Study Title: Operational evaluation of Mass Screening and Treatment using ultrasensitive rapid detection tests to reduce *P. falciparum* incidence in a malaria elimination program in Eastern Kayin State, Myanmar.

Internal Reference Number / Short title: MAL 17011 / Mass Screening and Treatment for reduction of falciparum malaria

Date and Version No: 20 Feb 2018 – v2.0

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Mass Screening and Treatment for reduction of falciparum malaria

Operational research study protocol

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Chief Investigator Signature:	

The investigators declare no conflict of interest.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the authorised individuals from the University of Oxford, the Investigator Team and members of the Oxford Tropical Research Ethics Committee (OxTREC), unless authorised to do so.

TABLE OF CONTENTS

1. SYNOPSIS

Study Title	Operational evaluation of Mass Screening and Treatment using ultrasensitive rapid detection tests to reduce *P. falciparum* malaria incidence and prevalence in a malaria elimination program in Eastern Kayin State, Myanmar.	
Internal ref. no.	MAL 17011	
Study Design	Stepped wedge intervention	
Study Participants	Populations of villages with high *P. falciparum* incidence located in Eastern Kayin State, Myanmar.	
Planned Sample Size	60 villages (group 1) 60 villages (group 2)	
Planned Study Period	3 years	
	Objectives	**Outcome Measures**
Primary	To measure the impact of URDT-based MSAT on *P. falciparum* incidence at village-level (group 1 & 2)	Adjusted incidence rate ratio before/after MSAT
Secondary	1) To measure the impact of MSAT on prevalence of P. falciparum infection in malaria hotspots (group 1)	1) Prevalence of *P. falciparum* infection measured in the village by URDT and by reference method
	2) To measure the impact of reactive MSAT on incidence of seasonal malaria peaks / outbreaks (group 2)	2) Change in the incidence dynamics over the transmission season
	3) Feasibility of MSAT as a programmatic tool and as a reactive strategy (group 1 and 2)	3) Coverage of village population with intervention
	4) % of infections treated compared to infections detected by reference method (group 1 only)	4) % of P. falciparum positive samples by reference method which were positive by URDT and treated; % of P. falciparum negative samples by reference method which were positive by URDT and treated

2. ABBREVIATIONS

ACT	Artemisinin Combination Therapy
AL	Artemether Lumefanthrin
CE	Community engagement
DP	Dihydroartemisin-Piperaquin
ELISA	Enzyme Linked Immuno Sorbent Assay
GCP	Good Clinical Practice
GMS	Greater Mekong Subregion
HRP2	Histidin Rich Protein 2 (Plasmodial antigen detected by lateral flow assays: RDT or URDT)
ICF	Informed Consent Form
MDA	Mass drug administration
METF	Malaria Elimination Task Force
MP	Malaria Post
MSAT	Mass Screening and Treatment
NMCP	National Malaria Control Program
OxTREC	Oxford Tropical Research Ethics Committee
PI	Principal Investigator
PIS	Participant Information Sheet
PMQ	Primaquine
Q7C7	Quinine 7 days; Clindamycine 7 days
RDT	Rapid Diagnostic Test
sld PMQ	single low dose Primaquine
SOP	Standard Operating Procedure
uPCR	Ultrasensitive quantitative polymerase chain reaction
URDT	Ultrasensitive Rapid Diagnostic Test

3. BACKGROUND AND RATIONALE

Malaria elimination has been undertaken in the Western Greater Mekong Subregion (Western Thailand, Eastern Myanmar) in an attempt to prevent the spread of artemisinin- and multidrug resistant falciparum parasites.

The malaria elimination task force (METF) has been operating in Eastern Kayin State since April 2014. METF is present in the four townships of Hpapun, Hlaingbwe, Myawaddy and Kawkareik, in a total of 158 village tracts. METF is providing access to early diagnosis and treatment of malaria in 1200 villages through community-based facilities "Malaria Posts" (MP) [1]. The monitoring of falciparum malaria incidence in the villages after the opening of a MP showed that incidence decreases quickly in most locations (approximately by 25% per quarter of MP activity, Figure 1A). As a result in April 2017 a total of 965 villages out of 1,222 villages (79%) corresponding to 104 village tracts were free of *P. falciparum* malaria for at least 6 months.

However the activity of the MP had more limited impact in villages with high prevalence of asymptomatic infections: a slower or no decrease of falciparum was observed, even after >24 months of MP activity (Figure 1B).

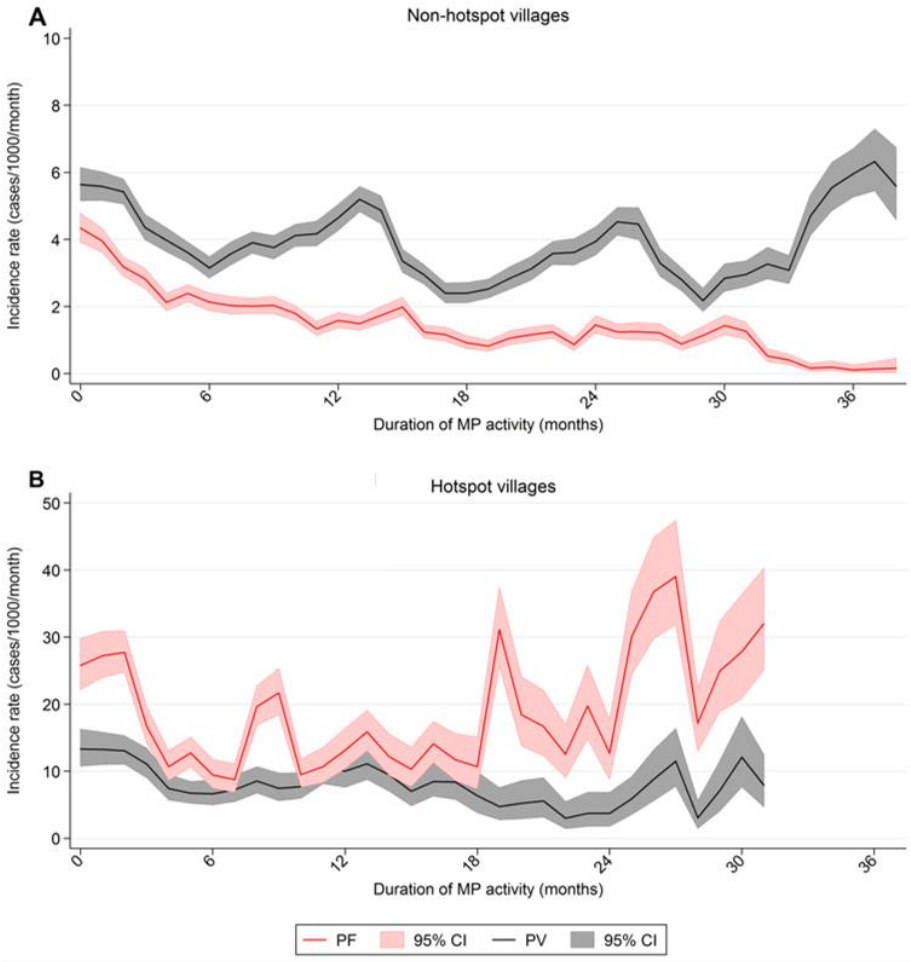

Figure 1: Malaria incidence (falciparum in red and vivax in black) since MP opening in the townships of Hpapun (n=468 MP) and Myawaddy (n=100) where hotspots were identified (52/69 located in Hpapun Township, 7/69 in Myawaddy). *Different y-scales are used in each graph.*

A: non-hotspot villages. *The widening confidence intervals after 24 months indicate that fewer MP have been active for 2 years or more. The oscillations in P. vivax incidence are related to seasonal peaks occurring in the same locations in Myawaddy Township.*

B: hotspot villages, without intervention. *Only 16 hotspots contribute to follow-up for durations of MP activity above 18 months. These high incidence locations were only identified during the final campaign of baseline surveys (Nov. 2016 to Jan. 2017) and had not been addressed by April 2017. The median duration of MP activity before intervention was 12 months (IQR=5-16). Over the period corresponding to a duration of MP activity between 0 and 18 months, a decrease can be observed in hotspots which is captured by the statistical model.*

Following a successful pilot study initiated in 4 villages in 2013 [2], METF has conducted surveys to measure the prevalence of asymptomatic malaria infections. Villages with high prevalence of malaria (>40%) in which falciparum represented at least 20% of malaria infections, were defined as hotspots and treated with a 3-month mass drug administration campaign. These campaigns were very successful at depleting the reservoir of asymptomatic carriage of *P. falciparum* (Figure 2), which translated in a significant decrease in clinical episode incidence (Figure 3). In spite of its success, this strategy is difficult to scale up due to numerous constraints and was not considered by the Myanmar NMCP. However, the successful decrease in case incidence obtained by treating the reservoir of asymptomatic carriers sparks interest for a simpler and faster intervention, relying on mass screening and treatment (MSAT) rather than MDA (Table 1). Such approach was previously impossible due to the lack of sensitivity of standard RDT to detect asymptomatic infections. A newly available ultrasensitive RDT (URDT) shows a 50% sensitivity and 99% specificity compared to uPCR. This sensitivity is sufficiently high to allow accurate detection of high prevalence villages, and to warrant evaluation of the impact of a URDT-based screening and treatment intervention. MSAT is a method approved by the Myanmar NMCP and recommended by the WHO.

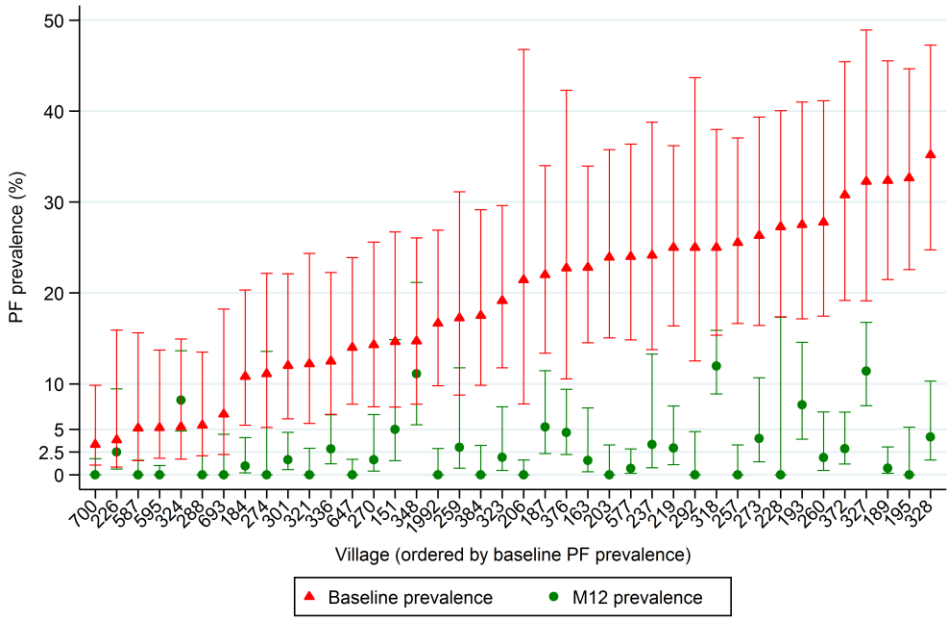

Figure 2: Impact of MDA on P. falciparum prevalence *(95% confidence interval) comparing baseline and 12 months after MDA (n=40 villages) in villages equipped with MP and addressed with MDA between January 2015 and April 2016.*

By design, an MSAT intervention using a field test will not identify all carriers. However, it is likely to decrease village level prevalence by 50%, and parasite biomass by >97%, since 87% of infections with parasite densities >1000 parasite/mL are expected to be identified. This depletion of the reservoir could be sufficient to durably modify the village-level transmission and initiate or accelerate the MP-driven decrease. Additionally, a URDT-based MSAT strategy could be an extremely useful tool to respond to malaria outbreaks. Indeed, by rapidly screening the population of a village where the incidence has reached alarm thresholds, parasite carriers could be rapidly identified and treated, leading to a rapid decrease in the reservoir and an interruption of the transmission.

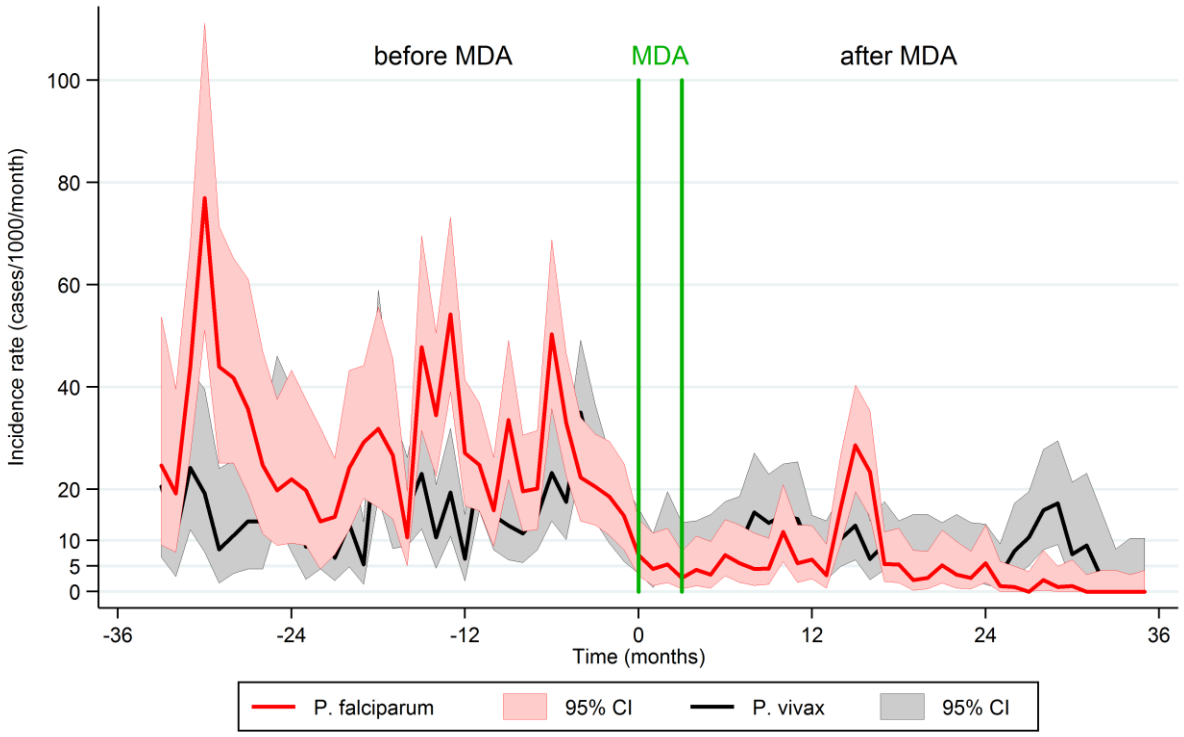

Figure 3: Average P. falciparum and P. vivax incidence in hotspots before and after MDA, centered on date of MDA. *A marked decrease in P. falciparum incidence after MDA can be observed, in spite of an increase in incidence around 15 months after MDA. This increase is related to 5 hotspots (out of 52 followed up to M18) showing an incidence above 50 case/1000 for 1 month during the second year after MDA. This increase did not persist further.*

In this project, we aim at an operational research deployment of URDT-based MSAT in the METF elimination program. This intervention will be tested in two types of setting, which correspond to two arms in this study. In group 1, MSAT will be used in a programmatic setting in order to decrease the reservoir of asymptomatic carriers in high incidence villages (following the same principles and objective as previously deployed MDA interventions). In group 2, we take advantage of the lighter framework of MSAT to use it as a reactive intervention in order to respond to malaria outbreaks in low to intermediate incidence villages. The MSAT intervention will be preceded with community-level consent and community engagement (CE) activities. MSAT will be conducted over a period of approximately 1 week in each hamlet, village or group of villages, and will consist in administering a *P. falciparum* URDT to all individuals agreeing to participate. A limited subgroup (expected 5-25%) will be found positive and receive supervised treatment over 3 days for the standard regimen (DP to cure asexual stage infection + single low-dose primaquine to destroy gametocytes). After this intervention, the incidence of clinical falciparum episodes will be monitored by the village MP. In group 1, a comparison of the prevalence at baseline and 12 months after MSAT intervention will be performed through a second URDT survey, in addition to which both baseline and 12-month surveys will include the collection of a 50µL capillary blood sample for reference detection in the laboratory.

The intervention will be evaluated primarily on its ability to reduce yearly cumulative incidence of clinical falciparum malaria compared to year before intervention. Additional evaluations of the impact of MSAT will include: in group 1, comparison of asymptomatic infection prevalence; and in group 2, modifications of the shape of the incidence curve following intervention.

Potential risks and benefits for participants

This study has limited risks for participants. 75 to 95% of participants will only undergo a malaria rapid diagnostic test (URDT) requiring a finger prick and receive a negative result. 5 to 25% of participants are expected to be found URDT positive. The specificity of the test is high compared to uPCR (99%) as well as predictive positive value (88%), ensuring that >9/10 of these positive individuals will be infected with *P. falciparum* and will benefit from the ACT treatment ([3], Appendix D). The safety of DP and single low dose primaquine are well-described, including among asymptomatic individuals [2,4]. The risk associated with treatment will be limited. All screening participants in group 1 will also be sampled for 50µL capillary blood using the same finger prick as used for the URDT, which does not aggravate the risk. All procedures on participants will be conducted by trained medical personal.

Participants will benefit from this study by acquiring knowledge of their infection status as individuals and as a community. Infected individuals will be treated, which will decrease the potential negative consequences of a long-lasting infection [5,6] without increasing the risk of a subsequent clinical episode [7]. Households where positive participants will be identified will receive additional guidance on measures to protect against vectors and be reminded to consult the village MP within 24h in case of fever onset. At the community level, treatment of asymptomatic carriers is expected in reducing significantly the number of infective individuals, thus limiting the infection of mosquitoes and decreasing the overall village transmission and malaria burden.

Interest of the research

The METF target area in Eastern Kayin State, Myanmar, is piloting strategies with potential applications for >20 countries in the 2020 horizon, and many more countries by 2030 since elimination is one of the Sustainable Development Goals. It is also a region of significant artemisinin resistance, and under the threat of the expansion of a multidrug resistant falciparum lineage spreading in the GMS. Strategies developed and tested in a context of antimalarial resistance are therefore extremely valuable for this region.

4. OBJECTIVES AND OUTCOME MEASURES

Objectives	**Outcome Measures**	**Timepoint(s) of evaluation of this outcome measure (if applicable)**
Primary Objective To measure the impact of URDT-based MSAT on *P. falciparum* incidence at village-level.	Adjusted incidence rate ratio before/after MSAT	Follow-up from MP opening to end of study
Secondary Objectives 1) To measure the impact of MSAT on prevalence of P. falciparum infection in malaria hotspots	1) Prevalence of *P. falciparum* infection measured in the village by URDT and by reference method (group 1)	1) Comparison between M0 survey (during MSAT) and M12 survey (12 months after)
2) To measure the impact of reactive MSAT on incidence of seasonal malaria peaks / outbreaks.	2) Change in the incidence dynamics over the transmission season (group 2)	2) Duration and height of seasonal peak before, during and after reactive intervention
3) Feasibility of MSAT as a programmatic tool and as a reactive strategy.	3) Coverage of village population with intervention	3) coverage based on census conducted before/during MSAT
4) % of infections treated compared to infections detected by reference method	4) % of P. falciparum positive samples by reference method which were positive by URDT and treated; % of P. falciparum negative samples by reference method which were positive by URDT and treated (group 1)	Group 1 only
Tertiary Objectives Retrospective comparison of MSAT versus MDA on prevalence and incidence of P. falciparum malaria	Cumulative incidence of falciparum malaria episodes before and after MDA/MSAT; prevalence of falciparum infection before and after MDA/MSAT	Entire incidence series

5. STUDY DESIGN

Stepped-wedge open-label, non-randomized, cluster intervention.

This study will be performed in clusters (hamlet (isolated group of household, village, or group of village). The intervention will be conducted in two types of clusters, both corresponding to locations where an excess of case was detected.

Group 1: Sustained high incidence clusters, characterized by a yearly cumulative incidence >84 cases/1000/year

Villages in group 1 will be attributed an intervention a given year based on the cumulative incidence over the previous 12 months (METF stratification January, including the last 2 transmission seasons). The order of intervention will be decided based on logistic constraints and highest incidence.

Group 2: Focal transmission clusters, corresponding to locations where an epidemic alert has been signalled and confirmed (see definition of thresholds).

Villages in group 2 will be attributed an intervention based on P. falciparum incidence in the previous 4 weeks. In near-0 transmission area, an intervention will be conducted in each likely source location of transmission of a locally acquired case. In the other areas (METF1+METF2), the intervention will be triggered when the incidence is above the pre-defined epidemic threshold.

In each cluster, all inhabitants will be invited to undergo an URDT test to identify their infection status, and will receive the appropriate treatment according to their characteristics. Information on village inhabitants absent during the MSAT activities will be obtained from village population lists provided by the village headman and from household member declarations. During the URDT screening, all individuals will receive a unique identifying number that will be used to record demographic data in the MSAT paper logbook and to label URDT and reference sample.

Before and after MSAT intervention, incidence of clinical malaria episodes will be recorded at the MP (1 or several) serving the cluster receiving the intervention. No individual data will be collected to link clinical case participation, infection status and incidence of clinical episodes.

Participants from group 1 clusters will also be invited to participate in a prevalence survey during MSAT and 12 months after, in order to evaluate the impact of the MSAT campaign on the asymptomatic carriage prevalence. This will require collection of a 50µL sample during the MSAT campaign and a second round of URDT screening with the collection of a 50µL sample, 12 months after MSAT.

6. PARTICIPANT IDENTIFICATION AND RECRUITMENT

6.1. Study villages selection

The study village selection is based on the definition of three main strata of *P. falciparum* incidence corresponding to different probabilities of rapid elimination. Based on 2017 data, the townships of Myawaddy, Hlaingbwe and Kawkareik have almost interrupted *P. falciparum* transmission (95% of villages with 0 incidence), while heterogeneity persists in Hpapun township with diverse transmission and incidence profiles.

6.1.1. Group 1: sustained high incidence villages

Villages classified as high incidence, low probability of elimination (*P. falciparum* cumulative incidence >84 cases/1000/year, in spite of >1 year of functioning malaria post) will be eligible to be included in group 1. Villages in this group will be addressed by MSAT waves of 10-15 villages.

Villages will be included in a given wave based on accessibility to the team and proximity from each other, as well as requirements for the program impact. Wave 1 will include 5 villages and be conducted during first semester 2018, after which a maximum of two waves will be conducted per semester.

6.1.2. Group 2: seasonal focal transmission villages/locations

This group will follow the NMCP case/and foci investigation guidelines, but use URDT instead of standard RDT for screening. MSAT group 2 locations will be cluster of houses, villages or clusters of villages selected based on the results of case or foci/outbreak investigation.

6.1.2.1 Village inclusion after case investigation

Case investigation will be performed systematically in the three townships of Myawaddy, Kawkareik and Hlaingbwe, upon notification of ≥1 P. falciparum clinical case. The aim will be to determine the most likely location of transmission.

The village or cluster of houses corresponding to the most likely location of transmission will be included in group 2 it is located in Myawaddy, Kawkareik and Hlaingbwe.

The most likely location of transmission will not be included in group 2 if it is located outside METF area, or in Hpapun township.

However, upon evolution of the overall incidence, subdivisions of Hpapun township reaching 0-transmission will be included in the case investigation activities and therefore eligible for case investigation and inclusion in group 2 MSAT.

6.2.2.2 Village inclusion after outbreak investigation

Outbreak alarms will be generated from Hpapun township by the analysis of weekly *P. falciparum* incidence data by METF surveillance system (e.g. following thresholds such as cases>90th percentile at the village or cluster level). An investigation will be conducted to determine the context of transmission and assess the contribution of imported case in this increase.

The cluster will be included in group 2 if the investigation concludes to the existence of a local increased transmission episode.

6.2. Study Participants

Since the study will be performed at cluster/village level, the population of study villages will be included and monitored as a whole for the primary outcome on falciparum malaria incidence in the village. Likewise, change in prevalence will be measured by a follow-up survey at village level without matching individuals from MSAT screening to follow-up survey.

MSAT intervention and follow-up surveys will be conducted on individual participants. All population of the cluster/village will be eligible for participation in the MSAT campaign. For the follow-up survey, only residents living in the village for >1 months will be considered in order to assess the reservoir in the village population and limit the contribution of imported carriage.

6.3. MSAT intervention

6.3.1. Inclusion criteria

All persons living in the village or cluster of villages will be eligible for MSAT intervention. Individuals living in smaller settlements (permanent or temporary) within walking distance of a selected intervention village will also be eligible.

Large "work-related" settlements in the vicinity of a targeted village (military camps, logging camp, mining site) will be approached by the team to be included in the screening and treatment activity. They will be included in the analysis as a unit within a cluster of villages if all the study information can be collected (including follow-up survey for Group 1).

6.3.2. Exclusion criteria

- Individuals who do not provide informed consent for both URDT screening and treatment in case of positive result. Individuals will be given the possibility to refuse the collection of the 50µL reference sample or the DBS collection but participate to URDT screening and treatment.
- Children <1 year old
- Individuals with a documented Pf-positive malaria RDT who received treatment (AL+sld PMQ) during the previous 7 days.

NB: Individuals who were diagnosed infected with PF and received a treatment between 7 and 30 days before the intervention are still likely to be URDT positive due to the persistence of HRP2, and this will result in treatment of individuals who are likely uninfected. However, in a high prevalence area or in an outbreak context, previous infection signals exposure, and DP will provide a protection against a likely re-infection.

6.3.3. Specific treatment regimen

Screening can be conducted on all village inhabitants but some specific population groups will require a specific treatment if they are found positive (see 8. Intervention)

6.4. Follow-up at 12 months (group 1 only)

6.4.1. Inclusion criteria

Randomly sampled individuals living in the village.

6.4.2. Exclusion criteria

- Individuals who do not provide informed consent.
- Individuals who have lived in the village for less than 4 weeks (newcomers residing elsewhere before)

- Individuals who had a falciparum positive RDT and received a complete ACT-treatment course during the 3 weeks before the survey.

6.4.3. Follow-up MSAT

A follow-up MSAT will be triggered if the estimated URDT prevalence of the M12 survey is ≥5%. It will follow the same procedures as the baseline MSAT.

7. STUDY PROCEDURES

7.1. Recruitment

Villages will be allocated to group 1 (programmatic MSAT) based on incidence over the previous 12 months. This list will be revised twice per year after each transmission season (February and August). Villages will be allocated to group 2 (reactive MSAT) based on weekly analysis of malaria surveillance data (see details in 6.1).

Following the allocation of villages to a given group and the decision to conduct the intervention, the community engagement team will conduct meetings in the village/cluster to explain the purpose of the intervention and seek community approval, first with village and local authorities, then with the entire population. After the agreement from the community, a date will be set for the intervention and the mobilization and engagement of individuals will begin, involving CE team members and the MP worker from the village.

The MSAT intervention will be conducted by visiting participants at home or by inviting them to a central location, depending on the lay-out of the village and the preferences of the populations. Participant information will be discussed individually and participant will be recruited in the MSAT intervention after informed consent. The same procedure will be followed for M12 follow-up survey in Group 1.

7.2. Informed Consent

The MSAT intervention will first be explained to the community, through meetings with leaders and authorities, public meetings with all individuals and meetings and activities for specific groups of participants (school-age children…). Seeking community consent first will allow time for the population invited to participate to discuss with field team members and with each other, to ask questions and address concerns. Participant information will be presented under different forms during these activities.

At inclusion, written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the field team or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the

consent must be suitably qualified and experienced, and have been authorised to do so by the Chief Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the study site.

For children ≥ 10 to < 18 years old t, an assent will be obtained in addition to the consent of their parent or guardian. For children below 10 years, the consent of the parent or guardian will be obtained.

A second consent will be collected upon participation to the follow-up survey, 12 months after MSAT intervention.

7.3. Screening and Eligibility Assessment

All participants who consent to be tested and treated if falciparum positive will be eligible to participate in the MSAT intervention.

7.4. Randomisation, blinding and code-breaking

No randomization will be conducted. Villages with sustained high incidence will be assessed every 6 months and prioritized based on accessibility/feasibility. As much as possible, all villages from a given area will be addressed at the same time in order to maximize impact of interventions.

7.5. Baseline Assessments

The baseline assessment (D0) will occur immediately after inclusion. Participants will be asked baseline demographic data (age, sex, visitor status, occupation and activities during the previous month) and baseline clinical data (temperature and history of fever over the previous week), which will be recorded in a logbook where each patient will be identified by a unique number printed on a sticker that will also be used to label the URDT, the sample(s) collected from the participant.

In **group 1 only**, participants will undergo a finger-prick from which capillary blood will be collected using the lancet provided by the URDT manufacturer in order to realize the URDT. From the same finger-prick, 50µL of capillary blood will also be collected on an EDTA microtube, labelled with a participant sticker.

The URDT will be read by a trained field worker following the manufacturer's instructions. If the test is positive, a second finger prick will be conducted in order to collect an additional blood sample on filter paper (3x 1cm blood spots), labelled with a participant sticker.

If the test is positive, a physical and clinical examination will be conducted. Female participants of child-bearing age will be asked if they are or could be pregnant and proposed to take a pregnancy test if unsure. History of malaria diagnosis and treatment during the previous week will be collected. The weight of the participant will be measured and history of previous allergies to drugs and specifically antimalarials will be collected. A curative treatment for *P. falciparum* malaria will be administered to falciparum-positive participant following a weight-adjusted dosage and according to each participant characteristics (see 8. intervention).

The willingness and ability of eligible participants to take a complete treatment will be assessed by the fieldworker, and the importance of completing the treatment will be discussed with the participant if

necessary. The first dose of treatment will be administered directly, and recorded in the logbook. An appointment will be set for the next dose on the following day.

Participants who declare being unable to attend supervised treatment for the next takes will be given the remaining doses (expected <2.5% of treatments initiated).

7.6. Subsequent Visits

7.6.1. Treatment administration follow-up visits (visit 2, visit 3)

Subsequent visits will be conducted on the second and third day of standard DP antimalarial treatment for individuals receiving the standard DP course (D1, D2). Before administering the next dose, a small questionnaire will be used to collect potential adverse events or other complaints.

For 1st trimester pregnant women treated with Q/C, the village malaria post worker will be in charge of completing the follow-up of the 7-day treatment course as per his training. For patients receiving other types of treatment, a team medic will follow-up the completion of the treatment and monitor for AE daily.

7.6.2. Malaria incidence monitoring

Each village is equipped with a community-based malaria post, where a trained member of the community provides diagnosis and treatment to all fever cases occurring in the village. The MP records the result of all RDT conducted and reports weekly activity data, including number of fever consultations, number and result of RDT performed and number of clinical malaria episodes treated by sex and age-group of patient and by parasite species.

7.6.3. Follow-up prevalence survey (group 1)

Twelve months after the beginning of the MSAT campaign in group 1 villages (high incidence), a population list will be collected again and a prevalence survey will be conducted in a sample of village inhabitants to perform URDT and collect a sample of 50µL for reference testing (sample size based on ability to measure a 90% decrease from baseline, see 9.2).

After the subsample of inhabitants has been surveyed, if the URDT prevalence is above 5%, the survey will shift to an MSAT round following the same protocol as baseline. If the URDT prevalence is below 5%, the positive individuals will be referred to MP for treatment and the survey will finish.

This survey will identify households from the MSAT campaign, but not match individual identification codes.

Since the procedures and interventions are similar to

7.7. Sample Handling

From the same finger-prick used to perform the URDT, 50µL of capillary blood will also be collected on an EDTA microtube, labelled with a participant sticker and transported to SMRU laboratories or stored immediately at -80°C in a dry-shipper liquid-nitrogen tank before transport. Upon reception at SMRU laboratories, the samples will be and stored at -80° C until processed for reference malaria detection by Quansys ELISA.

If the test is positive, a second finger prick will be conducted in order to collect an additional blood sample on filter paper (3x 1cm blood spots), labelled with a participant sticker, dried and stored in a plastic blister with silica gel. The DBS will be sent to MORU laboratory in Bangkok for DNA extraction and analysis by GENRE Mekong (O. Miotto, Mahidol Oxford Research Unit & Sanger Institute, UK) or MIVS-ACT (M. Imwong, Faculty of Tropical Medicine, Mahidol University, Thailand and Mahidol Oxford Research Unit) projects.

The follow-up survey 12 months after MSAT will follow the same procedures. The samples will be transported to SMRU laboratories and stored at -80°C until processed for reference malaria detection by Quansys ELISA.

7.8. Discontinuation/Withdrawal of Participants from Study

The follow-up and study outcomes are defined at the village-level and apply to village populations rather than individuals. An eligible village will be excluded from the study if:

- the MSAT intervention can't be conducted (refused by the community, impossible to access)
- the population size changes by ≥50% between MSAT and follow-up survey.

Each participant has the right to withdraw from the study at any time.

In this study, systematic individual follow-up will occur during the MSAT intervention period and after the MSAT intervention to monitor demographics and clinical case incidence.

During the MSAT intervention individual follow-up will be conducted to ensure that a complete treatment is taken by all individuals found infected with *P. falciparum*. In the light of the threats of artemisinin-resistance, it will however be necessary to ensure that all falciparum-positive individuals are treated and that all individuals initiating a treatment take a complete curative course. Counselling will be provided to participants wishing to discontinue their participation. The counsellors will be members of the CE team with an extensive experience of interventions requiring the treatment of asymptomatic participants (filariasis and/or malaria mass drug administration campaigns in the region). In case the participant refuses to continue his/her antimalarial treatment course outside of the study, he/she will be offered to receive standard treatment for falciparum clinical malaria (AL+sld PMQ) from the village MP, or referred to the nearest health facility. The reason for withdrawal will be recorded in the MSAT logbook.

Individuals or households moving in or out of the village will be assessed during the M12 follow-up. Newcomers will contribute to the village follow-up and be included in the prevalence survey if they match inclusion criteria.

7.9. Definition of End of Study

The end of the study will be 12 months after the MSAT campaign if the estimated prevalence measured by URDT in the field is <5%, with the upper limit of the confidence interval below 10%. If the estimated prevalence is ≥5%, a new round of MSAT will be triggered. The follow-up will be conducted until 12 months after the last MSAT or 36 months after the first MSAT.

The threshold of 5% URDT prevalence corresponds to a 7.5% prevalence by uPCR, which was found to be indicating the presence of a substantial reservoir previously.

The MP will remain to continue diagnosis and treatment of fever cases as per the general METF procedures.

8. INTERVENTIONS / INVESTIGATIONS

For all participants in the two groups, investigation will consist in 1 URDT (Malaria Ag P.f ultrasensitive, SD/Alere, Republic of Korea).

For *P. falciparum* URDT positive individuals in the two groups:

- collection of 3x1cm dried blood spots on filter paper
- administration of a supervised antimalarial treatment course to individuals for which a P. falciparum will have been detected by URDT.

Specifically in **group 1**, a population list will be collected in each village prior to MSAT campaign and all participants will undergo:

- collection of a 50µL-aliquot of capillary blood for each participant to the screening during the MSAT intervention.
- 1 URDT + collection of a 50µL-aliquot of capillary blood for each participant to the follow-up survey at M12.

A safe, recommended treatment of *P. falciparum* malaria will be administered to URDT positive individuals based on participant's characteristics:

- The standard regimen for participants without known antimalarial allergy, not pregnant and not breastfeeding, will be a 3-day supervised weight-adjusted DP course and a single low dose PMQ. The single low dose PMQ will be administered on the first day.
- Pregnant women and in their 2nd or 3rd trimester, and breastfeeding mothers, will receive a DP course but no PMQ.
- Pregnant women in their first trimester will receive an oral course of quinine+clindamycin (7 days).
- Individuals with known drug allergy to piperaquine will be treated with AL (+/- sld PMQ as per their pregnancy/breastfeeding status)
- Specific/complex cases will be assessed by a medic and referred to a health facility for treatment if necessary.

The treatment will be directly observed by the MSAT team.

9. STATISTICS AND ANALYSIS

9.1. Description of Statistical Methods

Coverage of intervention: the coverage of the intervention will be defined as the proportion of individuals screened by URDT divided by the total number of individuals present in the village during the next

Before/after MSAT yearly cumulative incidence and 95% Poisson confidence intervals will be calculated using:

- Numerator: the total number of *P. falciparum* cases reported during the weeks with an MP report.
- Denominator: the village population size measured during the MSAT * number of weeks with an MP report during the year (number of weeks of MP activity).

Analysis including the entire follow-up available for malaria posts will be conducted in order to provide adjusted estimates on the

9.2. The Number of Participants (group 1 only)

The sample size can be calculated for group 1 only, since the baseline cumulative incidence in group 2 will depend on surveillance outcomes, and often correspond to responses to a single case.

9.2.1. Sample size for stepped-wedge comparison of incidence decrease following MSAT

MSAT will be implemented in 60 villages with high 1-year cumulative incidence of *P. falciparum* clinical cases in 6 waves of 10 villages (1 wave per semester). Assuming a baseline cumulative incidence of 250 cases/1000/year, a population of 190 inhabitants per village with a 0.49 coefficient of variation in cluster size and an intra-cluster correlation coefficient of 0.12, the expected detectable difference between villages with and without intervention is <25 cases/1000/year for alpha=0.01 & beta=0.9.

9.2.2. Sample size for prevalence comparison

9.2.2.1. Sample size for prevalence decrease evaluation at village-level

For each village, the sample size required for M12 survey will be calculated to measure a 90% prevalence decrease from baseline prevalence by reference method, with a 95%CI width of 200% of expected M12 prevalence if its value is expected <2% and of 100% of expected M12 prevalence if its value is expected>2%. This should result in a sample size of 60 to 120 samples per village, depending on village size.

9.2.2.2. Number of villages required for prevalence comparison

We assume an average *P. falciparum* prevalence of 16% in group 1 villages using reference assay. Before/after comparison at village level will use samples from the same village but not necessarily the same individuals (correlation between paired observations set to 0). The sample size was calculated assuming 60 samples per cluster (the minimal number of samples collected at M12), an intra-cluster correlation coefficient of 0.15, the sample size required to identify a 50% decrease 12 months after intervention compared to baseline is 43 clusters for alpha=0.05 and 1-beta=0.8.

9.2.3. Sample size for retrospective incidence comparison between MSAT and MDA

If comparing 50 MSAT villages and 50 MDA, we will be able to differentiate MSAT from MDA efficacy if MSAT achieves around 50% of MDA impact on incidence or less.

The average village size was 190 inhabitants (coefficient of size variation=0.64) with an intra-cluster correlation coefficient of 0.12 (estimated using incidence series) and MDA was already completed in 60 villages. The 10 villages in which MDA was conducted based on prevalence values that will be the furthest

from what will be measured during the MSAT campaign will be excluded. Villages receiving MSAT after MDA will be excluded from MSAT group. With a comparison of 50 MSAT, it will be possible to detect a difference in incidence rate of 50/1000/year between the two groups (with alpha=5% and power=80%). This would correspond to a reduction of incidence by only 40% after MSAT (70 cases/1000/year after compared to 125 before). With MDA, 80% incidence reduction was achieved: the average cumulative incidence during the first year after MDA was 20 case/1000/year, compared to 125 before.

9.3. Analysis of Outcome Measures

Primary outcome: stepped-wedge comparison of incidence.

The incidence comparison will be conducted over a 2-year series of weekly incidence data, corresponding to 12 months before and 12 months after MSAT intervention. A generalized additive multilevel mixed model will be used to adjust for location, season, and temporal trend due to MP activity using uni- (seasonality, duration of MP activity in the village) or bivariate (latitude and longitude) splines, including random intercept and slopes at village level. MSAT intervention will be included in the model as a 0-1 variable. The incidence rate ratio obtain for this variable will quantify the average decrease in incidence after MSAT campaign compared to before.

Secondary outcome: before-after comparison of prevalence

For each village, the fractional change in prevalence before MSAT and 12 months after will be calculated and compared to 0% change. This change will be compared to the fractional change observed after MDA.

10. DATA MANAGEMENT

10.1. Access to Data

Direct access will be granted to authorised representatives from the Shoklo Malaria Research Unit/Mahidol Oxford Research Unit and any host institution for monitoring and/or audit of the study to ensure compliance with regulations.

10.2. Data Handling and Record Keeping

All data collected specifically for the study (MSAT logbook, AE forms and where appropriate, follow-up survey logbook, demographic follow-up and individual falciparum clinical case data) will be recorded on paper forms in the field and entered in a secure Access database managed by SMRU data management team.

Laboratory results will be merged to this database using patient unique identifier which will be used to label the samples.

Analysis will be conducted on anonymized data.

11. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with relevant regulations and standard operating procedures.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

12.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

12.3. Approvals

The protocol, informed consent form, and participant information sheet will be submitted to the Ethics Review Committee on Medical Research Involving Human Subjects from the Republic of the Union of Myanmar, Ministry of Health and Sports, Department of Medical Research (Lower Myanmar), OXTREC for written approval. Furthermore, the protocol, informed consent form, and participant information sheet will be reviewed by community-based committees assembling members of the communities in which the study will be performed: the Karen Department of Health and Welfare and the Tak Community Advisory Board [8].

The Investigator will submit and, where necessary, obtain approval from the above parties for all amendments to the original approved documents.

12.4. Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all study documents and any electronic database, with the exception of the MSAT logbook, where participant name will be added to allow identification. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

12.5. Expenses and Benefits

No payments will be given to participants for their participation in the study.

12.6. Reporting

The CI shall submit an Annual Progress Report to OxTREC on the anniversary of the date of approval of the study. In addition, the CI shall submit an End of Study Report to OxTREC within 12 months of completion of the study.

13. FINANCE AND INSURANCE

13.1. Funding

This project is funded by a grant from the Bill & Melinda Gates Foundation to the Shoklo Malaria Research Unit, via Oxford University and the Mahidol Oxford Research Unit.

13.2. Insurance

The project is covered under the Oxford University sponsorship.

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

14. PUBLICATION POLICY

The results of this study will be published in peer-reviewed journal, following the standard policy of the Mahidol-Oxford University Research Unit.

The results will be communicated to the communities, by the Community Engagement team of the Malaria Elimination Task Force.

15. REFERENCES

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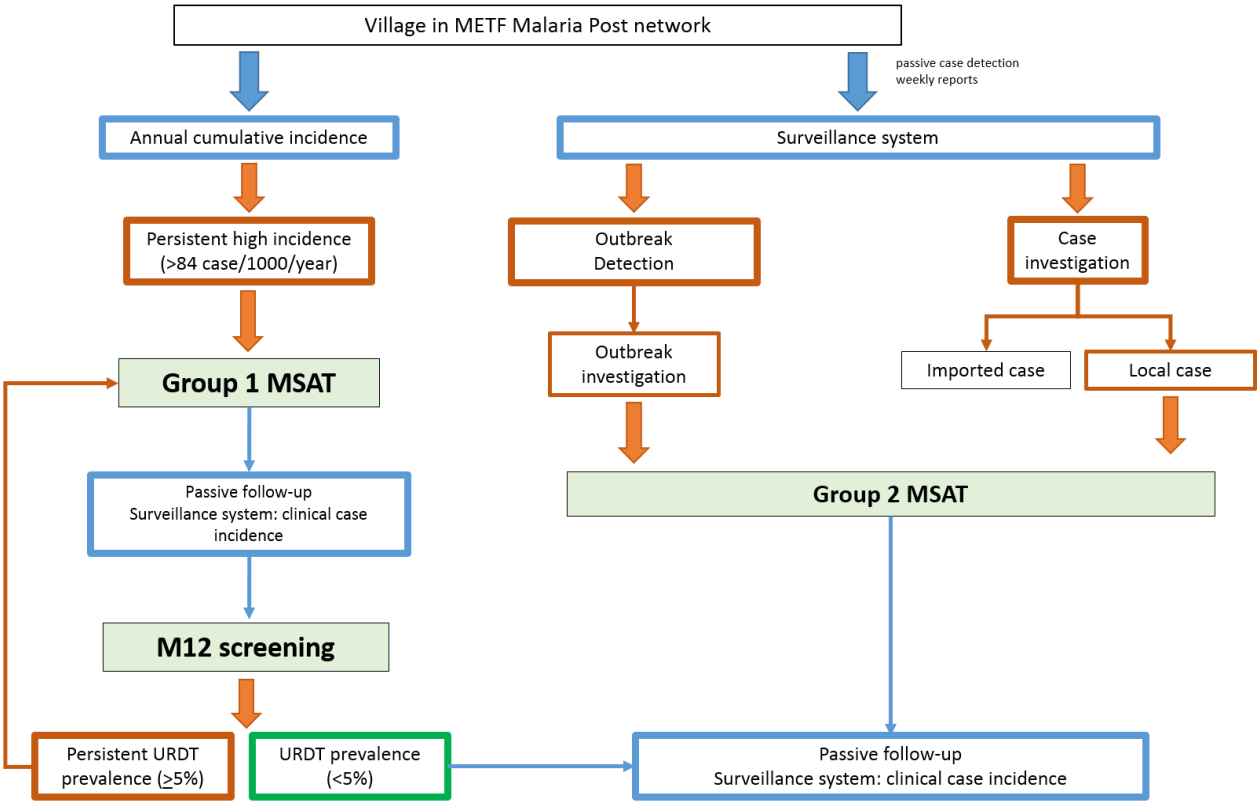
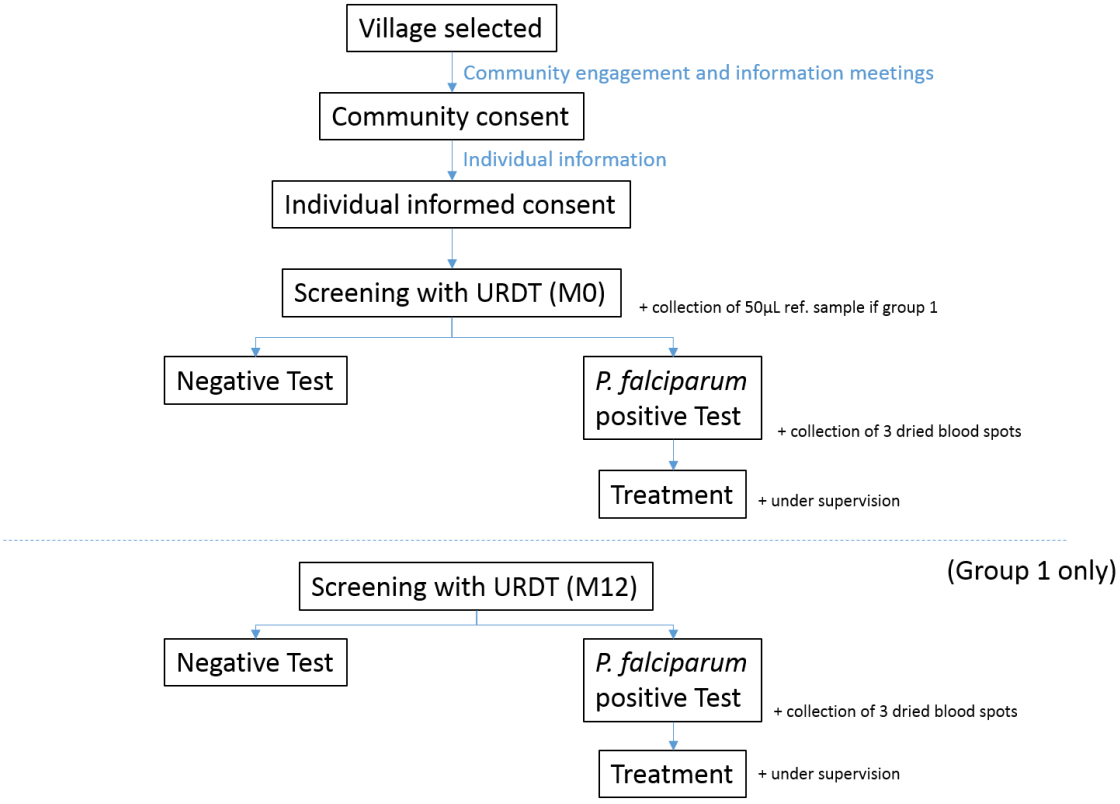
16. APPENDIX A: STUDY FLOW CHART

– by village



- by participant



17. APPENDIX B: SCHEDULE OF STUDY PROCEDURES

Procedures	**Visits (insert visit numbers as appropriate)**					
	Day 0					
Informed consent	Day 0					
Demographics	Day 0					
Malaria history over previous 3 weeks	Day 0					
Temperature	Day 0					
Falciparum URDT (+50µL sample collection in group 1)	Day 0					
Falciparum positive participant						
Clinical examination	Day 0					
Treatment	Day 0	Day 1	Day 2	*		
Passive AE follow-up (study team/MPW)	Day 0	Day 1	Day 2	Day 3	Day 4	
All participants						
Passive incidence data collection	Day 0				Daily	Day 365
Group 1 villages						
Demographic data collection	Day 0					Day 365
M12 follow-up survey in group 1: URDT+50µL sample collection						Day 365
*a longer follow-up may be required for patients receiving specific regimen other than standard MDA treatment (DP3+sldPMQ) or standard first-line treatment for clinical cases (AL3+sldPMQ).						

18. APPENDIX C: AMENDMENT HISTORY

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

List details of all protocol amendments here whenever a new version of the protocol is produced. This is not necessary prior to initial Ethics Committee submission.

19. APPENDIX D: UNPUBLISHED DATA ON URDT PERFORMANCE

This data was presented as a poster at the ASTMH Conference in November 2017.

Table 1: Comparison of the detection methods to uPCR

		uPCR result: Pf DNA+		Properties (%)		
		Neg	PF			95%CI
Lab uRDT result	**Neg**	**1469**	**91**	**Specificity (Sp)=**	**98.9**	**98.3-99.4**
	PF	**16**	**115**	**Sensitivity (Se)=**	**55.8**	**48.8-62.7**
Lab RDT result	Neg	1483	143	Sp=	99.9	99.5-100.0
	PF	2	63	Se=	30.6	24.4-37.7
Microscopy result	Neg	1483	148	Sp=	99.9	99.5-100.0
	PF	2	58	Se=	28.2	22.1-34.8
Field RDT result	Neg	1477	163	Sp=	99.9	99.5-100.0
	PF	2	43	Se=	20.9	15.5-27.1
Field uRDT result	**Neg**	**1413**	**122**	**Sp=**	**99.3**	**98.7-99.7**
	PF	**10**	**80**	**Se=**	**39.6**	**32.8-46.7**
Quansys ELISA	Neg	1427	35	Sp=	96.1	94.98-97.0
	PF	58*	171	Se=	83.0	77.2-87.9

* Plasmodium DNA found in 26/58 (45%) in the 1st step of uPCR, but PF was not characterized by the 2nd step.

Table 2: Reference test results in 16 uRDT positive, uPCR PF negative samples

Interpretation of combined HRP2- and parasite-reference tests

- Probable falciparum (or mixed) infections (Plasmodium DNA + PfHRP2).
- Insufficient evidence of falciparum infection (PfHRP2 without Plasmodium DNA or non-falciparum DNA without PfHRP2).
- No evidence of malaria infection (no DNA and no HRP2).

uPCR	Quansys HRP2		
	Neg	PF	Total
Negative	3	3	6
P. vivax	2	1	3
Plasmodium spp.	1	6	7
Total	6	10	16

Figure 1: Parasite densities detected by each method

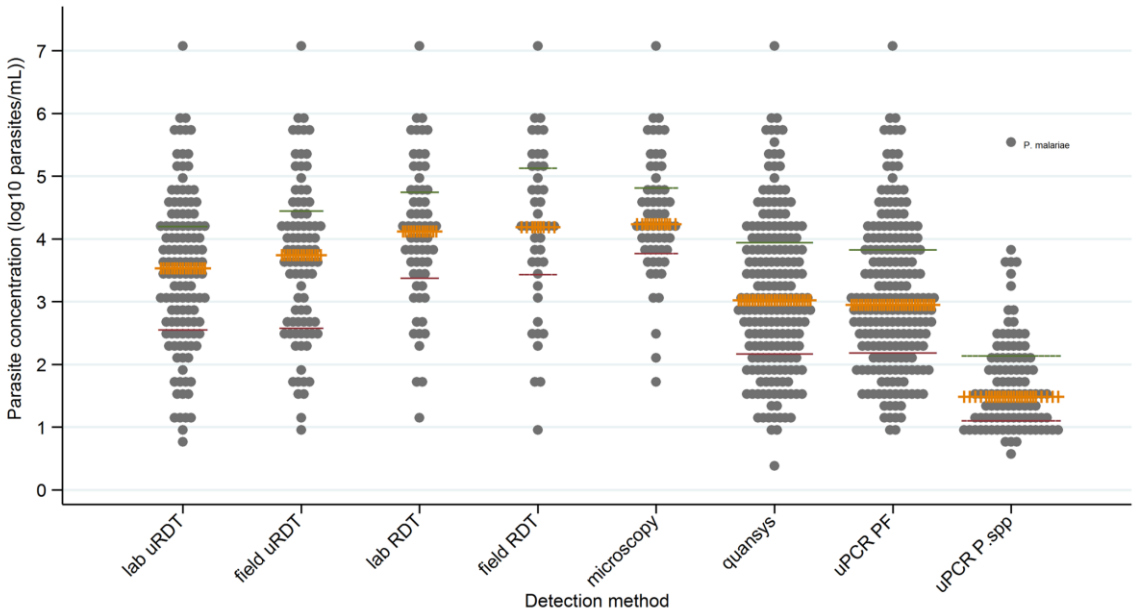


Table 3: Detection of asymptomatic PF infections according to parasite density.

Number of infections detected by method (for lab uRDT and RDT: corresponding % of uPCR infections detected).

	Parasitemia category (parasites/mL)						
Detected by:	$<10^2$	10^2-10^3	10^3-10^4	10^4-10^5	10^5-10^6	$>10^6$	Total
uPCR	41	67	52	32	13	1	206
Lab uRDT	5 (12%)	30 (45%)	38 (73%)	28 (88%)	13 (100%)	1 (100%)	115 (56%)
Lab RDT	2 (5%)	6 (9%)	22 (42%)	19 (59%)	13 (100%)	1 (100%)	63 (31%)