Study Title: Developing a methodology to assess 8-aminoquinoline associated haemolytic risk in females heterozygous for G6PD in endemic populations

Short Title: Assessing a risk model for G6PD deficiency

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Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, Data Safety Monitoring Board, host institutions, regulatory authorities, and members of the relevant ethics committees.

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1. LIST OF ABBREVIATIONS

AE	adverse event
AR	adverse reaction
CBC	complete blood count
CQ	chloroquine
CRF	case report form
EDTA	ethylenediaminetetraacetic acid
FST	fluorescent spot test
G6PD	glucose-6-phosphate dehydrogenase
GCP	good clinical practice
HCG	human chorionic gonadotropin
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	independent ethics committee
IRB	institutional review board
OxTREC	Oxford Tropical Research Ethics Committee
PI	principal investigator
PID	patient identification
PMQ	primaquine
PK	pharmacokinetic
RBCs	red blood cells
SAE	serious adverse event
SID	subject identification
SMRU	Shoklo Malaria Research Unit
SOP	standard operating procedure
WBC	white blood cell
WHO	World Health Organization
WIRB	Western Institutional Review Board

2. STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the International Conference on Harmonisation (ICH) E6 and the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator (PI): François Nosten

Signed: _____ Date: _____

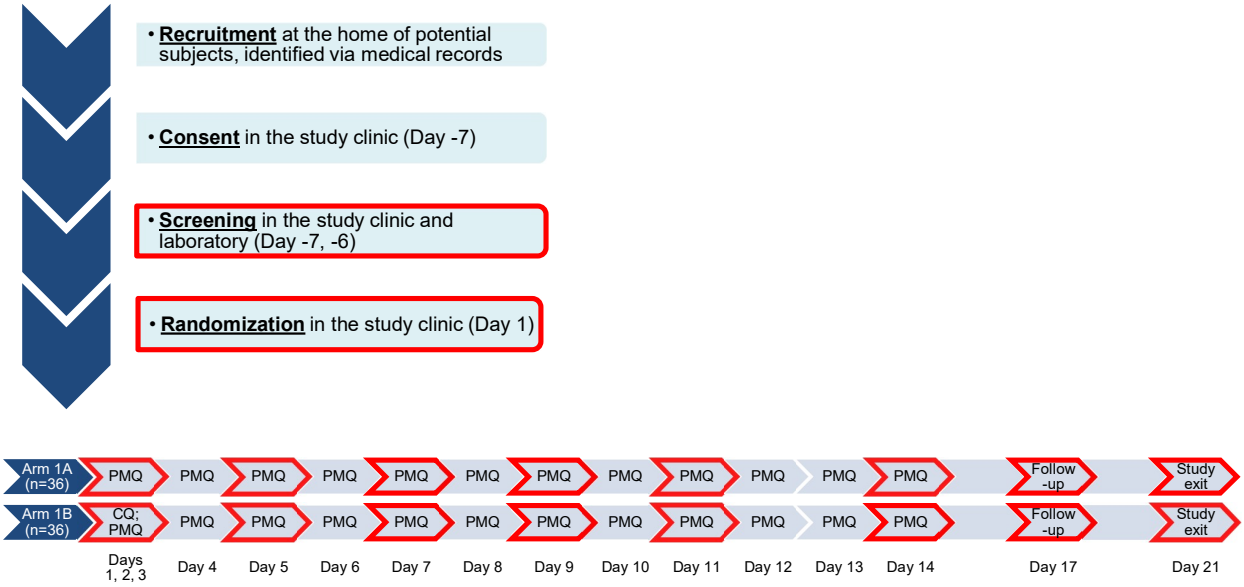
3. PROTOCOL SUMMARY

Study Title	Developing a methodology to assess 8-aminoquinoline associated haemolytic risk in females heterozygous for G6PD in endemic populations
Background	Open label, randomized trial with 72 total participants assigned to one of two treatment arms. Each arm will have 36 participants comprised of 12 males hemizygous for wildtype G6PD, 12 females homozygous for wildtype G6PD, and 12 females heterozygous for G6PD with a normal FST (G6PD genotype abnormal with G6PD activity ≥40% and ≤80% of normal). Arm 1a will receive primaquine for 14 days, and Arm 1b will receive chloroquine for 3 days and concomitant primaquine for 14 days. All participants will be healthy volunteers without severe G6PD deficiency who will be followed for two weeks after completing their study drug dosing. Pregnant women and those breastfeeding will be excluded. Venous blood samples will be taken at regular intervals for haematologic measures, G6PD quantification, and drug level assays.
Objectives	*Primary Objective:* To understand the risk of haemolysis in healthy G6PD heterozygous females receiving (i) primaquine for 14 days at 0.5 mg/Kg and (ii) chloroquine for 3 days and primaquine for 14 days at 0.5 mg/Kg. *Secondary Objectives:* • To determine if additional haematologic and biochemistry measures are critical inputs or determinants for drug-related haemolytic risk models. • To associate primaquine and chloroquine drug levels at the time of sampling with patient haematological and G6PD profiles. • To measure frequency of adverse events in women heterozygous for G6PD treated with current standard of care for *P. vivax* malaria.
Endpoints	*Primary Endpoints:* • The absolute haemoglobin reduction from baseline on exposure to primaquine for *P.vivax* treatment over treatment course. • Absolute reduction in haemoglobin-related change in intracellular G6PD concentration profiles over treatment course. *Secondary Endpoints:* • Incorporation of haematological and biochemical markers such as complete blood count, reticulocyte count, liver and renal function tests, urobilinogen level, and methemoglobin level into risk of haemolysis models. • Association of chloroquine and primaquine drug levels at the time of sampling and dextromethorphan assay results at baseline for haematological and G6PD profiles. • Frequency of adverse events in women heterozygous for G6PD.
Population	24 males hemizygous for wildtype G6PD, 24 females homozygous for wildtype G6PD, and 24 females heterozygous for G6PD deficiency. All healthy volunteers with ≥40% normal G6PD activity (defined as 3.00 IU/gHb on the quantitative G6PD spectrophotometric assay) recruited from the Thai-Myanmar border.

Phase	Phase I
Number of Sites	3 clinical sites along the Thai-Myanmar border through the Shoklo Malaria Research Unit (SMRU) in Mae Sot, Thailand
Description of Study Agents:	• Primaquine (oral, 15 mg tablets): 0.5 mg/kg daily for 14 days • Chloroquine phosphate (oral, 250 mg tablets): 10, 10, 5 mg/kg over 3 days
Study Duration	9 months (estimated). December 1, 2017 – August 31, 2018
Participant Duration of follow up	21 days after randomization (28 days from first day of screening)

4. SCHEMATIC OF STUDY DESIGN

Figure 1. Study Visit Flow Chart



Note: Visits outlined in red include a blood draw

5. KEY ROLES

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6. INTRODUCTION

6.1 Background Information

Infection with *Plasmodium vivax* accounts for 50-80% of all malaria cases in parts of South America, Southeast Asia and Oceania.(1) Radical cure of *P. vivax* infection involves both the clearing of blood parasitemia and the prevention of future relapse. *P. vivax* hypnozoites remain dormant in the liver and can result in frequent recurrence as the dormant hypnozoites are not affected by anti-malarial drugs that only target parasitemia in the blood. The only drugs that can clear the hypnozoites of *P. vivax* are 8-aminoquinolines, which include primaquine, bulaquine, quinocide, and tafenoquine. Currently, primaquine is the only available member of this drug class, although tafenoquine is in the late stages of clinical development. (2-4) Wide spread availability and prescription of the 8-aminoquinoline malaria drugs primaquine and (in the future) tafenoquine is critical in malaria elimination efforts in areas where *P. vivax* is prominent. However, individuals with a genetic glucose-6-phosphate dehydrogenase (G6PD) deficiency are at increased risk to develop haemolytic anemia following exposure to 8-aminoquinolines (5-7). Providing the best clinical treatment options for *Plasmodium vivax* malaria remains an enormous challenge. This is primarily due to (a) a knowledge gap on haemolytic risk of patients, particularly females, on exposure to primaquine (and 8-aminoquinolines in general) and (b) the absence of diagnostic tests that allow rapid assessment for risk at the point-of-care.

People with severe G6PD deficiency should not be prescribed 8-aminoquinoline-based drugs, or if so, under different dosage regimens that spread out exposure over many weeks. The severity of haemolysis is dependent on G6PD variant, drug dosing, patient status, and disease factors. The X-linked G6PD gene is perhaps the most polymorphic gene in the human genome (8). Possibly due to selective pressure, G6PD gene mutations resulting in reduced erythrocytic G6PD activity levels are typically most prevalent in malaria endemic regions. Thailand and South East Asia are no exceptions with G6PD deficiency reaching over 15% prevalence in certain populations (5-8). It is therefore critical to diagnose whether a patient is G6PD deficient prior to administering primaquine. Due to the long half-life of tafenoquine this may be even more critical for tafenoquine, and may even require identification of heterozygous women and their genotype.

Currently, the G6PD status of a patient is most often defined by the patient's G6PD phenotype, or the G6PD activity determined from blood lysate. While the gold standard is a quantitative spectrophotometric test, the most commonly performed test is a qualitative fluorescent spot test (FST). The former can clearly identify subjects with all ranges of activities (including intermediate levels associated with heterozygous genotypes which may also be at risk of severe haemolysis) while the latter can only discriminate gross deficiencies from all the other phenotypes. The World Health Organization (WHO) bases its recommendations for primaquine treatment on the threshold of detection for the qualitative test, so only individuals with 30% or greater of normal G6PD activity should be prescribed primaquine.(9) The WHO considers 30-80% of normal G6PD activity to be "intermediate activity" and appropriate for primaquine dosing with counselling on how to recognize the signs and symptoms of haemolytic anemia. Genotyping and cytometry based assays (which observe G6PD activity within individual cells) are the only available techniques able to unambiguously identify heterozygous females.(10-13) Nonetheless genotyping alone cannot predict the actual phenotype in this class of subjects and would not be a suitable indicator of the haemolytic risk associated to drug treatment.

For more than 60 years, chloroquine has been the first line treatment against *Plasmodium vivax* malaria. It is a 4-aminoquinoline agent with cidal activity against schizonts.(14) Plasma concentrations of chloroquine remain above the minimum effective concentration for at least 28 days and therefore, can delay relapse.(15) Side effects are mild when chloroquine is taken for a short duration. These include dizziness, headache, nausea, vomiting, difficulty with visual accommodation, itching, and skin rash. When given for prolonged periods or intravenously, chloroquine can cause hypotension and retinal, otic, cardiac or CNS toxicity. Chloroquine has also been linked to increasing oxidative stress on red blood cells (RBCs).

Primaquine is known to demonstrate synergistic activity with chloroquine against *Plasmodium falciparum* gametocytes.(16) Pukrittayakamee et al.(17), suggests a possibility of synergy of primaquine with chloroquine against *Plasmodium vivax* gametocytes, but more evidence is needed. Additionally, synergy of primaquine with chloroquine and quinine against *P. vivax* hypnozoites has been described, but this has not been studied recently.(18) Concomitant prescription of chloroquine and primaquine is the standard treatment regimen in Thailand.

Primaquine is recommended by the WHO as a 14-day course for the radical treatment of *Plasmodium vivax* malaria. The optimum dose is still debated and WHO recommendations range from 0.25 mg/kg/day to 0.5 mg/kg/day, depending on the geographic location.(9) Despite these recommendations primaquine is not widely used because of concerns over toxicity and continued uncertainty over efficacy.(19) Abdominal pain is a dose dependent side effect commonly caused by primaquine. This is typically alleviated by food even when high doses of primaquine are given (20), but the pharmacokinetics of primaquine have only been studied in fasting subjects. Methemoglobinemia may occur with usual doses of primaquine. Symptoms develop when methemoglobin levels reach 10% of the normal level of haemoglobin. Mean levels of methemoglobin with primaquine therapy have been documented to range between 6% and 11%, but symptomatic disease has only been noted in patients with inborn deficiency of methemoglobin reductase.(21) Importantly, primaquine can precipitate haemolysis in patients with G6PD deficiency. In areas where the burden of *Plasmodium vivax* is high, often G6PD screening is not available.

Apart from Oceania and parts of South America, the remainder of the *P.vivax* affected world has generally recommended a primaquine dose of 0.25mg/kg/day given for 14 days. This has generally been considered effective, although there have been relatively few studies. Primaquine tolerance or resistance in *P. vivax* remains an area of uncertainty and confusion.(22-26) High dose primaquine (420 mg total dose) alone and in combination with artesunate given over 7 days have shown efficacy.(27-28) Multiple countries in South America, including Peru and Brazil, have adopted P. *vivax* treatment guidelines to include 7 days of primaquine treatment at 0.5 mg/kg/day.(29-30) The efficacy of high dose primaquine over 7 days in combination with chloroquine has yet to be confirmed, although preliminary data suggests that a primaquine dose of 1.0 mg/Kg/day for 7 days is safe and effective for those without G6PD deficiency in Thailand.(31) The standard regimen in Thailand for treating P. *vivax* is chloroquine for 3 days along with primaquine dosing of 0.5 mg/Kg/day for 14 days. This is in accordance with WHO guidelines, which recommend s a total dose of 7 mg base/kg bw (0.5 mg/kg/day for 14 days) for tropical strains of P. *vivax*, known for frequent relapses, prevalent in East Asia and Oceania.(9)

The 2D6 isoform of the cytochrome cyp450 is required to metabolize primaquine into its active agent. CYP2D6 is also polymorphic with some mutations leading to slow metabolism of primaquine. Poor response to primaquine in terms of relapse is associated to slow metabolism of primaquine in people with slow metabolizing CYP2D6 alleles. (33-36) Primaquine metabolism by CYP2D6 also appears to impact the haemolytic toxicity of the drug.(37) Slow metabolizing CYP2D6 alleles can be identified either by identifying known mutations or phenotypically through the dextromethorphan assay described in the protocol.

The proposed research program seeks to address the challenges in providing appropriate *P. vivax* treatment through modelling the relationship between the individual red blood cell G6PD levels and risk of lysis, and the introduction of point-of-care diagnostic tests that reliably determine G6PD activity and haemoglobin concentration in patients to inform clinical management. A model for haemolytic risk based on detailed profiling of intracellular red blood cell G6PD activity levels, represents a totally innovative approach to investigate G6PD-associated risk for haemolysis, particularly in females. In this study, the haemolytic patterns in G6PD heterozygous females will be correlated to G6PD activity and primaquine drug levels. When outputs from this research are combined with novel G6PD diagnostic tests this will translate into clinical practice.

The outcomes of this study seek to develop a risk model for primaquine-associated haemolysis. This model could be used for many drugs beyond 8-aminoquinolines and has the potential to

improve patient care for *P. vivax* malaria by quantifying drug induced haemolytic response in patients who would already normally receive primaquine for radical cure.

6.2 Scientific Rationale

Exposure to 8-aminoquinoline anti-malarial drugs, such as primaquine or tafenoquine, that put oxidative stress on RBCs has been strongly associated with haemolytic risk—the rupture or destruction of RBCs—in individuals with a genetic G6PD deficiency. For radical cure of *Plasmodium vivax*, the standard treatment is to prescribe chloroquine and primaquine. Chloroquine has also been linked to an increase in oxidative stress on RBCs. It is generally accepted that males hemizygous for G6PD deficiency and females homozygous for G6PD deficiency should not be given high doses of 8-aminoquinolines. Qualitative tests for G6PD deficiency can reliably exclude these populations from treatment. Much less is understood about the risk associated with females heterozygous for G6PD deficiency, who have two distinct populations of RBCs: one with low G6PD activity and one with high G6PD activity. Quantitative tests for G6PD deficiency can identify patients with intermediate G6PD activity; however, these tests only provide a mean G6PD activity across the population of RBCs in a patient and are therefore not informative tools for understanding G6PD activity levels associated with risk of haemolysis. The relative proportion of each cell population can vary widely between heterozygous females, and the correlation of available quantitative tests to whole blood G6PD activity is not very precise. Here we are developing a model that can predict how at risk these females are to severe haemolysis when exposed to 3 days of chloroquine and 14 days of primaquine or 14 days of primaquine alone. This model is important to understand the risk profile of 8-aminoquinolines in this sub-population of females heterozygous for G6PD deficiency for the potential broadening of access to drugs for radical cure of *vivax* malaria.

7. OBJECTIVES

7.1 Study Objectives

The main focus of the proposal is to associate intracellular G6PD activity to likelihood of haemolysis. The aim of this study is to generate empirical data to inform G6PD deficiency and 8-aminoquinoline associated risk models for haemolysis, particularly in females heterozygous for G6PD deficiency with intermediate G6PD activity (approximately 1.8 to 5.0 IU/gHb at 30^{o}C).

7.1.1 Primary Objective

To understand the risk of haemolysis and intracellular G6PD activity in healthy G6PD heterozygous females receiving (i) primaquine for 14 days at 0.5 mg/Kg and (ii) chloroquine for 3 days concomitant with primaquine for 14 days at 0.5 mg/Kg.

7.1.2 Secondary Objectives

1. To determine if additional haematologic and biochemistry measures are critical inputs or determinants for drug-related haemolytic risk models.
2. To associate primaquine and chloroquine drug levels at the time of sampling with patient haematological and G6PD profiles.
3. To measure frequency of adverse events in women heterozygous for G6PD treated with current standard of care for *P. vivax* malaria.

7.2 Study Endpoints

7.2.1 Primary Endpoints

1. The absolute haemoglobin reduction from baseline on exposure to primaquine for *P.vivax* treatment over treatment course.
2. Absolute reduction in haemoglobin-related change in intracellular G6PD concentration profiles over treatment course.

7.2.2 Secondary Endpoints

1. Incorporation of haematological and biochemical markers such as complete blood count, reticulocyte count, liver and renal function tests, urobilinogen level, and methemoglobin level into risk of haemolysis models.
2. Association of chloroquine and primaquine drug levels at the time of sampling and dextromethorphan assay results at baseline for haematological and G6PD profiles.
3. Frequency of adverse events in women heterozygous for G6PD.

8. STUDY DESIGN

A total of 72 participants will be enrolled, consisting of 24 males hemizygous for wildtype G6PD, 24 females homozygous for wildtype G6PD, and 24 females heterozygous for G6PD deficiency. Participants will be equally distributed into 2 treatment groups: Arms 1a and 1b. Each arm will have 36 participants comprised of 12 males hemizygous for wildtype G6PD, 12 females heterozygous for wildtype G6PD, and 12 females heterozygous for G6PD deficiency (Table 1).

Table 1. Schematic of Study Design

Arm	Sub-group	Sample Size	Intervention
Arm 1A	Males hemizygous for wildtype G6PD	12	Primaquine
	Females homozygous for wildtype G6PD	12	
	Females heterozygous for G6PD deficiency	12	
Arm 1B	Males hemizygous for wildtype G6PD	12	Chloroquine + primaquine
	Females homozygous for wildtype G6PD	12	
	Females heterozygous for G6PD deficiency	12	

Participants will be all healthy volunteers who are not G6PD severely deficient (as confirmed by the quantitative G6PD spectrophotometric assay). One group (Arm 1a) will receive 0.5 mg/kg/day of primaquine for 14 days, and the second group (Arm 1b) will receive chloroquine for 3 days along with 0.5 mg/kg/day of primaquine for 14 days (Figure 1, Study Visit Flow Chart). After treatment is completed, study participants will be followed for 2 visits over the next 7 days. This is an open label, randomized trial and neither participants nor study staff will be blinded to treatment regimen allocation.

8.1 Study Site

This study will take place at Shoklo Malaria Research Unit (SMRU) in Mae Sot, Thailand. Mae Sot is located in western Thailand, along the Thai-Myanmar border. SMRU is a field site for the Mahidol University in Thailand and is part of the Mahidol-Oxford Research Unit (MORU). SMRU has three clinical sites (Wang Pha Clinic, Mawker Thai, and Moruchai Clinic), all are on the border near Mae Sot, and mainly serve a migrant population. Research procedures will be conducted at the haematology laboratory at SMRU in Mae Sot, Thailand; PCT Laboratory, Inc in Bangkok, Thailand; and at the Faculty of Medicine Universitas Indonesia (FKUI) in Jakarta, Indonesia. Study volunteers will be recruited from three SMRU clinical sites.

8.2 Study Period

Screening and recruitment will start as soon as approvals from the institutional review boards at the Western Institutional Review Board (WIRB), Oxford Tropical Research and Ethics Committee (OXTREC), and the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University are obtained. Study participant enrollment and data collection is expected to take up to 4 months. Laboratory sample analysis is expected to take an additional 2 months. Data analysis will take 3 months once sample processing is complete. The total study duration is expected to be 9 months.

The end of trial is the date of the last clinic or home visit of the last participant.

8.3 Study Population

The SMRU beneficiaries include refugees and other migrants, as well as permanent residents of the Mae Sot area. These individuals may have higher prevalence of health outcomes associated with poverty and transience such as anemia, malaria, TB, and HIV. The SMRU clinics are intended to treat the specialized needs of this population.

This study is intended to enroll only healthy subjects, so potential participants will be screened to meet all eligibility criteria.

8.3.1 Inclusion Criteria

All participants:

- Previous G6PD test at SMRU clinic with one of following results:
    - G6PD homozygous wildtype females (G6PD genotype normal)
    - G6PD heterozygous females with a normal FST (G6PD genotype abnormal with G6PD activity ≥40% and ≤80% of normal)
    - G6PD hemizygous wildtype males (G6PD genotype normal)
- Willing to participate and sign informed consent form
- Willing to allow donated samples to be used in future research
- Aged ≥18 years
- Ability (in the investigators' opinion) and willing to comply with all study requirements

8.3.2 Exclusion Criteria

All participants:

- Malaria or other illness
- Recent history (within 20 days) of anti-malarial treatment
- History of allergy or adverse reaction to chloroquine or primaquine
- Blood transfusion in the past 3 months
- G6PD activity less than 40% normal activity or 3.00 IU/gHb by the quantitative G6PD spectrophotometric assay
- Haemoglobin ≤10 g/dL
- Presence of any condition which in the judgment of the investigator would place the subject at undue risk or interfere with the results of the study

Female participants only:

- Pregnancy at the time of screening
- Breastfeeding

8.4 Sample Size

A total of 72 volunteers will be randomized in this study. Each arm (Arm 1a and 1b) will each have 36 participants, each with 12 males hemizygous for wildtype G6PD, 12 females homozygous for wildtype G6PD, and 12 females heterozygous for G6PD deficiency. We estimate that at least a third of those approached through recruitment will not be interested in participation and that up to 20% of those who consent for screening may be ineligible, so an estimated 135 individuals will be recruited for this study to yield 72 participants eligible for randomization in the study.

This study is a pilot study and the analyses of haemolysis will be mainly descriptive. As such, it is not powered to provide definitive evidence of treatment-induced effects; rather it is based on smaller numbers of participants and will only indicate trends, feasibility, or size of effect and variability of measurements to inform the development of a risk model.

The change in haemoglobin and haematocrit levels over time after exposure to primaquine will be used as measures of treatment effect in women heterozygous for G6PD deficiency. We assume

initial haemoglobin and haematocrit levels in the study population are normally distributed, mirroring a general population sample from the Thai-Myanmar border region, with a mean haematocrit among adult males of 38.1% (standard deviation [SD] 3.5) and a mean among adult women of 35.6% (SD 2.9).(32) With an alpha of 0.05 for a one-sided t-test comparing the mean haemoglobin levels between treatment arms, a sample size of 12 per study group in each treatment arm will have >80% power to detect a difference in means greater than 10% after treatment between women heterozygous for G6PD and women with wildtype G6PD in each treatment arm. This calculation accounts for a 5% loss to follow-up rate.

Table 2 shows sample size implications for safety in terms of the ability to detect haemoglobin levels dropping >3.0 g/dL (considered an adverse event), i.e., the probabilities of observing 0, 1+ (1 or more), 2+ or 3+ such events among a group of n=12 for a range of possible true event rates.

Table 2. Probabilities of Detecting Events for n=12

Event rate (Absolute haemoglobin drop >3.0 g/dL)	Probability (0/12 Events)	Probability (1+/12 events)	Probability (2+/12 events)	Probability (3+/12 events)
0.010	0.89	0.11	0.006	0.0002
0.025	0.74	0.26	0.03	0.003
0.035	0.65	0.34	0.06	0.007
0.050	0.54	0.46	0.12	0.02
0.100	0.28	0.72	0.34	0.11
0.150	0.14	0.86	0.56	0.26
0.200	0.07	0.93	0.73	0.44
0.250	0.03	0.97	0.84	0.61

9. STUDY PROCEDURES

Table 3. Study Procedures

Study Procedure		Visit Day															
		Day -7	Day -6	Day 1	Days 2, 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Days 12, 13	Day 14	Day 17	Day 21
Arm 1A																	
Clinic	Enrolment consent	√															
	Randomization			√													
	Primaquine (PMQ) dose*			√	√	√	√	√	√	√	√	√	√	√	√		
	Blood draw	√		√**	√		√		√		√		√		√	√	√
	Dextromethorphan urine assay	√	√														
	G6PD florescent spot test	50 µl															
	Pregnancy test	√		√					√		√				√		√
	Malaria smear***	√		√	√		√		√		√		√		√	√	√
	Haematocrit***	√		√	√		√		√		√		√		√	√	√
	Methemoglobin level			√	√		√		√		√		√		√	√	√
	Urine urobilinogen			√	√		√		√		√		√		√	√	√
	Study exit																√
Laboratory	PMQ PK sample			1.5 ml	1.5 ml		1.5 ml		1.5 ml		1.5 ml		1.5 ml		1.5 ml	1.5 ml	1.5 ml
	CBC	2 ml		2 ml	2 ml		2 ml		2 ml		2 ml		2 ml		2ml	2 ml	2 ml
	Quantitative G6PD																
	Reticulocyte count																
	G6PD flow cytometry																
	Buffy coat saved for sequencing																
	Liver function tests			5 ml	5 ml		5 ml		5 ml		5 ml		5 ml		5 ml	5 ml	5 ml
	Kidney function tests																
Arm 1B																	
Clinic	Enrolment consent	√															
	Randomization			√													
	Chloroquine (CQ) dose*			√	√												
	PMQ dose*			√	√	√	√	√	√	√	√	√	√	√	√		
	Blood draw	√		√**	√		√		√		√		√		√	√	√
	Dextromethorphan urine assay	√	√														
	G6PD florescent spot test	50 µl															
	Pregnancy test	√		√					√		√				√		√
	Malaria smear***	√		√	√		√		√		√		√		√	√	√
	Haematocrit***	√		√	√		√		√		√		√		√	√	√
	Methemoglobin level			√	√		√		√		√		√		√	√	√
	Urine urobilinogen			√	√		√		√		√		√		√	√	√
	Study exit																√
Laboratory	CQ PK sample			1.5 ml	1.5 ml		1.5 ml		1.5 ml		1.5 ml		1.5 ml		1.5 ml	1.5 ml	1.5 ml
	PMQ PK sample			1.5 ml	1.5 ml		1.5 ml		1.5 ml		1.5 ml		1.5 ml		1.5 ml	1.5 ml	1.5 ml
	CBC	2 ml		2 ml	2 ml		2 ml		2 ml		2 ml		2 ml		2 ml	2 ml	2 ml
	Quantitative G6PD																
	Reticulocyte count																
	G6PD flow cytometry																

	Buffy coat saved for sequencing																
	Liver function tests			5 ml	5 ml		5 ml		5 ml		5 ml		5 ml		5 ml	5 ml	5 ml
	Kidney function tests																

NOTE: PK = pharmacokinetics, CBC = complete blood count
*Study drug dosing may be observed by study staff in the clinic or in the home
**Blood draws on day 1 include one blood draw before initial treatment and one blood draw after the treatment
***Laboratory test will be assessed from blood taken for CBC

9.1 Recruitment

Potential participants will be recruited from SMRU based on the G6PD status noted in their medical records. In SMRU, any patient presenting at the clinic with malaria receives a G6PD test prior to treatment. The G6PD status is recorded in the patient's clinical record and they are given a card to keep for their own records. Test results are entered into an electronic database that is shared among all SMRU clinics and is accessible by SMRU research and clinical staff. Prior to searching the database and contacting the patients for study screening, permission will be requested from the Director of SMRU, Professor François Nosten. For this study, we will search the database to identify up to 72 healthy patients with the following characteristics: 24 males hemizygous for wildtype G6PD, 24 women homozygous for wildtype G6PD, and 24 women heterozygous for G6PD deficiency. In some instances, patients will have already been asked for their permission that we contact them if there is a new study applicable to their G6PD status. For patients who haven't been previously given verbal consent, we will ask them if they are interested to be contacted about research. We will not contact any patients who have already declined to be contacted.

When approaching the potential participants to introduce them to the study, the staff member will explain to the volunteers that our electronic records contain their G6PD status, that we identified them by searching our database and that this was done for study purposes only. The local communities are already aware that SMRU conducts malaria research and this will be emphasized to the volunteers. We will also emphasize that access to their record is restricted and in this instance, we reviewed it specifically because of this study. Computer searches for specific patients is not routinely done. By using retrospective record review we seek to limit our initial recruitment to only healthy volunteers, although final determination of good health will be made at the time of screening enrollment.

Patients who meet the criteria for recruitment will be contacted at their home by a clinic staff member whose responsibilities include home visitation. The home visitor will explain the study, provide the patient with a Participant Information Sheet (Appendix A) and invite the patient to participate. If the patient expresses interest in participating in the study, she or he will be directed to visit within the following month the clinic where they usually receive care.

9.2 Screening and Enrolment

At the time that the potential participant visits the clinic after recruitment, he or she will be directed to the study area. Clinic staff assigned to informed consent duties will review the study details with the volunteer. If the potential participant remains interested in participating, patients will be screened to assess for inclusion and exclusion criteria that can be determined with a brief medical history. Any potential participant determined to be eligible will continue with the informed consent process for further screening and enrolment procedures. The volunteer will be given adequate time and opportunity to review the informed consent form and ask questions. Once the volunteer has agreed, the study staff proceed with the consent and enrollment process.

For additional details on informed consent, see Section 9 Ethical Considerations.

Following completion of informed consent process, the study staff (who are trained in phlebotomy) will draw approximately 7 ml of venous blood. The venous blood specimen will be taken using the standard venipuncture kit used at the SMRU clinics, and blood will be collected in the EDTA treated tube. The tube will be labelled with the patient's study and clinical ID numbers as this is routine

procedure at the SMRU clinics for all research specimens. The samples will be transported refrigerated (within 8 hours) to the central haematology lab of SMRU. These phlebotomy procedures will be followed for any study-related blood draw.

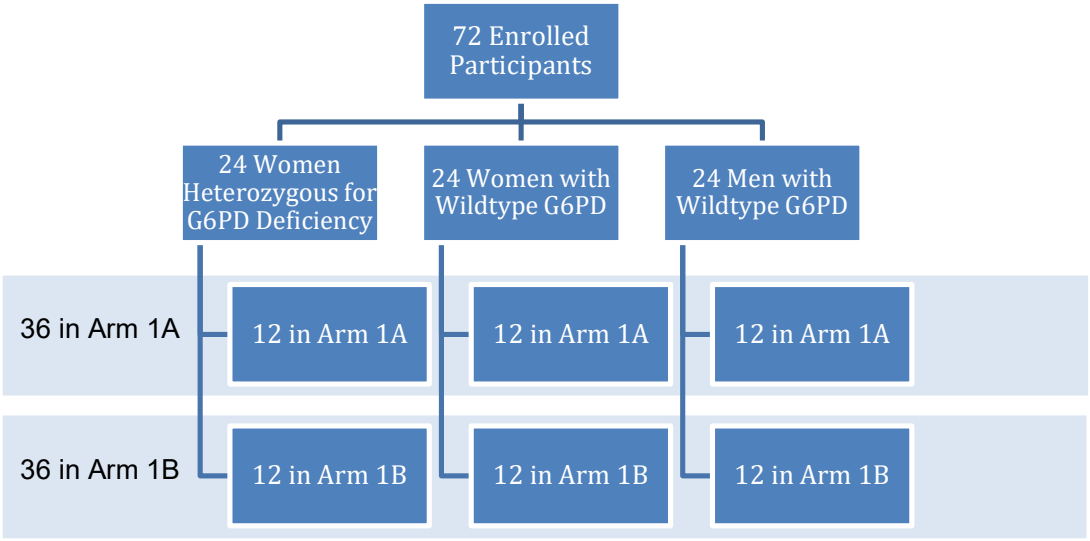
To conduct the dextromethorphan urine assay, participants will need to fast for 8 hours prior to the dose. Participants will be housed within the SMRU inpatient ward for 2 nights to ensure 8 hours fasting and pre and post dose urine collection. Prior to the start of fasting, participants will begin the pre-dose urine collection and fed one meal. Water is permitted during the 8 hour fasting period and vital signs will be monitored every 6 hours (as per routine inpatient care). If the participant does not tolerate fasting (i.e. low glucose or weakness) the fasting will be stopped and the patient will be withdrawn from the study. Following the 8 hour fasting period, dextromethorphan as a 30 mg dose will be administered to the participant. They will remain fasting for an additional 4 hours after the dose. During the following 24 hours all the urine samples will be collected for the detection of dextromethorphan and its metabolite in the urine. The analysis of dextromethorphan and its metabolite will be carried out at the end of the study. The participant will then be asked to return to the study clinic in 6 days' time for randomization and the initiation of study treatment.

If any laboratory test results determine that a volunteer is not eligible for the study, based on the inclusion and exclusion criteria, that individual will be informed and will not be asked to return to the study clinic for randomization and any other study procedures.

The results of CBC using venous blood will be included in the participant's clinic record. The results of the other tests will be kept in a separate research database unless clinically relevant, in which case they will also be recorded in the volunteer's clinical record and reported to the volunteer during their next visit to the clinic. At that time, the appropriate counseling for the given conditions will be provided. Health workers at the SMRU clinics have had training in counseling for medical conditions as part of prior SMRU research protocols. Additionally, SMRU is advised by a community advisory board on the most appropriate practices. For ineligible volunteers, any specimens will be discarded and the research record will be kept for administrative purposes, but the data will not be used. If the study subject withdraws after informed consent, the reason will be recorded in the study record.

9.3 Randomisation

Figure 2. Randomization Schematic



On day 1 (a week after informed consent was signed), participants who have met all the inclusion and exclusion criteria and return to the clinic will be randomized. Study participants will be distributed in blocks and assigned to each of the 2 treatment regimens Arm 1a and Arm 1b as

diagrammed in Figure 2 above. Arm 1a treatment regimen will be primaquine for 14 days to a total of 36 participants: 12 males hemizygous for wildtype G6PD, 12 females homozygous for wildtype G6PD, and 12 females heterozygous for G6PD deficiency. Arm 1b treatment regimen will be chloroquine for 3 days and primaquine for 14 days to a total of 36 participants: 12 males hemizgyous for wildtype G6PD, 12 females homozygous for wildtype G6PD, and 12 females heterozygous for G6PD deficiency.

A randomization list stratified by the 3 subject groups (males with wildtype G6PD, females with wildtype G6PD, and females heterozygous for G6PD deficiency) will be prepared prior to study start. Randomization envelopes will be prepared accordingly and will be distributed in sequence to participants according to their G6PD genotype and gender.

9.4 Direct Observation of Drug Administration

In order to ensure adherence to the prescribed drug regimens, all drug administration will be directly observed by study staff. Study staff will provide the participant with the appropriate dosing of the drug for that day and observe the participant taking the drug. For days in which a blood draw is scheduled (see Table 3), the drug administration will occur in the study clinic during the study visit. For days in which study drug is to be administered but no other study procedures are scheduled, direct observation of the study drug may be conducted in the participant's home or the study clinic.

The following procedures are to be performed for each direct observation of drug administration subsequent to the first dosing:

- Record adverse events as reported by participant or observed by study staff
- Record vital signs
- Administer the study drug
- Record of the place and time of study drug administration

Study staff will review any adverse events and available laboratory findings prior to drug administration. The investigator will determine if any adverse event requires discontinuation of the study medication or results in inability to continue to comply with study procedures.

9.5 Follow-up Visits

After signing informed consent on day -7, participants will be scheduled to return to the clinic ten times total on day -6, day 1, day 2, day 3, day 5, day 7, day 9, day 11, day 14, day 17, and day 21. Refer to Table 3 for associated study procedures.

Before dosing with primaquine, all study participants will be counselled on symptoms of haemolysis and anaemia. They will be instructed to visit the clinic if these symptoms or any other symptoms occur during the study duration.

The visit window for the day 1 visit is a 90 hour window (i.e., two days before or after day 1), with the subsequent study visits for drug administration and monitoring scheduled based on the date of the first dose given. An appointment card will be prepared and provided to the participant with the full drug administration and follow-up schedule based on the day of the first dose. Each subsequent visit for drug administration will have a 24 hour study visit window. The day 16 follow-up visit has a 48 hour visit window and the day 21 follow-up visit has a 72 hour visit window centred on the target date (i.e., a day before or after day 21).

The following procedures are to be performed for each visit with a sample collection:

- Record adverse events as reported by participant or observed by study staff
- Record vital signs
- Review all available laboratory results, including any haematology findings

- Collect blood and urine samples for laboratory tests outlined in Table 3. Blood and urine samples on day 1 will be collected before treatment is given. On day 1 an additional blood sample will be taken after treatment is administered.

At study exit on day 21, any participant with ongoing, unresolved AEs will be referred to care for appropriate clinical follow-up in the outpatient department.

9.6 Missed Visits

In case of a no-show at the clinic for a scheduled study visit, study personnel will call the participant if the participant has a phone. Study personnel will also visit the participant's home to remind the participant of the study visit. Maximum efforts will be made to ensure complete follow-up in the trial, including routine education for participants on follow-up schedules. For participants who do not complete a scheduled visit within the visit window, that visit will be documented as "missed" but study staff will still attempt to complete the appropriate assessments from that visit, if possible.

Participants who miss a visit, for other than a protocol-mandated reason for discontinuation, are permitted to continue with any subsequent study treatments that can still be scheduled in the time interval specified by the protocol.

Based on our current experience, we expect that fewer than 5% of the participants will be lost to follow-up at the time of primary outcome assessment. We think it is unlikely that attrition rates will differ between randomization groups. Loss to follow-up has been accounted for in the sample size calculations.

9.7 Participant Withdrawal or Termination

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

- Ineligibility (either arising during the study or retrospective having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study medication or results in inability to continue to comply with study procedures, including an absolute haemoglobin reduction of 3.0 g/dL or absolute haemoglobin below 7.0 g/dL or any significant signs or symptoms of haemolysis such as dizziness, feeling tired, difficulty breathing, pallor or low blood pressure
- Consent withdrawn
- Lost to follow up

The reason for withdrawal will be recorded in the case report form (CRF). A participant is considered to be withdrawn prematurely from the study if they do not complete the Day 21 assessment.

If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilized. Prior to study initiation, the trial's Data Safety and Monitoring Board (DSMB) will establish and approve stopping rules specifying adverse events that would necessitate discontinuing study drug for a participant.

If the participant is withdrawn because they are not able to tolerate the 12 hour fasting period, another participant will be recruited in their place.

9.8 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be promptly provided by the suspending or terminating party to the sponsor, Data Safety Monitoring Board, and institutional review boards (IRBs)/independent ethics committees (IECs).

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, and/or data quality are addressed and satisfy the sponsor and IRBs/IECs.

10. LABORATORY TESTS

All laboratory results will be reviewed by a qualified staff member and recorded in the CRF.

Any leftover EDTA-treated venous blood will be spun down for 5 minutes at 3000 rpm, and buffy coat and packed red blood cells (RBCs) will be stored in labeled tubes for later DNA extraction and genotyping of G6PD and CYP genes. Leftover plasma will also be stored at SMRU for up to 10 years for possible use in this or future studies, although currently there is no foreseeable use for plasma under this protocol. In the event the stored specimens are used, SMRU will be responsible for obtaining the necessary ethical approvals prior to any future testing or analysis.

Samples stored for future use will be labelled with the appropriate SID. Stored samples will be destroyed after a maximum of 10 years.

Laboratory tests will be performed according to the schedule outlined in Table 3 of study procedures. Per that table, some tests will be performed in the clinic as point-of-care tests and some tests will be carried out on specimens that have been transport to either the SMRU or the University of Mahidol laboratory. Each of these laboratory tests is described in greater detail below.

10.1 G6PD Assays

- **Quantitative G6PD spectrophotometric test** (30µl from blood taken from CBC)

  The quantitative spectrophotometric assessment of G6PD activity in blood is based on the measure of the increase in absorbance at 340nm due to the production of NADPH during incubation of haemolyzed blood with G6P and NADP- substrates. Available commercial kits will be used for this test. Quantitative G6PD enzyme activity will be assessed on every blood draw as per study design. The quantitative G6PD spectrophotometric test will be performed as part of screening procedures. 100% normal G6PD activity for this population using this assay in this laboratory has been determined previously to be 7.51 IU/gHb (38). The 40% normal activity threshold used for inclusion is therefore 3.00 IU/gHb.

- **G6PD flow cytometry** (100µl from blood taken from CBC)

  Whole blood specimens will be characterized for intracellular G6PD activity by flow cytometry as described previously Shah et al. 2012. Specimens will be analyzed using an Accuri™ C6-UV flow-cytometer (BD Biosciences); 10,000 events will be recorded in the FL1 channel 533 +/− 30 nm.

  G6PD flow cytometry testing will be assessed on every blood draw as per study design.

  The cytofluorometric data analysis tool (already developed by PATH and validated with SMRU) will be further refined to improve assessment in changes of red blood cell distribution. This will be refined to include a model that describes how sub populations of red blood cells express and change in G6PD activity in time due to treatment with primaquine.

- **G6PD fluorescent spot test** (50µl from blood taken from CBC)

  Most field deployable G6PD tests are qualitative and the standard of care for case management is the G6PD fluorescent spot test (FST). The G6PD FST will be performed on recruitment as a comparator to the quantitative test. An EDTA tube will be used to acquire blood for glucose-6-phosphate dehydrogenase semi-quantitative fluorescent spot test for initial screening in the field. The fluorescent spot test (R&D Diagnostics) will be performed as per manufacturer's instructions. Additional qualitative tests such as the CareStart G6PD test may be performed depending on availability.

10.2 Haematology Tests

- **Complete Blood Count** (2 ml)

  Whole blood will be collected by venipuncture and placed in an EDTA tube. Samples will be sent to the centralized haematology laboratory at SMRU. Automated CBCs will be analyzed using the CeltacF MEK-8222K haematology analyzer (Nihon Kohden, Tokyo, Japan). Machine maintenance and calibration will be performed in accordance with SMRU SOP's.

- **Reticulocyte count** (5µl from blood taken from CBC)

  Reticulocyte counts will be determined either by standard slide microscopy on 1000 red blood cells stained with new methylene blue stain or by Flow cytometry using Thiazole Orange staining.

- **Methemoglobin Level Testing**

  Methemoglobin levels will be measured with a finger sensor using a Masimo Radical 57 oximeter. This procedure is non-invasive.

10.3 Urine Tests

- **Urine urobilinogen**

  Urine samples will be collected in sterile containers and tested using Roche® Combur-10 test strips.

10.4 Biochemical Tests

- **Liver and kidney function tests** (5 ml)

  Whole blood will be collected by venipuncture and placed in a plain tube. At the field site, the blood will be centrifuged to obtain the serum for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total and direct bilirubin concentration, total protein, albumin and lactate dehydrogenase. Kidney function tests will include blood urea nitrogen, creatinine, and potassium.

10.5 Pharmacokinetic Tests

- **Chloroquine drug levels** (1.5 ml)

  Blood samples will be obtained by venipuncture and placed in an EDTA tube. At the field site, the blood will be centrifuged and the plasma will be frozen at -20°C and processed at the Faculty of Tropical Medicine, Mahidol University for chloroquine PK analysis.

- **Primaquine drug levels** (1.5 ml)

  Blood samples will be obtained by venipuncture and placed in an EDTA tube. At the field site, the blood will be centrifuged and the plasma will be frozen at -20°C and processed at the Faculty of Tropical Medicine, Mahidol University for primaquine PK analysis.

  PK samples will be collected at 6 hours after drug administration and the time recorded. This will ensure that for all patients there will be a 6, 30, 54 PK value for the first three days of treatment and then a 6 hour PK value on days of full blood analysis. PK samples are required for the duration of the study because the PQ (and in Arm 1B CQ) metabolite concentrations will be required for the model. The purpose of the PK tests is to collect drug and metabolite concentrations at the time of sampling as an input for the modelling.

10.6 Other Testing

- **CYP2D6 phenotyping via dextromethorphan urine assay**

  All patients will be phenotyped for CYP2D6. The dextromethorphan assay for assessment of CYP2D6 phenotypes (manuscript submitted for publication) will be performed on all volunteers before primaquine treatment. The major metabolizer of primaquine is the 2D6 isoform of the CYP450 which is also responsible for metabolising other compounds. A 10 hour urine sample will be collected before dexamethasone dosing (this time includes the 8 hour fasting period). After a supervised 8 hours fast, dextromethorphan is given to volunteers in a 30mg dose and measured in a 24 hour urine sample together with its metabolite (dextrorphan); the relative proportion of the two compounds allows for characterization of CYP2D6 phenotype (e.g. fast metabolizer, slow metabolizer, etc).

- **Malaria smear** (20µl from blood taken from CBC)

  A thick and thin malaria smear will be made at each scheduled visit with a blood draw and participant will be excluded if malaria is suspected. Thick and thin blood films will be prepared and stained with Giemsa stain. Stained thick and thin blood smears will be examined by qualified laboratory technicians. Quality assurance will be performed in accordance to SMRU SOPs.

  Parasite density will be calculated by counting the number of asexual parasites per 500 leukocytes in the thick blood film, based on an assumed white blood cell (WBC) count of 8,000 /µl. The parasite density per microliter will be calculated using the following formula:

  $$\text{Parasite density / µl} = \frac{\text{Number of parasites counted x 8,000 (or WBC)}}{\text{Number of leukocytes counted}}$$

  Or per 1000 RBC on the thin film for higher parasitemias:
  Parasite density = Number of *P. vivax* trophozoites per 1000 RBC x Haematocrit x 125.6

- **Urine β-human chorionic gonadotropin (HCG) pregnancy test**

  A urine β-HCG pregnancy test will be performed in all women of childbearing age (unless menstruating) during initial screening. Bioline HCG test strips or equivalent will be used. After initial screening, the test will be performed during follow up visit. Pregnancies occurring after initial treatment with the study drugs will be withdrawn from the study. Any study participants identified as being pregnant will be informed and referred for antenatal care.

Table 4. Specimen collection for the study.

Study Procedure	Visit Day															
	Day -7	Day -6	Day 1	Days 2, 3	Day 4	Day 5	Day6	Day 7	Day 8	Day 9	Day 10	Day 11	Days 12, 13	Day 14	Day 17	Day 21
Blood draw	~2 ml		10 ml x 2	10 ml		10 ml		10 ml		10 ml		10 ml		10 ml	10 ml	10 ml
Urine	√	√	√	√		√		√		√		√		√	√	√

11. STUDY DRUGS

11.1 Formulation of Study Drugs

Primaquine will be provided in 0.5 mg/kg daily doses. Chloroquine will be provided in 25 mg/kg total dose.

Chloroquine will be dosed as a 25 mg/kg base given in divided doses of 10 mg/kg orally on days 1 and 2, and in 5 mg/kg dose on day 3. Tablets will be obtained from Government Pharmaceutical Organization (GPO) in Bangkok, Thailand and supplied as 250 mg tablets (155.3 mg base). Side effects are mild when chloroquine is taken for a short duration. These include dizziness, headache, nausea, vomiting, difficulty with visual accommodation, itching, and skin rash.

Primaquine will be dosed at 0.5 mg/kg daily orally as a single dose for 14 days. Doses will be based on real body weight. Doses will be given to the nearest ¼ tablet. Tablets will be obtained from Government Pharmaceutical Organization (GPO) in Bangkok, Thailand and supplied as 15 mg tablets. Abdominal pain is a dose dependent side effect commonly caused by primaquine and is usually alleviated with food prior to administration.

11.2 Administration of Study Drugs

After randomization, the study participant will be assigned 1 of 2 study drug regimens:

- Arm 1A: Participants will receive primaquine in a 0.5 mg/kg dose once daily for 14 days. The first dose will be administered on day 1 and the last dose on day 14.
- Arm 1B: Participants will receive chloroquine on days 1, 2, and 3 in 10, 10, 5 mg/kg dose once daily. Participants will receive primaquine in a 0.5 mg/kg dose once daily for 14 days. The first dose will be administered on day 1 and the last dose on day 14.

Doses are measured based on weight and dosing charts as per SMRU malaria guidelines are available for reference. Doses will be given to the nearest ¼ tablet.

Treatment will be observed either in the clinic or at the participants' village or work site by a member of the study team. The investigator may withdraw a participant if dose compliance is considered unsatisfactory.

11.3 Storage of Study Drugs

Storage of study medication will be in accordance with the manufacturers' instructions and dispensed by an authorised staff member.

11.4 Study Drug Accountability

All movements of study medication between pharmaceutical or distributor companies and SMRU will be documented. Study medications will be transported from the SMRU office to the outpatient clinics. All treatment doses will be observed, therefore, all unused medication at the outpatient clinics will be collected by a member of the Investigator team and returned to SMRU.

11.5 Concomitant Medication

If needed, study investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate care for any adverse event during the study period. Any medication taken during the study will be recorded in the CRF.

12. SAFETY ASSESSMENTS AND REPORTING

12.1 Adverse Event (AE)

An AE or adverse experience is:

Any untoward medical occurrence in a study participant administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication).

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study medication, whether or not considered related to the study medication.

12.2 Adverse Reaction (AR)

Adverse reactions include all untoward and unintended responses to a medicinal product related to any dose.

The phrase "responses to a medicinal products" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

12.3 Serious Adverse Event (SAE)

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

A serious adverse event (SAE) or serious adverse reaction (SAR) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires inpatient hospitalisation or prolongation of existing hospitalisation, except for routine hospitalization for *Plasmodium vivax* or *falciparum* malarial disease
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

12.4 The difference between an SAE and an SAR is the relatedness attributed to the study treatment. An SAR has the same standard for determining causal relationship as outlined above in the section describing adverse reactions.Expected Serious Adverse Reactions

An expected serious adverse reaction (SAR) is a serious adverse reaction, the nature or severity of which is consistent with the applicable product information. Primaquine can cause significant haemolysis in G6PD deficient persons (<30% of normal G6PD activity or 2.25 IU/gHb by the quantitative G6PD spectrophotometric assay). Only individuals with 40% or greater G6PD activity (3.00 IU/gHb) will be enrolled in the study, therefore significant haemolysis is unlikely to occur.

If haematocrit is <30%, the participant will receive the standard anemia treatment (ferrous sulphate 200 mg twice daily, folic acid 5 mg daily, vitamin B12 100 mg twice daily and Vitamin C 100 mg twice daily) until the haematocrit rises above 30%. If the fractional drop in haematocrit is >25%, an adverse event sheet will be completed and the patient will be followed as clinically indicated. Methemoglobin levels increase in normal persons taking primaquine but are usually asymptomatic. Symptoms may consist of cyanosis, lethargy or dyspnea. During study drug administration, symptoms of methemoglobinemia and methemoglobin levels (using a Masimo Radical 57 oximetry machine) will be monitored. If a participant develops an increase in SpMet (3-20%) and does not have symptoms, the study medication will be continued and the participant will be monitored until levels normalize. If a participant develops symptomatic methemoglobinemia or if SpMet >20%, the study medication will be stopped, patient admitted and treated with ascorbic acid.

12.5 Suspected Unexpected Serious Adverse Reactions

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information.

12.5.1 Management of SAR

We do not expect to see subjects with profound falls in haemoglobin because all subjects will have a normal G6PD phenotype. However, as a safety net, individual subject stopping criteria are in place (see section 9.7). If a subject requires a blood transfusion, they will be managed by the research team. Subjects will be referred to Mae Sot General Hospital for further management, if determined to be clinically unstable.

The clinical indications for a blood transfusion are:

- Haemoglobin <6 g/dL or Haematocrit <18%, or
- Clinically significant symptoms & signs of anaemia (e.g. dizziness, feeling tired, difficulty breathing, pallor or low blood pressure).

12.6 Reporting Procedures for All Adverse Events

All AEs occurring during the study observed by the investigator or reported by the participant, whether or not attributed to study medication, will be recorded on the CRF up to day 21.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

AEs considered related to the study medication as judged by a medically qualified investigator or the sponsor will be followed until resolution or the event is considered stable. All AEs will be managed by the clinical study site team in accordance with good medical practices and the standard clinical practices in place at the hospital. The clinical team will assess and treat or refer the participating child for medical care as appropriate, which may include additional study visits, if necessary. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment (see Section 6.4). A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant will be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

The severity of events will be assessed on the following scale:

- 1 = mild (asymptomatic or mild symptoms requiring clinical or diagnostic observations only; no intervention indicated)
- 2 = moderate (symptoms limit age appropriate instrumental activities of daily living; minimal, local or non-invasive intervention indicated)

- 3 = severe (symptoms are medically significant but not life threatening, disabling, or limit self care activities of daily living; hospitalization or prolongation of hospitalization indicated)
- 4 = life threatening (symptoms are life threatening; urgent intervention indicated)
- 5 = death

A medically qualified investigator will assess the relationship of AEs to the study medication. The relatedness of AEs to the study product administration will be assessed on the following scale:

- Definitely related: adverse event and administration of study product are related in time, and a direct association can be demonstrated with the study product
- Probably related: adverse event and administration of study product are reasonably related in time, and the adverse event is more likely explained by the study product than by other causes
- Probably not related: a potential relationship between administration of study product and adverse event could exist, but is unlikely, and the adverse event is most likely explained by causes other than the study product
- Not related: the adverse event is clearly explained by another cause unrelated to administration of the study product. Reportable events must have documentation to support the determination of "not related"

Any pregnancy occurring during the clinical study and the outcome of the pregnancy will be recorded and followed up for congenital abnormality or birth defect.

12.7 Reporting Procedures for Serious Adverse Events

All serious adverse events will be reported to the PI and sponsor within 1 working day and to the Ethics Committee as required. SAEs will be reported to the Data Safety Monitoring Board as part of interim or final reports. SARs, whether expected or unexpected, will be reported to the DSMB within 72 hours of the PI and sponsor's notification.

12.8 Study Halting Rules

Administration of study drug will be halted if severe haemolysis (defined as absolute haemoglobin reduction of 3.0 g/dL or absolute haemoglobin below 7.0 g/dL or any significant signs or symptoms of haemolysis such as difficulty breathing or low blood pressure) occurs at the following frequencies among study participants:

- Heterozygous women
    - 3/12 individuals in Arm 1A meeting criteria of severe haemolysis
    - 3/12 individuals in Arm 1B meeting criteria of severe haemolysis
- Wildtype men/women
    - 6/48 individuals total from either Arm 1A or 1B meeting criteria of severe haemolysis

The PI will notify the study sponsor and co-investigators immediately when the threshold in any of the above groups of participants is met for severe haemolysis and enrollment screens will stop accepting new study participants. Prior to study initiation, the DSMB will identify and approve any additional study halting rules to be followed for the study. When any event occurs that meets a study halting rule, the study sponsor will inform the DSMB members within 24 hours of notification and will provide the DSMB with AE listing reports. The DSMB will convene an ad hoc meeting by teleconference or in writing as soon as possible.

The objective of the safety review is to decide whether the study (or study drug for an individual or study cohort) should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrolment (for a particular group or for the entire study) is a potential outcome of a safety review. The DSMB will provide its recommendations to the study sponsor. The study sponsor will inform the IRBs/IECs of the temporary halt and the disposition of the study.

12.9 Safety Oversight (Data Safety Monitoring Board)

Safety oversight will be under the direction of a Data Safety Monitoring Board (DSMB), an independent group of experts that advises the study investigators. The DSMB will be composed of at least three voting members, including at least one malaria expert and one biostatistician. One of the DSMB members must be a clinician. The primary responsibilities of the DSMB are to 1) periodically review and evaluate the accumulated study data for participant safety and study conduct and progress, and 2) make recommendations concerning the continuation, modification, or termination of the study. The DSMB will operate under the rules of an approved charter that will be reviewed at the organizational meeting of the DSMB held before the study begins recruitment. At this time, each data element that the DSMB needs to assess and the roles and responsibilities of DSMB members will be clearly defined.

The DSMB will meet once approximately half of the target sample size has been enrolled or three months after recruitment begins, whichever is sooner, and then again at the end of the study to assess safety data by randomization arm. Prior to each DSMB meeting, a study report will be distributed to the DSMB for review. Adverse events will be presented in aggregate summary, whereas any SAEs will be presented individually in DSMB reports. DSMB findings will be reported to the PI and sponsor within a reasonable amount of time specified in the approved charter.

13. DATA HANDLING AND RECORD KEEPING

13.1 Data Collection

All study data will be recorded on standard paper Case Report Forms (CRFs). Data will be entered on a secure database in accordance with standard operating procedures (SOPs).

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarized into the CRF), clinical charts, laboratory, correspondence, log books, and CRFs. For most variables, the CRF will be considered the source document.

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent and screening logbook, the participant will be referred to by the study participant number/code, not by name.

13.2 Data Access

The participants will be identified by a study identification number and the patient (clinical) identification number in the SMRU clinical database. The name and any other identifying detail will NOT be included in any study data electronic file. The database linking the volunteer's clinical identification number to the study identification number will be kept by the staff at SMRU and PATH will not have access to the link. All records will be kept locked and all databases will be password protected such that clinic staff and study staff will have access to their respective databases.

Direct access will be granted to authorized representatives from the sponsor, host institutions and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13.3 Data Storage

SMRU will maintain, and store securely, complete, accurate and current study records throughout the study. In accordance with regulations, study staff will retain all study records on site for at least five years after study closure. Study records will not be destroyed prior to receiving approval for record destruction from the sponsor. No study records will be destroyed while study specimens are still being stored. Applicable records include source documents, site registration documents and reports, informed consent forms, and notations of all contacts with participants.

13.4 Quality Control and Quality Assurance

The study will be conducted in accordance with the current approved protocol, International Conference on Harmonisation (ICH) good clinical practices (GCP), relevant regulations and standard operating procedures.

Regular monitoring will be performed according to ICH GCP. A SMRU data monitoring team visits each clinical site roughly once per month to review completion of consent forms, CRFs, and study file management. The monitor collects all study-related forms and brings them to the SMRU offices for database entry and storage. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Study data will be aggregated into a database and an electronic monitoring report will be generated every month summarizing key indicators for study compliance. These indicators include but are not limited to the number of participants consented, the number of samples acquired, any deviations from study procedures, and corrective actions taken. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. In addition, a PATH representative will conduct site monitoring visits as needed to ensure compliance with the protocol and relevant SOPs.

14. STATISTICAL ANALYSIS

Primary aim: *To understand the risk of haemolysis in healthy G6PD heterozygous females receiving (i) primaquine for 14 days at 0.5 mg/Kg and (ii) chloroquine for 3 days and then primaquine for 14 days at 0.5 mg/Kg.*

This will be done by studying survival rates of RBC populations with known G6PD by method of flow cytometry which will measure the G6PD activity of a random selection of 10,000 RBCs throughout various time points in the study. Descriptive statistics will characterize the haematological markers collected, including absolute haemoglobin reduction from baseline on exposure to primaquine over treatment course as well as absolute reduction in haemoglobin-related change in intracellular G6PD concentration profiles over treatment course. Additional descriptive statistics characterizing haematocrit levels, absolute and fractional haematocrit reduction from baseline, and changes in intracellular G6PD concentration populations over time (as measured by flow cytometry) will be determined for each study group in each treatment arm. Estimates of lysis from haematocrit levels and from flow cytometry will be checked for concordance.

Correlations among these exposure metrics between arms will be evaluated. Normality of measurements will be assessed and non-parametric tests or log transformations will be used as appropriate.

Secondary aims: (1) *To determine if additional haematologic measures are critical inputs or determinants for a drug-related haemolytic risk model, (2) to associate primaquine and chloroquine drug levels at the time of sampling with patient haematological and G6PD profiles, and (3) to measure frequency of adverse events in women heterozygous for G6PD treated with current standard of care for P. vivax malaria.*

Descriptive statistics characterizing dextromethorphan assay results, CBC, methemoglobin, reticulocyte count, and bilirubin and urine urobilinogen levels; drug levels of chloroquine and primaquine; and adverse events will be determined for each study group in each treatment arm. Correlations between haematology measures and drug levels at the time of sampling between arms will be evaluated. Normality of measurements will be assessed and non-parametric tests or log transformations will be used as appropriate.

A mixed affects regression model will be developed to understand the dynamic relationship between predictors such as collected haematological markers, gender, gross G6PD activity, G6PD zygosity, Dextromethorphan assay results, PQ concentration, bilirubin levels and their effect on a patient's absolute drop in haemoglobin and haematocrit.

A mixed affects regression model will be developed to understand the dynamic relationship between predictors such as collected haematological markers, gender, gross G6PD activity, G6PD zygosity, Dextromethorphan results, PQ concentration, bilirubin levels and their effect on the drop in the number of cells within specified bins of G6PD activity measured by G6PD cytometry assay.

15. ETHICAL CONSIDERATIONS

15.1 Principles for Clinical Research

The investigator will ensure that this clinical trial is conducted in compliance the International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP) or the Declaration of Helsinki, whichever affords the greater protection to the subject. Additionally, the investigator assures that all activities of this protocol will be guided by the ethical principles of The Belmont Report, 45 CFR 46 and all of its subparts (A, B, C and D).

All study staff will be trained and certified in the protection of human subjects.

15.2 Community Engagement

The populations served by the SMRU clinics are predominantly migrant workers and refugees. These individuals have lower levels of income and education, poor health outcomes, and migratory housing arrangements. SMRU has been serving this population for over 20 years and has experience involving vulnerable populations. SMRU medics, nurses and health workers are recruited from the same areas as the patient population and are sensitive to their needs. All communication will be conducted in the preferred language of the subject and consent forms will be translated into both Burmese and Karen.

We have chosen to conduct this research within this population through SMRU because this patient population has high rates of G6PD deficiency and is representative of a spectrum of regional G6PD deficiency traits in addition to other haemoglobinopathies such as haemoglobin E and thalassemia.

We will ensure that participant participation is voluntary and willing by conducting a two-step recruitment and consent process. The volunteer will have plenty of time to decide whether or not to participate after the recruitment process, which takes place at his or her home. At each step, the study staff (recruiter or study nurse) will emphasize that participation is voluntary and that future care from the clinic will not be impacted by choosing not to participate.

Consultation with the Community Advisory Board of Tak Province will be undertaken. This Board comprises representatives from the communities that SMRU serves. Study objectives and procedures will be presented (with translation as necessary into Karen or Burmese) to the Community Advisory Board and discussed in detail.

15.3 Recruitment

Targeted recruitment will involve screening the clinical records of patients who have previously received G6PD testing at the SMRU clinics. For this study, we will search the database to identify 24 males hemizygous for wildtype G6PD, 24 women homozygous for wildtype G6PD, and 24 women heterozygous for G6PD deficiency. By screening retrospectively we seek to limit our initial recruitment to only healthy volunteers, although final determination of good health will be made at the time of enrollment. In addition, by targeting enrollment to those individuals who have already been tested for G6PD deficiency we eliminate the extra blood draw which would otherwise be necessary to screen general population patients who do not have clinical indications for G6PD testing.

Patients who meet the recruitment criteria will be contacted at their home by a clinic staff member whose responsibilities include community outreach. Health workers are recruited from the local communities. The SMRU health worker is trained to provide outreach services in the community served by the clinic. Health workers attend training courses during their orientation to work. These courses include basic medical, laboratory skills as well as care for patients. The health worker will explain the study, provide a printed copy of the Participant Information Sheet for the patient to

review (Appendix A), and invite the patient to participate in the study. It will be clearly explained that participation is optional and not participating will not affect future care at the clinic. If the patient expresses interest in participating in the study she or he will be directed to visit within the following month the clinic where they usually receive care.

15.4 Informed Consent

Clinic staff assigned to study duties will meet individually with the volunteer in a private space and review the study details, using the Participant Information Sheet (Appendix A) to help summarize the main points of the study. They will emphasize that participation in this study will be voluntary, and under no circumstances will the clinical management of the volunteer be affected by their decision to participate or not in the study. Following summary of the study, the study staff will review the Informed Consent Form (ICF) with the potential participant (Appendix B). The ICF will review the study purpose, the procedure involved, the volunteers' rights to withdraw, confidentiality, and benefits and risks of participating in the study. Volunteers will be given the opportunity to ask questions about the study, procedure, and the consent process. Volunteers will be consented using an ICF in the language which they understand; the ICF will be prepared in English and translated into both Burmese, and Karen.

Volunteers will be asked to sign the ICF as a confirmation of willingness to enroll in the study. Volunteers who are unable to read and write will be provided an independent witness who will attend the briefing session and sign and date the consent form prior to entry of the subject to the study.

If an amendment to the study protocol is submitted and approved, the PI will ensure that the ICF is revised to reflect changes relevant to the volunteer's participation in the study. Any modification to the ICF will be approved by the appropriate ethics committees before being used with participants.

15.5 Volunteer Confidentiality

The investigator will ensure that each volunteer's confidentiality is maintained. Volunteers will not be identified in any publicly released reports of this study. All records will be kept confidential by SMRU. PATH will not have access to records that identify the subjects.

SMRU data management practices record clinical data on Case Report Forms with only the study identification number (SID). Both the SID and the patient (clinical) identification number (PID) will be entered into the SMRU clinic database. This is standard practice for all SMRU research. The link between study ID and patient ID will be maintained in the main SMRU clinical database. Prior to sending data to PATH, SMRU will scrub the PID from all study records, leaving only the SID-associated study records.

15.6 Benefits & Risks

15.6.1 Benefit to study volunteers

There is no direct benefit of this study to the volunteers, but this research will be advantageous for academic study and overall community. Nonetheless, participating in the study will include a brief medical consultation based on symptoms/complaints at time of enrollment, which may prompt the volunteer to raise any health concerns at that time and counselling about G6PD deficiency.

15.6.2 Benefit to participating study sites

There is no direct benefit to the study sites. Nonetheless, the additional time interacting with patients who have previously been seen at the clinic may help strengthen the relationship of the clinic with its community members. In addition, the training and monitoring that will accompany the introduction of the study SOPs may improve the overall technical capacity at the sites.

15.6.3 Risk Considerations

The proposed study does not involve the use of an investigational drug or article. All study procedures are routine and pose no additional risk to the study participant beyond clinical procedures that might be performed outside of a study setting.

Primaquine can cause significant haemolysis in G6PD deficient persons. Only individuals with ≥40% of normal G6PD activity or 3.00 IU/gHb by the quantitative G6PD spectrophotometric assay will be enrolled in the study, therefore significant haemolysis is unlikely to occur. Nonetheless, enrolled women heterozygous for G6PD deficiency may be at higher risk of haemolysis when exposed to primaquine at the doses proposed in this study or when followed by chloroquine administration. Enrolled participants will be closely followed with safety monitoring of clinical and laboratory signs to promptly identify and manage any adverse events.

Study procedures do not represent significant risks to the participants beyond those that are associated with normal blood draw, such as pain, discomfort, and infection at the site of fingerstick or venipuncture. In the unlikely event of a research related injury, cost of treatment will be covered by PATH.

Home visiting for clinical care and follow up is common practice at the SMRU clinics. SMRU health workers are known in the community and often provide medication at home if the patients are unable to visit the clinic. It is possible that our patients will feel their privacy compromised and during screening, the health workers will be trained to address this issue. If volunteers have more concerns, the PI will become involved only after consent has been taken or refused. If volunteers do not want to accommodate home visits, they will not be recruited into the study.

The study staff are at risk for exposure to blood-borne pathogens in the course of their work. All research team members will follow their institutions' standard procedures for infection control. Research staff exposed to blood-borne pathogens during the course of their study roles will follow their institutional guidelines for post-exposure prophylaxis.

15.7 Compensation

Volunteers will be compensated at least 500 baht for the time/labor costs of participation; this is equivalent on average to one day's wages in this area and will cover transportation costs for most of the catchment area. If a participant lives far enough away from the study clinic that 500 baht will not fully reimburse transportation costs, additional compensation may be provided to make up the difference, determined on a case-by-case basis. Compensation will be provided to participants at enrollment, for each day they stay at SMRU clinic during the observation period for the dextromethorphan urine testing, and each scheduled follow-up study visit.

Patients who say no at the time of recruitment will not be provided 500 Baht. If the patient comes to the clinic and says no during Informed Consent, or withdraws after consent, he or she will still be compensated 500 Baht for that visit.

15.8 Ethical Review

The protocol, informed consent form, participant information sheet, and recruitment materials will be submitted to WIRB, OXTREC, and the Faculty of Tropical Medicine Ethics Committee at Mahidol University, for written approval.

15.8.1 Amendments

All amendments and modifications to the protocol, ICF, participant information sheet, and recruitment materials will be submitted to the above institutional review boards (IRBs)/independent ethics committees (IECs) for review and approval. No changes in protocol conduct will be implemented until approvals by all IRBs/IECs are obtained.

15.8.2 Continuing Review Reports

The PI will be responsible for submitting the required continuing review report and associated documents to the relevant IRBs/IECs, allowing sufficient time for annual review and continuation determination prior to the established continuing review date. A closeout report will be submitted at the end of five years, or upon completion of the study, whichever comes first.

15.8.3 Deviations

Any deviation from the protocol that may have an impact on the safety or rights of the subject or the integrity of the study will be reported to the sponsor within 24 hours of identifying the deviation and to the appropriate IRBs/IECs within 72 hours. All other deviations will be reported in the annual continuing review report.

15.8.4 Unanticipated Events

Non-serious unanticipated problems will be reported in the annual continuing review report. All unanticipated problems involving risk to subjects or others, including breach of confidentiality and incidents of non-compliance will be promptly reported by phone or e-mail to the SMRU research coordinator. A complete written report will follow the initial notification.

16. FINANCING AND INSURANCE

This study is funded by [redacted] under the award titled, "Development of a point-of-care test for G6PD deficiency" (Reference number: [redacted]).

The sponsor has taken out insurance from ACE/Illinois Union Insurance Company in the event of a screening or study-related injury. PATH will pay to treat any injury suffered as a result of participation in the trial.

17. CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

18. PUBLICATION POLICY

The data generated by this study will inform the design of future evaluations of G6PD tests and may inform programmatic decisions around testing for G6PD deficiency.

All data will be published in the open medical literature with the identity of the subjects protected. This trial will be registered in a web based protocol registration scheme at http://www.ClinicalTrials.gov.

19. REFERENCES

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20. APPENDICES

20.1 Appendix A – Participant Information Sheet

[Sample Participant Information Sheet]

20.2 Appendix B – Informed Consent Form

[Sample Informed Consent Form]