of transport for the patients. The schedule of treatment and follow-up examinations given in this protocol must be followed to ensure data integrity. Patients who fail to return on days 1 and 2 and miss one dose of the treatment will be withdrawn from the study definitively. After day 3, patients who fail to return on day 7 but are present on day 6 or 8 (likewise days 13/15, days 20/22, days 27/29, days 34/36 and days 41/43) may still be included in the analysis. Deviation from the protocol of more than 1 day should, however, be avoided (see also section 3.7).

7. DATA MANAGEMENT

The principal investigator will ensure that the study protocol is strictly adhered to and that all data are collected and recorded correctly on the case report form. Laboratory and clinical data will be recorded on a daily basis on the case report form designed for the study. Data derived

from source documents should be consistent with the source documents, or the discrepancies should be explained. Any change or correction to a case report form should be dated and explained and should not obscure the original entry. All case report forms will be checked for completeness.

After the study has been completed, data will be entered into a database by double independent data entry, according to WHO standard procedures.[5] The study data will be stored in a computer database, maintaining confidentiality.

The principal investigator is responsible for keeping all screening forms, the case report form and the completed subject identification code list in a secure location. These documents will be destroyed immediately after the analyses have been completed. The sponsor will provide instructions to the principal investigator regarding destruction procedures of documents collected during the study.

8. STATISTICAL METHODS

8.1 Minimum sample size

As the treatment failure rate to ASMQ or ASP in the area is 10%, 10% has been chosen. At a confidence level of 95% and a precision around the estimate of 8%, a minimum of 54 patients must be included. With a 10% increase to allow loss to follow-up and withdrawals during the 42-day follow-up period, 60 patients should be included in the study per site for *P. falciparum* and 60 *P. vivax* and per species.

8.2 Analysis of data

Standard WHO spreadsheet will be used for data management and analysis. Data will be analysed by two methods: the Kaplan-Meier method and per-protocol analysis. In addition to the reasons for withdrawal listed in section 3.8, patients will be considered withdrawn from the analysis if the PCR results are unclassifiable or if the results of PCR indicate that the failure is due to reinfection with *P. falciparum.*

The final analysis will include:

- a description of all patients screened and the distribution of reasons for non-inclusion in the study;
- a description of all the patients included in the study;
- the proportion of adverse events and serious adverse events in all the patients included in the study;
- the proportion of patients lost to follow-up or withdrawn, with 95% confidence intervals and a list of reasons for withdrawal;
- the cumulative incidence of success and failure rates at day 28 and 42, PCR-uncorrected and PCR-corrected; and
- the proportion of early treatment failure, late clinical failure, late parasitological failure and adequate clinical and parasitological response at day 28 and 42, with 95%

[5] WHO/GMP. *Standardized data entry for therapeutic efficacy tests*. Geneva, World Health Organization (http://www.who.int/malaria/resistance).

confidence intervals, PCR-uncorrected and PCR-corrected.

Guidelines on calculating the cumulative success or failure rate, the proportion of adequate clinical and parasitological response and treatment failure are given in Appendix 9.

8.3 Dissemination of results

At the end of the study, the principal investigator will submit a report on the study and its main outcome. This report will be shared with the national malaria control programme and the Ministry of Health. The results will seek for the publication in the peer review journal. The meeting with provincial health supervisors and also local community leaders will be conducted after the preliminary results are available after the completion of the study at each study site.

8.4 Amendments to the protocol

After the protocol has been accepted, no change may be made without the agreement of the principal investigator, the sponsor(s) and the institutional review boards.

9. ETHICAL CONSIDERATIONS

9.1 Approval by the national ethical committee

Before study initiation, official approval to conduct the study will be obtained from National Ethics Committee at Institute of Public Health, Cambodia. The study key information will be posted on a public clinical trial registry (http://www.ANZCTR.org.au).

9.2 Informed consent

Patients will be included in the study only if they (if adults included) or parents or guardians of children give informed consent. The consent request is available in English and translated into Khmer, will be read entirely to the patient, parent or guardian. Details about the study and its benefits and potential risks will be explained. Once any questions have been answered, a signature will be requested on the document (Appendix 10). If the patient is illiterate, a literate witness must sign; if possible, the signatory will be selected by the prospective participant and will have no connection to the research team. The principal investigator must also obtain and document the assent of children over the age of 12 years and 18 years, but their assent should be accompanied by the consent of a parent or guardian. A child aged between 12 and 18 years who does not agree to participate will not be enrolled in the study and will be referred the health facility staff to be treated according to the standard of care established by the Ministry of Health. Written consent statement for the pregnancy test and the need for contraception is also required for female participants of child-bearing age who are sexually active.

9.3 Confidentiality

All information on patients will remain confidential and be shared only by the study team. Unique identifiers will be used for computer-based data entry and blood samples. In all cases, the principal investigator will ensure that screening forms, the case report form and the completed identification code list are kept in locked files.

9.4 Health-care services

Free health care throughout follow-up for any illness related to malaria will be provided to the study patients regardless of treatment outcome; this includes any expenses related to hospital admission and to adverse medicine reactions, if required.

When prospective or actual participants are found to have diseases unrelated to malaria, the principal investigator should advise them to obtain, or refer them for, medical care.

Any person who decides not to participate or who cannot be enrolled into the study because he or she does not meet the criteria will be referred to the health facility staff. Such people will be treated with antimalarials recommended in the first line treatment in the national treatment guideline and followed-up according to the standard of care established by the Ministry of Health. The principal investigator will ensure that this antimalarial drug is available at the health center.

If a patient is withdrawn from the study before he or she has completed the full course of the treatment, the physician must make all necessary arrangements to provide the patient with the full dose of the medicine being tested or with a full course of antimalarials—ASMQ or ASP (Annex 3) based on the areas where the study site is— recommended in the national treatment guideline.

9.5 Reimbursement and compensation

Subjects shall be reimbursed for their transport to attend all visits to the health center. Insecticide treated nets will be provided to participants. No other gifts or payments will be made.

9.6 Community

Even though the study is health facility-based the community will be informed about the presence of and study purposes. The researchers will meet with the provincial health departments and ask for the collaboration and coordination between the researchers and VMWs/VHVs in the catchment areas of HCs under the study. The researcher will send one of research team members to attend the VMWs/VHVs monthly meeting to inform the community about the progress study and seek for further collaboration from the community.

9.7 Clinical trial registration

As required by WHO, it is necessary to register all therapeutic efficacy studies being done by the country. The clinical trial registration will be done by the Ministry of Health through NMPE on these recommended websites: http://www.ANZCTR.org.au or www.clinicaltrials.gov

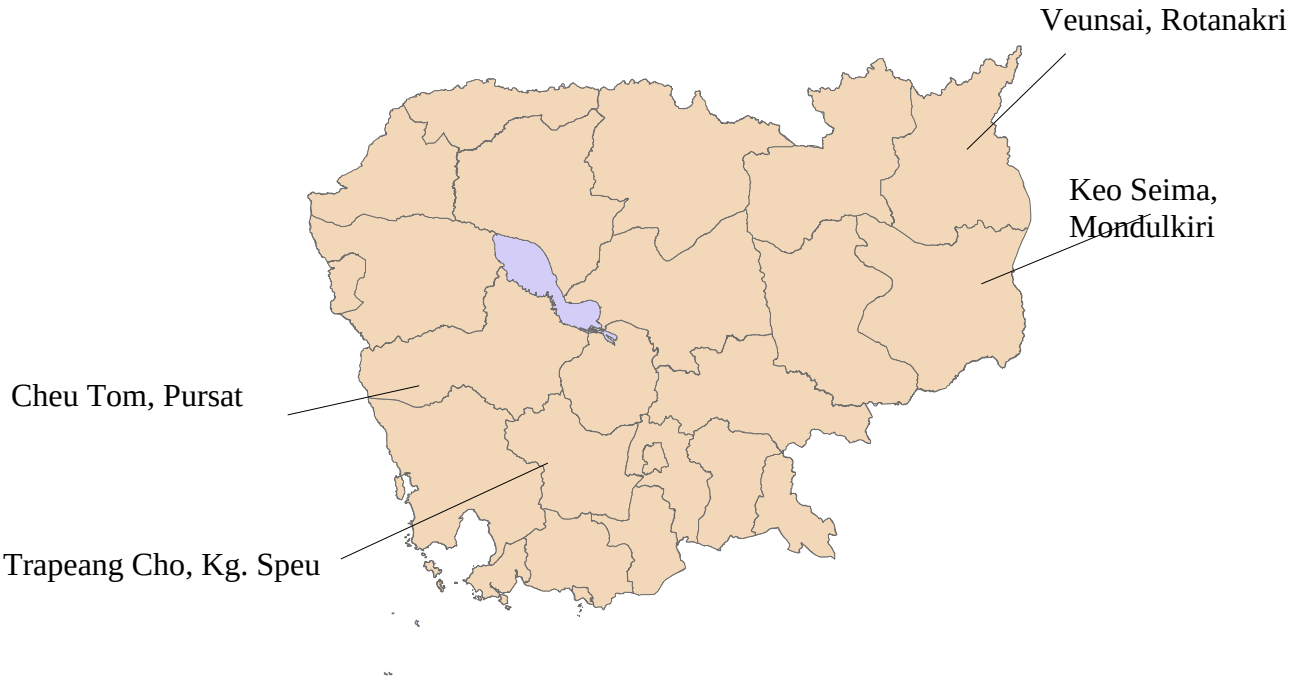

Figure 1: Study sites for 2020

Appendix 1. Definition of severe falciparum malaria[6]

Severe manifestation of *P. falciparum* malaria in adults and children

Clinical manifestations

- prostration,
- impaired consciousness,
- respiratory distress (metabolic acidosis),
- multiple convulsions,
- circulatory collapse,
- pulmonary oedema (radiological),
- abnormal bleeding,
- jaundice,
- haemoglobinurea.

Laboratory findings

- severe anaemia (haemoglobin < 5 g/dl, haematocrit < 15%),
- hypoglycaemia (blood glucose < 2.2 mmol/l or 40 mg/dl),
- acidosis (plasma bicarbonate < 15 mmol/l),
- hyperlactataemia (venous lactic acid > 5 mmol/l),
- hyperparasitaemia (> 4% in non-immune patients),
- renal impairment (serum creatinine above normal range for age).

Classification of severe malaria in children

Group 1: children at increased risk for death

- prostration
- respiratory distress

Group 2: children at risk for clinical deterioration

- haemoglobin < 5 g/dl, haematocrit < 15%
- two or more convulsions within 24 h

Group 3: children with persistent vomiting

[6] World Health Organization. Severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2000, 94(Suppl. 1):1–90.

APPENDIX 2. MEDICATIONS (WITH ANTIMALARIAL ACTIVITY) THAT SHOULD NOT BE USED DURING THE STUDY PERIOD

- chloroquine, amodiaquine;
- quinine, quinidine;
- mefloquine, halofantrine, lumefantrine;
- artemisinin and its derivatives (artemether, arteether, artesunate, dihydroartemisinin);
- proguanil, chlorproguanil, pyrimethamine;
- sulfadoxine, sulfalene, sulfamethoxazole, dapsone;
- primaquine;
- atovaquone;
- antibiotics: tetracycline*, doxycycline, erythromycin, azythromycin, clindamycin, rifampicin, trimethoprim;
- pentamidine.

* Tetracycline eye ointments can be used.

APPENDIX 3. DOSING CHART OF SURVEILLANCE ACT

ASMQ (Artesunate + Mefloquine co formulated)

The adult dose tablet contains 100mg Artesunate plus 200mg Mefloquine.

Weight (kg)	D0	D1	D2
18-29	1p	1p	1p
≥30	2p	2p	2p

Pyronaridine-artesunate (Pyramax®, Shin Poong Pharmaceuticals). One tablet contains 60mg artesunate+ 180mg pyronaridine. Dosing will be according to body weight.

Table 1: Pyronaridine-artesunate dosage by body weight

Body Weight(kg)	Daily dose(mg)		Number of tablets
	PYR	AS	
20–<24 kg	180	60	1 tab
24–<45 kg	360	120	2 tabs
45–<65 kg	540	180	3 tabs
65 ang above	720	240	4 tabs

Pyronaridine-artesunate will be taken orally with water, once daily for 3 days. Each dose will be administered under supervision in the clinic or if not possible by a home visitor to the patient's home. A dose will be repeated in full if vomiting occurs within 30 minutes of

administration of the first day of administration only. This event will be documented in the case record form (CRF).

APPENDIX 4. CLASSIFICATION OF TREATMENT OUTCOMES[7]

Early treatment failure

- danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia;
- parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature;
- parasitaemia on day 3 with axillary temperature ≥ 37.5 ºC;
- parasitaemia on day 3 ≥ 25% of count on day 0.

Late treatment failure

Late clinical failure

- danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 42) in patients who did not previously meet any of the criteria of early treatment failure;
- presence of parasitaemia on any day between day 4 and day 42) with axillary temperature
  ≥ 37.5 ºC (or history of fever) in patients who did not previously meet any of the criteria of early treatment failure

Late parasitological failure

- presence of parasitaemia on any day between day 7 and day 42) with axillary temperature
  < 37.5 ºC in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure

Adequate clinical and parasitological response

- absence of parasitaemia on day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure

[7] WHO. *Susceptibility of* Plasmodium falciparum *to antimalarial drugs. Report on global monitoring 1996–2004*. Geneva, World Health Organization, 2005 (WHO/HTM/MAL/2005.110) (http://www.who.int/malaria/resistance).

APPENDIX 5. CASE SCREENING FORM

Case screening form	
Health centre name: Locality: District: Province:	Study number: Patient screening number: Date of visit (dd-mm-yyyy):
Demographic data	
Date of birth (dd-mm-yyyy): or estimated age: in: ☐ months or ☐ years Height (cm): Weight (kg): Sex: ☐ Male ☐ Female If female, is the patient pregnant? ☐ Yes ☐ No ☐ Not sure **(If yes, patient is not eligible)** If pregnant, provide the date of the last menstrual period (dd-mm-yyyy):	
Pre-treatment temperature	
History of fever in previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary	
Thick and thin blood smears for estimation of *P. falciparum* parasite counts	
Species: ☐ *P. falciparum* ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae* Were species other than *P. falciparum* present? ☐ Yes ☐ No **(If yes, patient is not eligible).** Approximate number of *P. falciparum* asexual parasites: Presence of 1–200 parasites / 12 white blood cells? ☐ Yes ☐ No **(If no, patient is not eligible)** Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No	
Has a blood sample for PCR been collected? ☐ Yes ☐ No	
Haematocrit: %	
Urinary analysis (pregnancy test for female patients)	
Result of pregnancy test: ☐ Positive ☐ Negative **(If positive, patient is not eligible)**	
Inclusion criteria	
• age between2 and 60 years • mono-infection with *P. falciparum* confirmed by positive blood smear (i.e. no mixed infection) • parasitaemia between 500 and 100,000/µl of asexual forms • measured temperature (depending on method of measurement) or history of fever within previous 24 h • ability to swallow medication	

- ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule
- absence of severe malnutrition (defined as per protocol)

Does the patient meet all the inclusion criteria? ☐ Yes ☐ No **(If no, patient is not eligible)**

Case screening form (page 2)

Exclusion criteria

- signs and symptoms of severe or complicated malaria requiring parenteral treatment according to WHO criteria (Appendix 1)
- mixed or mono-infection with another *Plasmodium* species detected by microscopy
- severe malnutrition
- febrile conditions caused by diseases other than malaria or other known underlying chronic or severe diseases
- regular medication which interferes with antimalarial pharmacokinetics
- history of hypersensitivity reactions or contraindications to the medicine tested
- positive pregnancy test or breastfeeding
- unable to or unwilling to take contraceptives.

Does the patient meet any of the exclusion criteria? ☐ Yes ☐ No **(If yes, the patient is not eligible)**

If yes, please specify the reason for exclusion:

Patient informed consent and assent

Consent form signed: ☐ Yes ☐ No Assent form signed: ☐ Yes ☐ No	Patient identity number: Date (dd-mm-yyyy):

APPENDIX 6. SCHEDULE OF FOLLOW-UP ACTIVITIES

	Day										
	0	1	2	3	7	14	21	28	35	42	Any other
Procedure											
Clinical assessment	X	X	X	X	X	X	X	X	X	X	(X)
Temperature	X	X	X	X	X	X	X	X	X	X	(X)
Blood slide for parasite count	X		X	X	X	X	X	X	X	X	(X)
Urine sample	(X)										
Blood for:											
genotyping	X				X	X	X	X	(X)	(X)	X
haemoglobin or haematocrit	X					X		(X)		(X)	(X)
molecular markers	X				(X)	(X)	(X)	(X)	(X)	(X)	(X)
in vitro test	X										(X)
Treatment											
Medicine to be tested	X	X	X								
Rescue treatment		(X)	(X)	(X)	(X)	(X)	(X)	(X)			(X)

Parentheses denote conditional or optional activities. For example, treatment would be given on days 1 and 2 only for 3-day dosing. On day 1, the patient should be examined for parasitaemia if he or she has any danger signs or if parasite clearance needs to be calculated. Rescue treatment could be given on any day, provided that the patient meets the criteria for treatment failure. Extra days are any days other than regularly scheduled follow-up days when the patient returns to the facility because of recurrence of symptoms. On extra days, blood slides may be taken routinely or at the request of the clinical staff.

Day 0

Screening

- clinical assessment, including measurement of weight and height; referral in cases of severe malaria or danger signs;
- measurement of temperature;
- parasitological assessment;
- pregnancy test (if necessary);
- informed consent.

Enrolment

- treatment, first dose;
- blood sampling for genotyping.

Optional

- urinary test to detect antimalarial drugs;
- haemoglobin/haematocrit;
- molecular markers of drug resistance;
- in vitro test;
- antimalarial drug blood concentration.

Day 1

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment in cases of severe malaria or danger signs or if parasite clearance needs to be calculated.;
- treatment, second dose or alternative treatment in case of early treatment failure.

Day 2

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment;
- treatment, third dose or alternative treatment in case of early treatment failure.

Day 3, day 7, day 14, day 21, day 28, day 35 and day 42 and any other day ()

- clinical assessment; referral in cases of severe malaria or danger signs;
- measurement of axillary temperature;
- parasitological assessment;
- alternative treatment in cases of treatment failure;
- pregnancy test at the end of follow-up (if necessary);
- blood sampling for genotyping to distinguish between recrudescence and reinfection in cases of treatment failure after day 7 and in vitro susceptibility.

Optional (after day 7)

- haemoglobin/haematocrit;
- blood sampling for molecular markers for drug resistance

APPENDIX 7. CASE REPORT FORMS

Case report form: follow-up day 0	
Health centre name: Locality(Village): District: Province:	Study number: Patient identity number: Date of visit (dd-mm-yyyy):
Demographic data	
Date of birth (dd-mm-yyyy):	or estimated age: in: ☐ months or ☐ years
Height (cm): Weight (kg):	Sex: ☐ Male ☐ Female
If female, is the patient pregnant? ☐ Yes ☐ No ☐ Not sure **(If yes, patient is not eligible).** If pregnant, provide the date of the last menstrual period (dd-mm-yyyy):	
Pre-treatment temperature	
History of fever in previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary l	
Thick blood smears for *P. falciparum*: quantitative parasite counts and qualitative gametocyte counts	
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No **(If yes, patient is not eligible).** If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*	
Has blood sample for PCR been collected? ☐ Yes ☐ No	

Case report form: follow-up day 0 (page 2)				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 1				
Study number: Patient identity number: Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No If yes, perform thick blood smear. Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 2				
Study number: Patient identity number: Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 3				
Study number: Patient identity number: Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 7				
Study number:				
Patient identity number:				
Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
History of fever within previous 24 h? ☐ Yes ☐ No				
Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 14				
Study number: Patient identity number: Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 21				
Study number:				
Patient identity number:				
Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
History of fever within previous 24 h? ☐ Yes ☐ No				
Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: day ___(any other day that is not part of regular follow-up)				
Study number: Patient identity number: Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 28				
Study number:				
Patient identity number:				
Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
History of fever within previous 24 h? ☐ Yes ☐ No				
Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 35				
Study number: Patient identity number: Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No History of fever within previous 24 h? ☐ Yes ☐ No Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/µl: Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No Were species other than *P. falciparum* present? ☐ Yes ☐ No If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No If yes, name the adverse event: Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	

Case report form: follow-up day 42				
Study number:				
Patient identity number:				
Date of visit (dd-mm-yyyy):				
Clinical status				
Presence of danger signs or signs of severe or complicated malaria? ☐ Yes ☐ No				
History of fever within previous 24 h? ☐ Yes ☐ No				
Temperature: ºC ☐ Axillary				
Thick blood smears for estimation of *P. falciparum* parasite counts				
Average number of asexual *P. falciparum* parasites/μl:				
Presence of *P. falciparum* gametocytes? ☐ Yes ☐ No				
Were species other than *P. falciparum* present? ☐ Yes ☐ No				
If yes, which species? ☐ *P. vivax* ☐ *P. ovale* ☐ *P. malariae*				
Has a blood sample for PCR been collected? ☐ Yes ☐ No				
Adverse events				
Presence of an adverse event? ☐ Yes ☐ No				
If yes, name the adverse event:				
Is it a serious adverse event? ☐ Yes ☐ No. If yes, inform the sponsor.				
Medication administration				
Name(s) of antimalarial drug(s)	Time of dose (hh:min)	Number of tablets	Did the patient vomit?	Time of vomiting (hh:min)
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Name(s) of other medicine(s)				
			☐ Yes ☐ No	
			☐ Yes ☐ No	
Urinary analysis (pregnancy test for female patients)				
Patients with a positive pregnancy test must be followed up for 6–8 weeks after delivery				
Result of pregnancy test: ☐ Positive ☐ Negative Date of test (dd-mm-yyyy):				

If the patient is pregnant, follow-up of the pregnancy is required, including: clinical examination of the infant at birth and 6-8 weeks after birth. Please provide comments below. If needed fill in the serious adverse event report form):

Case report form: final day of follow-up day 42

Overall assessment

Outcome:

- ☐ adequate clinical and parasitological response
- ☐ early treatment failure
- ☐ late clinical failure
- ☐ late parasitological failure
- ☐ lost to follow-up
- ☐ withdrawn (complete section below: Reason for withdrawal)

Outcome occurred on follow-up day: (e.g. 1, 2, 3, 7, 14, …)

PCR:

- ☐ *P. falciparum* recrudescence
- ☐ *P. falciparum* reinfection
- ☐ other species
- ☐ mixed with *P. falciparum* recrudescence
- ☐ mixed with *P. falciparum* reinfection
- ☐ unknown

PCR corrected results:

- ☐ adequate clinical and parasitological response
- ☐ early treatment failure
- ☐ late clinical failure
- ☐ late parasitological failure
- ☐ lost to follow-up
- ☐ withdrawn

Reason for withdrawal:

Other comments:

APPENDIX 8: SERIOUS ADVERSE EVENT REPORT FORM

Serious adverse event report form	
Health centre name: Locality: District: Province:	Study number: Patient identity number: Date of visit (dd-mm-yyyy): Follow-up day:
Demographic data	
Date of birth (dd-mm-yyyy): or estimated age: in: ☐ months or ☐ years Height (cm): Weight (kg): Sex: ☐ Male ☐ Female If female, is the patient pregnant? ☐ Yes ☐ No ☐ Not sure If pregnant, provide the date of the last menstrual period (dd-mm-yyyy):	
Serious adverse event	
Type of event: ☐ Death ☐ Life-threatening ☐ Hospitalization or prolongation of hospitalization ☐ Permanent disability ☐ Congenital anomaly or birth defect Date of occurrence (dd-mm-yyyy):	
Describe the serious adverse event (include all relevant laboratory results):	
Describe how the reaction was treated:	

Serious adverse event report form (page 2)
Comments (e.g. relevant medical history, drug allergies, previous exposure to similar drugs, other laboratory data, whether reaction abated after stopping the drug, whether reaction reappeared after reintroduction):
Outcome
☐ Recovered completely ☐ Not yet recovered ☐ Recovered with long-term consequences If patient recovered, provide date of recovery (dd-mm-yyyy):
Medicines (list the **medicine suspected of causing** the serious adverse event as well as all **concomitant medicines**)

Brand name, batch number, manufacturer name (list suspected medicine first)	Daily dose	Route	Start date	End date	Indications for use

Reporting officer	
Name:	
Qualification:	
Address:	
Phone:	
Fax:	
Email:	
Signature:	Date:

APPENDIX 8. GUIDELINES FOR ANALYSIS OF RESULTS

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

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

* WHO. *Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations.* Geneva, World Health Organization, 2008 (http://www.who.int/malaria/resistance

NOTE:

COMBINED SITE

HC	**COMBINDED HC**
TRAPEANG CHO	**ORAL**
VEUNSAI	**KACHUON HC & VIRAKCHEY**
KEO SEIMA	**SEN MONOROM**