Protocol

Targeting high-risk populations with enhanced reactive case detection: a study to assess the effectiveness and feasibility for reducing *Plasmodium falciparum* and *P. vivax* malaria in Southern Lao PDR

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1 Protocol summary

Aim	To determine the effectiveness and feasibility of enhanced reactive case detection (RACD) targeting high-risk villages and forest workers for reducing *Plasmodium falciparum* and *Plasmodium vivax* transmission in southern Lao PDR
Primary research question and hypothesis	1. Null hypothesis: Compared to standard of care, which includes case management and RACD with conventional RDTs and is directed at villagers, there is no additional benefit of enhanced community-based RACD, which is conducted by village malaria workers (VMWs), uses HS-RDTs, and targets forest workers in addition to villagers, for reducing *P. falciparum* and *P. vivax* confirmed case incidence and parasite prevalence over an 18-month period in Lao PDR. Research hypothesis: Enhanced community-based RACD will be more effective than standard of care case management and RACD at reducing *P. falciparum* and *P. vivax* confirmed case incidence and parasite prevalence over an 18-month period in Lao PDR.
Research objectives	**Primary Objective:** To evaluate the effectiveness of reactive case detection (RACD) using HS-RDTs, targeting both village and forest working populations, compared to control for reducing the health center catchment-level incidence and prevalence of *P. falciparum* and *P. vivax* within two provinces in Lao PDR. **Secondary Objectives:** 1. To determine the operational feasibility, cost-effectiveness, and acceptability of targeting high-risk populations with community-based reactive case detection strategies aimed at reducing *P. falciparum* and *P. vivax* transmission among village and forest working populations. 2. To determine the operational feasibility and safety of conducting G6PD testing and PQ administration and follow-up at the community level. 3. To determine factors associated with G6PD testing after referral to district or provincial-level facilities for persons with P. *vivax* positive RDT results, and subsequent adherence to Primaquine treatment. 4. To assess malaria vector species composition and bionomics in both village and forest settings
Study site and target populations	Total population of approximately 120,000 in 32 health center catchment areas of two Provinces in Lao PDR (Champasak and Saravan Province)
Study design	A cluster randomized controlled trial design will be used to evaluate the enhanced RACD intervention against standard of care
Primary outcome measures for assessing impact	1. Cumulative incidence over the study period at the health facility level 2. Prevalence of infection (*P. falciparum* and/or *P. vivax*) using qPCR 3. HS-RDT and RDT test positivity rate for RACD
Data collection and sampling period	18 months from start date (June 2019 – November 2020)

Sample size	32 health center catchment areas in Lao PDR
Estimated enrollment	1) Confirmed case incidence across 32 health center catchment areas: population of approximately 145,000 2) Village-based reactive case detection: estimated 14,400 3) Forest worker reactive case detection: estimated 2,880 4) Endline household survey: 14,080 persons at end-line 5) Qualitative surveys: 6 Focus group discussions (FGDs) and 18 Key informant interviews (KIIs) at baseline, 9 FGDs and 18 KIIs at end-line. 6) Competency checklists with 32 VMWs at baseline, midline, and endline 7) Entomological human landing catches: 20 collectors over three nights at baseline
Statistical and analytical plan	Rapid reporting of microscopy or RDT and HS-RDT positivity will be analyzed as interventions are implemented (or shortly thereafter) to provide preliminary assessment of interventions. Negative binomial (for incidence) and generalized linear mixed effects (for prevalence) models will be used to test the effect of each intervention independently (main effects) relative to control (standard of care). For the enhanced RACD vs. control comparison, both country-specific and pooled analyses will be conducted.

2 Background and introduction

A standard and widely practiced method to identify infections at the community level is reactive case detection (RACD), whereby household and community members of a passively identified index case are tested with either an RDT or by microscopy [1–3]. However, traditional household-based RACD methods using current diagnostics have shown limited effectiveness for identifying additional infections, especially in settings of forest malaria transmission such as the GMS. Results from a recently conducted UCSF-MEI study of RACD in two Thailand border areas (Chanthaburi and Kanchanaburi Provinces) identified only 0.2% parasite prevalence by PCR in household and community members tested residing around passively identified index cases (n=27); all additional secondary infections identified were *P. vivax* (n=4). Furthermore, a higher risk of malaria infection was associated with being male, aged 31 years and older, and having worked in or near the forest fringe. While there have been several published small-scale observational studies examining the yield of household-based RACD using conventional RDTs in the GMS [4], none have examined its impact on transmission. Recent pilot studies in Cambodia suggest targeting high risk groups (using PCR) will substantially increase the infections found and cleared [5]. Preliminary results of an observational study of RACD targeted to high-risk forest workers (using LAMP) in Aceh Province, Indonesia carried out by UCSF-MEI suggest that substantially more secondary infections will be identified when RACD is targeted to high-risk groups compared to standard RACD in the neighborhood of cases. None of these studies have used the newly available HS-RDTs for *P. falciparum* [6]; evidence suggests these will increase the sensitivity of active case detection over standard RDTs and have greater utility for community-based approaches where PCR or LAMP are not likely to be feasible, but rigorous testing is needed in routine operational settings, especially in *P. vivax* co-endemic settings.

Recent results from an observational trial in Myanmar demonstrate the effectiveness of early diagnosis and treatment through village-based malaria posts and mass drug administration for *P falciparum* in hotspot villages [7]. In 2018, the UCSF-MEI conducted a community randomized control trial of mass test and treat (MTAT) in villages combined with focal test and treat (FTAT) in forest workers led by forest-working peer navigators using HS-RDTs in Champasak, Lao PDR, which helped inform community-based approaches for targeting RACD to high-risk forest workers in this proposal. Additionally, the UCSF-MEI is conducting formative ethnographic and epidemiological research to understand forest worker and other high-risk populations in several provinces in southern Lao PDR, working closely with Health Poverty Action (HPA) within their Malaria Post/village malaria worker (VMW) networks. These studies have highlighted the importance of involving the community, especially high-risk individuals, as part of community-based active surveillance and response strategies, and further operational research is essential to assess feasibility and challenges associated with scaling up such community-based approaches.

Safe and effective management of *P. vivax* cases requires addressing glucose-6-phosphate dehydrogenase (G6PD) deficiency and ensuring patients with G6PD normal function complete a 14-day treatment regimen with primaquine. The heterogeneity and severity of G6PD deficiencies in SE Asia and associated risk of severe hemolysis with oxidative drugs such as primaquine is an especially serious public health issue [8]. The majority of *P. vivax* cases in most transmission settings are due to relapses [9], making primaquine (and tafenoquine when available) a critical component of elimination activities. However, treatment adherence for full 14-day primaquine is generally sub-optimal, and standard of care use of primaquine in health systems has shown minimal effectiveness in preventing relapses [10]. There are limited data on primaquine use in Lao PDR; a study conducted by WHO and CMPE in 2015 enrolled 1,577 *P. vivax* cases (363 women) for G6PD testing of which 67 (4.2%; 95% CI: 3.3 to 5.3%) were classified as deficient.[11] A total of 1,225 G6PD non-deficient individuals with *P. vivax* infection were enrolled for PQ administration, of which 889 (72.5%) completed follow-up hematocrit testing, and only one individual experienced a serious drop in hematocrit (below 25%). However, treatment adherence and parasite clearance were not measured in the study. This study and others

suggest that there remain key gaps in knowledge to inform safe and effective programmatic use of primaquine drug regimens to target the hypnozoite reservoir, especially with regards to ensuring adequate follow-up and safety monitoring at all levels of the health system including the community level.

2.1. Rationale

The Center for Malariology, Parasitology, and Entomology (CMPE) in Lao PDR over the past two years has completed the National Strategic Plan 2016-2020[12], and new National Surveillance Guidelines that includes case-based surveillance and focal response including RACD. After some setbacks from 2011-2015, there has been major progress and incidence has recently fallen dramatically in several southern provinces, making foci-based active surveillance strategies now feasible in these areas.

Under the current national and regional artemisinin initiative 2 elimination (RAI2E) funding, VMWs and Malaria Posts will be expanded to improve coverage across these provinces. Household-level RACD has yet to be operationalized in southern Lao PDR, but is planned for roll-out over the next year by teams from national, provincial, and district levels. In order to maximize sustainability and impact, this strategy will need to be community-based and target the highest risk populations.

The current management of *P. vivax* cases and G6PD deficiency varies by country; in Laos, current policy states that all *P. vivax* cases are to be tested for G6PD deficiency and only those without deficiency receive a 14-day course of primaquine. Rigorous follow-up of cases to monitor safety and adherence is not conducted, and the use of G6PD testing and primaquine treatment has not yet been widely operationalized.

The research proposed here will evaluate the effectiveness and feasibility of a community-based reactive case detection strategy when combined with the use of HS-RDTs, targeting high-risk villages and forest populations, and *P. vivax* testing treatment adherence after referral to district or provincial-level facilities. Results of this research will have direct implications for continued roll out and sustainability of community-based foci management, and provide practical guidance that other malaria programs can utilize.

2.2. Significance

We propose to conduct this operational research in a large number of the remaining active foci across in two of the remaining higher burden provinces in southern Lao PDR. The findings will help Laos assess the benefit and potential challenges of community-based approaches to active foci management including RACD for *P. falciparum* and *P. vivax*, and the effectiveness of RACD using HS-RDTs in this context, which will provide a rigorous evidence base for other countries in the GMS applying these approaches. Importantly, this trial will incorporate community-based approaches for targeting high-risk forest workers, which are a uniquely prevalent high-risk population in the GMS.

2.3. Primary aim

The primary aim of this project is to determine the effectiveness and feasibility of enhanced community-based reactive case detection (RACD) in Lao PDR, targeting high-risk villages and forest workers, for reducing *P. falciparum* and *P. vivax* transmission.

2.4. Primary research questions and hypotheses

This project aims to answer the following research question and hypotheses:
Is community-based RACD using HS-RDTs targeting both village and forest working populations more effective than the standard of care (community case management, and RACD conducted by district surveillance teams) in reducing P. falciparum and P. vivax confirmed case incidence and parasite prevalence over an 18-month intervention period?

Null hypothesis 1: Compared to standard of care, which includes case management and RACD with conventional RDTs and is directed at villagers, there is no additional benefit of enhanced community-based RACD, which is conducted by VMWs, uses HS-RDTs, and targets forest workers in addition to villagers, for reducing *P. falciparum* and *P. vivax* confirmed case incidence and parasite prevalence over an 18-month period in Lao PDR.

Research hypothesis 1: Enhanced community-based RACD will be more effective than standard of care case management and RACD at reducing *P. falciparum* and *P. vivax* confirmed case incidence and parasite prevalence over an 18-month period in Lao PDR.

2.5. Study overview

To test this hypothesis, this study will employ a cluster randomized controlled trial design with two comparison arms:

1. Control: standard of care - passive case management provided through community-based VMWs and existing health facilities; includes village-based RACD with conventional RDTs conducted by district surveillance teams.
2. Enhanced community-based RACD: RACD conducted by community-based VMWs using both HS-RDTs and conventional RDTs within villages and among forest workers.

The primary outcome measures to assess effectiveness include *P. falciparum* and *P. vivax* confirmed case incidence over the study period; PCR-based *P. falciparum* and *P. vivax* prevalence at endline; and HS-RDT test positivity rate in village and forest worker RACD. Secondary outcomes measures will examine the operational feasibility, safety, and acceptability of VMW-led reactive approaches and G6PD testing, referral to district or provincial-level facilities, safety and treatment adherence for *P. vivax* cases. The trial will be implemented between June 2019 and December 2020 in Lao PDR. The primary evaluation will be conducted following an end-line cross-sectional survey in November/December 2020.

2.6. Summary of ethical issues

Prior to implementation, the protocol and all related project activities will be reviewed and approved by the institutional review boards in Lao PDR and at UCSF. For other partnering institutions (i.e., University of Texas Southwestern, University of Massachusetts, Tulane University), requests will be made for reliance on UCSF IRB. The protocol will be submitted to CDC Human Subjects for non-engaged review. Participation in either the research or intervention is voluntary. Individuals will be included in the study only if they or their parents/guardians provide written informed consent. The consent process will be conducted in appropriate local languages at the start of every new contact with an individual. For individuals under 18 years of age, informed consent will be obtained from a parent or guardian. Oral assent for adolescents 12 to <18 years of age will be obtained in addition to consent from a parent or guardian. As part of the informed consent, specific consent for the pregnancy test will also be required for female participants of child-bearing age.

The safety risks associated with participation in this trial are expected to be minimal. The study drugs, which are first-line treatment regimens for the respective areas in Lao PDR, artemether-lumefantrine (AL) and PQ in G6PD non-deficient individuals, are well tolerated and safe. Prior to drug administration, participants will be asked about known contraindications, and if such contraindications are reported, participants will be restricted from taking the relevant medication. PQ administered as a single low dose has been found to be safe in individuals with any of the G6PD variants and is recommended by WHO without G6PD testing [13]. The 14-day course of PQ will only be given to G6PD non-deficient individuals after G6PD testing with a qualitative RDT. Should a subject be misclassified as G6PD non-deficient when in actual fact they are G6PD deficient and had begun their 14-day PQ regimen, participants will be asked to stop treatment with primaquine immediately and a

robust follow-up and severe adverse event management system will pick up early cases of hemolysis, stop treatment with primaquine and if necessary refer for further clinical management.

The risks associated with loss of privacy in this study are likely to be low. To ensure confidentiality is maintained, all information will be treated as private by study personnel, and records kept securely in locked filing cabinets and offices. Electronic records will be kept on a secure, firewall- and password-protected server. For all data collected as part of the study, participants will be assigned a unique identification number. No personal identification information such as names will be used in any reports arising out of this research. All project staff will be trained on procedures for maintaining confidentiality.
Study participants with malaria infections identified by RDTs/HS RDTs will benefit directly from treatment with recommended first line treatment.

Participants will not be paid to take part in this study. Most assessments will be conducted at households or working sites, which will eliminate the need for participant travel and minimize opportunity costs for the participants. Any diagnosis and treatment associated with the study will be provided free of charge.

3 Methodology

3.1. Study sites

The selected study sites include health facility catchment areas in two of the highest burden provinces in Southern Laos. All selected health facilities had at least 3 malaria cases in the previous year. The districts included in Laos are shown in Table 1 and Figure 1. In addition to the number of health facilities included in the study, the total number of villages and population in those selected areas, and the *P. falciparum* and *P. vivax* annual parasite index (API) for Oct 2017 – Sep 2018 is shown. The anticipated period for the study is June 2019 – December 2020.

Table 1. Overview of incidence and population within selected health facility catchment areas in Lao PDR

District	Province	Total health facilities	Total villages	Total population	*Pf* 2017-18 (API)	*Pv* 2017-18 (API)	Total cases 2017-18 (API)
Khong	Champasak	4	32	22764	3.43	2.81	6.24
Mounlapamok	Champasak	4	23	18005	0.56	1.94	2.5
Pathoumphon	Champasak	3	34	22975	3	4.61	7.62
Sukhuma	Champasak	1	8	10300	0.58	1.65	2.23
Samouay	Saravan	3	28	6177	0.65	13.11	13.76
Saravan	Saravan	3	35	17877	1.51	1.29	2.8
Taoi	Saravan	5	57	12713	16.6	1.65	18.25
Toumlan	Saravan	6	46	18366	6.81	1.47	8.28
Vapy	Saravan	3	37	18621	3.11	2.09	5.21
Total		32	300	147,798	3.98	2.79	6.77

Figure 1. Selected study districts in Lao PDR (highlighted in orange)

3.2. Study population

The study population includes all villagers and forest workers in the selected health facility catchments. Inclusion and exclusion criteria for the interventions are described below.

Inclusion and exclusion criteria

Inclusion criteria

Subjects must fulfill the following inclusion criteria to be eligible for enhanced RACD:

- **Index cases:**
  - Presented as a confirmed malaria case to an intervention health facility or village malaria worker, and lives in a village within a selected intervention health facility catchment area, or worked or spent at least one night at a forest or forest-fringe site in the past 30 days located within an intervention health facility catchment area
- **Village residents:**
  - Lives in a village within a selected intervention health facility catchment area and in one of the five households closest to the residence of an index case of malaria
- **Co-worker/traveler referral:**
  - Worked or traveled and spent at least one night in forest in past 30 days in same location within an intervention health facility catchment area as an index case of malaria
- **All participants:**
  - Willing and available to participate in the study
- Informed consent for participant under the age of 18 will be provided by the parent or guardian.

Exclusion criteria:

- **All RACD referrals:**
  - Previous participation in the study as a result of any RACD event in the past 30 days.

- Individuals with severe disease or drug contra-indications will be excluded from the treatment component only (detailed below)

3.3. Interventions

Each intervention cluster (i.e., health facility catchment area) will be randomly assigned to either the RACD intervention or control arm (in a 1:1 ratio) before the study begins. The randomization process is described in more detail in section 3.4.2.2 below. Interventions (RACD) will be conducted by VMWs and/or community volunteers in response to an index case (presenting to VMW, malaria post, health facility, or other testing facilities) occurring anywhere within a selected intervention health facility catchment area. At presentation, all index cases will initially be asked about recent forest exposure. The intervention response for cases with no history of forest exposure in the past two months will be conducted only at the village level, while the response for cases with history of recent forest exposure will include both village and forest-worker populations. Forest-worker RACD will be conducted at recent work sites if accessible and located within the health facility catchment and/or amongst peer forest-goers referred by the index case. VMWs in intervention villages will maintain a register of forest workers and locations to assist them with tracking. In Lao PDR, health center staff will assist with RACD during the first six months of the study only, after which point VMWs will operate independently.

Index case capture

Interventions will be initiated after an individual from a cluster assigned to an intervention is diagnosed with malaria and notified to the study team. Suspected malaria cases attending local health services (hospital, health center, health post, or VMW) will be tested with an RDT or microscopy according to routine practice. All cases parasitologically confirmed by RDT will be treated according to national guidelines and asked for informed consent to participate in the study. If the patient gives informed consent, the VMW or health staff will promptly notify the study team using mobile phone. A short screening questionnaire will be administered to determine location of residence and recent forest or other travel. Additionally, finger prick blood will be collected on DBS from the index case for subsequent analyses.

3.3.1. Reactive case detection (RACD)

Household RACD

Within 7 days of the index case notification, the VMW will conduct the investigation visit at the residence of the index, and the index case will be interviewed using a standard case investigation form. All members of the index case's household will then be invited to participate in the study. A GPS point will be captured for the index house, and the nearest five households around the index case's household will be identified by the VMW. In the neighboring five households, a GPS point will be captured and all household members, including temporary visitors, will be invited to participate in the study. Household members and parents/guardians of children will be verbally informed of the general purpose of the intervention and the study, and the possible risks and potential benefits associated with participation. Individual written consent will be obtained at each household and for each blood sample collected for testing. For children <6 to 12 years old, consent will be obtained from the parent or guardian. For children 12 to <18 years of age, the child's oral assent will also be required in addition to parents/guardians' consent. All informed consent procedures will be conducted in the appropriate local language. All consenting participants will be interviewed using the participants' questionnaire to assess history of malaria in previous year, travel information, and utilization of malaria prevention measures.

A finger stick blood sample will be collected for each consenting individual for testing with the HS-RDT for *P. falciparum,* a standard combination RDT, and four blood spots on filter paper. Individuals will be told of their test result and a positive test result on either RDT will prompt treatment as per the national treatment guidelines. See tables 2 and 3 below for treatment guidelines.

In the event that household members are not present at the time of testing, the VMW will ask if any absent household member has been ill with fever during the previous two weeks. If any absent household members are reported as having recent fever history or if any other household members test positive for malaria, the VMW will schedule a time to revisit the household. At this time, the VMW will administer the informed consent form to any previously absent household members and then test these individuals (and treat positive cases). Febrile individuals testing negative by RDT(s) will be referred to the nearest health center.

RACD of cases' co-workers/co-travelers

Index cases will be screened by the VMW at their households at the time of case investigation to determine if they have traveled or worked in a forest or forest-fringe area within the past 30 days. If eligible, the case will trigger two reactive recruitment strategies to screen and treat others who recently traveled or worked with the case in a forest or forest-fringe location:

1. *Peer-referral RACD (PR-RACD)*: In this strategy, the case will identify specific co-travelers or co-workers resident in their village who had spent the night with the case at a forest or forest-fringe area and spent the night there in the past 30 days.
2. *Venue-based RACD (VB-RACD)*: In this strategy, co-workers will be recruited directly from (accessible) forest or forest-fringe work sites where the index case worked and spent at least one night in the past 30 days.

Participating index cases who meet eligibility criteria will be asked to provide a list of all people who have worked or traveled in the forest or forest-fringe with him/her in the past 30 days between dusk and dawn and their contact information (names, phone numbers, home addresses) (peer-referrals). The questionnaire will elicit from index case all forest and forest-fringe work sites where the case has spent the night in the past 30 days, as well as contact information for any respective employers.

For PR-RACD, VMWs will contact peer-referrals by phone, at their home, or through village leaders and invite them to participate in a survey interview and malaria testing at a time and place that is most convenient. This may be the referral's place of residence, a health facility, or another location in the village that provides sufficient confidentiality, such as a coffee shop.

a. Peer referrals will be screened for eligibility by the VMW and, if eligible, informed consent will be conducted in the appropriate local language.
b. Consenting referrals will provide a fingerstick blood sample for testing with the HS-RDT, standard combination RDT and four blood spots on filter paper. If infection is present according to any RDT, they will receive treatment following the guidelines in the following section.
c. All consenting referrals will complete an interviewer-administered survey questionnaire.

For VB-RACD, if available, employers will be contacted by the VMW to coordinate a potential visit to identified work sites.

a. Work sites considered for VB-RACD will meet the following criteria:
    - Case reports that the work site is currently active
    - Case reports there are at least five other workers who work or sleep there (the minimum threshold ensures that screening there is worthwhile, given logistic constraints, and will be specified in SOP)
    - The work site is safe to visit and accessible
    - The nearest VMW has necessary permissions to travel to site

b. The nearest VMW will travel to any such work sites identified
c. All workers present at the site(s) will be screened for eligibility and, if eligible, informed consent will be conducted.
d. Consenting referrals will provide a finger stick blood sample for testing with the HS-RDT, standard combination RDT, and four blood spots on filter paper. If infection is present according to any RDT they will receive treatment following the guidelines in the following section.
e. All consenting referrals will complete an interviewer-administered survey questionnaire.

All participants will receive a small token of participation such as a bar of soap or phone credit.

Forest workers may be difficult to reach at their places of residence as they are often away from the household. When following-up with PR-RACD referrals, VMWs will attempt to contact them directly by phone multiple times and visit the household if the address was provided or they are familiar with the location. When referrals are unreachable by phone or the residential location is unknown, VMWs will elicit the help of the village leader to identify where they live or how best to contact them. VMWs will record each contact into a register of all individuals in their community known to travel to forest, the location of their residence, and location of work-site if possible.

Case Management and Follow-up

All individuals who test positive by either HS-RDT or Standard RDT will be told of their results and treated on site per national guidelines:

- Individuals with *P. falciparum* infection will be treated with an age-appropriate course of artemether-lumefantrine (AL) and a single low dose of primaquine (SLD-PQ). Weight-based dosing is described in Tables 2 and 3 below.
- At all study sites in Lao PDR, patients with a *P. vivax* infection identified by RDT (both febrile and asymptomatic) will be given a unique coded and signed informational letter directing them to the nearest district hospital (or other testing facility) for G6PD deficiency testing and possible radical cure administration depending on results. Study contact information will also be included if the participants present at other health facilities. At the health facility, G6PD normal individuals will be treated with AL and a 14-day course of PQ, whereas G6PD deficient individuals will receive AL alone as per the national guidelines and referred to a hospital for further primaquine management decisions. The study staff will meet with district hospital and health facility staff monthly from all study areas and nearby district hospitals and health facilities to collect these forms, with small incentives for health staff to report receiving a study card (phone top up card, etc.) to maximize data completeness. After six months of implementation, a subset of VMWs will begin conducting qualitative G6PD testing and treatment administration at the community-level with the assistance of a health facility staff member.

Table 2. AL weight-based dosing

Body weight (kg)	Tablet strength (mg)		Tablets/ dose	Mg of drug per dose		Tablets/day
	Artemether	Lumefantrine		Artemether	Lumefantrine	
5 to 14	20	120	1	20	120	1 tablet given twice* per day for 3 consecutive days

15 to 24	20	120	2	40	240	2 tablets given twice* per day for 3 consecutive days
25 to 34	20	120	3	60	360	3 tablets given twice* per day for 3 consecutive days
35 or greater	20	120	4	80	480	4 tablets given twice* per day for 3 consecutive days
*Approx. 8 hours between doses 1 and 2. Approx. 12 hours between all other consecutive doses.						

Table 3. Weight-based dosing of SLD- Primaquine (.25 mg/kg) for *P. falciparum*

Body weight (kg)	**SLD-PQ dose (7.5 mg tablet) (as base)**	**Tablets**
10 to < 25	3.75	1/2
25 to < 50	7.5	1
50 to 100	15	2
***Given as SLD in addition to AL for P *falciparum* positive**		

Table 4. Age-based dosing of Primaquine for *P. vivax*

Items	Dose	No. of tablet/day	Frequency	Duration
G6PD Normal				
Children				
4-8 years old	5 mg/day	1/3	Once per day after meal	14 days
9-14 years old	10 mg/ day	2/3		
>14 years old (adults)	15 mg/ day	1		

Per national guidelines in Laos, AL will not be given to women who are pregnant or may be in their first trimester. All women of reproductive age with a last menstrual period greater than four weeks positive HS RDT or Standard RDT will be offered a pregnancy test. Pregnancy testing and consent will be embedded in the participant questionnaire. Any woman who acknowledges pregnancy in the first trimester or confirmed by a positive pregnancy test or refuses to take a pregnancy test will be excluded from receiving AL and will be referred to the nearest health facilities for treatment.

Contraindications to primaquine include pregnancy (any trimester), age less than 12 months, infants weighing less than 10 kilograms, women in the first 12 months of breastfeeding, and prior allergic reaction to primaquine. Participants with contraindications to primaquine will not be excluded from the study and will receive first-line treatment alone, provided they are eligible.

All individuals with suspected severe malaria or other severe illness (including those with symptoms of severe anemia, prostration, impaired consciousness, respiratory distress, convulsions, circulatory collapse, abnormal bleeding, jaundice or passing dark urine) will be referred to the nearest health facility for clinical assessment and treatment.

3.3.2. Adherence and safety monitoring

To ensure participants' adherence to their medication and monitor safety, participants will be informed on how to take their medications, about the side effects of the treatment and the importance of compliance to the treatment. In addition to DOT on day 0, The VMW will conduct a day

3, day 7, and day 14 adherence and safety monitoring visit for all individuals treated with primaquine for *P. vivax*, and the VMW will ask a series of questions to determine adherence with each dose, number of remaining tablets, potential adverse events, and reasons for non-adherence or treatment refusal. Participants with phone number will be sent a daily text message to remind them to adhere to their treatment and remind them about any symptoms suggesting serious adverse events. All participants and their family members will be provided a chart to record and track their adherence as well as symptoms that may indicate an adverse event.

The safety risks associated with participation in this trial are expected to be minimal. The study drugs, AL and PQ, are well tolerated first-line treatments and safe for G6PD non-deficient individuals. Prior to drug administration, participants will be asked about known contraindications, and if such contraindications are reported, participants will be restricted from taking the medication.

Drug	Contraindications
AL	Pregnancy, infants < 5kg, prior allergic reaction, any severe disease
PQ	Pregnancy, age < 12months, infants < 10kg, women in first 12 months of breastfeeding, prior allergic reaction, any severe disease, G6PD deficient

The common adverse events reported for each drug are as below:

- AL: headache, dizziness, loss of appetite, generalized weakness, fever, chills, arthralgia, myalgia, nausea, vomiting, and abdominal pain
- PQ: nausea, headache, fatigue, dizziness, hives, chest pain, painless darkening of urine, jaundice of skin and vomiting.

The Primaquine Roll Out Pharmacovigilance Tool Data Collection Forms (PROMPT) will be used to track the status of individuals treated with PQ. Identification of serious adverse events (SAEs) will occur both passively and actively. As part of the consent process, participants will be instructed to call or visit VMW to report any adverse events that occur between VMW visits. During adherence and follow up visits, the VMW will also ask participants for specific adverse events related to hemolysis. SAEs as well as serious unexpected serious adverse reactions (SUSAR) will be reported to the study management and PI who will report to the data safety monitoring board (DSMB). At each study site, a local physician will be recruited (Local Safety Monitor, LSM) to determine causality and grade of the SAE. All SAEs will be reported to the Pharmacovigilance center of the Ministry of Health (Lao PDR) and the respective IRBs. All potential AEs or SAEs will be referred by VMW to the nearest health center or district hospital for management and documented as per 4.2 below, and the study will reimburse all patient travel and hospitalization resulting from study drug administration.

3.4. Study evaluation methods

3.4.1. Overview of evaluation activities by study objectives, outcomes, and data collection activities

Table 4. Overview of evaluation activities

Study activity	Research objective	Primary outcome measures	Data collection activity
Activity 1: Impact evaluation of enhanced RACD	1. Evaluate the relative effectiveness of enhanced community-based RACD using HS-	Total confirmed outpatient (OPD) *P. falciparum* and *P. vivax* malaria case incidence among all ages by health facility catchment	Routine malaria data from all reporting VMWs, health centers and district hospitals

	RDTs in villages and forest-based HRPs, compared to control	*P. falciparum and P. vivax* prevalence in all individuals	Cross-sectional parasite survey November-December 2020
		HS-RDT and RDT test positivity rate	HS-RDT and RDT positivity data from RACD intervention data
Activity 2: Assessment of the feasibility and acceptability of community-based RACD	1. Assess the operational feasibility of community-based RACD	Population coverage of RACD and interventions	RACD intervention data
		Proportion of VMWs who rate conducting the RACD interventions as very easy, somewhat easy, somewhat difficult, and very difficult, and changes in proportions over time	Entrance, mid-intervention, and exit competency checklists with VMWs; focus group discussion and interviews with VMWs at end-line
		Costs of RACD as unit and total costs; cost-effectiveness will be assessed as an incremental cost effectiveness ratio (ICER)	Program data on costs combined with estimates of program effectiveness
	2. Assess the acceptability of community-based RACD	Proportion of targeted individuals refusing RACD intervention	RACD intervention data
		Proportion of survey respondents who strongly disagree, disagree, are ambivalent, agree and strongly agree on the importance and acceptability of community-based RACD	Cross-sectional survey and qualitative studies at endline with villagers, forest workers, VMWs and other health sector staff
Activity 3: Assessment of the operational feasibility and safety of conducting G6PDd testing, referral, and treatment adherence among positive *P. vivax* RDT cases	1. Assess the operational feasibility of G6PD referral at community level	Proportion of VMWs who rate conducting G6PD referral as very easy, somewhat easy, somewhat difficult, and very difficult, and changes in proportions over time	Entrance, mid-intervention, and exit competency checklists with VMWs; focus group discussion and interviews with VMWs at end-line
		Proportion of *P. vivax* cases with valid recorded G6PD result	VMW and health facility records
	2. Determine risk factors for non-attendance at hospitals or health centers after referral for G6PD testing	Proportion of referred cases presenting at hospitals or health centers for G6PDd testing.	Cross-sectional survey and qualitative studies at endline with villagers, forest workers, VMWs
	3. Assess treatment adherence among positive *P. vivax* RDT cases	Proportion of patients with physical evidence of full adherence with the prescribed drug regimen	VMW follow-up visits on day 3, 7, and 14
		Proportion of *P. vivax* cases who relapsed in the six months following treatment	VMW and health facility records
	4. Assess the safety of community-level G6PD testing, referral, and *P. vivax* treatment follow-up	Adverse event rate per treated individual	RACD intervention data and VMW follow-up visits; health facility adverse event reporting forms
Activity 4: Assessment of the risk of malaria vector biting in forest and village settings	1. Describe local vector species composition and bionomics in forest and village settings	Vector occurrence and density by species; vector biting behaviors; human behavioral observations in relation to human biting rate (HBR)	Human Landing Catches (HLCs) vector sampling at baseline

3.4.2. Impact evaluation of enhanced RACD

3.4.2.1. Study design

This study will employ a two-arm cluster-randomized control trial design with randomization of clusters into either RACD or control.

3.4.2.2. Randomization

A total of 32 health center catchment areas (HCCAs) in Laos will be selected for inclusion based upon incidence per 1000 population between October 2017 and September 2018. HCCAs with higher incidence will be prioritized to improve power; where possible, directly neighboring HCCAs will not both be included to reduce contamination. Restricted randomization of the HCCAs into either RACD or control arms will be conducted, whereby sets of randomizations (with 16 HCCAs in each arm) will be generated that achieve balance across arms on incidence per 1,000 over the prior year, population size, and amount of forest cover. From these sets of potential randomizations, a single randomization will be randomly selected to assign 16 HCCAs each to the RACD or control group. Restricted randomization in this way will ensure balance across intervention and control groups on potential confounding factors. The 'sample' command in Stata v14 (StataCorp, College Station, TX) will be used to implement randomization.

Table 5. Study arms

Study arm	Description	Lao PDR
RACD	Reactive case detection led by VMWs in response to cases in study area HCCA, with follow up testing with HS-RDTs/RDTs in both villages and forest workers; referrals for qualitative G6PD testing for *P. vivax* cases and 14-day PQ for G6PD non-deficient	16
Control	Standard of care including case management through health facilities and malaria posts/VMWs; village-based RACD conducted by district staff in some areas	16

3.4.2.3. Primary outcome measures

1. Confirmed *P. falciparum* and *P. vivax* malaria parasite incidence: defined as the number of OPD malaria confirmed and suspected cases per person per year for each HCCA, as ascertained from the health facility registers, utilizing administrative catchment population size estimates for the exposure denominator.
2. PCR-based *P. falciparum* and *P. vivax* parasite prevalence in sampled HCCAs: defined as the proportion of individuals ≥18 months old with *P. falciparum* or *P. vivax* infection (detected by PCR) out of all individuals ≥18 months tested within the endline survey (2020).
3. HS-RDT/RDT -based test positivity rate in village and forest-based reactive case detection: defined as the proportion of all individuals tested by HS-RDT/RDT in response to an index cases, with a positive HS-RDT/RDT, among the population older than 18 months.

3.4.2.4. Sample size

3.4.2.4.1. Confirmed P. falciparum and P. vivax malaria parasite incidence

Assuming an average baseline transmission of 2.5 per 1000 population per year (all species, from 2017 health system data across study sites and a 50% reduction), an average population of 4000 per health facility catchment, and coefficient of variation of 0.6 (based upon 2017 health system data), in order to detect a 50% reduction in cumulative infection incidence (as measured by routine health system surveillance data over an 18-month period) in a random effects Poisson or negative binomial model, a minimum of 16 randomization units will be required in each study arm. A total of 32 health facility catchment areas will be included in Lao PDR, with 16 allocated each to enhanced RACD and control.

Only health facility catchment areas with at least 3 malaria cases in the past year will be considered for inclusion.

3.4.2.4.2. Population-level P. falciparum and P. vivax malaria parasite prevalence

Assuming 16 health catchment areas per arm, a baseline control prevalence by qPCR (all species) of 2.5%, and a coefficient of variation of 0.5, a minimum of 400 individuals per catchment area will be required in order to detect a 50% reduction in prevalence at endline. Assuming a 10% refusal rate, a total of 14,080 individuals will be required across both arms at the end-line survey. Assuming 4 individuals per household, this will result in a total of 3,520 households sampled, or 110 per health facility catchment area.

3.4.2.4.3. HS-RDT/RDT test positivity during RACD intervention

Test positivity by HS-RDT and RDT will be monitored throughout the intervention period. There is no formal sample size calculation for this outcome, but based upon an estimated 720 cases in Lao PDR over the study period, an average of 5 households per response event, 4 people per household, and 4 forest-workers per case, we estimate a total of 17,280 individuals will participate in testing during RACD in Laos.

3.4.2.5. Data collection

3.4.2.5.1. Confirmed malaria case incidence

The confirmed parasite incidence from all reporting units (including HCs, district hospitals, VMWs with RDTs, and PPM sites) will be captured throughout the study with support from study or health center staff. In the months prior to the start of study activities, all villages within each health facility catchment area will be mapped, and trainings conducted with health facility staff to systematize the collection of village names at health facilities for confirmed malaria cases. Routine supervision of health facility staff will be conducted by study staff to ensure accurate recording of village names in health facility registers throughout the trial. Forest sites visited by malaria cases will be recorded and mapped where possible.

3.4.2.5.2. Cross-sectional surveys

An endline survey will be conducted in November/December 2020 to obtain an unbiased estimate of *P. falciparum* and *P. vivax* malaria parasite prevalence in each study arm, as well as assess intervention coverage, treatment seeking, and mobility and forest going activities. The endline survey will include testing with HS-RDTs/RDTs and collection of DBS for qPCR-based testing.

<u>Sampling frame and sampling strategy</u>

Within each of the HCCAs selected for study inclusion, survey staff will work with village authorities to update household ledgers, and then all households will be enumerated and a GPS point captured. All households will be given a study ID card and household sticker with a unique barcode, which will be used throughout the study to identify repeat visits at each household, as well as for individuals to present at health facilities if they report for care.

In order to reduce potential contamination due to proximity to intervention areas (for the control arm), households within 2 km of a neighboring study village will be removed from the sampling frame. Households will be selected via simple random sampling from the remaining HH lists.

<u>Questionnaires and human specimen collection</u>

During the endline survey, selected households will be visited and the head of household interviewed by a study staff member using a tablet or paper form. To capture any household members not present at time of survey, study staff will plan for an overnight stay whenever feasible to schedule visits for early morning or late evening, but will have a maximum of four visits to each HH. The survey questionnaire used for interviewing will be developed in English with input from local health staff. This

will then be translated to Lao, and back-translated by a fluent bilingual health expert prior to field testing.

The survey questionnaire will capture household-level demographics, and assess potential risk factors for malaria infection. Information collected will include age, gender, pregnancy, nationality, ethnicity, occupation, socioeconomic status, travel history, history of malaria, treatment seeking for fever in the past two weeks, individual and household use of vector control measures, housing structure type, proximity to forest and forest-fringe, and frequency of overnight sleeping in forest or forest-fringe areas.

At the endline survey, all household members (residents and temporary visitors) aged 18 months and older will be invited to participate in an RDT and blood collection component. Informed consent will be obtained from all participants, including parental consent for any participant younger than 18 years of age. After consenting, the study team will capture axillary temperature, and test each individual using a standard combination RDT and an HS-RDT (SD Bioline Malaria Ag P.f High Sensitive Cat# 05FK140), and Hemocue kit, followed by collection of four DBS on filter paper.

If found positive by RDT or HS-RDT, treatment will be administered as in Section 3.1.1. Testing results from the collected DBSs will not be provided to the participants.

3.4.2.5.3. Intervention implementation data

Data collection during RACD activities is described below.

Questionnaires and human specimen collection: reactive case detection (RACD)

For all household members and co-worker referrals from forest workers in RACD intervention areas, after obtaining informed consent, a short demographic and malaria risk factor survey will be conducted to obtain information on individuals' occupations, recent forest work or travel, ITN usage, and recent care-seeking behavior for fever. Household members and referrals will be linked to the index case through unique codes in order to facilitate follow-up and allow for statistical adjustments, such as clustering by index case.

In the intervention areas, all patients with a *P. vivax* positive RDT will be given a coded and signed informational letter directing them to the nearest health center of hospital for G6PD testing and follow-up treatment. All *P. vivax* patients will be recorded into a registry and given an identification card for presentation and treatment and follow-up visits.

Sites where interviews occur will be geo-located if all parties agree. VMWs may also obtain contact information from consenting persons to allow for follow-up on positive cases and potential creation of support networks for other HRPs.

3.4.2.6. Data management and analysis

Data will be collected via ODK-based tablet application with internal range checks or paper-based with subsequent double-entry, and will be stored in Microsoft Excel or Access. The number of malaria cases, based on RDT and PCR results, will be mapped by village or sub-village and compared between surveys.

Primary outcomes

Outcome 1: Community-level confirmed *P. falciparum* and *P. vivax* malaria parasite incidence

Data pertaining to this outcome will be analyzed on an intention-to-treat basis at the level of health facility catchment area (randomization unit). Cumulative counts of confirmed malaria cases from the health facility registers over the study period and the previous year will be analyzed in a Poisson or

negative binomial regression model with random intercepts at the health center catchment level and an offset for the estimated population size of the catchment area (from administrative data). The models will include a fixed effect for each study arm and a fixed effect for time period (pre- and post-intervention start). The interaction between these two terms will be the primary effect measure (also known as the difference-in-differences estimator). Pre/post-intervention will be determined as all time periods before the start date of the intervention in the areas considered as being pre-intervention and all time periods after (and including the start date of the intervention in the area considered as being post-treatment).

Outcome 2: Community-level PCR-based *P. falciparum* and *P. vivax* parasite prevalence in sampled villages

The effectiveness of the interventions will be assessed as *P. falciparum* and *P. vivax* prevalence via PCR at end-line (post only) using generalized linear mixed effects models with separate random intercepts to allow for clustering within health center catchments. The binomial distribution will be used to analyze prevalence outcomes (logistic regression). All main analyses will be analyzed as intention-to-treat, and all survey clusters will be analyzed within the intervention group assigned at randomization, regardless of adherence. The primary effect estimate will be evaluated using the fixed effects for RACD. Secondary analyses will include adjustment for age, sex, and other potential confounders, and a per-protocol analysis of the primary effect estimate.

Outcome 3: HS-RDT and RDT-based test positivity rate in village-based and forest-based samples

The HS-RDT and RDT test positivity rate will be estimated for the RACD areas during each intervention. This will be done as soon as data on HS-RDT results are available. Differences in test positivity measures over time and between village and forest base samples will be assessed using a χ^2 test, as well as logistic regression models to account for potential confounding factors.

3.4.2.7. Laboratory analysis

The laboratory procedures described below will be followed for all laboratory-based activities conducted during this project. The laboratories performing these tests include the Pasteur Institutes in Phnom Penh and Paris, and UCSF.

Rapid diagnostics tests

Standard combination RDT, HS-RDTs and G6PD Carestart RDT will be used during field activities. Standard combination RDT and HS-RDTs will be used to determine malaria infection status and will be performed on participants every time a blood sample is collected. The standard combination RDT can detect *P falciparum* and *P vivax* malaria infection while HS-RDT can detect whether *P falciparum* infection is present or not and is estimated to be several times more sensitive than standard RDTs; new HS-RDTs including detection of *P. vivax* will be used if they become available over the study period. The VMW will use a finger prick blood sample to run the Standard SD Bioline and HS RDTs in parallel with results available within 20 minutes and recorded by study staff. The G6PD Carestart RDT will be used by health center and hospital staff to determine the G6PD status of all individuals positive for P *vivax* by standard RDT. All RDTs will be used according to the manufacturer's instructions. The results of these tests will be provided to the participant. For quality assurance of qualitative G6PD tests, quality assurance processes including user training, proficiency testing, and regular quality control of tests using control reagents will be introduced in intervention facilities. Images of G6PD RDTs conducted over the first three months of the study will be reviewed by laboratory technicians at the district hospital.

Filter paper sample collection

Dried blood spots (DBS) will be collected onto filter paper for future molecular analysis including parasite and human (i.e., G6PD) genotyping for research purposes only. These results will not be provided to the participants. Filter paper (Whatman 3MM) will be pre-cut into individual squares and

stapled to a thick card that will serve as its cover. Blood spots will be collected onto the filter paper in volumes of approximately 25 µl aliquots per blood spot (4 blood spots per card). Filter paper samples labelled with the individual's barcodes or ID number on the covering cardboard and will be allowed to dry at ambient temperature and relative humidity before closing the card over the filter paper. Filter paper samples will be transported from the field in a Ziploc bag then placed in a stock card filter paper box with desiccant and humidity indicator card and stored at 4˚C within one week, and at -20˚C within one month. Dried blood spots (DBS) will be regularly transported to the district or provincial offices for refrigerated storage prior to bulk transport and shipment to designated laboratories.

Serology

Serology, a test of past infection as assessed by the presence of antimalarial antibodies, will be used to improve the identification of hotspots and estimate current and historical transmission intensities[14]. Using DBS, ELISA assays will be performed using previously described methods. Briefly, antibodies will be eluted from DBS and assayed to detect antibodies against the *P. falciparum* blood stage antigens including merozoite surface protein-1 (MSP-1) and apical membrane antigen-1 (AMA-1), both biomarkers of *P. falciparum* exposure[15]. Markers for *P. vivax* exposure will include Pvmsp-1 and Pvcsp [16]. Other antigens that are sensitive and specific for recent exposure (currently undergoing evaluation) for *P. falciparum* and *P. vivax* may also be used. ELISA assays will be performed in duplicate and optical densities recorded with an ELISA reader. Other serological and antigenic platforms (bead array, protein microarray) may be used to analyze responses to multiple antigens/antibodies, if available.

Genotyping

Genotyping of *P. falciparum* infections to assess transmission networks will consist of a panel of microsatellites located throughout the genome. Briefly, DNA samples will be amplified in a multiplex pre-amplification step followed by amplification of microsatellites in individual reactions using fluorescently tagged primers and sized using denaturing capillary electrophoresis. Multilocus genotypes from mixed infections will be reconstructed, where possible, by quantifying alleles at each locus. Genotyping of additional loci including for HRP2 deletion will be performed as needed.

Individual microsatellite amplifications will be undertaken using single round or nested PCR assays with fluorescently labeled primers, and the amplicons sized by denaturing capillary electrophoresis with internal size standards. Allele-calling will be undertaken with the aid of the GeneMapper v4.0 software. The potential for effective multilocus haplotype reconstruction in polyclonal infections will be explored. Additional informative SNP markers identified in whole genome sequencing efforts may also be genotyped as necessary to improve sample fingerprinting.

Genotyping for markers of drug resistance will include PCR and/or sequencing to identify markers of resistance to artemisinin-based combination therapies (ACTs), in particular artemether-lumefantrine, artesunate-amodiaquine, dihydroartemisinin-piperaquine (including K13 and plasmepsin), and the antifolate combination sulfadoxine-pyrimethamine (SP).

Human DNA may be used in the future to screen for genetic risk factors for malaria, such as inherited blood disorders and drug metabolism e.g., G6PD variant, CYP2D6 variant. In subjects who experience an SAE and where hemolysis is suspected, G6PD genotype will be examined as well as a number of control subjects of people who were given primaquine but did not hemolyse or have an SAE. A random sample from the cross-sectional surveys may be examined for G6PD genotype in order to assess the prevalence of G6PD deficiency in study areas. No additional human genetic testing unrelated to malaria will be performed.

3.4.3. Assessment of the feasibility, acceptability, and safety

Qualitative key informant interviews (KII) and focus group discussions (FGDs) will be conducted at baseline and endline in Laos PDR.

3.4.3.1. Implementation procedures

The feasibility, acceptability and safety evaluations will be conducted using a mix of quantitative data collection and key informant (KI) interviews and focus group discussions (FGDs) of key study and health sector personnel including provincial, district, and health facility staff, VMWs, forest workers/employers and villagers/community members. In addition, participatory research methods including a process-mapping workshop at a subset of health facilities and a workshop based on the co-design of training and counselling messages for G6PD deficiency and risk factors for radical cure treatment will be conducted at baseline.

3.4.3.2. Primary outcomes measures

1. Population coverage of enhanced RACD interventions: this indicator will be measured in two ways. Operational program coverage will be defined as the proportion of individuals ≥18 months old and households visited and offered the RACD interventions within the target areas per time period. Effective program coverage is defined as the proportion of individuals (≥18 months old) that agreed to participate in the RACD intervention among all individuals ≥18 months old eligible to participate in the intervention in the target population per time period.
2. Feasibility of conducting enhanced RACD intervention at the community level: feasibility will be determined based upon a combination of population coverage data, responses of provincial, district, and health staff, VMWs, and community members to KIIs and FGDs at baseline and endline, VMW competency checklists at baseline, midline, and endline, and cost data.
3. Acceptability of community-based RACD approaches: acceptability will be determined based upon refusal rates during interventions and responses of community members and VMWs to endline questionnaire, interviews, and focus groups.
4. Safety of community-based RACD and treatment follow-up: safety measures will include the adverse event rate amongst treated individuals and hemoglobin measurement pre and post treatment for individuals receiving PQ.
5. Operational feasibility of G6PD referral: operational feasibility of G6PD referral will be determined by the results of the process-mapping and co-design workshops at baseline, responses of health staff and VMWs to interviews and focus groups at baseline and endline and competency checklists at baseline, midline, and endline, the proportion of referred *P. vivax* cases presenting at a health facility for G6PD testing, and the proportion of *P. vivax* cases with a valid recorded G6PD result.
6. Assessment of *P. vivax* treatment adherence: treatment adherence will be determined by the proportion of *P. vivax* cases with physical evidence of adherence through pill count, the *P. vivax* relapse rate across study arms, and the proportion of *P. vivax* cases with successful parasite clearance on day 14.

3.4.3.3. Sample size

Population coverage data for RACD interventions will be collected routinely and estimated numbers of individuals included are described in section 3.4.2.4.3 above. Focus group discussion (FGD) and key informant interview (KII) activities for feasibility and acceptability outcomes will aim to reach saturation and be comprehensive. At baseline and endline there will be a total of 1 FGD and 2 KIIs per district with provincial, district or study staff (36 KIIs total), VMWs, and recent malaria cases reporting forest travel. Supervisors will observe and complete a competency checklist on a random subset of 32 VMWs at three different time points: within the first month of intervention launch, at the midpoint of the intervention, and within the final month of the intervention. Sample sizes for quantitative (Likert

scale) acceptability data derived from cross-sectional surveys will be based on end-line sample size of 3,520 households.

3.4.3.4. Data collection

Baseline and endline KIIs with province and district authorities and FGDs with health center staff, VMWs, and high-risk populations (HRPs) will be conducted to qualitatively assess the feasibility, acceptability and safety of community-based RACD practices, including G6PD deficiency and primaquine knowledge, attitudes, and practices. The outputs of a process-mapping workshop and co-design workshop at two purposively selected health facilities will be documented and used to inform downstream training materials and counselling messages.

Standardized tools including interview/discussion guides and a competency checklist will be developed using best practices in qualitative research, and translated and back-translated prior to implementation. Study staff and an independent FGD/KII moderator will be trained on the appropriate tools, and all interviews will be conducted in the local language. The end-line FGDs and KIIs will explore constraints and any issues related to all the topics identified from the quantitative survey, field experience implementing the RACD intervention, and barriers to implementation of malaria control efforts. Informed consent will be obtained from all FGD, KII, and HRP participants in the local language prior to any data collection. FGDs and KIIs will be audio-recorded, transcribed, and the transcripts translated into English.

Themes to be explored during the KIIs and FGDs include the following: Demographics of HRP groups; Occupations of HRP groups, including seasonality of work; Forest-going behaviors; Migratory patterns; Health-seeking patterns and behaviors; Social networks and congregation/frequented sites (locations and days/times visited); Ideal locations and times to access HRPs for case management and surveillance activities; Operational feasibility and attitudes towards VMW-led active case detection; Capacity to conduct active case detection in forest workers; Knowledge, attitudes, and practices related to *P. vivax* infection, G6PDd testing, and primaquine.

FGDs and KIIs will be grouped as follows:

1) High risk populations (HRPs), including recent malaria cases who have worked or traveled at least one night at a forest or forest-fringe site located outside of a permanent settlement in the past 30 days
2) Health facility-based staff involved in malaria diagnosis and treatment
3) Village Malaria Workers and other community members
4) Provincial and district health officers
5) Other health sector and ministry personnel
6) Process-mapping and co-design workshops. Participation will include a cross-section of supervisors, health center staff, VMWs, and community members at 2 health centers. The health centers will be purposively selected based on malaria case volume.

At endline, FGDs and KIIs will be conducted with health center staff, VMWs, and HRPs. Themes to be explored during KIIs and FGDs include: Forest-going behaviors; Migratory patterns; Health-seeking patterns and behaviors; Operational feasibility and attitudes towards VMW-led active case detection; Capacity to conduct active case detection in forest workers; Knowledge, attitudes, and practices related to *P. vivax* infection, G6PDd testing, and primaquine; Facilitating factors and challenges to scale-up of the interventions.

These KIIs, FGDs and participatory research methods will focus on assessing the operational feasibility and acceptability of community-based active case detection in both villages and forest workers, G6PDd referral, and treatment follow-up of radical cure for *P. vivax* infection using 14-day primaquine.

The costs of RACD will be collected. Total costs as a unit as well as cost-effectiveness will be assessed as an incremental cost effectiveness ratio (ICER). The types and potential sources of expenditure data are shown in Table 6.

Table 6. Types and potential sources of expenditures

Expenditure category	Types of expenditure needed	Potential sources of information
Personnel	All human resource expenditures and time contributions • Salary/wage payments • Value of benefits • Volunteer labor	• Annual program budgets for salaries and benefits • Work logs or reports from volunteers
Commodities and Services	All supplies and services used toward RACD activities • In-kind donations • Purchased commodities (including acquisition costs) • Utilities • Travel/transit costs: fuel, transit fees and services, airfare • Reproduction costs, postage • Staff trainings: per diem payments, trainer fees, consultants • Current rental value of property	• Printing and postage receipts • Training budgets and receipts • Vehicle travel logs, fuel receipts • Utility bills • Consultant invoices

Focus groups will be facilitated by two members of the research staff: 1 moderator and 1 note-taker. A locally-appropriate location will be used to hold the focus group discussions (i.e., community center, health facility, etc). After a brief introduction, the moderator will obtain informed consent separately from each participant. All sessions will be audio recorded. During the sessions, the research staff will generate notes as the discussion unfolds to help formulate follow-up questions and probes, with no identifying information.

For interviews and focus groups, the note taker and moderator will discuss interview notes/similarities, and responses will be recorded/finalized at the end of each interview. The moderator for each group will transcribe the recording and once all focus groups have been conducted and data transcribed, and results disseminated, all audio recordings will be destroyed. A professional translator will translate the transcript to English. The local study coordinator will supervise and review the local language and English transcripts to ensure accuracy. Each focus group discussion will take approximately 1-2 hours total and occur at an appropriate time for the study respondents (i.e., weekday vs weekend; morning vs afternoon).

Data from the interviews and focus groups will be entered into Dedoose or a similar program for simple thematic analysis and stored on a password protected device. All data will be stored on a password-protected database and will only be available to the study personnel. All data from participants will be coded using a study identification number in place of the individual's name. Data will be analyzed to provide preliminary descriptive statistics on the study population and recurrent themes within the assessment. Data will only be used for the purposes of this study.

All participants will receive locally-appropriate compensation for participating in the focus group or interview to compensate for travel costs and time, and will be determined following discussions with

local partners. Light snacks and non-alcoholic refreshments will be provided during the focus group session.

3.4.3.5. Analytical plan

Outcome 1: Village-based population coverage of test and treat interventions

The operational coverage will be estimated at the individual and household level as the percent of the population that received a visit from the intervention teams to offer the RACD intervention, among those eligible for inclusion. This will be obtained from a combination of RACD program data and enumeration data. Additionally, the proportion of individuals accepting the RACD interventions, among those eligible for inclusion in the intervention, will be estimated, providing an estimate of the effect coverage of each program. Data for the denominator of individuals and households targeted for the intervention will be ascertained from the household enumeration for the sampling frame. Additionally, to validate the enumeration, attempts will be made to use remote sensing data, and/or Google Earth, to enumerate household structures. To the extent possible, individual, household and community level factors associated with coverage will be assessed using mixed effects logistic regression.

Outcome 2: Feasibility of conducting RACD interventions

The operational feasibility of VMWs to conduct RACD interventions will be estimated at the individual level as the percent of VMWs and health center staff who rate the intervention activities as very easy, somewhat easy, somewhat difficult, and very difficult. Supervisors will conduct a competency checklist on 2 VMWs per intervention area (32 total) at baseline, midline, and end-line; the capacity of following case investigation, recording, management, and follow-up SOPs will be assessed over time. Qualitative surveys will be conducted with VMWs and health center staff at endline. The costs of RACD and cost-effectiveness will be assessed as an incremental cost effectiveness ratio (ICER). Program data on costs/expenditure for RACD will be collected and combined with estimates of program effectiveness to determine ICER.

Outcome 3: Acceptability of community-based RACD approaches

The acceptability of a community-based approach for RACD will be assessed as the proportion of targeted individuals refusing RACD intervention, among those eligible for inclusion. Qualitative data collection methods such as focus groups and key informant interviews with villagers, forest workers, VMWs and other health sector staff will be implemented.

Outcome 4: Safety of community-based RACD and treatment follow-up

The adverse event rate for RDT positive participants will be assessed using intervention data and health facility adverse event reporting forms. Study staff and VMWs will be trained on detection of adverse events for positive cases receiving treatment.

Outcome 5: Operational feasibility of G6PD referral, testing and non-adherence

The operational feasibility of G6PD referral, testing and non-adherence at the community and facility levels will be evaluated. For testing, interview data at baseline and endline will be collected from district health staff and community members, as well as focus group discussions with both groups at end-line. Risk factors for non-adherence to G6PD testing after referral will be determined through comparison of factors captured at enrollment for individuals who were tested at a facility versus those who were not.

Outcome 6: Assessment of *P. vivax* treatment adherence

The treatment adherence of *P. vivax* positive study participants will be assessed through physical evidence of adherence during VMW follow-up visits on day 3, 7 and 14 and comparison of relapse rates across study arms.

3.4.4. Assessment of the risk of malaria vector biting in forest and village settings

A baseline entomological investigation will be conducted along with the baseline qualitative activities in Aug/Sep 2019. The investigation will involve collection of vector occurrence and density data (by species), human landing catches (HLC) to assess vector biting behaviors (indoor vs outdoor and forest sites), and household structure type, and human behavioral observations. All collections will take place over a one-week period. HLC collections will occur in 4-5 sleeping structures each in the forest and village, with 2 HLC collectors by structure. At the end of each HLC collection hour, collectors will also record human behavior (sleeping (or awake) under net/sleeping without net/awake), both indoors and outdoors. Field collectors will be community volunteers that will have been properly informed about the sampling methods and their implications, and who have consented to participating by signing consent forms. Prior to the start of collections, volunteers will also need to be tested for malaria. Depending on national policy, they may also be required to take malaria prophylaxis. HLC data collection forms are found in the Appendix.

4 Ethical issues related to human subjects research

4.1. Adequacy of protection against risks

We will administer an informed consent form both verbally and in writing to all participants in the local language for participation in all study activities. These forms will be read or will be given to participants to read themselves and will include a full description of voluntary participation, the right to withdraw from the study at any time, and the right to not answer any question or participate in any component of the research.

These forms will also address the risks, benefits, and purpose of the study and what we hope to learn, with a specific focus on the potential risks associated with the administration of SLD-PQ. We will train all interviewers extensively on the consent procedure, and each form will be co-signed by a team member to ensure all participants have consented. Checks in the field by the PI and project leaders will further ensure the consent process is followed in all cases. Data collection team members will provide the contact information for study coordinators who can be contacted for any further information on the topics brought up in the interview, or for additional treatment if necessary. The confidentiality procedures are designed to meet all contingencies to ensure the confidentiality of participant data and the privacy of the participants is preserved.

Our proposed strategies to reduce risks to privacy or of disclosure of confidential information include:

1. Identifying information will be recorded only in secure database software on password protected computers, and data collectors will only have access to the data that they themselves directly collect which will be cleared from their devices after all follow-up visits are completed. All data will be stored only in password-protected files on password-protected computers in locked offices.
2. Prior to analysis, data will be de-identified with the exception of geo-location codes, which are necessary for specific per-protocol analyses. The absence of individual identifying information will protect subject confidentiality.
3. All paper records will be stored in a locked location.

The potential risks of drawing blood from a finger-prick include temporary discomfort, pain, transient bleeding, bruising, skin infection, and fainting. The volumes of blood taken will be too small to produce any adverse physiologic effects from blood loss anemia and overall the aforementioned risks associated with blood draws are likely to be low. Study staff will be trained in the proper conduct of a finger-prick according to standard operating procedures to minimize the risk of discomfort and infection.

4.2. Protection against risks associated with administration of PQ

The main safety concern for primaquine administration is the risk of acute hemolytic anemia in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency[17]. In a context where G6PD testing is not available, a dose of 0.25mg/kg of PQ has been found to be safe in individuals with any of the G6PD variants and is recommended by WHO [13]. All individuals RDT+ for *P. vivax* will be referred to and tested at a health facility using a G6PD Carestart RDT and a 14-day course of primaquine will only be given to G6PD non-deficient individuals. After the first six months, a subset of VMWs will conduct G6PD testing under supervision by a study staff member for *P. vivax* cases diagnosed in the community. Primaquine is typically well tolerated but possible side effects include nausea, headache, fatigue, dizziness, hives, chest pain, painless darkening of urine, jaundice of skin, vomiting and syncope (fainting). In line with national guidelines, PQ will not be administered to pregnant women in any trimester or to women unable to undergo pregnancy testing, children less than 6 months of age, infants weighing less than 10 kg, women in the first 6 months of breastfeeding, or individuals with prior allergic reaction to PQ or related drugs.

The key to managing side effects and any potential adverse events (AEs) or serious AEs (SAEs) is thorough training of VMWs/VHVs, health facility staff and supervisors, and intensive sensitization of the community. HC workers and VMWs/VHVs will passively monitor their respective communities for AEs and refer any potential SAEs to designated health facilities. Health workers at these facilities will be informed of the project and potential side effects, and undergo training on completion of AE reporting forms (8.14 and 8.15). Cases that cannot be treated at health centers will be referred to district or provincial hospitals.

All participants receiving study drug will be provided with an informational sheet listing potential side effects and instruction to seek care at the nearest health center should they experience any of the defined symptoms or other adverse events. Community sensitization events and targeted IEC/BCC materials will also help increase community awareness of potential adverse events and encourage early care-seeking if events arise.

A passive case detection system will be employed in both intervention and control villages to help detect and refer any potential SAEs and active follow-ups will be conducted by VMWs on days 3, 7, and 14; VMWs/VHVs will be oriented on potential drug side effects and asked in addition to passively monitor their communities and refer any potentially serious or unexpected AEs. All public health facilities in the nine target districts will be trained on AE reporting procedures prior to implementation of any study activities. A data safety monitoring board (DSMB) will be established to oversee and report on any SAEs that are potentially linked to the administration of AL or SLD-PQ, as outlined in further detail in Section 4.5. The following steps will be taken for all AEs:

The health facility will complete an AE reporting form (Appendix 8.14). The completed form will then be submitted to the Local Safety Monitor (a medical doctor), who with the support of the UCSF project manager will assess if the event is an SAE or unexpected AE based upon specific criteria determined by the DSMB. If determined to be an SAE or unexpected AE, the UCSF project manager will submit the AE reporting form and any accompanying documentation (clinical records, laboratory reports) to the DSMB and the UCSF PI within 48 hours from the identification of the potential SAE.

Upon receipt of the AE reporting form, the DSMB will complete an AE investigation form (Appendix 8.14) within 24 hours to a) confirm if the event is an SAE, and 2) to determine if the SAE was caused by the administration of AL with SLD-PQ as part of this study. If the event is confirmed to meet the criteria of an SAE, the DSMB will submit the completed AE investigation form to the PI, who will submit to the research ethics committees (RECs) at UCSF and NIOPH. The respective RECs will be notified within 24 hours of the DSMB's completion of all potential SAE investigation forms, and within 48 hours of DSMB notification if deemed an AE, but not fulfilling criteria for an SAE.

Travel and hospitalization costs for any SAEs resulting from PQ administration will be covered by the study.

4.3. Potential benefits of the proposed research to the participants and others

The proposed research may benefit patients in direct and indirect ways. Participants will directly benefit from detection of low-level parasitemia, and the curative effects of AL administration on existing malaria infections in the RACD intervention arm. Furthermore, patients may directly benefit due to community-wide reductions in malaria transmission that are expected to occur after the application of the interventions. Finally, entire target village populations will benefit from SLD-PQ, which targets the parasite sexual stages thereby decreasing overall transmission.

Participants may also indirectly benefit, as the information gained from this research will be used to help establish the safety and efficacy of new malaria control interventions in both countries. The research will benefit the scientific and malaria control communities more generally by expanding the evidence base on RACD targeting high risk populations.

4.4. Alternatives to participation

Participation in the research study is voluntary. Individuals electing not to participate in the research study may still receive testing and treatment as part of the routine malaria case management. Individuals who do not wish to receive testing and treatment or presumptive treatment during the intervention campaigns may visit local health facilities for malaria testing and treatment.

4.5. Data and safety monitoring plan

The project will follow US National Institutes of Health (NIH) guidelines for establishing a data safety monitoring board (DSMB). The DSMB will be established prior to any data collection as part of this study. The members of the DSMB will serve in an individual capacity and provide their expertise and recommendations. The primary responsibilities of the DSMB will be to periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate make recommendations to investigators concerning the continuation, modification, or termination of the trial. DSMB will consider study-specific data as well as relevant background knowledge about malaria, adverse events from drugs used in the study, drug resistance, and the participant population under study.

Membership of the DSMB will consist of five independent experts in malaria control, diagnosis, case management and epidemiology, and one member of the research team to advise and clarify study activities for the independent experts. No independent member of the DSMB shall have any conflict of interest with the study team, the organizations funding or conducting the research, or the results of the study. The DSMB will be comprised of experts in the following areas, with an emphasis on local Lao expert participation:

- The study population in Lao PDR (Lao representatives)
- Malaria diagnosis and case management
- Malaria epidemiology
- Biostatistics
- Conduct of clinical trials
- Malaria drug resistance

No data on futility or benefit of the intervention will be estimable during the course of the trial as the timing of outcome data collection precludes developing stopping rules based on outcome data collected during implementation. Safety concerns associated with the wide-scale use of AL + SLD-PQ, although unexpected, will form the basis of development of a stopping rule. The stopping rules

for this trial will be based on detection of a significantly higher rate of mortality, hospitalization for possible drug-related events, or any other SAE in the intervention villages versus rates recorded at health facilities for the control villages. Further, any investigated SAE that results in death that is found to be due to the administration of one of the study drugs will be grounds for stoppage.

The Local Safety Monitor (physician) will review all SAEs, hospital admissions and deaths in the study areas, give an opinion on causality, and give regular feedback to the DSMB.

4.6. Collection of specimens

RDT kits for malaria using finger prick blood samples will be collected during the research and intervention. These samples and their byproducts will be collected for disposal according to local disposal standards for biohazard and sharps waste at the nearest health facility (generally incineration). DBS will also be collected for PCR analysis of malaria parasite infection. These blood spots will be disposed of after the research and not stored for future use. The research period will be defined by the approval dates from the local research ethics committee, the UCSF, and ministry of health, and blood samples will not be stored for any longer than three years past the date the research study end. The informed consent documents will specify the uses and storage plans for dried blood specimens. Patient identifying information will be replaced with an unrelated unique identifier on each filter paper. All appropriate universal and site-specific safety precautions will be used in handling sharps, RDTs, and filter paper blood spots. All survey workers will be trained in the proper storage and handling of blood samples prior to fieldwork.

4.6.1. Racial and ethnic origin

A diverse range of ethnic groups will be included in the study, including several marginalized ethnic minorities. Our study will be conducted outside of the U.S., and no racial/ethnic group will be excluded. We do not expect to find race/ethnicity differences in the intervention effect, but refusal rates may differ.

4.6.2. Inclusion of vulnerable subjects – children and pregnant women

All age groups will be included in this study, except for those < 18 months. All children that participate must have the consent of the parent or guardian. Children older than 6 years and less than 18 must also provide oral assent before participation. All women who are pregnant or believe they may be pregnant will be assessed appropriately before treatment.

4.7. Participant consent/assent

Written informed consent will be obtained from the household members, neighbors, and co-workers eligible for the study. For the field procedures (survey, blood testing, treatment and follow-up visits), people will be asked to consent each time they are eligible to be part of the study. Consent will not be required for collection of de-identified, used RDTs from health facilities inside and outside of the study areas, if this collection is deemed feasible.

Consent will be conducted in the participant's household or in a private area prior to study activities. Consents may be collected from all members of the household and/or co-workers once. Parents will be able to sign one form to consent for themselves and all of their participating children (under 18 years old) at once. Each additional adult member of the house will sign separate consent forms. Each minor (12-<18 years old) will sign a separate assent form.

Informed consent will be conducted in local languages. If the participant is unable to read or write, an X will substitute for a signature.

As part of the informed consent process, study personnel will assess participants' understanding of the study procedures that were explained by using a checklist comprised of key components of the study. Participants who pass will be allowed to sign the written consent form. If the participant does not pass, the consent discussion will be repeated, before asking for a signature. In this case, the consent form will be read again, focusing on areas where understanding was limited, and encouraging

the subject to ask questions. Up to five attempts will be permitted per participant. If, despite five consecutive attempts (each incorrect one followed by the team's correction with explanation), the participant still has not answer a minimum number of questions correctly, then he/she will not be allowed to take part in the study.

5 Potential risks, limitations, data quality assurance, and dissemination plan

5.1. Potential risks from participation

Detailed discussion of potential AEs related to drug regimens is discussed above in section 4.2. Finger pricks for RDTs and DBS are associated with small risks of bleeding, hematoma, and infection. To minimize these risks, the skin will be cleaned with alcohol prior to puncture, and sterile unused lancets will always be used, and pressure will be placed on the puncture site after removal of the lancet using sterile gauze. Although the quantity of blood drawn would not lead to any ill effects on the participants' health, some adults and rarely children feel faint from the blood during the finger prick. The risks will be minimized by having trained health staff perform all procedures, and all untoward effects will be evaluated by health center staff.

For those subjects with *P. vivax* diagnosis that are tested for G6PD deficiency, there is a risk of misclassification and wrongly ascribed to being G6PD non-deficient and then being treated with 14 days of PQ. In this study, the team will ensure a high-quality of training for all those using the G6PD RDTs, in addition subjects will be given information on what to do if they experience adverse events, and on days 3 and 14 all subjects given PQ will be actively followed up. If hemolysis is suspected PQ treatment will be stopped and the subject monitored to at least day 14. Should the hemolysis event require further clinical management, the study team will ensure access to appropriate health services.

5.2. Limitations

Several limitations have the potential to compromise study outcomes.

Malaria declines

There may be large-scale changes in malaria incidence and prevalence throughout the study area over the course of the 18-month implementation period, which could compromise study power.

Malaria increases

Any large increases in malaria burden or other diseases (e.g., dengue) in target districts could increase overall caseloads at health centers, potentially impacting availability of VMWs or other staff to support test and treat campaigns, especially in areas with ethnic minorities where their expertise is crucial.

Safety of PQ

Accidental administration of PQ to G6PD deficient individuals could result in an SAE and study stoppage.

Adherence to treatment

Poor study outcomes could result from poor adherence to the 14-day PQ course.

Widespread HRP2/3 deletions

There have been no surveys for *P. falciparum* HRP2/3 deletions in Lao PDR, and only limited data from other settings in the GMS: in China and Myanmar deletions were detected in 4/87 samples (unpublished data WHO MPAC, 2017). If these deletions are common in the study sites, the impact of HRP2-based test and treat could be severely compromised. *P. falciparum* positive DBS from the baseline cross-sectional survey will be rapidly analyzed to assess the level of HRP2/3 deletions in Southern Lao PDR.

Changes in forest-going activity patterns

The Lao government decree (May 2016) is believed to have had a major impact on the total number of illegal and semi-legal forest-goers. However, the future status is unknown; if there are changes to the decree itself or to its enforcement, the VMWs could potentially be overwhelmed with interviews or sampling sites to target.

Mobility of target populations

The fluxional nature of HRPs targeted in this intervention and the mobility inherent in forest-based economic activities has the potential to contaminate the HCCA-level randomization. While HRPs may freely transit between intervention and non-intervention arms, and thereby 'carry-over' community-based interventions, the use of unique IDs will allow the impact of these movements to be assessed and adjusted for in exploratory analyses.

Other partners' interventions impact study outcomes

The implementation of diverse programs by other partners across the study area has the potential to impact study outcomes if implementation is differential across the study arms. The nature of the randomization should minimize biases from partner activities, and a detailed matrix of other project activities will be created, and used to assess and adjust outcomes for exploratory analyses.

5.3. Data quality assurance plan

Data quality and management

Data collection will occur in multiple locations, and differences in data collection systems may exist at different locations, which could potentially bias results. However, study teams and regular health staff will receive comprehensive training, as well as ongoing evaluation, supervision, and supplementary capacity building as necessary to ensure data quality and completeness.

Procedures to minimize biases

A survey instrument based on the formative work (carried out in December 2016) will be developed in English with input from collaborators at CMPE. This will then be translated to Lao and back-translated by a fluent bilingual health expert before field testing. Any ethnic minority language interviews will be conducted in the appropriate language, and translated into Lao. Formative work will assess the feasibility of using tablets for data entry versus paper questionnaires. A pilot study will test the utility of the survey instruments; these data will then be discarded if significant changes are made to the survey instrument. Study coordinators will be responsible for monitoring data quality to ensure that questionnaires are completed and entered correctly.

Potential changes to this protocol based on the piloting of tools and methods

The organization and supervision of the peer navigators may be changed based on initial feasibility studies during field-testing of survey instruments.

5.4. Dissemination Plan

The finalized protocol will be filed at http://www.clinicaltrials.gov/ prior to implementation, and will conform to CONSORT recommendations for cluster randomized trials[18].

The results of the baseline survey, as well 'hotspots' identified during community-based RACD activities will be shared on a real-time basis with national, provincial, district and village-level partners as well as other key stakeholders (i.e., WHO, PMI, CHAI, PSI) throughout implementation to ensure that the most up-to-date information about malaria is available in the target districts.

6 Timeline

Activities	2019				2020				2021
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
Study Preparation									
Finalize protocol									
• Consent forms, questionnaires, SOPs, training materials									
IRB submissions									
Partner engagement									
Develop subcontracts with local institutions									
Hire Staff									
Procurement of materials									
• RDTs, HS-RDTs, G6PD RDTs, AL, PQ, laboratory supplies, field supplies									
Electronic data collection tool development									
Meeting with provincial and district staff									
Pilot & Training Staff									
Revisions electronic data collection tool									
Baseline qualitative data collection, mapping, and surveillance data collection									
Data collection (Intervention)									
Midline review and competency checks									
Data collection (Endline survey)									
Laboratory analysis									
Data analysis									
Prepare final manuscripts and reports									

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8 Appendices

8.1 Schematic of study design
8.2 Household survey informed consent
8.3 Household survey questionnaire
8.4 Blood collection informed consent
8.5 Blood collection results
8.6 RACD household informed consent
8.7 RACD HH and Forest Questionnaire
8.8 RACD household blood collection informed consent
8.9 RACD forest informed consent
8.10 VMW forest registry form
8.11 VMW G6PD referral form
8.12 HF G6PD register form
8.13 HF Case investigation form
8.14 Adverse event reporting form
8.15 Severe adverse event investigation form
8.16 FGD/KII informed consent form
8.17 VMW FGD guide
8.18 VMW interview guide
8.19 VMW competency checklist
8.20 HF FGD guide
8.21 PAMS and DAMS KII guide
8.22 District and supervisor KII guide
8.23 HRP interview guide
8.24 Treatment adherence follow-up form
8.25 PROMPT Primaquine treatment data collection form
8.26 Entomological data collection form
8.27 Human Behavior Observations form
8.28 Informed Consent Form for Persons Conducting Human Landing Catches
8.29 Informed Consent Form for Heads of Household
8.30 RACD Household Survey Oral Assent