Spring 2018

Evaluation of the CareStartTM Glucose-6-phosphate dehydrogenase (G6PD) Rapid Diagnostic test at Community and Health Center level, Cambodia

Bertha Nyagaya-Wojnarski, DNP, FNP-BC, MPH
George Washington University

Evaluation of the CareStart™ Glucose-6-phosphate dehydrogenase (G6PD) Rapid Diagnostic test at Community and Health Center level, Cambodia

Presented to the Faculty of the School of Nursing

The George Washington University

In partial fulfillment of the requirement for the degree of Doctor of Nursing Practice

Bertha Nyagaya-Wojnarski, FNP-BC, MPH

Project Team

Dr. Zhou (Pearl) Qiuping

Dr. Ashley Darcy-Mahoney

Author:

Bertha Nyagaya-Wojnarski, FNP-BC, MPH

Email:bnyagaya@hotmail.com

Date of Degree: Spring, 2018

G6PD CARESTART™ RAPID DIAGNOSTIC TESTING

Abstract

Background: Primaquine (PQ) is the only FDA-approved drug for radical cure of Plasmodium vivax (P.v) malaria, but treatment can result in life-threatening hemolysis if given to a glucose-6-phosphate dehydrogenase deficient (G6PD***d***) patient. Therefore, the G6PD status of the patient with P.v must be known prior to prescribing PQ. However, patient G6PD status in rural malaria endemic settings is generally unknown, illuminating the need for reliable point of care G6PD diagnostic tests as a prerequisite to safely administer PQ. To increase community PQ access in Cambodia, performance of CareStart™ G6PD rapid diagnostic tests (RDTs) needs to be evaluated in healthcare workers (HCWs) and village malaria workers (VMWs).

Methods: Training materials on G6PD and PQ were developed for HCWs and VMWs and each performed G6PD RDT test on 8-12 adult male volunteers, with pre- and post-training questionnaires completed by trainees and G6PD test volunteers. The performance of CareStart™ RDT for G6PD***d*** screening was assessed against a quantitative G6PD lab test (Pointe Scientific, Inc. MI, USA). Descriptive and inferential statistics were used to analyze the data.

Results: 94 trainees and 960 G6PD volunteers were recruited in Oddar Meanchey province, Cambodia from December 2017 to February 2018. Of the 960 volunteers, 156 (16%) were G6PD deficient based on a quantitative test activity threshold of 30%. The sensitivity, specificity, PPV and NPV of CareStart™ RDT were 96.8%, 95.5%, 80.2%, 99.4% for HCW/VMW trainees vs. 96.2%, 97.2%, 86.7%, and 99.3% for trained study staff in the field and 94.2%, 98.8%, 93.6% and 98.92% for experienced laboratory staff, with no statistical difference among the groups. The mean knowledge score pre-training was 33.9% (VMWs) and 56.4% (HCWs), with improvement to 89% and 90% post training ($p < 0.001$). Improvement in the HCW/VMW's knowledge scores was independent of years of experience, profession and education level. Perception of PQ risk based on RDT results was assessed pre-and post-training.

Conclusions: With minimal training, CareStart™ RDT is highly specific, feasible and a practical option for the identification of G6PD***d*** male patients and its use may enable safer prescribing of PQ to decrease the burden of P.v relapse.

Keywords: Malaria, G6PD, Primaquine, Rapid Diagnostic Test

Background

Despite joint efforts by the national malaria control program and various international partners working towards malaria elimination in Cambodia, these efforts continue to be a challenge in this resource-limited setting. Progress has been hampered by antimalarial drug resistance, and lack of access to curative doses of Primaquine for the treatment of *Plasmodium vivax*. However, Primaquine (PQ) which is currently the only Food and Drug Administration (FDA) approved drug that can eliminate hidden reservoir of *P.v* infections in the liver and prevent frequent relapses, can also cause significant life threatening hemolysis if wrongly given to a glucose-6-phosphate dehydrogenase (G6PD) deficient patient (Seidlein et al. 2013; Roca-Feltrer A, Khim N et al. 2014).).

The World Health Organization (WHO) estimates that one million people are infected annually with malaria (Population Reference Bureau, 2016). Among different malaria species in Southeast Asia, *Plasmodium vivax* (*P.v)* accounts for 46% (26,183) of all malaria cases in Cambodia (Sovannaroth, S et al. 2016). Cambodia's National Malaria program (CNM) estimates that the number of malaria outbreaks decreased. However, malaria remains as a major cause of morbidity in Cambodia and requires a robust system and malaria management program to ensure a successful elimination strategy (Population Reference Bureau, 2016).

G6PD*d* is one of the most common defective human enzymes affecting close to 400 million individuals worldwide. It is an X-linked genetic disorder that results in impaired enzyme activity in erythrocytes (Gonzalo et al. 2013). G6PD enzyme protects erythrocytes against oxidation by generating a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). G6PD***d*** a mutated G6PD gene, affects erythrocyte cell stability, especially when exposed to 8-aminoquinoline drugs such as PQ required for cure of patients with *P.v*. Easy to

use, reliable, and easily accessible screening for G6PD*d* is needed before PQ can be administered safely (Amoah et al. 2016; Sovannaroth et al. 2016).

At the moment, the Cambodian National Strategic Plan for Elimination of Malaria (CNM, 2011-2015) has procedures and strategies in place for training providers in the diagnosis and treatment of malaria, but not for G6PD***d*** screening, or the use of G6PD rapid diagnostic tests (RDTs) at the community level (CNM, 2011). Despite reports of decreasing *Plasmodium falciparum (Pf)* malaria in Cambodia, *P.v* elimination poses continued challenges, and the country's economic status is not well equipped to handle the needed costs for implementing needed strategies and carrying out sustainable practices to ensure *P.v* elimination by 2025, the goal set by the Cambodian government and WHO (WHO, 2015). Inexpensive G6PD screening tests could increase access to radical curative doses of PQ but their reliability needs to be assessed in the field settings to ensure safe deployment at appropriate level of care.

Demographics of the country

Cambodia is located in Southeast Asia, covering an area of 181,040 sq. km, and shares borders with Vietnam, Laos and Thailand. Current population is 15,840,251. It has 25 provinces and 13,406 villages (WHO, 2003; CNM, 2011). Public health centers provide basic medical care for a small charge, such as $0.12 per visit for the out-patient department and a $5 charge for in-patient care (Chu et al. 2017). Neither private nor government health insurance is generally available, particularly in rural areas (Sovannaroth et al. 2016) (Appendix A).

Geographical distribution

Currently, malaria elimination is a national priority with goals of eliminating *P.f by* 2020 and *P.v* malaria by 2025 (Canavati et al. 2013). The prevalence of G6PD***d*** in Cambodia has been

reported to be between **13-26% in males** and 3-4% in females (Khim et al. 2013; Sovannaroth et al. 2016).

Problem Statement

G6PD status of *P.v* patients must be known prior to prescribing PQ treatment in order to avoid potentially life-threatening hemolysis from standard doses of PQ. The safe treatment for *P.v* malaria depends on the reliability of results for G6PD screening, yet available screening tests have not been evaluated in the field settings (Jalloh et al. 2004). In addition, the number of studies evaluating the performance of G6PD RDT in Cambodia are relatively few, and prior kits were only formatted for laboratory and temperature controlled settings (USAID, 2012). The national malaria treatment guidelines for Cambodia recommended treatment of *P.v* patients with PQ since 2012, with precise guidelines updated in 2014 (Sovannaroth et al. 2016). Despite these recommendations, PQ is still not widely used in Cambodia due to the unavailability of reliable G6PD diagnostic-field tests, unavailability of PQ, and insufficient data on how to train staff to perform G6PD RDT tests safely.

Recent published data suggested that a new 3rd generation CareStart™ RDT may be more suitable for field-testing, which might allow treatment implementation with PQ in patients with *P.v* malaria (Roca-Feltrer, A. et al, 2014). However, to date, most testing of CareStart™ have only been done in the laboratory settings by trained laboratory staff or by research investigators (Gonzalo et al. 2013). In addition, the level of training that is required for novice users of G6PD testing kits has not been established.

Purpose

The purpose of the study was to assess the performance of the CareStart™ in the hands of HCW/VMWs in the field settings and compare it with the results from trained study staff

(expert/observers) in the field and lab RDT, ***vs.*** the quantitative reference test (Ponte Scientific, Inc. MI, USA). To achieve this, training materials were developed to teach HCWs, VMWs, and lab technicians on how to reliably screen patients for G6PD using the CareStart™, and provide standardized counseling to patients based on their G6PD results. The study also intended to evaluate HCW/VMWs based on their ability to correctly perform and interpret the G6PD RDT results, as well as their effectiveness in counseling G6PD test volunteers.

Specific Aims

- **Develop training materials** to improve knowledge and acceptability of PQ use in G6PD normal patients and assess the performance of the CareStart™ for G6PD*d* screening in the field settings in the hands of HCWs and VMWs.
- C**ompare the performance** of CareStart™ for G6PD*d* screening in the field setting in the hands of the HCW/VMWs vs. trained study staffs (expert observers) in both field and laboratory settings.
- Develop PQ treatment related **counseling materials** to educate patients regarding risks and benefits of PQ in malaria case management.
- Assess HCW/VMW's **knowledge of PQ,** and their acceptability to assess the G6PD status of malaria patients seen at their health centers, and **willingness** to prescribe PQ.

Research Questions

Among HCW and VMW with varied levels of education:

- Does standardized training and education on the safe use and deployment of CareStart™ for G6PD screening result in comparable rates of correctly tested and interpreted RDTs, and improve their knowledge?
- How do the field RDT results compare with the results obtained in controlled settings in the laboratory and against the gold standard quantitative testing?
- Does education and training improve the willingness of HCWs/VMWs to give PQ post training?

- Does education and training improve acceptability levels of malaria workers to test for G6PD among malaria patients

Significance

PQ is the only FDA-approved drug that can provide radical cure from *P.v*. However, to receive PQ, patients must be G6PD normal to prevent drug-induced hemolysis. Due to the limited availability of diagnostic testing for G6PD in Cambodia, most patients only receive treatment that eliminates malaria from the blood, without killing the liver stage parasites (hypnozoites) (Khim et al. 2013). There is insufficient data in regards to modified PQ dosing regimens for G6PD***d*** patients in Cambodia that would make PQ safe for use in a wider scale. Therefore, in order to use PQ in Cambodia, patients must be screened for G6PD***d,*** and the performance of these tests must be evaluated in the field and at the level of HCW/VMW responsible for treating patients with malaria, before deploying PQ at these lower levels of care.

There is therefore an urgent need to identify G6PD screening tests that can be deployed at the point of care, often at the village level, where patients with malaria seek care. Although a field study assessing the performance of the available CareStart™ G6PD rapid diagnostic test (RDT) was conducted in Cambodia in 2012, this was done in controlled laboratory setting, and therefore uncertainty remains on how well CareStart™ RDT will perform when deployed in 'real world' setting (Roca-Feltrer A, Kim S et al. 2014, 2012). The level of training and supportive supervision required to effectively train village malaria workers (VMWs) and health center staff on the correct use and interpretation of the RDTs have also not been fully assessed. Neither has the acceptability among HCW/VMWs to incorporate G6PD test at the community level and their acceptability to prescribe and give PQ given its potential risks. In addition, since G6PD deficiency is bimodal with most G6PD deficient males having G6PD activity levels of <30%,

WHO recommends that appropriate G6PD point of care tests should be able to detect all G6PD deficient males with <30% activity. There is insufficient data if this can be achieved with CareStart™ in the field settings, despite the promising results from the laboratory settings. Given the potential risks associated with incorrect diagnosis of G6PD status, it is possible that use of G6PD RDTs in a community setting might not be appropriate, but additional data is needed.

The WHO and National Center for Parasitology, Entomology and Malaria Control (CNM) cautioned that the deployment of RDTs for G6PD should be piloted to evaluate RDT performance in real-life field settings, which will ensure safe generalization of the program to the Cambodian public. In addition, WHO (2015) stated that there *is a need to evaluate feasibility, effectiveness and acceptability of CareStart use at the HCW and VMW implementation levels in the field settings, to address the knowledge gap*.

Literature Review

Scopus, PubMed and Web of Science were searched for peer-reviewed studies published between 2001-2017 in the English language using the key terms “malaria or P. vivax”, “G6PD deficiency”, “RDT testing”, “CareStart RDT G6PD testing”, Point-of–care systems”, “healthcare worker or malaria village worker training”, “Primaquine”, “PQ”, “Cambodia”, “malaria elimination”. Studies were selected if they addressed or evaluated healthcare provider training and G6PD RDT testing to assess for G6PD*d*. A total of 48 articles met search criteria.

Von Fricken et al. (2014) conducted a study to test the sensitivity, specificity, positive predictive value (PPV), and negative predicative value (NPV) of ***2nd generation*** CareStart™ in the Haitian community. They screened a total of 456 participants, 267 females and 189 males all healthy subjects. The kit generated sensitivity of 90% and NPV of 98.2%. This study concluded that the 2nd generation testing kit was highly specific and effective at determining those with

normal G6PD enzymatic activity in controlled temperature settings. However, the study only used venipuncture blood samples, not finger prick samples, and only reported the results on a very small number of deficient samples.

Another qualitative study was done by Roca-Feltrer et al. (2014) using (FST) fluorescent Spot test. The study discovered that G6PD***d*** prevalence rates and enzyme activity in malaria patients are consistent with those in healthy individuals. This is important as these findings suggest that testing for deficiency can be done at the time of acute malaria or before the diagnosis of malaria is made. In addition, Khim et al. found that 2nd generation CareStart™ reliably detected deficiencies if the enzymatic activity was less than 30%. The results yielded a sensitivity of 84.8%, specificity 93.7%, NPV 98.2% and PPV 60% noting that "the low PPVs… were affected by the relatively low prevalence of severe G6PD*d* … in the sample population and should not represent an impediment to the use of this RDT" (Von Fricken et al. 2014). Despite the authors not stating temperature settings of this study, they recommended that HCW and VMW should be equipped with tools to test for G6PD***d***. Both Khim et al and Brenden & Jitthai (2012) agreed that the potential cost of RDT implementation into malaria management might be a deterrent to meeting this goal. None of these tests evaluated the performance of CareStart at the proposed end user level.

Cost

Kyaw et al. (2016) discussed the importance of cost analysis during a pilot study in Myanmar to assess sustainability of the program. The authors concluded that annual cost of supporting similar studies though sustainable in comparison with previous testing kits are affected by the design of study, location (remoteness) and the ability to carry out surveillance activity. In their study, up to 60% of the study finances were allocated to the supervisory work.

Brenden and Jitthai (2012) also conducted a study on *Evaluation of Malaria control in Cambodia*. The study assessed the cost of point of care (field) testing, and suggested that cost should be leveraged based on provinces and not calculated for the whole country as this might increase the overall cost. The authors suggested that the sustainability of VMW should be assessed before training to ensure there is longevity of benefit to the healthcare facilities for those trained. Kyaw et al. (2016) explained that despite the seemingly minimal cost of CareStart™ kit and cost of treatment, (US$ 0.3 per RDT performed and US $0.5 per treated patient) this cost is still not easily attainable for low income earners who make an average of $30 (thirty) dollars to $140 per month (Kyaw et al; Carmichael, 2015).

CareStart™ RDTs

Kim et al's conducted a study in Pailin, west Cambodia using CareStart™ 2nd generation. The test was carried out in a temperature-controlled research setting. At the time, CareStart™ falsely identified thirty-one (31) patients as "normal" when the subjects were G6PD deficient based on the quantitative test done. The sensitivity and specificity was 68% and 100% respectively. In 2012, the ***3rd generation*** of CareStart™ was tested against the lab controlled gold standard (quantitative test), and it showed sensitivity of 100% and specificity at 97.7% compared to the quantitative assay among people presenting with G6PD***d*** and <3.6 U/g Hg enzymatic activity (Roca-Feltrer et al. 2014). Based on the results of this study and other literature reviews, CNM made plans to deploy *this 3rd generation* RDT. Therefore, this study will help CNM implement *3rd generation* CareStart™ if the results of this study can be replicated in the field setting, in the hands of the HCW/VMWs. Roca et al. previously noted that the reliability of CareStart can be compromised if done outside of the manufacturers' specified temperatures of 18 °C to 32 °C (64.4°F- 89.6°F).

G6PD Testing

G6PD***d*** is widespread across malaria endemic areas like Cambodia (up to 26%). WHO; Roca-Feltrer et al. 2014; Harvey and Bell, 2008; and Kim et al. (2011) all emphasized the importance of screening for G6PD***d*** in Cambodia. The authors stressed the importance of ensuring feasibility and establishing appropriate endpoints for evaluating interventions. Roca-Feltrer et al's study included 938 adults with variable G6PD***d*** levels, from moderate to severe. The authors concluded that the newer, more user friendly tests (*3rd generation CareStart*™) can effectively identify G6PD***d*** subjects and aid in the efforts of eradicating *P.v* as it allows treatment for majority of patients with *P.v* malaria, thus lowering *P.v* re-infection rates in the country.

In addition, Khim et al. enrolled 2,408 patients mainly from W. Cambodia in a bid to study their G6PD status. Selected mean age was 26.7 years (range 2–81), ratio of males to females was 3.9:1, of which 40.1% had *P.v*. The mean G6PD activity was 11.6, with close to 14% being G6PD***d*** (335/2,408). This study concluded that deploying PQ in Cambodia should be preceded by PQ safety studies paralleled with evaluations of easy-to-use tests to detect G6PD***d*** (Khim et al. 2013).

Theerathananon, Francois, Zongram & Kanchanakhan (2016) also surveyed 102 subjects for G6PD***d*** screening and assessed various variables including "demographic data, current illness, malaria knowledge, malaria infection history, symptom, medication and prevention by using questionnaire and in-person interview". This study concurred with Khim et al's claim that men are 20% more likely to be G6PD***d*** as opposed to females (13%). The study also indicated no statistical difference in malarial infection between patients with normal G6PD versus those who are G6PD***d***.

Health worker Training (Appendix B)

Seidlein et al. (2013) assessed educational barriers and efficiency in RDT testing as this is crucial for *P.v* elimination. The study showed that the performance of RDTs for G6PD***d*** screening has improved over the years. After training some malaria workers in Cambodia, USAID (2010) concluded that approaching healthcare trainers as future trainers, and instructing them as potential educators yielded the most participation and scored higher post-training in teach-back. USAID noted that engaging the private sector when projects target low-income areas caused a higher turn-out from local healthcare staff and patients. Greater sustainable results were also achieved when healthcare trainees were encouraged to give feedback. As part of their input to healthcare training, the President's Malaria Initiative (PMI) developed several objectives for Cambodia to be met by 2015, a process that is still pending. Some of these objectives include advising the national malaria program on how to "perform risk-benefit analysis to inform decision-making on administering or withholding radical cure when a patient's G6PD status is unknown, … testing for and interpreting G6PD tests and managing risk of hemolysis when PQ is administered when G6PD status in not known" (WHO, 2015). Therefore, PMI supports training of HCW and VMW to carry out the said objectives with the hope of making G6PD RDT testing standard practice for malaria diagnosis and treatment (PMI, 2016).

Theoretical foundation for the development of study

The behavioral change communication (BCC) framework is a methodology that emphasizes the use of dialogue and attempts to explain and predict health behaviors by focusing on the attitudes and beliefs of individuals. To effectively implement the objectives of this study, both BCC and Roll Back Malaria (RBM) initiatives should be engaged (RBM, 2012).

Roll Back Malaria (2012) objectives employ the concepts of BCC methodologies that emphasize the use of dialogue and participation. RBM "uses communication as an overarching term to describe a planned process for influencing actions or responses among specific groups of people" to enhance program effectiveness (RBM, 2012). One of the ways RBM will be utilized is by increasing the health care provider's' knowledge on malaria, G6PD***d***, risks and benefits of PQ treatment, and how to use RDTs for G6PD screening through standardized teaching materials and hands on training (Acosta et al. 2014) (Appendix C).

Behavioral Change Communication (BCC)

The success of BCC goals relies on effective coordination and organization. BCC theoretical framework is useful in bridging the gaps between successfully *P.v* eradication and elimination through the training of both HCW and VMW. BCC promotes individual behavior change, supportive environments… [and is a] key component of many malaria programs (Acosta et al. 2014). BCC is also a collaborative process that helps create a bridge in communicating with the communities. These steps in communication promote positive behaviors and are conducive for positive outcomes. However, some authors are conflicted by studies that have incorporated BCC, as these studies showed inadequate proof in regards to the benefits of BCC. However, Acosta et al. claim that BCC is more beneficial when incorporated with malaria eradication frameworks such as Roll Back Malaria. In the context of BCC, the success of this project relies on the program organizers directly engaging the community and having the HCW/VMW lead in the activities and distribution of educational materials. The benefit of this process relies on the trust and communal bond that already exists between the villagers and malaria workers. As an additional level of complexity, it was unknown at the time what role VMW can play in G6PD screening, and if they can grasp the deployment of RDTs for G6PD without compromise of test

results and thus affecting participant's safety. Hopefully, communication and engaging community involvement might help when the pilot study is implemented (Appendix D).

Identifying Variables

Assessing the strategies that contribute to malaria elimination and improving clinician's adherence to standards of practice based on national guidelines is vital for the project's success. Some of the variables included were demographic data, knowledge on G6PD and PQ, malaria infection history, medication prescribing practices, and effectiveness of education using questionnaire and teach-back (Appendix E); (Figure I).

Methods

Design

Quasi-experimental study was conducted in the *P.v* prevalent villages of west Cambodia, where deployment of PQ is planned by CNM. Two primary reasons for choosing this design are: 1) it was a good fit to answer the proposed research questions; and 2) it was realistic and feasible. The study assessed the feasibility and performance of VMWs and HCWs in using and interpreting the *3rd generation* CareStart™ over an 8-week period. At the workshop, we assessed HCW/VMWs' hands-on-practice using CareStart G6PD RDT. The trainees were observed and evaluated four to six times at the end of the 2-day training, with each G6PD test performed using commercially available G6PD deficient and G6PD normal control samples. Post-training in the field setting, each trainee tested 8-12 G6PD volunteers. VMWs and HCW completed a survey (questionnaire) on day 1, Day 2 and at the end of each G6PD testing day to assess their ability to retain the knowledge they received during training, explore their willingness to use CareStart™ RDT and make PQ treatment decisions based on the RDT results.

Study Sites and Population

This study was conducted in West Cambodia whose ethnic composition is primarily Khmer. The location is relatively poor, agrarian (rice, rubber, corn, vegetables, cashews, tapioca), with almost 75% being farmers and loggers. Cambodians average annual income is approximately 3,735 USD. Most of the people living in this area have either a primary and secondary level of education (Grade 1-6 and Grade 7-9), and statistics show that 100% of individuals aged 15 or older can read and write. (CIA, 2008).

The target population: The study population consisted of two groups: (1) VMWs, HCWs ***(nurses, laboratory technicians)*** and (2) G6PD blood donor (G6PD test) volunteers who reside in these malaria endemic villages of west Cambodia.

Sampling Plan

The study utilized convenience sampling method.

Inclusion criteria:

- HCWs/VMWs working in Cambodia, Oddar Meanchey Province (Anlong Veng and Trapang)
- Healthy male volunteers at least 18 years old. Only male test volunteers were screened, since females can have 2 populations of red blood cells simultaneously, G6PD normal and G6PD***d***, and the distribution of G6PD***d*** and G6PD normal red blood cells (RBCs) is determined at random. CareStart™ kit cannot identify "intermediate" females, who have a mix of normal and deficient cells. RBCs with G6PD normal activity produces G6PD normal result, however following PQ administration, the fraction of G6PD***d*** RBCs are subject to drug-induced hemolysis, which can result in a drop in hemoglobin level (Thriemer et al. 2017; Malar J. 2017). Presently, only quantitative tests can provide

results on the actual G6PD levels and thus reliably identify those females who are G6PD***d*** with activity of 30 - 70% (WHO, 2004).

Exclusion criteria:

- HCWs or VMWs not able to understand spoken and written Khmer
- Patients with severe illness requiring immediate hospitalization and treatment
- HCWs/VMWs not able to stay for the duration of the study

Sample size

Formal sample size calculations are not required for feasibility studies. However, to calculate the ideal sample size, assuming a small effect size (Cohen's d of 0.30) (we did not expect to see greater difference in knowledge score or ability to correctly test and interpret CareStart G6PD RDT results between HCWs/VMW and expert reading as per Khim et al. 2013), the power of 80%, with alpha of 0.05, the study would require G6PD test volunteer sample size of 139. For this study, up to 150 health-worker trainees were recruited and104 enrolled.

However, previous studies conducted in Cambodia reported G6PD prevalence in males of 15%. The sensitivity of G6PD RDT tests to detect G6PD***d*** (<30% enzyme activity) in ideal lab conditions was expected to be 99 to 100% based on Roca-Feltrer (2014). Therefore, to detect a difference in the test sensitivity from 99% (ideal) to 95% (trainees), it required a sample size of 1560 (80% power using 5% two-sided test) and 15% prevalence of G6PD***d*****.** In this population of 15% G6PD***d*****,** to detect 4% difference in the prevalence of G6PD***d*** (15% vs. 11%), when the test is conducted by expert readers (trained study staff) vs. trainees, the minimum sample is 1274 volunteers (80% power, 5% two-sided test).

Setting

West Cambodia, border of Thailand, a malaria-high zone area. The targeted healthcare facilities and village areas have approximately 800-1000 patients seen at these facilities on a monthly basis. Participants were enrolled from the district referral hospital, approximately 11 health centers, 1 Health Post and multiple VMW posts at or near Anlong Veng and Trapang Prasath district, Oddar Meanchay Province in Cambodia. The healthcare facilities have 33 HCW and the province has 174 VMW (covering 50 villages).

Study duration

The entire study was planned to be carried out over a 12-month period to allow for development of training manuals, Data Collection Forms, consent forms, survey forms and training of malaria workers; however, individual participation of HCW/VMW lasted no more than 3 months for trainees, and one day for G6PD test volunteers (Table A).

Recruitment of participants

The Armed Forces Research Institute of Medical Sciences (AFRIMS) team in Cambodia advertised the screening on public notice boards with the authorization of the village chiefs. Meetings were held with the ministry of health and Malaria Elimination program representatives. G6PD test volunteers were screened, consented and enrolled by AFRIMS study team. Patients seen at the health centre who met the inclusion criteria were recruited as G6PD test volunteers by study team and trainees. Trainees were recruited from there working stations (VMW locations and healthcare centres). Each trainee was required to obtain a signed supervisors note to enrol in the study (Appendix I; Appendix F).

Informed consent Process

Written Informed Consent form (ICF) was obtained from HCW/VMWs and every G6PD test volunteer prior to study participation. The completed ICFs were retained by the investigators as part of the study records and a copy was provided to each study volunteer. All ICFs were translated into the local language (Khmer). For the illiterate volunteers, the consent form was read to them in Khmer in the presence of a witness who then signed and dated the consent form in the presence of the participant. The participant then thump printed the ICF in the allocated line (Appendix G).

Intervention

The study was carried out in 2 phases. During Phase 1: training materials were developed and training provided to the HCWS/VMWs using the tools developed. All trainees had to pass the knowledge test (Appendix H). In Phase 2: the trainees screened G6PD volunteers using CareStart™ RDT. All trainees had to correctly read and interpret the results for each test done.

Instrument and Measurement

A Power-Point training manual on G6PD and PQ (dose, risks and benefits) was developed for use at the workshop together with a standardized questionnaire to help assess knowledge, acceptability and perceptions of the trainees in regards to PQ use and CareStart RDT. AFRIMS professional medical linguists translated all questionnaires from English to Khmer. The translations were authenticated and certified. The questionnaires had both structured and semi-structured questions, using 7-point Likert scale with varying responses such as *not willing* to *willing* or *not comfortable to comfortable on a scale of 0 to 7*. The demographics gathered included participants age, occupation, gender, current residence, and level of education (Appendix J).

How to scale and score G6PD RDT

Field-tested G6PD status was based on a-Yes or a-No result depending on color change (Yes/deficient: white color, or No/normal: purple color (Appendix: Figure 2). However, quantitative test was used as a reference to classify volunteers as having a normal or deficient G6PD status based on the lab values (Appendix J).

1) Performance was assessed by:
    i) % of tests interpreted correctly by HCWs/VMWs (RDT colour result meaning deficient or normal)
    ii) % of CareStart™ correctly interpreted (against trained study staff and quantitative test)
2) Feasibility of G6PD RDT use by HCW/VMWs and assess their response to administer hypothetical PQ by:
    a. % of HCW/VMWs stating they would be willing to prescribe/ or give PQ based on the RDT result and based on the counselling information provided *(Not willing =1, willing = 7).* The respondents were also given the option to comment if they answered "If yes" …, how likely) (Appendix J).
3) Acceptability to testing for G6PD and willingness to give PQ questionnaires were based on a 7-point Likert scale. The questionnaires consisted of a total of 10 Likert- scale questions. The scales assessed:
    a) % of HCW/VMWs willing to use G6PD RDT as a routine component of malaria case management. Also measured by responding *(Not-comfortable =1 to Comfortable =7 with using RDTs).*

The questionnaires consisted of 25 structured questions that assessed trainee knowledge on G6PD, CareStart™ and PQ. Each correct answer received one score. The HCW/VMW pre-training

questions were a total of 44 questions and Post-training questions were a total of 42 questions, excluding demographics.

Training and testing for G6PD

HCW/VMWs. A 2-day training was held in Khmer language using "Teaching Manual PowerPoint" that was developed for this project. The class consisted of 104 trainees (HCW/VMWs), but only 94 completed the study. The first day consisted of theoretical training that covered the topics on G6PD deficiency, CareStart™ G6PD RDT, PQ associated risks and benefits, and how to recognize side effect. The teaching manual also covered the current treatment guidelines for malaria in the country. PQ Counseling script (Appendix M) was reviewed with the trainees to aid them in effective counseling of G6PD test volunteers.

Following the theoretical training, a practical hands-on workshop was conducted using CareStart G6PD RDT on 2nd day of workshop (Appendix N). To accurately evaluate the results, *control samples* of known G6PD activity (normal and deficient samples) were purchased from ACCESSBIO (the maker of CareStart™) for use during the hands-on training sessions. The overall goal of the 2- day training and subsequent follow-up was to ensure HCW/VMWs could reliably screen the volunteers and effectively counsel them based on their G6PD results, and make hypothetical treatment recommendations based on the national treatment guidelines (Appendix O).

After the 2-days of training, each HCW/VMW was assigned a total of 16 G6PD files to use in field. Each trainee was assigned an expert observer (who is trained study staff) to evaluate their procedures in the field setting. A Checklist was used to ensure that accurate steps were carried out, plus accurate recording of results; and that questions regarding hypothetical dosing of PQ were answered. CareStart performance was assessed in the hands of HCWs/VMWs, and results

compared against the expert readers(observers) in the field vs expert in lab using RDT, then all the three tests were compared against the quantitative lab test (Pointe Scientific, Inc.) which is the current gold standard "quantitative" method of G6PD screening in the laboratory.

However, the volunteers were not informed of the G6PD RDT results even if the results indicated deficiency until all the quantitative tests were done as a "gold standard" to confirm the results. At the end of the study, every G6PD volunteer who had consented to knowing their G6PD status was informed of their status by a designated study team member.

Trainees also answered a set of questions, using pre and post-training questionnaires. The pre-training questionnaires consisted of 44 semi-structured questions but only 25 knowledge questions were scored for knowledge assessment. The other questions were 7-point Likert scale that assessed participant's perception of their willingness to prescribe/give PQ or to test the G6PD status of patients coming to the health centers with malaria. The post-training questionnaire consisted of 42 questions; the same 25 knowledge questions were scored post training to assess if HCW/VMWs retained the information given during training. This was assessed at 2-days post training, and at two, four, six and eight weeks. The study staff probed HCW/VMWs understanding of each major topic area covered on G6PD and PQ. A checklist was used. Trainees who failed to receive 100% understanding on any given topic received additional instruction on the specific topic missed. HCW/VMW underwent this process and repeat counseling script training until they were able to mark "yes" to every queried section of the counseling DCF (Appendix N).

Blinding

HCW/VMWs were blinded to the (expert's) trained study staff's G6PD test results until both results were recorded and locked to avoid untoward bias. The Trained study staff performed

the test first then observed HCW/VMWs do the test. However, both HCW/VMW and observer were blinded to the quantitative results until RDT results were "locked" in the Data Collection form.

Blood sample collection

Currently, CareStart™ is the only "field friendly" test available for field G6PD testing. It is also less temperature sensitive than other qualitative G6PD tests. Enzyme activity is shown through a visual dye colorization, (white = deficient; purple = normal), but is limited by the lack of a built in control signal. The CareStart™ does not require refrigeration with recommended storage temperature of 18-32°C.

Process of Sample Collection. The blood samples for G6PD RDT testing in the field was collected via finger prick, the CBC and G6PD testing in the laboratory were performed on venous blood samples. All sample collections were performed under sterile technique. For field test using RDT kit, 2µl blood was pipetted from a finger prick, [and from EDTA vacutainers in lab]. The pipetted drop of blood was added to the sample well using the pipette provided. Then, two drops of buffer supplied by the manufacturer was added to the buffer well. The results were then read in 10 minutes according to the manufacturer's guidelines. HCW/VMWs carried out all the procedural steps for testing for G6PD***d***, but the trained study staff performed blood test first, and HCW/VMWs' then performed second. In addition, a 2 ml venipuncture blood sample was obtained from each 'patient' in Vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ) containing EDTA (K2 Ethylenediaminetetraacetic acid) as an anticoagulant, for CBC, lab RDT and quantitative G6PD testing (Von Fricken et al. 2014; Gonzalo et al. 2013). RDTs from the same batch were used for all participants. All field participants were blinded to the results from the spectrophotometric analysis (quantitative test).

Data Collection

Data collected were recorded in data collection forms (DCF) and transferred to the electronic database, which was password protected. Demographic data was gathered using study developed questionnaires. The study collected data consisted of:

a. Questionnaires to assess acceptability of PQ treatment and effectiveness of training, completed by HCW and VMW; and willingness to test G6PD status of patients with malaria, and knowledge assessment.

b. CareStart™ G6PD test results (RDT) from the field and quantitative lab results from a reference laboratory (G6PD, CBC)

Validity and Reliability of Instrument

HCW/VMWs evaluation. Post training, each HCW/VMW performed four to six G6PD RDTs using known control samples (deficient and normal). Trainees were observed by an expert reader (trained study staff) during the workshop training held over a 2-day period. Subsequent to workshop training, HCW and VMWs performed G6PD RDT test in the field setting where they were also observed by trained study staff to ensure correct reading of results.

Reference Standard. The G6PD spectrophotometric quantitative analysis used Pointe Scientific, Inc. (MI, USA) G6PD reagent kit. Samples collected were analysed within 24hours. G6PD activity was calculated and reported using the international units, corrected for haemoglobin levels (Appendix L).

Acceptability survey. At the end of each field day, to help explore trainee' knowledge and understanding of G6PD and PQ, HCW/VMW completed questionnaires regarding their willingness to use the G6PD RDT routinely and acceptability to prescribe/give PQ, and also their understanding of PQ risks (Appendix J).

Data analysis

The analyzed data was retrieved from case report forms (CRF) developed for this study. This raw data was then entered into the electronic database, and transcribed to excel spreadsheet for analysis and reporting using STATA 15 program, SPSS and Graphpad Prism 7. ***p***-values <0.05 indicated statistically significant differences. Sensitivity, specificity, PPV and NPVs for RDTs in the field and lab setting were calculated against the population adjusted median of 30% on reference quantitative test. Descriptive and inferential statistics were also used to analyze data collected. Data was downloaded directly into SPSS once it was recorded for final analysis**.** Test performance at the field was compared between samples collected from the finger prick and from venous samples analyzed in the lab.

The experts assessed trainees' (HCW/VMW) level of understanding about G6PD RDTs and PQ, which was indicated by the percentage of accurately performed and correctly interpreted tests. Any missing data was assumed as failed result. Lost to follow-up was low based on single visits required for the G6PD test volunteers and the trainees worked from their usual working stations.

Data Dissemination. The results of this study will be presented to CNM and AFRIMS. In addition, key findings will be presented to key country stakeholders, during AFRIMS sponsored workshop.

Ethical Considerations

The study received approval by the National Ethics Committee for Health Research of the Ministry of Health of Cambodia and WRAIR IRB. In addition, the protocol was submitted for review by The George Washington University IRB (GW) to assure compliance with any GW institutional policies. We submitted all amendments for review and approval by all institutional

review boards. There were no unanticipated problems involving risks to volunteers or others, and no significant deviations. Informed consent was obtained from the volunteers before inclusion in the study and before any study procedures. The participants' records were kept confidential with limited access to records by study staff only. G6PD volunteer participants were informed of their G6PD status based on agreeing to receive results signed on the consent form. Result received were based on the gold-standard quantitative test results.

Results

From December 2017 to February 2018, 94 trainees completed the 2-day training workshop, including 21HC staff and 68 VMWs. In this eight-week period, 960 adult male volunteers were assessed for G6PD deficiency. The median age of the trainees was 37 years, with a range of 18 to 69 years old (Appendix Q).

I. Knowledge Assessment and Acceptability Survey

Trainee knowledge about G6PD and PQ was assessed based on their professional titles, educational levels and years of experience at their current position. At baseline, nurses had the highest knowledge score of 56%, but all the trainees showed improvement in their knowledge scores from pre- to post- training, with statistical significance in results post training ($p<0.001$).

Table 1. Knowledge score: Pre and Post-training based on trainee background

	Pre-Training % mean score of each profession	**Post-Training % mean score of each profession**	**Statistics, p value**
Trainee profession			
• VMWs (n=68)	34%	88.71%	
• Nurses (n=21)	56.4%	90.0%	
• Other HC workers (n=5)	34%	96%	
TOTAL (t, p-value)			**t=16.6 p <0.001**
Trainee level of Education			
• College graduates	53%	95%	
• High school graduates	48.9	92.4	
• Primary	33%	89.8%	

• Secondary	38%	84.4%	
TOTAL (t, p-value)			**t= 16.30, p <0.001**
Years in Experience	**% mean pre-training**	**% mean post-training**	
< 1 year	38%	91.33%	
1- < 3 years	37.5%	89.5%	
3- < 5 years	41%	78.67%	
>=5 years	37.33%	91. %2	
Total (t, p-value)			**t= 16.30, p< 0.001**

Pre-training, knowledge scores based on education level indicated that college graduates had the leading percentage mean score of 53% with primary-level educated volunteers scoring 33%. Post-training, college graduates had mean score of 95%, volunteers with secondary school education scored at 84.4%, and primary level scoring 89.8%, which was not statistically significant between the groups. VMWs with primary school level education, and 1 to 3 years of experience, scored the highest (p=0.38). Post training, scores for willingness to test for G6PD deficiency, and comfort in prescribing PQ for *Pf* and *P.v* significantly improved. In relation to years of experience, there were no statistical differences in the mean knowledge scores based on profession (p=0.57) and education level (p=0.38).

Table 2. Willingness to perform the test and level of comfort in giving treatments

	Pre-Training	Post-Training
Willingness to test for G6PD when a patient is diagnosed with malaria	56.3% (53/94)	80.8% (76/94)
Level of comfort in giving PQ based on G6PD RDT results	39.3% (37/94)	72.3% (68/94)
Level of comfort in giving PQ to a patient with *P.v* and normal G6PD test	36.1% (34/94)	80.0% (76/94)
Willingness to give a patient PQ single dose for *P.f* without G6PD screening	20.2% (19/94)	84% (79/94)

II. CareStart G6PD RDT Results

Calculating the G6PD*d* <30% cut-off of adjusted male median: 1,127 adult males with at least 18 years of age were included in analysis. The G6PD median value (IQR) was 8.30 (7.11, 9.61). Mean (±SD) of G6PD activities was 7.72 (± 3.35 U/g Hb. Nineteen males who had G6PD quantitative value equal or less than 10% (0.83 U/g Hb) were exclude in order to calculate "adjusted G6PD male median value". Adjusted G6PD male median value (IQR) was then estimated from 1,108 volunteers was **8.35** (7.22, 9.655). **G6PDd was defined as <30% of adjusted male median value which is <2.51" U/g Hb.** The distribution of G6PD enzymatic activity of our sampling population according to lab spectrophotometry ranged from 0.05 to 22.57. Sensitivity was described as the probability that the RDT test classified an individual as G6PD*d*, who is also classified as deficient by the quantitative assay; detecting <30% of enzymatic activity (<2.51U/g). Specificity was described as RDT results concurring with lab results as G6PD normal (>2.51U/g).

CareStart G6PD RDT Results. We used IBM SPSS to analyze G6PD RDT test results. Of the 960 volunteers, 156 (16%, 95% CI 14-18) were G6PD deficient based on a quantitative test activity threshold of 30%. The results of field-tested CareStart™ were compared with the spectrophotometric methods in the laboratory at the threshold of 30% adjusted male median activity. There was no statistical difference in test sensitivity, specificity, PPV and NPV for CareStart™ at the field site (**96.53%,** 95.69%, 80.81%, 99.32%) compared to the results by the expert in the same field conditions (**95.86%,** 97.40%, 87.42%, and 99.21%), and **93.79%,** 98.96%, 94.44%, 98.83% for experienced laboratory study staff, with no statistical difference among the groups. HCW/VMWs classified 35/960 (3.64%) volunteers incorrectly as being G6PD***d*** (when they were G6PD normal), compared to 20/960 (2.08%) expert in the field, and 8

(0.8%) by the expert in the lab. HCW/VMWs missed to identify 5/960 G6PD***d*** volunteers, compared to 6/960 misclassifications by the experts in the field, and 9/960 by the expert in the laboratory (Appendix P).

Discussion

This is the first study that has evaluated CareStart™ G6PD RDT test in the field settings in Cambodia in the hands of village malaria workers and other health care workers, who were mostly nurses working at health centers. This is also the first report of the established normal G6PD activity levels based on Point Scientific Quantitative Test, which is currently the recommended reference test following discontinuation of the Trinity kits from production. No prior studies evaluated community perception on PQ, G6PD screening, and provider comfort with prescribing PQ based on RDT results using Carestart™, the only point-of-care testing available. The training also allowed us to assess how the perceptions about PQ risk and limitations of G6PD screening can change, which is important in rolling out radical cure treatment for *P.v* in Cambodia.

The results of the study showed that trainees showed much higher willingness to screen patients for G6PD deficiency following the 2-day workshop training likely due to increased understanding of the benefits and risks of PQ. CareStart G6PD RDT offered at point-of-care testing performed reasonably well even at the peripheral level of care, at the community level by village malaria workers. Making this test available at this level could increase access to testing and radical cure treatment. However, it remains to be seen if VMWs can be given this new responsibility to screen patients for G6PD deficiency and prescribe PQ which is not without risks. Based on our results, the tendency of trainees was to over diagnose G6PD deficiency,

which explains the lower PPV. However, no group was free from missing the diagnosis of G6PD deficiency.

The implication of this is important for the policy makers as PQ deployment will not be without risks, even with G6PD screening. It is possible that new generation tests for G6PD deficiency could become more reliable, but this is yet to be demonstrated in the field trials for the next generation G6PD kits that are in development. CareStart RDT is currently the only point-of-care test that is available commercially and that can provide an inexpensive way to test male patients for G6PD deficiency with little time required to make trainees proficient in interpreting the test results.

Kim et al. (2011) study indicated that CareStart had 90% sensitivity with a NPV of 98.2% in the controlled laboratory settings. Our study showed higher sensitivity and better NPV of 99.29, likely reflective of the training provided, which was effective across all education levels and years of experience. Given the acceptable level of performance in the field settings even among novice users post training, it makes the results widely applicable for implementation in this malaria endemic areas. Implementation of the CareStart kit may be feasible at the community level, with limited training required and with added benefit of the low cost of CareStart in comparison to the quantitative tests that can only be done in temperature controlled laboratories.

Study Limitations

Wide applicability of our findings should be taken with caution when similar approaches are considered for implementation in other countries. Some of the trainees had difficulty with reading the digital clocks, which is required to indicate the reading of results at10 minutes mark. This resulted in the switch from digital clocks to using timers, with audible sound at the 10 mins

mark. The smaller size timers were difficult to set and were later replaced by another brand of the timers. These details in implementation should not be overlooked.

In regards to pre- and post- training questionnaires, some HCWs/VMWs stated that it was too long and therefore, took long to complete. They also stated that despite the fact that the questionnaires were translated into the local language (Khmer), some of the questions were still reported as "technical" and required explanation by the (experts)study staff, which could have introduced some degree of bias in the results. The trainees also provided us with feedback that the pictures in the counseling script were more helpful than reading of the script to the G6PD test volunteers. Most trainees and G6PD test volunteers found the pictures and explanations of the pictures sufficient for understanding the concepts, and a better method for them to learn as opposed to reading the whole script. One limitation to using CareStart™ was lack of the control line, which was previously recognized as a significant limitation from prior studies in the lab setting. In this case, supervision of testing will be beneficial, possibly in the form of re-training to make sure that trainees read the tests correctly, follow proper procedures, and record the results within the specified time of 10 minutes; otherwise, delayed reading can result in wrongful interpretation of the results.

Implications for practice, policy and research

The outcomes from this study show that with limited training, novice users can become quite proficient at reading and interpreting CareStart™ RDT results. This test is only suitable for use in male patients. However, with males being the predominant population affected by malaria in Cambodia, deployment of this test can increase access to radical curative doses of PQ. Increasing access to G6PD screening will allow more patients to be cured from *P.v* and this can help with prompt implementation of malaria elimination goals that have been set to eliminate *P.v*

in Cambodia by 2025 (MESA, 2016). The piloted G6PD counseling tools were highly effective and could be modified and adopted for wider use in clinical practice in Cambodia.

Conclusions

CareStart RDT shows promising results in terms of test sensitivity and ruling out G6PD deficiency. This study indicated that point-of-care screening for G6PD deficiency at the community level is feasible. However, incorrect interpretation of a few G6PD RDT test results will occur, and monitoring must be put in place before deployment of PQ treatment in Cambodia. Willingness of patients to be treated with PQ despite risks will likely result in lower cases of *P.v* malaria recurrence, and malaria morbidity from frequent *P.v* relapses in Cambodia.

Disclaimer: Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25.

This project was supported by a team of AFRIMS staff in Thailand and AFRIMS staff and field team in Cambodia:
Bertha Wojnarski[1,2], Chanthap Lon[1], Darapiseth Sea[1], Somethy Sok[3], Sabaithip Sriwichai[1], Soklyda Chann[4], Sohei Hom[4], Sokna Ly[4], Chandara Sok[4], Samon Nou[4], Pheaktra Oung[4], Nareth Kong[5], Vannak Pheap[3], Khengheang Thay[5], Vy Dao[3], Panita Gosi[1], Worachet Kuntawunginn[1], Jimee Hwang, Mitra Feldman[1], Nillawan Buathong[1], Mali Ittiverakul[1], Nichapat Uthaimongkol[1], Threechada Boonchan[1,] Huy Rekol[5], Lek Dysoley[5], Michele Spring[1], Philip Smith[1], Mariusz Wojnarski[1], Mar Fukuda[1]

1.US Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, 2. The George Washington University, School of Nursing, Washington, DC, 3. Ministry of National Defense, Department of Health, Phnom Penh, Cambodia, 4. US Armed Forces Research Institute of Medical Sciences, Phnom Penh, Cambodia, 5. National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia
*: equal contribution

References

Acosta, A., Keating, J., Storey, J, D., Koenker, H., Prosnitz, D., Doyle, S., & Butts, J. (2014). Roll Back Malaria: Communication Community of Practice. Retrieved from http://www.healthcommcapacity.org/wp-content/uploads/2014/11/RBM-CCoP-Reporting-Guide-for-Malaria-Communication-Evaluations.pdf

Amoah, L. E., Opong, A., Ayanful-Torgby, R., Abankwa, J., & Acquah, F. K. (2016). Prevalence of G6PD deficiency and plasmodium falciparum parasites in asymptomatic school children living in southern Ghana. Malaria Journal, 15(1) doi: 10.1186/s12936-016-1440-1

Brenden, N., & Jitthai, Nigoon. (2012). Evaluation of the Malaria Control in Cambodia Project. U.S Agency International Development (USAID). Retrieved http://pdf.usaid.gov/pdf_docs/pdacu493.pdf

Carmichael, R. (2015). Cambodia Raises Monthly Minimum Wage to $140. Retrieved from http://www.voanews.com/a/cambodia-raises-monthly-minimum-wage-to-140/2996627.html

Chu, C. S., Bancone, G., Moore, K, A., Win, H, H., Thitipanawan, N., Po, C., Chowwiwat, N., …White, N. (2017). Hemolysis in G6PD Heterozygous Females Treated with Primaquine for Plasmodium Vivax Malaria: A Nested Cohort in a Trial of Radical Curative Regimens. Plos Med 14(2): e1002224. Doi: 10.1371/journal.pmed.1002224

Gonzalo, D, J., Satyagraha, W, A., Anvikar, A., Baird, K., Bancone, G., Bansil, P., & …Carter, N. (2013). G6PD testing in support of treatment and elimination of Malaria: recommendations for evaluation of G6PD tests. Malaria Journal (12):391. Retrieved from doi: 10.1186/1475-2875-12-391

Harvey, S, A., & Bell, D. (2008). Malaria: How to use a rapid diagnostic test (Generic Pf). A Guide for training at a village and clinic. World Health Organization level. Retrieved from http://www.who.int/malaria/publications/atoz/generic_pf/en/

Jalloh, A., Tantular, I, S., Pusarawati, S., Kawilarang, A, P., Kerong, H., Lin, K., Ferreira, M, U., Matsuoka, H., Arai, M,, Kita, K,, Kawamoto, F. (2004) Rapid epidemiologic assessment of glucose-6-phosphate dehydrogenase deficiency in malaria-endemic areas in Southeast Asia using a novel diagnostic kit. Tropical Med International Health, 9:615–623.Retrived from https://www.ncbi.nlm.nih.gov/pubmed/15117307

Khim, N., Benedet, C., Kim, S., Kheng, S., Siv, S., Leang, R., & ... Ménard, D. (2013). G6PD deficiency in Plasmodium falciparum and Plasmodium vivax malaria-infected Cambodian patients. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23714236

Kim S, Nguon C, Guillard B, Duong S, Chy S, et al. (2011) Performance of the CareStart™ G6PD Deficiency Screening Test, a Point-of-Care Diagnostic for Primaquine Therapy Screening. *PLOS ONE* 6(12): e28357. https://doi.org/10.1371/journal.pone.0028357

Kim, S., Nguon, C., Guillard, B., Duong, S., Chy, S., Sum, S., … Menard, D. (2011). Performance of the CareStartTM G6PD Deficiency Screening Test, a Point-of-Care Diagnostic for Primaquine Therapy Screening. PLoS ONE 6(12): e28357. doi: 10.1371/journal.pone.0028357

Kyaw, S, S., Drake, T., Thi, A., Kyaw, M, P., Hlaing, T., Smithuis, F, M., White, L, J., & Lubell, Y. (2016). Malaria community health workers in Myanmar: a cost analysis. DOI: 10.1186/s12936-016-1102-3

Malaria Eradication Scientific Alliance (MESA) (2016). Cambodia Malaria Elimination Action

Framework (2016-2020). Retrieved from http://www.malariaeradication.org/knowledge-hub/cambodia-malaria-elimination-action-framework-2016-2020

National Center for Parasitology, Entomology and Malaria Control (CNM). (2011). National Strategic Plan Elimination of Malaria in Cambodia (2011-2025). Retrieved from http://www.cnm.gov.kh/userfiles/file/N%20Strategy%20Plan/National%20Strtegyin%20English.pdf

Presidential Malaria Initiative (2016). Fighting Malaria and Saving Lives: Operational Research. Retrieved https://www.pmi.gov/how-we-work/cross-cutting-technical-areas/operational-research-reports?select=research#filterPjts

Population Reference Bureau (PRB) (2016). Fewer Malaria Cases in Cambodia. Retrieved from http://www.prb.org/Publications/Articles/2002/FewerMalariaCasesinCambodia.aspx

Roll Back Malaria Partnership (RBM), (2012). The Strategic Framework For Malaria Communication at the Country Level 2012–2017. Retrieved from http://www.fhi360.org/sites/default/files/media/documents/Strategic-Framework-Malaria-Communication-RBM.pdf

Roca-Feltrer, A., Khim, N., Kim, S., Chy, S., Canier, L., Kerleguer, A., & ... Menard, D. (2014). Field trial evaluation of the performances of point-of-care tests for screening G6PD deficiency in Cambodia. Plos One, 9(12), e116143. Retrieved https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4277465/

Seidlein, L., Auburn, S., Espino, F., Shanks, D., Cheng, Q., McCarthy, J. … Price, R. (2013). Review of key knowledge gaps in glucose-6-phosphate dehydrogenase deficiency detection with regard to the safe clinical deployment of 8-aminoquinoline treatment regimens: a workshop report. Malarial Journal. 12:112. DOI: 10.1186/1475-2875-12-112

Sovannaroth, S., Roco-Feltrer, A., Vinjamuri, S, B., Bouth, D, M., Lek, D., Rashid, M, A., … Menard, D. (2016). Plasmodium vivax Malaria in Cambodia. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5201228/

Theerathananon, W., Francois, J, J., Zongram, O., & Kanchanakhan, N. (2016). Prevalence of G6PD deficiency in Malaria endemic area: Case Study in Bongti Sub-district, Sai Yok district, Kanchanaburi Province, Thailand. Retrieved from https://www.researchgate.net/publication/267987299

United States Agency for International Development (USAID) (2010). Report on Training of Health Workers on Malaria Case Management and Treatment Policy Rollout in Coast Province. Retrieved from http://pdf.usaid.gov/pdf_docs/PNADU433.pdf

United States Agency for International Development (USAID) (2010). Malaria Control in Cambodia: Building a Community-based Response. Retrieved from http://www.urc-chs.com/sites/default/files/MalariaControl.pdf

Von Fricken, M, E., Weppelman, T, A., Eaton, W, T., Masse, R., Beau de Rochars, M, V, E., & Okech, B, A. (2014). Performance of the CareStart Glucose-6-Phosphate Dehydrogenase (G6PD) Rapid Diagnostic Test in Gressier, Haiti. American Journal of Tropical Medicine and Hygiene, 91(1), pp. 77-80. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4080573/

World Health Organization. (2003). Malaria Rapid Diagnosis: Making it Work. Retrieved from http://www.who.int/malaria/publications/atoz/rdt2.pdf

World Health Organization (2004). Informal Consultation on Laboratory Methods for Quality

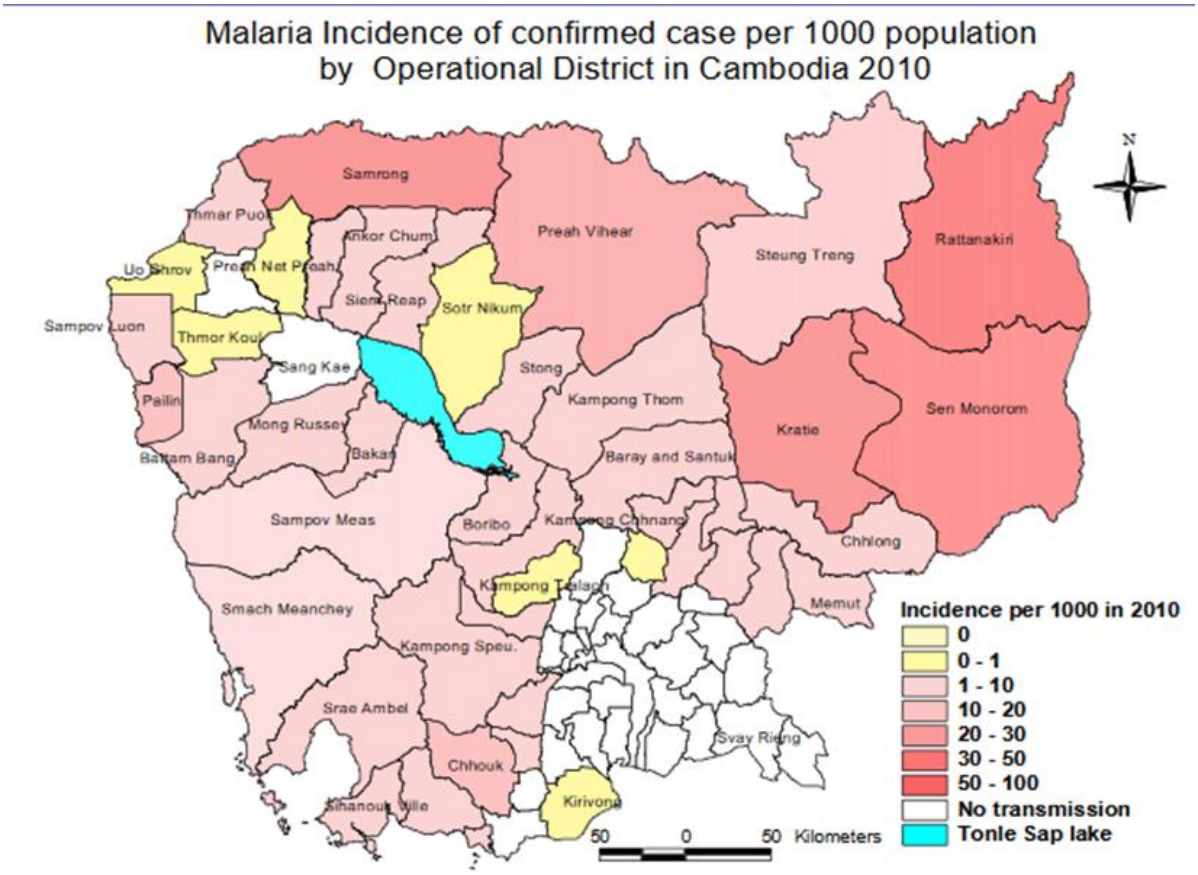
Assurance of Malaria Rapid Diagnostic tests. Retrieved from http://www.wpro.who.int/malaria/internet/resources.ashx/RDT/docs/pdf_version/QARDT_Jan05.pdf

World Health Organization (2015). Malaria Rapid Diagnostic Test Performance: Summary Results of WHO product testing of Malaria RDTs: rounds 1-6 (2008-2015). Retrieved from apps.who.int

WHO. (2015). Guidelines for the treatment of malaria (3rd edition). Geneva, World Health Organization (WHO) 2015. Retrieved from http://www.who.int/malaria/publications/atoz/9789241549127/en/

WHO. (2015). Control and elimination of Plasmodium vivax malaria –A technical brief. Geneva, World Health Organization (WHO) 2015. Retrieved from http://www.who.int/malaria/publications/atoz/9789241509244/en

Appendix A: Current Map of Malarial Incidence in Cambodia



Appendix B: Training Manual (English, also translated to Khmer).

PowerPoint Slide that covered several Objectives

- Provided a rationale for recommending treatment with Primaquine
- Educated on risks and benefits of Primaquine treatment following G6PD deficiency screening
- Reviewed recommendations on who needs G6PD screening
- Provided training on the correct use and interpretation of RDTs for G6PD deficiency screening
- Provided an overview of the current limitations of available point-of-care testing for G6PD deficiency
- Trained healthcare workers using a formulated “Counseling Script” on Primaquine and G6PD screening

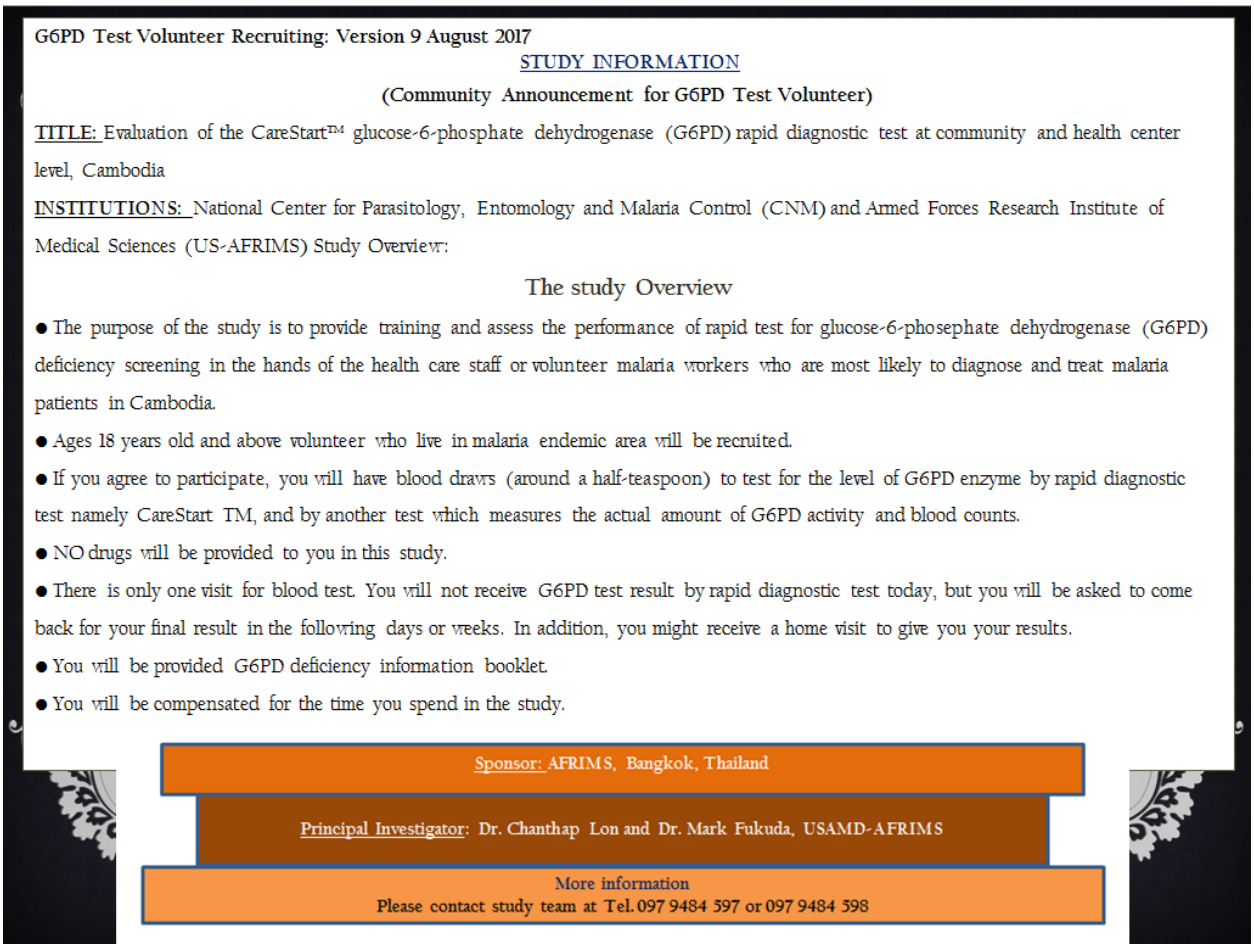
Appendix C: Communication planning model developed by NIH and CDC (RBM, 2012)

Appendix D: Variable Table with Theoretical and Operational Definitions

Variable	Theoretical Definition	Operational Definition
	Demographics	
Gender	Patient's biological sex	1=Male 2=Female
Age	Chronological age in years	Years of age as defined as current date minus birth date. Defined as: 1=18-<30, 2=30-<45, 3=45-<60, 4=>60
Race	Biological or genetic traits based on various sets of physical characteristics	1=Caucasian 2=Black 3=Hispanic 4=Asian 5=other
Educational level	Level of educational level completed	1=Grade school 2=High school 3=College 4=Graduate school
	Dependent	
Primaquine 1	Recommended as part of treatment for *P.vivax* (radical cure course)	Yes=1 No=2
Primaquine 2	Recommended as part of treatment to block malaria transmission (single dose)	Yes=1 No=2
Perception of threat	Based on demographics and recent history of malaria	Is patient G6PD deficient Yes=1 No=2
Susceptibility to malaria	Based on demographics and recent history of malaria	Yes=1 No=2
RDT G6PD test results	Concordance of RDT results by VMWs and trained study staff	Yes=1 No=2
Effective counseling of PQ safety	Effective communication on PQ safety, as assessed by volunteers level of understanding of risks and benefits	1=Volunteers not able to clearly state side effects of PQ or signs or symptoms of hemolysis 2=Volunteer able to cite one symptom of hemolysis but reports lack of understanding

		when to seek medical career 3=Volunteers reports understanding of the side effect profile of PQ, can state when he/she needs to seek medical care, and can recite clearly most signs and symptoms of hemolysis
Healthcare training	Effective teach-back	Yes=1 No=2
Independent		
Level of education/literacy	Education level of trainee	1=less than 12th grade 2=12 grade or higher 3=Literate (able to write and read) 4=Not able to write or read Khmer 5=VMW 6=Nurse 7=Physician
Training module	Type of training received and teach-back	1=hands-on training with the use of RDTs only 2=attended comprehensive training workshop 3=Video training
Malaria *P. vivax* control and prevention practices	Healthcare staff education (nurses, medics, malaria village workers) and patients are counselled on malaria management	Have mosquito nets Yes=1 No =2 Tested for malaria/G6PD deficiency Yes=1 No =2
RDT testing	G6PD screening with RDTs	2=Storage conditions 3=Equipment and reagents used correctly 4=Time to results 5=Interpretation of results

Appendix F: Study Information-Community Announcement



G6PD Test Volunteer Recruiting: Version 9 August 2017

STUDY INFORMATION

(Community Announcement for G6PD Test Volunteer)

TITLE: Evaluation of the CareStart™ glucose-6-phosphate dehydrogenase (G6PD) rapid diagnostic test at community and health center level, Cambodia

INSTITUTIONS: National Center for Parasitology, Entomology and Malaria Control (CNM) and Armed Forces Research Institute of Medical Sciences (US-AFRIMS) Study Overview:

The study Overview

- The purpose of the study is to provide training and assess the performance of rapid test for glucose-6-phosephate dehydrogenase (G6PD) deficiency screening in the hands of the health care staff or volunteer malaria workers who are most likely to diagnose and treat malaria patients in Cambodia.
- Ages 18 years old and above volunteer who live in malaria endemic area will be recruited.
- If you agree to participate, you will have blood dravrs (around a half-teaspoon) to test for the level of G6PD enzyme by rapid diagnostic test namely CareStart TM, and by another test which measures the actual amount of G6PD activity and blood counts.
- NO drugs will be provided to you in this study.
- There is only one visit for blood test. You will not receive G6PD test result by rapid diagnostic test today, but you will be asked to come back for your final result in the following days or weeks. In addition, you might receive a home visit to give you your results.
- You will be provided G6PD deficiency information booklet.
- You will be compensated for the time you spend in the study.

Sponsor: AFRIMS, Bangkok, Thailand

Principal Investigator: Dr. Chanthap Lon and Dr. Mark Fukuda, USAMD-AFRIMS

More information
Please contact study team at Tel. 097 9484 597 or 097 9484 598

Appendix G: Informed Consent Form (Translated to Khmer)

I. STUDY INFORMATION AND INFORMED CONSENT FORM

(Trainee volunteers)

PROTOCOL TITLE: Evaluation of the CareStart™ glucose-6-phosphate dehydrogenase (G6PD) rapid diagnostic test at community and health center level, Cambodia

INTRODUCTION

You are being asked to participate in a research study entitled "Evaluation of the CareStart™ glucose-6-phosphate dehydrogenase (G6PD) rapid diagnostic test at community and health center level, Cambodia". In this study, you will be trained to use a new kind of rapid diagnostic test (RDT), called CareStart™, a test that is similar to a malaria rapid diagnostic tests. The test requires a finger prick to collect a small amount of blood from volunteers who desire testing in order to determine the G6PD activity (normal or reduced) in their blood. Since the testing for G6PD deficiency is being recommended by national malaria program, in order to provide antimalarial drug Primaquine safely, we need to identify suitable tests that are easy to use and can be used in the field settings. Screening of patients for G6PD deficiency is needed in order to minimize the risks from taking Primaquine. So far CareStart™ has been used successfully only in special settings, like a lab or hospital. It is hoped that this research will give us better information about how well the test works when used at a health center or at the village level when the test is performed by a village malaria worker (VMW) or by military medics. We hope this research will assist the Cambodia Ministry of Health in determining if G6PD testing can be done properly at the community level. This study will help establish the appropriate level of care delivery where this test can be performed successfully following appropriate training.

STUDY DESCRIPTION Your participation will involve taking part in the 2-day training workshop focusing on testing for G6PD deficiency, use of Primaquine for malaria treatment and how to conduct the G6PD screening with RDTs. We anticipate that approximately 104 VMWs and other health workers will participate in this study. We will conduct training in smaller groups to facilitate learning environment. Following the workshop training, you will be observed conducting the G6PD screening tests on patient volunteers in your usual place of work. Each trainee will test approximately 16 volunteers during the course of study participation, for anticipated total of 1,600 patient volunteers tested by all trainees who participate in this study. Each trainee will also be asked to complete pre- and post- training questionnaires to help us understand current knowledge gaps about the drug Primaquine and about G6PD screening, and the willingness and acceptability of trainees to use Primaquine and G6PD screening tests in their place of work. The feedback we get from this study will help us optimize training materials for healthcare staff who may use Primaquine or G6PD screening tests. Based on the test performance, we will also be able to assess if the available G6PD screening tests (such as CareStart™) are appropriate tests for use in the field settings.

Who is eligible to participate? You have been asked to participate because you are a village malaria worker or health center staff or medic who manages malaria patients, and work within the study location. You should seek permission from your supervisor before you participate in the study. You can participate in this study if: • You are employed as village malaria worker or healthcare staff likely to diagnose and/or treat patients with malaria (nurses, medics, physicians are eligible) • You are able to participate in the 2-days workshop training • You agree to a minimum of 4 days of G6PD screening/testing of volunteers • You have no plans to move outside study area over the course of next 3 months You cannot participate in this study if: • You

have known color blindness • Judged by the investigator to be unsuitable for study participation In order for you to be enrolled in the study, you will also have to sign the consent form stating your willingness to participate.

What if I do not want to take part in this study? Participation in this study is strictly voluntary. If you decide not to participate in this study, you will NOT lose any medical or other benefits for you or your family. If you do not participate in this study, this will not have any negative effect on your ability to continue working at your current place of work.

How long will this study last: Your participation will last approximately 3 months. This includes the 2 days' workshop training and the time needed to recruit sufficient number of volunteers who will be tested by you for G6PD deficiency. You will be asked to test approximately 4-8 patient volunteers in one day, for a total of approximately 16 volunteers, during your study participation. So your active participation is expected to be approximately 6 days (2 days for workshop and 4 days for screening of patients – 4 volunteers per day), spread over the three-month period. These timelines can be adjusted if recruitment is slower than anticipated. You should also know that the test takes approximately 10 minutes to complete, per patient. However, you will also be asked to complete pre- and post-training questionnaires, and may be asked to participate in a group discussion, which will require additional time. However, your time participation on any given day will not exceed 8 hrs.

Study Procedures After enrollment you will be asked to provide information about your prior experience with malaria treatment and asked questions to assess your current knowledge about Primaquine and G6PD screening tests (pre-training questionnaire). Then you will receive training on G6PD, screening tests, and Primaquine. Training will also include hands-on workshop on how to use CareStartTM. After training you will be evaluated using G6PD RDTs

and you will be asked to answer a few questions about the training and training materials. If needed, you will receive re-training before going out to check volunteer patients' G6PD status using the RDTs at your village.

Check volunteer patients' G6PD status Within a few days of completing the training, you will be observed conducting the test on patient volunteers in your usual place of work. You will conduct the test on 4-8 patients per day to complete approximately 16 G6PD screening tests during this study. The total number of patients screened may differ slightly between trainees based on the recruitment of volunteers at each village. If you screen 4 patients a day, it will take 4 days to reach the target of 16. You will also be asked to collect details on patient's age, sex, level of education, and contact information for follow-up visit. Then you will be asked to complete additional post questionnaires about G6PD screening, Primaquine, and RDTs During your study participation you may be asked to participate in group discussions so we can better understand current knowledge gaps and the responses in the questionnaires.

Blood Tests During the Study As part of the study, you (trainee) will be asked to do a finger prick (1 or 2 drops of blood) on another trainee, during the workshop training. This means that during the workshop training, another trainee may practice finger prick on you as well. Following the training, you will be asked to do a finger prick to check volunteer patients' G6PD status using the RDT, during the observation visits by study team. Separate to the finger prick, a study team member will collect additional blood (approximately 2 ml) from the patient volunteer and send it for testing at a lab close to the treatment facility for confirmatory G6PD screening (for comparison of test results to those with RDTs).

Optional You will NOT be photographed and/or videotaped unless you provide us with consent allowing us to do so. This part is optional and not required to be able to take part in this study but

this can be helpful to us in development of future training and education materials.

NOTE: There will be NO photos or videos taken on military installations at any time.

STOPPING THE STUDY The entire study or a part of the study may be stopped at any time based on the judgement of the study researchers, the study sponsors, or the ethical committees.

POTENTIAL RISKS Taking blood from your finger during training will cause a brief sharp pain. The pain is usually mild, and can be described as pinching or pricking your skin with a sharp object only when we try to collect the blood. This discomfort lasts only a short time, usually less than 1 minute. All standard precautions will be taken to reduce any potential risk from accidental exposures to infected blood during finger prick procedure. This risk will be mitigated by requiring that all trainees wear surgical gloves when performing the finger prick on volunteers and this will be strictly enforced.

ANTICIPATED BENEFITS There are no other direct benefits to you from participating in this study other than potentially increasing your medical knowledge. The information from this study may help other people in the future who have malaria, a disease the kills more than 400,000 people worldwide every year. The study will benefit the community as a whole by providing up-to-date information on G6PD diagnosis and malaria treatment which will be provided to the National Malaria Control program, and other appropriate Cambodian government authorities.

SUBJECT RIGHTS Your participation in this study is voluntary. You may withdraw from the study at any time without penalty. You must inform the researcher of this decision as soon as possible. Deciding to leave the study will not influence the availability of future medical care or other benefits to which you are otherwise entitled. You are not waiving any legal rights by participating in this study. If you have any questions concerning the study, you may contact Dr.

Chanthap Lon, Armed Forces Research Institute of Medical Sciences (AFRIMS), No. 5 St. 550, Phnom Penh, Cambodia. Tel.: +855 12 976 799.

OR

Study team contact number 097 9484 597 or 097 9484 598 If you have any questions about your rights as a research participant in Cambodia or wish to speak to a person not associated with this research, you may contact: Dr. Vonthanak Saphonn, Secretary of the Nation Ethics Committee Health Research IRB#1(NECHR: IRB# 3143) #2 Kim Il Sung Blvd, Khan Tuol Kok, Phnom Penh. Cambodia, Tel.: 023 880 345, Fax: 023 880 346, E-mail: research03@nchads.org. The results of this study will be published in scientific journals and the data will be made available to health care officials in Cambodia, but there will be no specific effort to inform you of the results of this study. Your name or other identifying information about you will not be made publicly available except for those trainees who consented to be photographed or videotaped.

MEDICAL CARE FOR RESEARCH RELATED INJURY If you are injured as a **direct result of your participation in this study**, you should inform study team immediately. You will receive medical care for that injury at the appropriate local medical facility, following current standard of care, at no cost. The sponsor of this study is responsible for the medical costs. This medical care provision does not constitute a waiver or release of your legal rights. You will not receive additional compensation for this injury. If you have questions about this medical care, talk to the Principal Investigator for this study.

COMPENSATION For VMWs, HC staff, and/or medics the compensation will be 56,000 Cambodian Riel (approximately US $14/day) for each day of training (2 full days) and the same amount (~$14) on the days they are screening volunteers for G6PD deficiency (estimated as 4

days for each trainee). **If the trainee requires more than 4 days to screen 16 volunteers, the compensation on days of screening will be limited to 4 days only.** The compensation for VMWs/HC staff and medics who will screen volunteers for G6PD deficiency, takes into consideration the government set local per diem rates that is approximately $14/day. Compensation for all volunteers also takes into consideration lost earnings, meals and incidentals arising from participation.

CONFIDENTIALITY Your research record and personal information such as name, address, date of birth, etc., will be kept confidential and will not be made available to individuals or organizations who are not involved with the study, unless required by applicable law. If the results of this study are published in the medical literature, your identity will not be revealed. By signing this consent form, you are granting permission for your medical records collected under this study, to be reviewed by the study staff, authorized representatives of ethical committees who have approved this research, other regulatory authorities reviewing this study, and the study Sponsor's representatives. All of these are responsible to ensure that the research is conducted in an ethical, safe, and legal manner to protect your rights and your safety. Your record includes participation date, study code, name, age, sex, contact address and the reason if you cannot participate in the study will be kept in a secure location by the investigators for 5 years at AFRIMS office in Cambodia once the study is completed. The study data may be entered into an electronic database, which will be password protected. The review of these medical records may be in respect to this study and any further research that may be conducted in relation to it. Your records may be reviewed, as described above, even if you withdraw from the study. For military volunteers (medics), complete confidentiality cannot be guaranteed because information bearing on a soldier's health may be required to be reported to appropriate medical or command

authorities. However, this risk is mitigated as no highly sensitive information is being asked of the volunteers except general question about overall health status.

CERTIFICATES OF CONSENT I, the undersigned, have read, or have had read to me in language understandable to me, the above information. Before signing this consent form, a member of the research team has explained to me in detail, and I understand, the objectives, procedures, and risks possibly associated with this study as well as the benefits. The research team has answered all my questions willingly, clearly, without concealment and to my satisfaction. I have a right to withdraw from the study at any time. My participation in this research is voluntary. I can choose not to participate. Refusal or discontinuation of my participation will involve no penalty or affect the standard care that I would otherwise expect to receive. If I join this project, I can ask the researcher or his representative at any time about the project. I will be provided with a copy of this consent form. I understand that I can choose to be photographed and/or videotaped during this study. If refused, I can still take part in the study. The research team has confirmed to keep any data concerning myself confidential and my name and identity will not be revealed in any reports. Only authorities involving in the support or monitoring of this project may be permitted to examine my data. I consent of free will for the following (check one):

All required procedures to include permission to be photographed and/or videotaped

All study procedures WITHOUT being photographed and/or videotaped

_____

_____ _____

Signature of the volunteer Date Print name I have explained the purpose of this study to the volunteer and have answered all of the questions. _____

_____ _____

Signature of person obtaining consent Date Print name

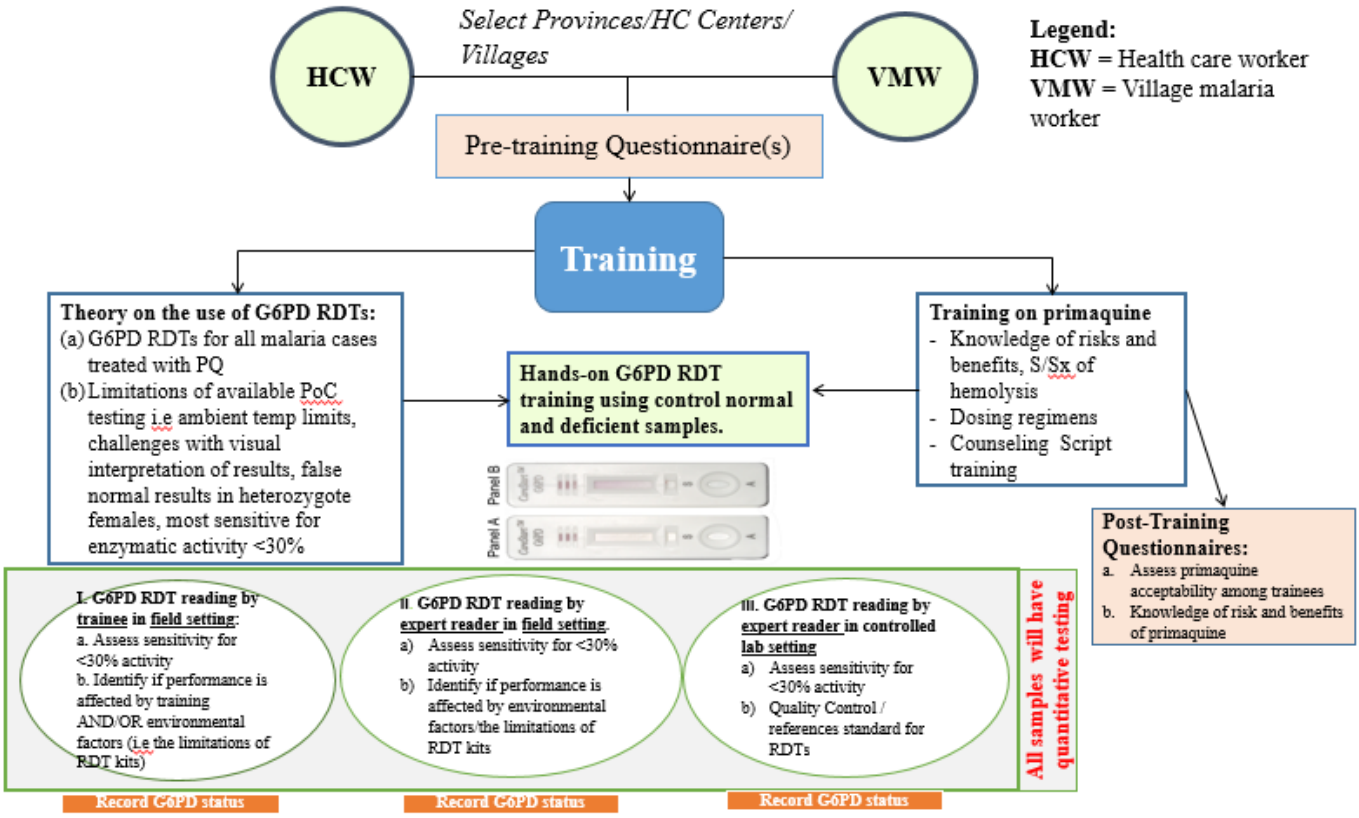
WITNESS I have witnessed the accurate informed consent process to the volunteer, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely. _____

_____ _____

Signature of the witness /ombudsman Date Print name

Copy provided to the participant _____ (*initials of person obtaining consent*)

APPENDIX H: Training Steps



Appendix I: Supervisor Permission Approval form (Khmer).

Translated from English Supervisor Approval Form-Trainee: version 13 October 2017

ការអនុញ្ញាតរបស់អនកគ្រូ

ខ្ញុំង់ចូលរួមកម្មវធីសិក្សាន េះលមាចុំងន ើងថ “ ការតម្េលលើលតស្របស្សស្រីក្ខិភីឌី

(G 6PD)ម្យប្រក់ គ្រ់ ឈាគ្រមលោតាស្គម៉ែ េ ងមណ្ឌ លស ខភា ក ង ន គ្រ ស កម ព

ជា ’ ។

● ខ្ញុំតស្សពិ ធីយឿលវិភាន តស្ររីណ៍សតា ់ឿសិក្សា ន យន ឿជា ឿចូលរួមរបស់ខ្ញុំង

មិ ឿ េល់រួនា ីរបស់ខ្ញុំទ។

● ខ្ញុំពិ ធីយន ើងវិញ វឿសិក្សា ធឿលវិភារបស់ខ្ញុំយ ងនមបញ្ហេឿ េចៀងនត្ន ខ្ញុំវ

ឿឿរអ ញ្ញាពីេនៃមើបីចូលរួមកម្មវធីសិក្សា។

● ចៅប់ចមលងន ទត់ន េះ ងតស្របាដា ៅក្ខងឯក្សារឿសិក្សារបស់ខ្ញុំ ។

— —

រថនលៀរបស់ស៊ីខឿម ឿលបរិនចេទ(d /MM/y)

— —

ន ម េះបស់ស៊ីខឿម

— —

— — — — — — — — — — — — — — — — —

អនកគ្រូតាបា ធ ឹក្តគ្រ:

● ខ្ញុំល់ថ ឿរចូលរួមកម្មវធីសិក្សាន េះ ងតស្រឿរនពលនវលាបស់បុគគលិកនលើអាមាផលប េ

ពា់ េលឿរអ វរត ៍របស់ពួកគ

● ខ្ញុំ ញ្ញានលឿរចូលរួមរបស់បុគគលិកនលើកម្មវធីសិក្សាន េះ

— —

រថនលៀរបស់អនកគ្រូ ់ត ឿលបរិនចេទ

Appendix J: Pre-Training and Post Training questionnaires

TRAINEE (HCWs/VMW/Medic)

(Complete PRIOR to training)

Visit Date: _____

(dd/MM/yy)

[Please Check (√) or Mark (X) to select your answer; if more than one answer can be selected, it will be stated in the question; for all other, only ONE answer can be selected]

NOTE: You are not expected to know the answers to all the questions. If you do not know the answer, you can select, I do NOT KNOW. The information you provide will help us develop future teaching materials to address the knowledge gaps.

DEMOGRAPHICS

1. Age: _____ (Years)

2. Sex: ☐ Male ☐ Female

3. Occupation:

☐ Physician ☐ Nurse ☐ Lab technician
☐ Village Malaria Worker ☐ Medic
☐ Other_____

4. Highest education level completed:

☐ Primary School (grades 1-6) ☐ Secondary (grades 7-9)
☐ High-school (grades 10-12) ☐ College
☐ Post-graduate degree (for example PhD or MD)

5. The number of years I have been working at my current position (i.e nurse/VMW/medic)

☐ <1 year ☐ 1 year to <3 years
☐ 3 years to <5 years ☐ 5 years or more

6. In what setting do you normally work?

☐ Hospital

☐ Health center/Health post
☐ Community/malaria village worker
☐ Military

KNOWLEDGE ASSESSMENT PRIMAQUINE AND G6PD SCREENING

[Please Check (√) or Mark (X)]

7. Have you heard about drug Primaquine (PQ)?

☐ Yes ☐ No

8. Do you know what Primaquine is used for?

☐ Yes (specify): _____
☐ No

9. Do you know of any benefits of Primaquine for people with ***P. vivax* malaria**?

☐ Yes (specify): _____
☐ No

10. Do you know of any benefits of Primaquine for people with ***P. falciparum* malaria**?

☐ Yes (specify): _____
☐ No

11. Do you know of any risks or side effects associated with taking Primaquine?

☐ Yes (specify): _____
☐ No

12. Have you ever prescribed Primaquine?

☐ Yes, for what disease: _____
☐ No

13. Have you ever treated a patient with acute hemolysis or severe anemia?

☐ Yes ☐ No

14. Have you heard of a condition known as Glucose-6-phosphate dehydrogenase (G6PD) deficiency?

☐ Yes ☐ No

G6PD CARESTART™ RAPID DIAGNOSTIC TESTING

15. Primaquine should **NOT** be used if the patient has (**select all that apply**):

☐ Headache ☐ Fever ☐ Malaria
☐ Mild abdominal pain ☐ G6PD deficiency ☐ I do not know

16. When taking Primaquine, patient should be monitored for the following? (**select all that apply**):

☐ Pallor ☐ Dizziness ☐ Yellow skin or eyes

☐ New onset or worsening fatigue ☐ Back pain ☐ Shortness of breath

☐ Change in color of your urine to dark, tea color

☐ Monitoring is NOT needed ☐ I do not know

17. Per current treatment recommendations in Cambodia, who should be treated with Primaquine? (**select all that apply**):

☐ Anyone with falciparum malaria (including G6PD deficient patients)

☐ Anyone with falciparum malaria (MUST have G6PD normal results)

☐ Anyone with vivax malaria (including G6PD deficient patients)

☐ Anyone with vivax malaria (MUST have G6PD normal results)

☐ I do not know

18. What is the **benefit** of treatment with Primaquine? (**select all that apply**):

☐ Primaquine kills the parasites that hide in the liver
☐ Primaquine can lower the risk of having vivax malaria recurrence
☐ Primaquine can be used for malaria treatment as monotherapy
☐ I do not know

19. What may happen if patient accidentally takes Primaquine when he/she is **G6PD deficient? (select all that apply)**:

☐ Patient may become sick from the side effects of Primaquine
☐ Patient may develop weakness or sudden change in the color of the urine
☐ Red blood cells that carry oxygen can get damaged

☐ Nothing will happen because Primaquine is very safe
☐ I do not know

20. The risk of side effects with Primaquine can be reduced with G6PD testing:

☐ True ☐ False ☐ I do NOT know

21. Primaquine is recommended for cure in patients with vivax malaria if they have **normal G6PD test**:

☐ True ☐ False ☐ I do NOT know

22. Primaquine is REQUIRED to be cured of falciparum malaria:

☐ True ☐ False ☐ I do NOT know

23. If a patient has **falciparum malaria,** they can take 1 (one) dose of Primaquine without checking their G6PD status.

☐ True ☐ False ☐ I do NOT know

24. Who should be tested for G6PD deficiency using the Rapid Diagnostic Test (i.e CareStart™)?

☐ Males ☐ Females ☐ Both males and females

25. How will you prescribe Primaquine for adult patient with **vivax malaria** (G6PD normal)?

☐ 14 tablets to take in one day
☐ One tablet taken every day, until patient feels better
☐ 1 tablet a day for 14 days to **complete full 2-week course**, even if all malaria symptoms disappear at the end of week one
☐ 1 tablet on the first day of treatment only
☐ I do not know

26. After I take Primaquine for vivax malaria, which of the following is true:

☐ I can still get vivax malaria again if I get bitten by an infected mosquito in the future
☐ I am permanently protected from vivax malaria after I take Primaquine
☐ I do not know

27. Taking 1 dose (1 tablet) of Primaquine if patient has falciparum malaria helps the community by lowering the risk of malaria spread.

☐ True ☐ False ☐ I do NOT know

28. Taking **1 dose (1 tablet) of Primaquine** by a patient with falciparum malaria is safe, without need for G6PD testing.

☐ True ☐ False ☐ I do NOT know

29. Taking **2 weeks course of Primaquine** by a patient with vivax malaria is safe, without G6PD testing.

☐ True ☐ False ☐ I do NOT know

30. If urine suddenly becomes dark, cola-colored (shown below), the patient should:

☐ Seek medical care immediately
☐ Monitor this situation over the next 3-5 days
☐ Continue taking Primaquine to make sure can be cured from malaria

31. Pregnant women and women who are breastfeeding can take Primaquine safely.

☐ True ☐ False ☐ I do NOT know

32. What is the benefit of Primaquine treatment for a patient with ***P. falciparum***?

☐ No benefit, treatment is NOT recommended

☐ Benefit is small

☐ The community can benefit by lowering the risk of malaria transmission to others

☐ I don't know

33. How beneficial is Primaquine treatment for a patient with ***P. vivax***?

☐ No benefit, treatment is NOT recommended

G6PD CARESTART™ RAPID DIAGNOSTIC TESTING

☐ Benefit is small

☐ Majority of patients can benefit from treatment (it is required for cure)

☐ I don't know

34. How long do you have to wait before reading G6PD RDT results (CareStart™)?

☐ 2 min ☐ 5 min
☐ 10 min ☐ 15 min
☐ I don't know

35. What can affect the test performance of G6PD screening test such as CareStart™ (**select all that apply**):

☐ Temperature >32 degrees
☐ Novice user not familiar with the color interpretation
☐ Reading of the test at 15 mins rather than 10 mins
☐ I don't know

36. When interpreting the G6PD RDTs, the patient is **G6PD deficient when**: (**SELECT ALL THAT APPLY)**?

☐ The color in the reading window is WHITE
☐ The color in the reading window is PURPLE
☐ The color in the reading window is VERY FAINT PURPLE
☐ I don't know

37. A male patient has G6PD result shown below, what do you recommend next?

☐ Treatment with Primaquine
☐ Counsel the patient that he/she is G6PD deficient
☐ Repeat the test
☐ I don't know

38. A male patient has G6PD result shown below, would you recommend Primaquine?

☐ Yes
☐ No
☐ I don't know

39. A mother comes to the clinic and informs you that **she is feeling tired** and **her lips look pale** after taking her husband's anti-malaria treatment. On inquiry, you discover he was prescribed Primaquine a week ago and felt better quickly, so he shared his remaining medications with his wife who he thought also had malaria. What is the most important step you should take at this time?

☐ Prescribe additional tablets of Primaquine so the patient has enough to finish 2 weeks' course
☐ Discharge home with instruction to return if she develops a fever
☐ Immediately refer to the nearest hospital for urgent evaluation
☐ I don't know

ACCEPTABILITY OF G6PD TESTING AND PRIMAQUINE TREATMENT

[Please Check (√) or Mark (X) to select your answer]

G6PD TESTING

40. How reliable is G6PD screening test for male patients (Rapid Diagnostic Test such as CareStart™)?

Not reliable						**Very Reliable**
1	**2**	**3**	**4**	**5**	**6**	**7**

☐ I don't know

41. How reliable is G6PD screening test for female patients (Rapid Diagnostic Test such as CareStart™)?

Not reliable						**Very Reliable**
1	**2**	**3**	**4**	**5**	**6**	**7**

☐ I don't know

42. G6PD screening tests should be made available at the following locations (**select all that apply**):
☐ In the village, testing by Village Malaria Worker
☐ Health clinic
☐ Hospital

43. Are you willing to test patients for G6PD deficiency every time they have malaria?
☐ Yes ☐ No ☐ I don't know

G6PD CARESTART™ RAPID DIAGNOSTIC TESTING

44. How comfortable are you providing Primaquine treatment based on the G6PD Rapid Diagnostic Tests only:

Not comfortable **Very comfortable**

1	2	3	4	5	6	7

☐ I don't know

45. How willing are you to use G6PD screening tests that can misclassify few patients who are deficient as being normal (due to limitations, some tests many give incorrect result)?

Not acceptable **Acceptable**

1	2	3	4	5	6	7

☐ I don't know

46. In your view, what is the likelihood of G6PD rapid test showing wrong result?

Very low **Very high**

1	2	3	4	5	6	7

☐ I don't know

47. What are your thoughts about G6PD screening with RDTs?

☐ I am supportive, reason:

☐ I am not supportive, reason:

☐ I am not sure

PRIMAQUINE RISK

48. How willing are you to give Primaquine to a patient with **P. vivax malaria** and **normal G6PD** test?

Not willing **Willing**

1	2	3	4	5	6	7

☐ I don't know

49. How willing are you to give Primaquine single dose treatment for ***P. falciparum*** for a patient **NOT screened** for G6PD deficiency?

Not willing **Willing**

1	2	3	4	5	6	7

☐ I don't know
Comments:
_____

50. How willing are you to give **2 week course** treatment of Primaquine to MALE patients with vivax malaria and **normal G6PD status** (based on CareStart™ RDT)?

Not willing **Willing**

1	2	3	4	5	6	7

☐ I don't know

51. How willing are you to give **2 week course** treatment with Primaquine to a FEMALE patient with vivax malaria and **normal G6PD status** (based on CareStart™ RDT)?

Not willing **Willing**

1	2	3	4	5	6	7

☐ I don't know

52. In your view, what is the risk of **single dose treatment** with Primaquine, when test shows you are **G6PD deficient (based on CareStart™ RDT)**?

No risk **Very high risk**

1	2	3	4	5	6	7

☐ I don't know

53. In your view, what is the risk of **single dose treatment** with Primaquine, when test shows you are **G6PD normal (based on CareStart™ RDT)?**

No risk **Very high risk**

1	2	3	4	5	6	7

☐ I don't know

54. In your view, what is the risk of **2 week course treatment** with Primaquine when test shows you are **G6PD deficient (based on CareStartTM RDT)**?

No risk **Very high risk**

1	2	3	4	5	6	7

☐ I don't know

55. In your view, how high is the risk of **2 week course treatment** with Primaquine, when test shows you are **G6PD normal (based on CareStart™ RDT)**?

No risk **Very high risk**

1	2	3	4	5	6	7

☐ I don't know

56. Please select if the treatment with Primaquine results in greater risk or benefit, for the following scenarios:

Malaria	Primaquine	G6PD status	Greater benefit	Greater risk	I don't know
P.falciparum	single dose (1 tablet)	deficient			
P.falciparum	single dose (1 tablet)	normal			
P.vivax	2 week course	deficient			
P.vivax	2 weeks course	normal			

57. How confident are you in **performing RDTs** for G6PD screening?

Not confident **Very confident**

1	2	3	4	5	6	7

G6PD CARESTART™ RAPID DIAGNOSTIC TESTING

☐ I don't know

58. How confident are you in your **interpretation** of the results of the G6PD screening test based on the color change (CareStart™ RDT kit)

Not confident **Very confident**

1	**2**	**3**	**4**	**5**	**6**	**7**

☐ I don't know

59. Do you have any additional comments about Primaquine safety or G6PD testing?

☐ Yes:

_____

☐ No

60. Which questions did you find confusing or in your view, should be removed (list by number)?

_____

Completed by: _____
(Trainee)

Reviewed form completeness by: _____
(Researcher or Designee)

TRAINEE (HCWs/VMW/Medic)

(Complete AFTER training)

Visit Date: _____

(dd/MM/yy)

Please Check (√) or Mark (X) to select your answer; if more than one answer can be selected, it will be stated in the question; for all other, only ONE answer can be selected

REVIEW OF THE TRAINING

1. The scope of the material and training was relevant to my job

Disagree **Strongly Agree**

1	2	3	4	5	6	7

2. The material was well organized and easy to understand

Disagree **Strongly Agree**

1	2	3	4	5	6	7

3. The examples presented helped me understand the content

Disagree **Strongly Agree**

1	2	3	4	5	6	7

4. The hands-on training workshop on the use of G6PD RDTs added value to my learning

Disagree **Strongly Agree**

1	2	3	4	5	6	7

5. The use of **known G6PD controls** added to my learning and I would recommend it in future workshops

Disagree **Strongly Agree**

1	2	3	4	5	6	7

6. What is the OPTIMAL number of known G6PD controls that should be used for practice in the workshop?

☐ 3 normal, 3 deficient, 3 intermediate
☐ Fewer number of controls can be used for training purposes
☐ Greater number of controls would be beneficial to become more familiar with the possible color changes

7. The use of AccessBio **Reference Color Card** is helpful in test interpretation of CareStart™ RDT

Disagree **Strongly Agree**

1	2	3	4	5	6	7

8. I would recommend this training to others

Disagree **Strongly Agree**

1	2	3	4	5	6	7

9. The content was presented in a way that allowed for sufficient learning

Disagree **Strongly Agree**

1	2	3	4	5	6	7

10. The length of training was:

☐ Too long ☐ Too short ☐ Just about right

G6PD CARESTART™ RAPID DIAGNOSTIC TESTING

KNOWLEDGE ASSESSMENT PRIMAQUINE AND G6PD SCREENING

[Please Check (√) or Mark (X)]

11. Do you know what Primaquine is used for?
☐ Yes (specify): _____
☐ No

12. Do you know of any benefits of Primaquine for people with *P. vivax* malaria?

☐ Yes (specify): _____
☐ No

13. Do you know of any benefits of Primaquine for people with *P. falciparum* malaria?

☐ Yes (specify): _____
☐ No

14. Do you know of any risks or side effects associated with taking Primaquine?

☐ Yes (specify): _____
☐ No

SCORED QUESTIONS 15 TO 39

15. Primaquine should **NOT** be used if the patient has (**select all that apply**):

☐ Headache	☐ Fever	☐ Malaria
☐ Mild abdominal pain	☐ G6PD deficiency	☐ I do not know

16. When taking Primaquine, patient should be monitored for the following (**select all that apply**):

☐ Pallor	☐ Dizziness	☐Yellow skin or eyes
☐ New onset back pain	☐ New onset or worsening fatigue	
☐ Shortness of breath	☐ Change in color of your urine to dark, cola color	
☐ Monitoring is NOT needed	☐ I do not know	

17. Per current treatment recommendations, who should be treated with Primaquine (**select all that apply**):

☐ Anyone with falciparum malaria (G6PD normal and G6PD deficient patients)

☐ Anyone with falciparum malaria (MUST have G6PD normal results)

☐ Anyone with vivax malaria (G6PD normal and G6PD deficient patients)

☐ Anyone with vivax malaria (MUST have G6PD normal results)

☐ I do not know

G6PD CARESTART™ RAPID DIAGNOSTIC TESTING

18. What is the **benefit** of treatment with Primaquine (**select all that apply**):

☐ Primaquine kills the parasites that hide in the liver
☐ Primaquine can lower the risk of having vivax malaria recurrence
☐ Primaquine can be used for malaria treatment as monotherapy
☐ I do not know

19. What may happen if patient accidentally takes Primaquine when he/she is **G6PD deficient?**

☐ Patient may become sick from the side effects of Primaquine
☐ Patient may develop weakness or sudden change in the color of the urine
☐ Red blood cells that carry oxygen can get damaged
☐ Nothing will happen because Primaquine is very safe
☐ I do not know

20. The risk of side effects with Primaquine can be reduced with G6PD testing:

☐ True ☐ False ☐ I do NOT know

21. Primaquine is recommended for cure in patients with vivax malaria if they have **normal G6PD test**:

☐ True ☐ False ☐ I do NOT know

22. Primaquine is REQUIRED for cure from falciparum malaria:

☐ True ☐ False ☐ I do NOT know

23. If a patient has **falciparum malaria,** they can take 1 (one) dose of Primaquine without checking their G6PD status.

☐ True ☐ False ☐ I do NOT know

24. Who should be tested for G6PD deficiency using the Rapid Diagnostic Test? (i.e CareStart™):

☐ Males ☐ Females ☐ Both males and females

25. How will you prescribe Primaquine for adult patient with **vivax malaria** (G6PD normal):

☐ 14 tablets to take in one day
☐ One tablet taken every day, until patient feels better
☐ 1 tablet a day for 14 days to **complete full 2-week course**, even if all malaria symptoms disappear at the end of week one
☐ 1 tablet on the first day of treatment only
☐ I do not know

26. After I take Primaquine for vivax malaria, which of the following is true:

☐ I can still get vivax malaria again if I get bitten by an infected mosquito in the future

☐ I am permanently protected from vivax malaria after I take Primaquine
☐ I do not know

27. Taking 1 dose (1 tablet) of Primaquine if patient has falciparum malaria helps the community by lowering the risk of malaria spread.

☐ True ☐ False ☐ I do NOT know

28. Taking **1 dose (1 tablet) of Primaquine** by a patient with falciparum malaria is generally safe, for patients with normal and G6PD deficient status.

☐ True ☐ False ☐ I do NOT know

29. Taking **2 weeks course of Primaquine** by a patient with vivax malaria is safe, without G6PD testing.

☐ True ☐ False ☐ I do NOT know

30. If urine suddenly becomes dark, cola-colored (shown below), the patient should:

☐ Seek medical care immediately
☐ Monitor this situation over the next 3-5 days
☐ Continue taking Primaquine to make sure can be cured from malaria

31. Pregnant women and women who are breastfeeding can take Primaquine safely.

☐ True ☐ False ☐ I do NOT know

32. What is the benefit of Primaquine treatment for a patient with ***P. falciparum***?
☐ No benefit, treatment is NOT recommended
☐ Benefit is small
☐ The community can benefit by lowering the risk of malaria transmission to others
☐ I do NOT know

33. How beneficial is Primaquine treatment for a patient with ***P. vivax***?
☐ No benefit, treatment is NOT recommended
☐ Benefit is small
☐ Majority of patients can benefit from treatment (it is required for cure)
☐ I do NOT know

34. How long do you have to wait before reading G6PD RDT results (CareStart™)?
☐ 2 min ☐ 5 min ☐ 10 min
☐ 15 min ☐ I don't know

35. What can affect the test performance of G6PD screening test such as CareStart^{TM}? (**select all that apply**):
    - ☐ Temperature >32 degrees
    - ☐ Novice user not familiar with the color interpretation
    - ☐ Reading of the test at 15 mins rather than 10 mins
    - ☐ I do NOT know

36. When interpreting the G6PD RDTs, the patient is **G6PD deficient when**: (**SELECT ALL THAT APPLY)**?
    - ☐ The color in the reading window is WHITE
    - ☐ The color in the reading window is PURPLE
    - ☐ The color in the reading window is VERY FAINT PURPLE
    - ☐ I do NOT know

37. A male patient has G6PD result shown below, what do you recommend next?

- ☐ Treatment with Primaquine
- ☐ Counsel the patient that he/she is G6PD deficient
- ☐ Repeat the test

38. A male patient has G6PD result shown below, would you recommend Primaquine?

- ☐ Yes
- ☐ No
- ☐ I don't

39. A mother comes to the clinic and informs you that **she is feeling tired** and **her lips look pale** after taking her husband's anti-malaria treatment. On inquiry, you discover he was prescribed Primaquine a week ago and felt better quickly, so he shared his remaining medications with his wife who he thought also had malaria. What is the most important step you should take at this time?
    - ☐ Prescribe additional tablets of Primaquine so the patient has enough to finish 2 weeks' course
    - ☐ Discharge home with instruction to return if she develops a fever
    - ☐ Immediately refer to the nearest hospital for urgent evaluation
    - ☐ I do NOT know

ACCEPTABILITY OF G6PD TESTING AND PRIMAQUINE TREATMENT

[Please Check (√) or Mark (X) to select your answer]

G6PD TESTING

40. How reliable is G6PD screening test for **male** patients (Rapid Diagnostic Test such as CareStart™):

Not reliable **Very Reliable**

1	2	3	4	5	6	7

☐ I do NOT know

41. How reliable is G6PD screening test for **female** patients (Rapid Diagnostic Test such as CareStart™):

Not reliable **Very Reliable**

1	2	3	4	5	6	7

☐ I do NOT know

42. G6PD screening tests should be made available at the following locations (**select all that apply**):

☐ In the village, testing by Village Malaria Worker
☐ Health clinic
☐ Hospital

43. Are you willing to test patients for G6PD deficiency every time they have malaria?

☐ Yes ☐ No ☐ I do NOT know

44. How comfortable are you providing Primaquine treatment based on the G6PD Rapid Diagnostic Tests only:

Not comfortable **Very comfortable**

1	2	3	4	5	6	7

45. Are you willing to use G6PD screening test if it can misclassify few patients who are deficient as being normal (due to test limitations, some tests many give incorrect result)?

Not acceptable **Acceptable**

1	2	3	4	5	6	7

☐ I do NOT know

46. In your view, what is the likelihood of G6PD rapid test showing **wrong** result?

Very low **Very high**

1	2	3	4	5	6	7

☐ I do NOT know

47. What are your thoughts about G6PD screening with RDTs?

☐ I am supportive, reason:

☐ I am not supportive, reason:

☐ I am not sure

PRIMAQUINE RISK

48. How willing are you to give Primaquine to a patient with **P. vivax malaria** and normal G6PD test?

Not willing **Willing**

1	2	3	4	5	6	7

☐ I do NOT know

49. How willing are you to give Primaquine single dose treatment for ***P. falciparum*** for a patient NOT screened for G6PD deficiency?

Not willing **Willing**

1	2	3	4	5	6	7

☐ I do NOT know

Comments: _____

50. How willing are you to give **2 week course** treatment of Primaquine to MALE patients with vivax malaria and **normal G6PD status** (based on CareStart^{TM} RDT)?

Not willing **Willing**

1	2	3	4	5	6	7

☐ I do NOT know

51. How willing are you to prescribe **2 week course** treatment with Primaquine to FEMALE patients with vivax malaria and **normal G6PD status** (based on CareStart™ RDT)?

Not likely **Very Likely**

1	2	3	4	5	6	7

☐ I do NOT know

52. In your view, what is the risk of **single dose treatment** with Primaquine, when test shows you are **G6PD deficient (based on CareStart™ RDT)**?

No risk **Very high risk**

1	2	3	4	5	6	7

☐ I do NOT know

53. In your view, how high is the risk of **single dose treatment** with Primaquine, when test shows you are **G6PD normal (based on CareStart™ RDT)?**

No risk **Very high risk**

1	2	3	4	5	6	7

☐ I do NOT know

54. In your view, how high is the risk of **2 week course treatment** with Primaquine when test shows you are **G6PD deficient (based on CareStart™ RDT)**?

No risk **Very high risk**

1	2	3	4	5	6	7

☐ I do NOT know

55. In your view, how high is the risk of **2 week course treatment** with Primaquine, when test shows you are **G6PD normal (based on CareStart™ RDT)**?

No risk **Very high risk**

1	2	3	4	5	6	7

☐ I do NOT know

56. Please select if the treatment with Primaquine results in greater risk or benefit, for the following scenarios:

Malaria	Primaquine	G6PD status	Greater benefit	Greater risk	I don't know
P.falciparum	single dose (1 tablet)	deficient			
P.falciparum	single dose (1 tablet)	normal			
P.vivax	2 week course	deficient			
P.vivax	2 weeks course	normal			

57. How confident are you in **performing RDTs** for G6PD screening correctly?

Not confident **Very confident**

1	2	3	4	5	6	7

☐ I do NOT know

58. How confident are you in your **interpretation** of the results of the G6PD screening test based on the color change (CareStart™ RDT kit)

Not confident **Very confident**

1	2	3	4	5	6	7

☐ I do NOT know

59. Do you have any additional comments?

☐ Yes: _____

☐ No

60. Which questions did you find confusing or in your view, should be removed (list the corresponding numbers)?

Completed by: _____
(Trainee)

Reviewed form completeness by: _____
(Researcher or Designee)

Appendix K: CareStart™ Equipment

Specific steps:

— 2.1 —

As shown on the job aid, assemble all the supplies you will need, including:

- A new, unopened test packet
- A new, unopened alcohol swab
- A sterile lancet (new and unopened)

- Buffer

- A watch or clock to use as a timer

- A new pair of disposable examination gloves

The blood-transfer device — (a) capillary tube, (b) straw, (c) loop, (d) pipette or other — is used to collect blood and transfer it to the test cassette.

a.

b.

c.

d.

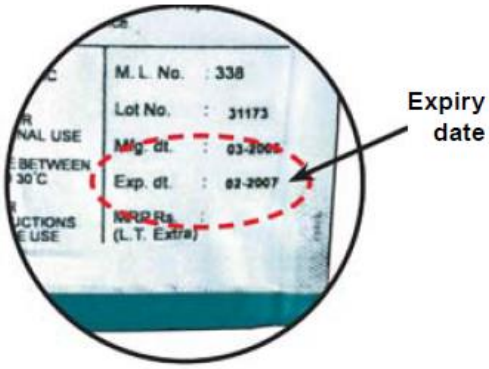
- Pencil

- A sharps disposal container

- A non-sharps disposal bin

— 2.4 —

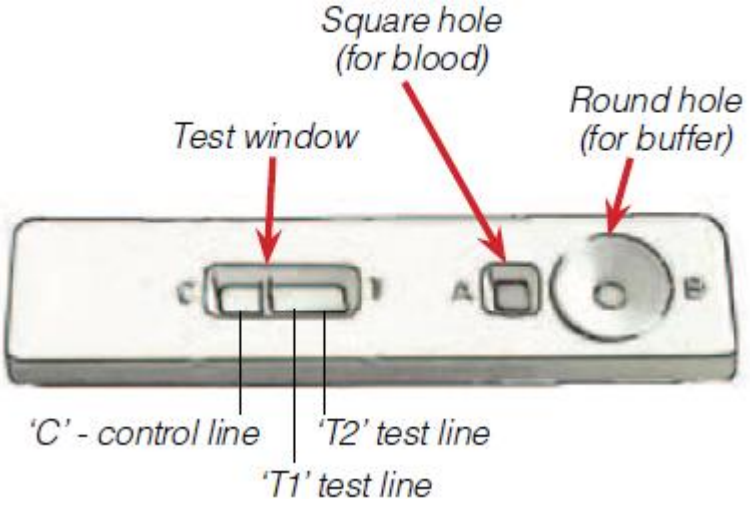
Explain the importance of the expiry date.



Point out the expiry date on the test packet, but do not read the date.

Pass the test packet around and ask each participant to look at it.

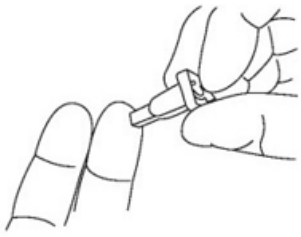
Allow participants to go through the process of hands-on training to ensure they can read expiration date and follow the steps for processing the sample.



APPENDIX

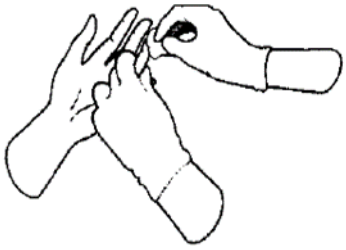
Steps for testing G6PD Status in field setting using CareStart™

Hold the end of a fingertip firmly and pierce the cleaned area of the fingertip using a lancet provided or venipuncture. Discard the lancet in the sharps box.

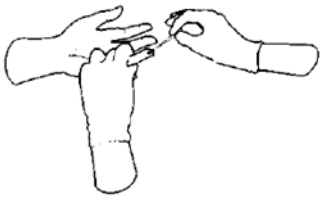


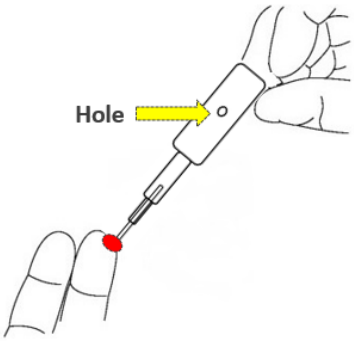
Puncture the skin

Wipe out the first drop of blood with sterile ga

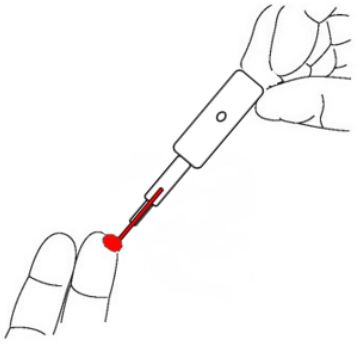


Collect the blood sample (2 µl) using the pipett micro-pipette.

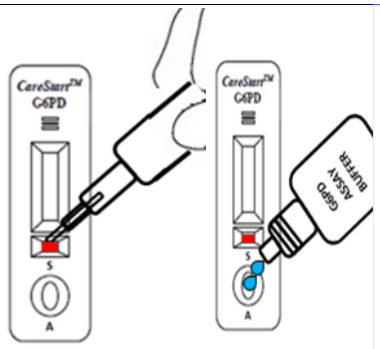


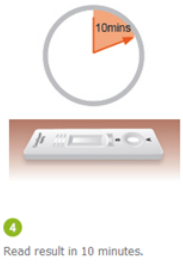


Touch the blood drop with the open end of the capillary tube (Do not cover the hole!)

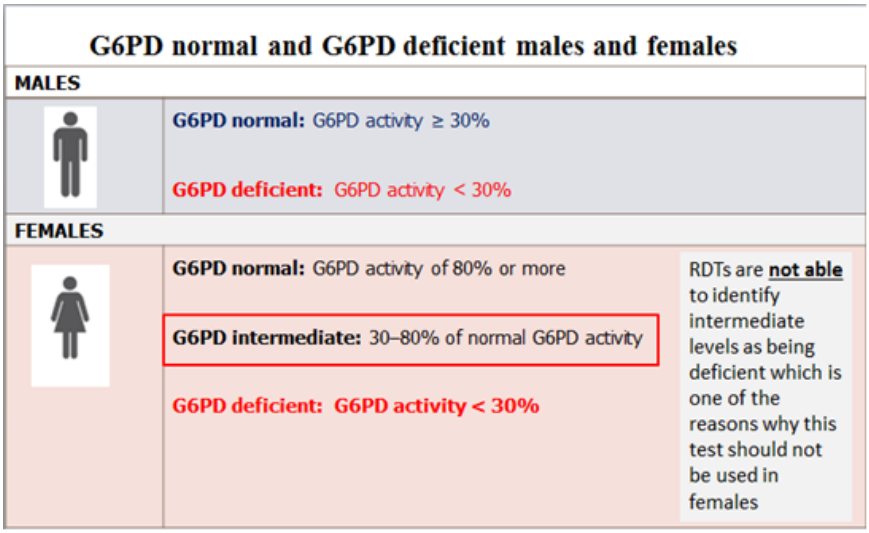


Let the blood flow into the capillary tube



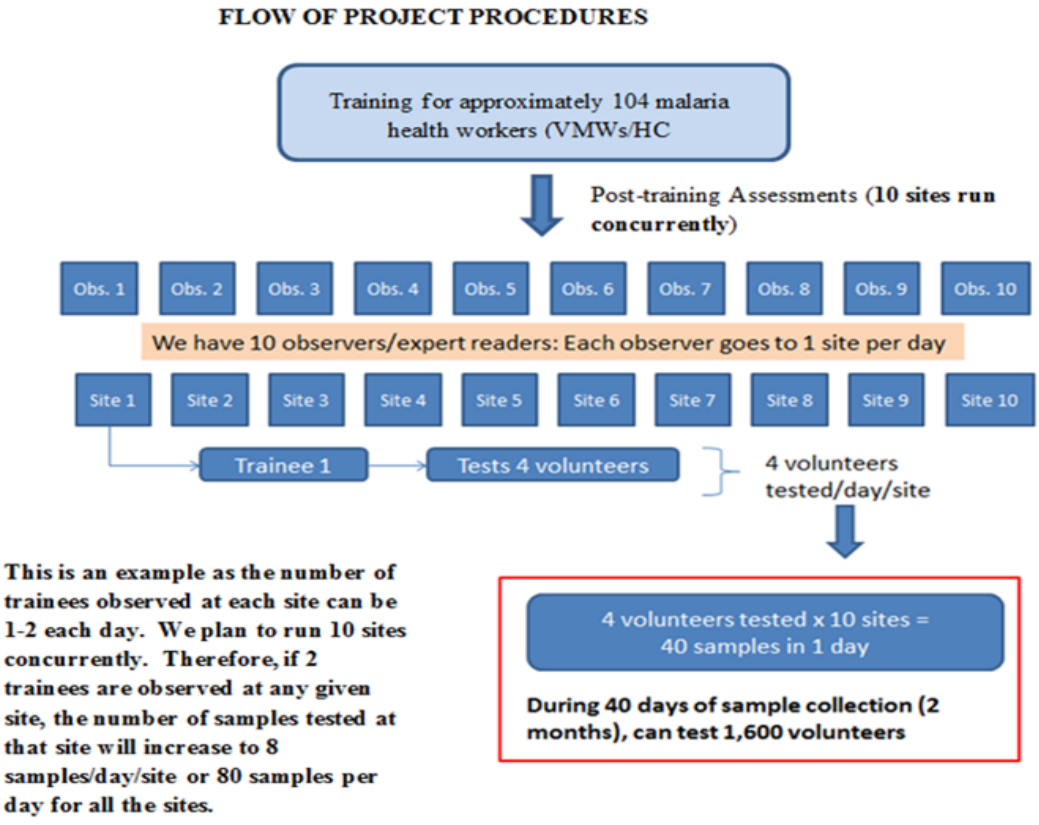


APPENDIX L: G6PD normal Vs deficient among Males/Females



(Global Malaria Program, 2016)

APPENDIX M: Flow Chart



Appendix N[1]: G6PD Trainee Checklist

CHECKLIST FOR G6PD RDT (CARESTARTTM)

No.	Task	Yes/No
1	Materials preparation	
	- Supportive materials (gloves, sharp box, trash bin)	
	- Blood collection set (alcohol pad, lancet, clean gauze)	
	- Timer	
	- RDT kit (RDT cassette, G6PD Assay Buffer, pipette): check the expiry date, and the RDT kit's name	
2	Patient preparation	
	- Staff, wash hands and wear gloves	
	- Identify patient's details and record patient's name on the RDT cassette	
	- Explain procedure to patient	
3	RDT procedure and reading results	
	- Collect an exact volume of blood using pipette	
	- Release the blood in the Sample Well	
	- Add 2 drops of G6PD Assay Buffer to the Buffer Well	
	- Read and record the result in 10 minutes	
4	Disposal of biohazard and non-biohazard waste	
	- Dispose of used cassette, transfer devices and other biohazard waste to biohazard trash bin	
	- Dispose of used lancet in a sharps container	
	- Dispose of used non-biohazard waste to trash bin	

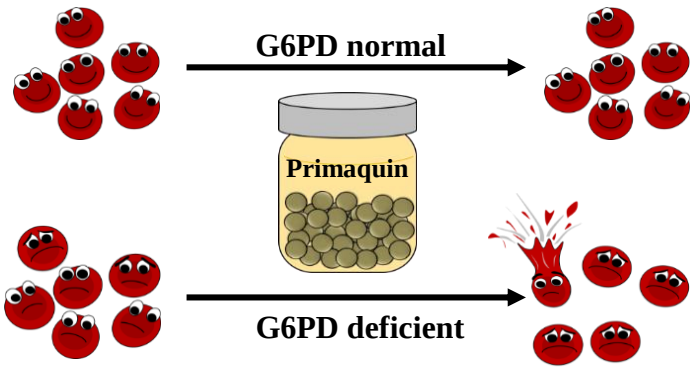
Recorded temperature at the time of test performance

Appendix O: PATIENT COUNSELING SCRIPT (ENGLISH)

INFORMATION ABOUT PRIMAQUINE & G6PD SCREENING

G6PD deficiency

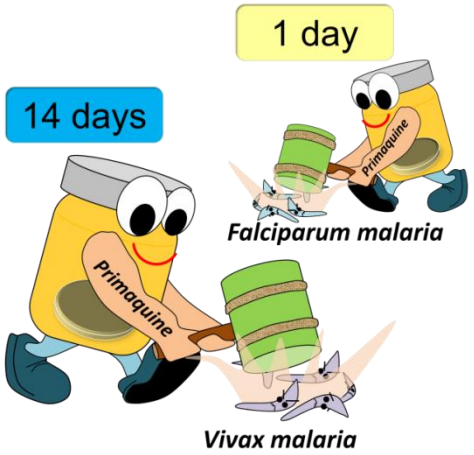
Some people have a low amount of a substance called glucose-6-phosphate dehydrogenase (G6PD) in the cells that carry oxygen in the blood around the body. These people have what is called G6PD deficiency. Most people with low G6PD levels never know they are different, and they do not show symptoms unless they take certain medications, such as Primaquine.



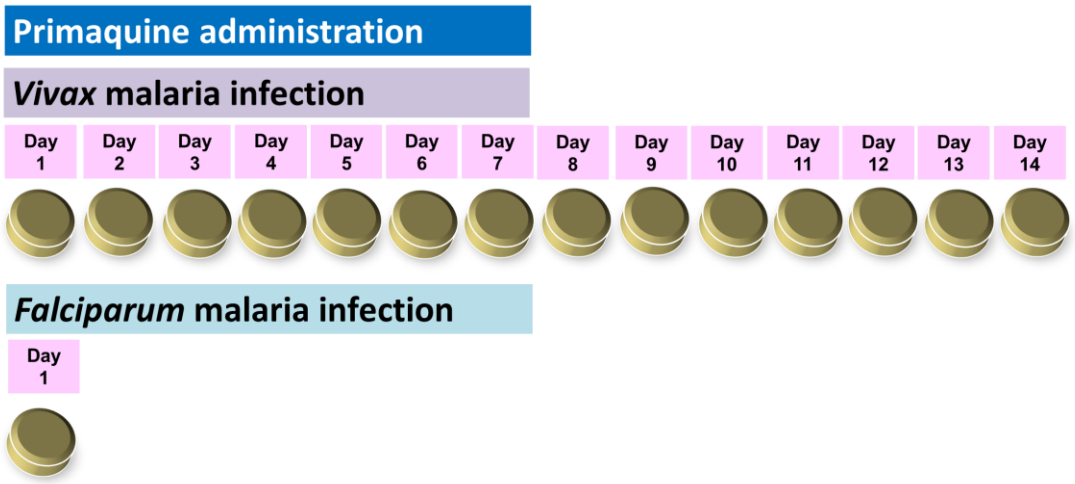
If the patient has a low amount of G6PD, their red blood cells can get broken or burst more easily.

Current malaria treatment recommendations in Cambodia

Current treatment guidelines in Cambodia recommend that all malaria patients receive antimalarial drugs called artemisinin-based combination therapy (ACT) for 3 days. Patients with malaria should also receive treatment with Primaquine, as long as they have normal levels of G6PD in their red blood cells.



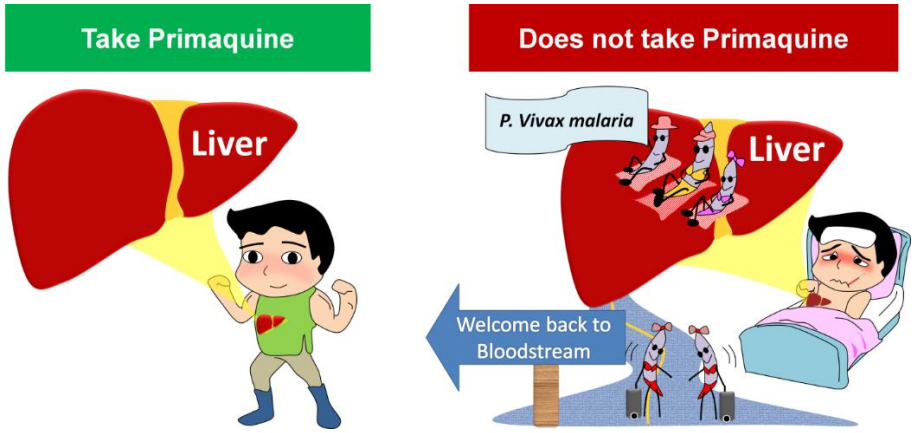
It is recommended that G6PD normal patients who have vivax malaria receive treatment with Primaquine every day for 14 days. Patients who have falciparum malaria should receive a single dose of Primaquine. However, all patients require testing to confirm they have normal G6PD levels, before receiving treatment with Primaquine.

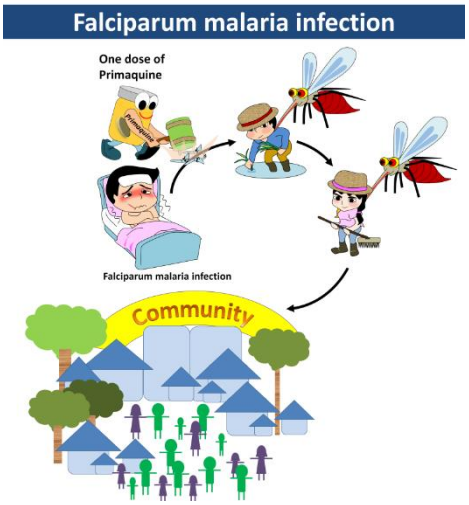


Why is Primaquine recommended for malaria treatment?

If a patient has vivax malaria and does NOT get treatment with Primaquine, they can have parasites that stay hidden in the liver and enter blood several months later, which will make the patient sick again with malaria symptoms.

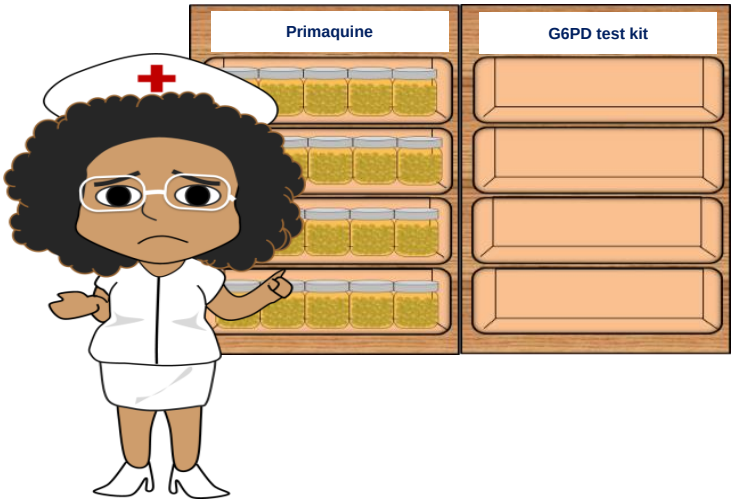
To get rid of these parasites from the liver, the patient needs to take Primaquine.





For patients who have **falciparum** malaria, it is recommended that they take **one dose** of Primaquine in order to reduce the risk of transmission of malaria to other people. This means fewer people will be able to transmit malaria in the community, so the risk of getting malaria when they get bitten by a mosquito may be **lower**.

G6PD testing was not available in Cambodia, so Primaquine was not used, but the National Malaria Program (CNM) would like to make testing and Primaquine more widely available for malaria patients, in support of Cambodia malaria elimination strategy.

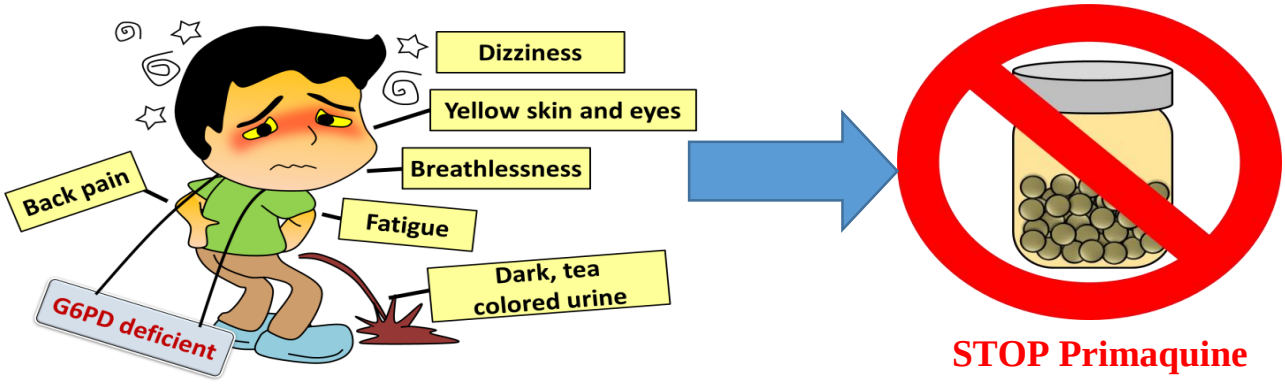


What are the risks of treatment with Primaquine?

For patients with NORMAL G6PD levels, taking Primaquine is generally very well tolerated without any issues.

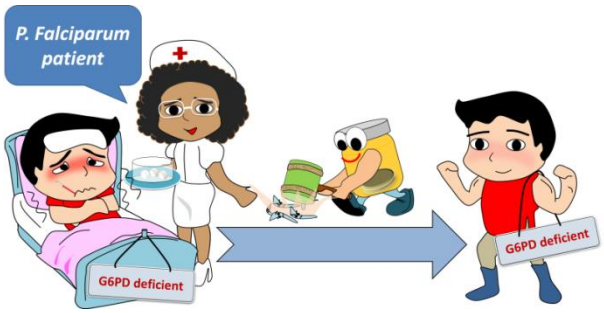
But taking Primaquine is NOT recommended for people with G6PD deficiency, as the breakdown of the red blood cells can make them ill. The most common symptoms are fatigue, shortness of breath after activity, rapid breathing, pallor, and increased heart rate, yellowing of skin and eyes, or dark, tea colored urine or back pain. In most cases, this can be corrected by stopping Primaquine treatment when these symptoms develop. **Patients with NORMAL G6PD levels are very unlikely to develop these symptoms with Primaquine.**

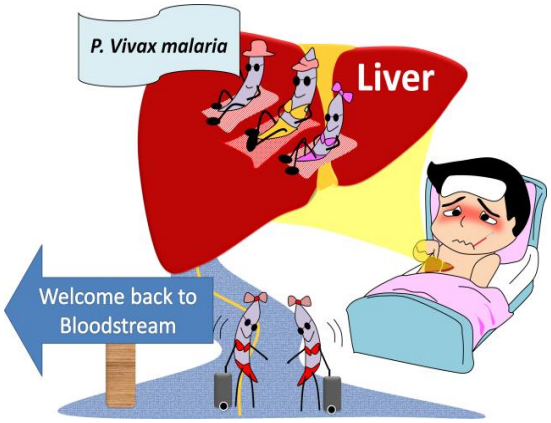
If someone's **G6PD levels are very low**, Primaquine treatment could result in a large number of their red blood cells being destroyed. Therefore, in rare cases, some patients treated with Primaquine may need a blood transfusion to replace the red blood cells that burst after Primaquine treatment. This is very unlikely for patients with normal G6PD levels



The risks of Primaquine treatment are based on patient's G6PD level, how many doses of Primaquine they take and other factors. Risk can be minimized if a patient has normal or near normal levels of G6PD.

The risk from Primaquine treatment is also considered very low for falciparum patients since they only have to take a single dose. Single dose treatment is unlikely to result in significant destruction of red blood cells, even for patients with low levels of G6PD. **It is therefore acceptable to take SINGLE dose of Primaquine for falciparum malaria without G6PD testing.**





Lack of treatment with Primaquine in patients with vivax malaria also poses risks to patients as this can result in frequent recurrences of vivax malaria. Having malaria recurrence frequently can affect your ability to work or take care of your family.

What are the benefits of PQ treatment?

It is suspected that many patients have *vivax* recurrence due to lack of treatment with Primaquine, which allows the hidden parasites in the liver to enter the patient's blood many times. Taking Primaquine when a patient is diagnosed with *vivax* will kill and eliminate hidden parasites in their liver and make them less likely to have recurrence of the infection. However, it is still possible for a patient to get vivax in the future if they get bitten again by an infected mosquito.

Taking Primaquine when a patient has *falciparum* malaria will make them less likely to transmit the infection to other people, which may reduce their own risk of getting infected again.

Limitations of the current tests for G6PD screening:

There are different tests for G6PD deficiency but most of them are not suitable for use outside of a highly specialized laboratory setting. The test for G6PD deficiency which is being used today comes with limitations as outlined below:

G6PD CARESTART™ RAPID DIAGNOSTIC TESTING

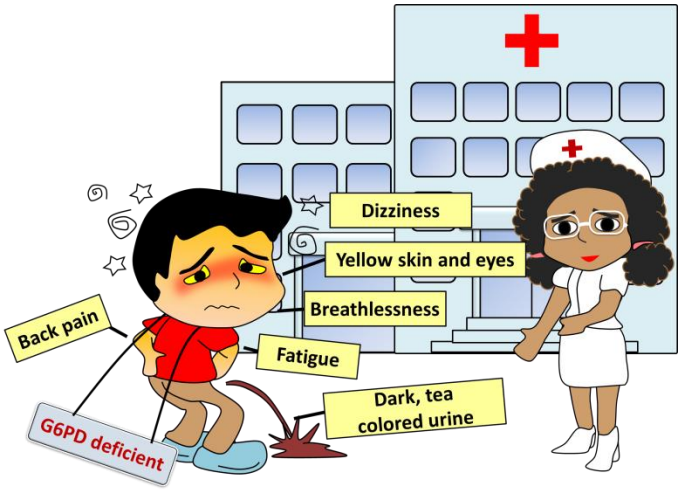
1. The rapid diagnostic test for G6PD deficiency requires interpretation of a color change, which can sometimes be difficult, or color change can be misinterpreted even by highly trained medical staff.
2. The reliability of the test may be compromised if the testing is done in the room with more than 32 degrees Celsius.
3. The rapid diagnostic test can only tell if a patient has NORMAL or LOW levels of G6PD. These tests cannot tell how much of the G6PD enzyme the patient has.

Therefore, it is recommended that even with NORMAL G6PD test result, all patients are monitored for possible side effects from taking Primaquine.

Understanding your test results:

If your test result is **normal** it means you should have sufficient levels of the G6PD enzyme to receive Primaquine safely

If the test shows you are **G6PD deficient**, this means that taking Primaquine for 14 days could damage some of your red blood cells and might make you feel sick. This can manifest as you being pale, having shortness of breath with activity, having a yellow pigmentation of the skin or eyes, or tea-colored urine. So treatment with Primaquine is not recommended for vivax malaria when you are G6PD deficient. However, single dose of Primaquine for falciparum malaria is normally tolerated even in patients with G6PD deficiency; therefore, it is recommended to reduce risk of transmission to others.



Given the limitations of the tests, if you are given Primaquine and develop the above mentioned symptoms or any new symptoms within 2 weeks of taking Primaquine, you should visit your doctor immediately. If your local health center is closed, then you should go to the nearest hospital.

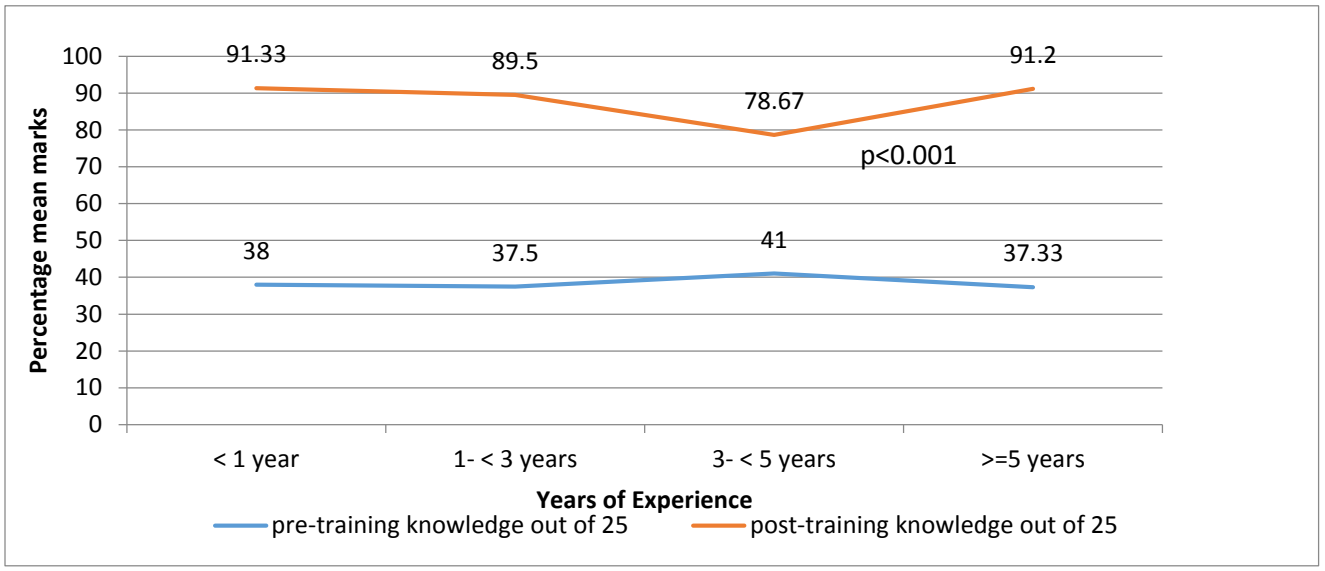
APPENDIX P: Descriptive Statistical Table for Trainees

Variables	**Total sample**	**(%)**
Trainee	94	100
VMW	68	72.3
Nurse	21	22.3
Other	5	5.4
Age Group Trainees		
<30yrs	29	30.9
31-50yrs	51	54.3
>51yrs	14	14.9
Gender		
Male	40	42.6
Female	54	57.4
Educational Level		
Primary grade (1-6)	39	41.5
Secondary	30	31.9
High school	14	14.9
College graduate	8	8.5
Medical Doctor	1	1.1

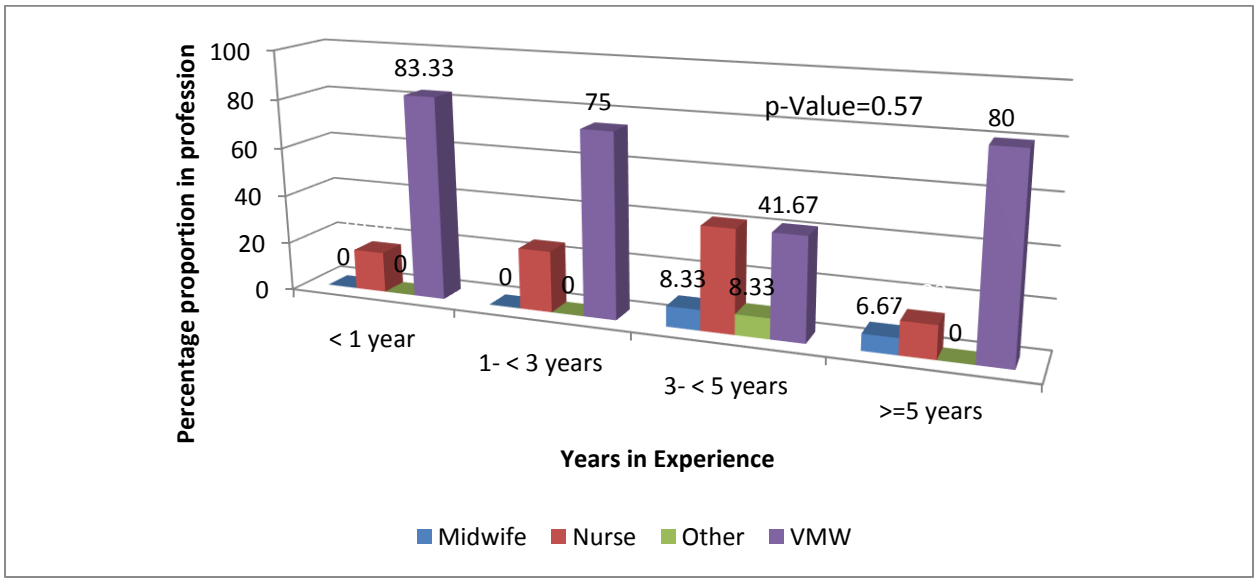
Appendix Q: Results from Study

Trainee score Pre and Post Training based on Years of Experience

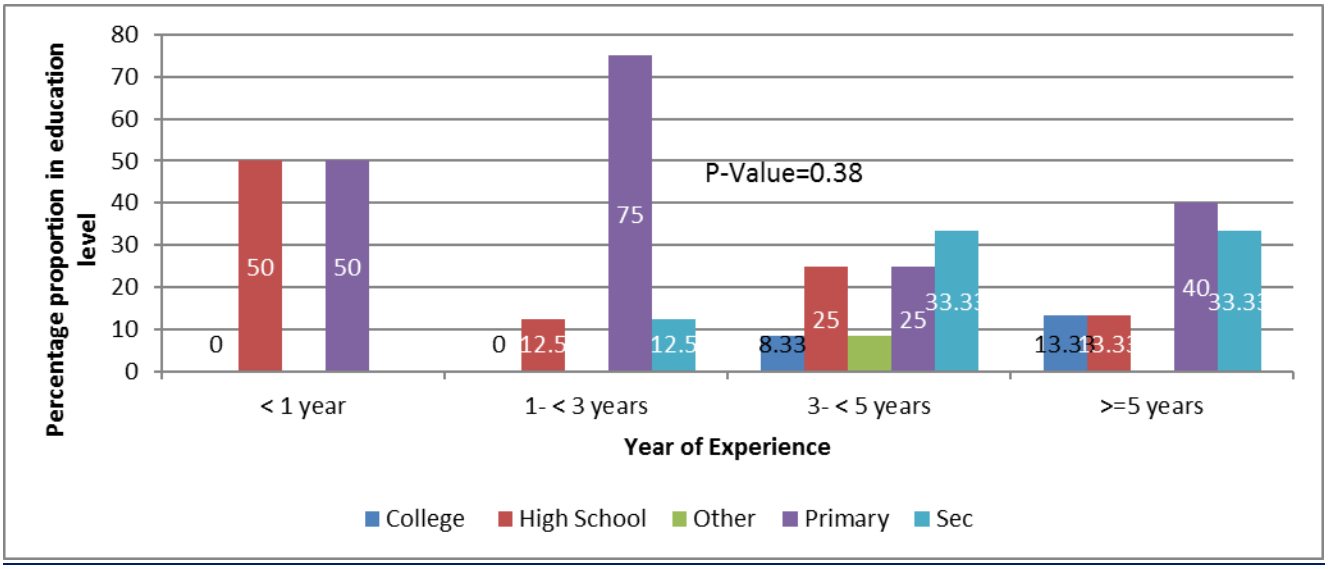
I.



II



III



IV

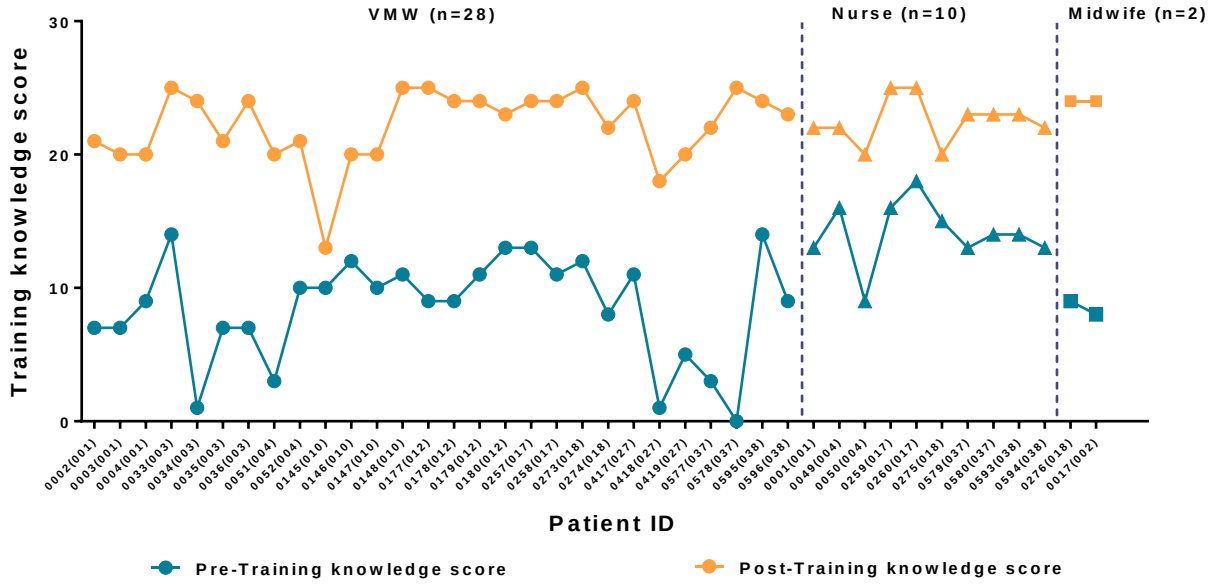
The percentage mean score knowledge pre-training and post-training (p<0.001)

	VMWs	**Nurses**	**Other**
Pre-Training	34%	56.4%	34%
Post-Training	88.71%	90%	96%

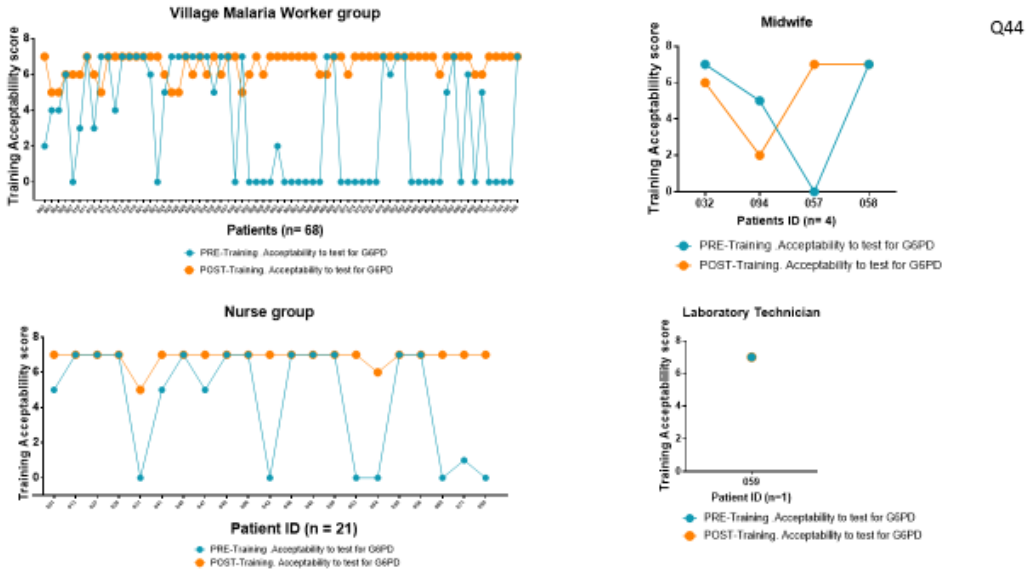
	PRE-TRAINING	**POST-TRAINING**
Willingness to test for G6PD when a patient is diagnosed with malaria	56.3% (53/94)	80.8%(76/94)
Level of comfort in giving PQ based on G6PD RDT results	39.3% (37/94)	72.3% (68/94)
Level of comfort in giving PQ to a patient with P.v and normal G6PD test	36.1%(34/94)	80.0% (76/94)
Willingness to give a patient PQ single dose for Pf without G6PD screening	20.2%(19/94)	84% (79/94)

V

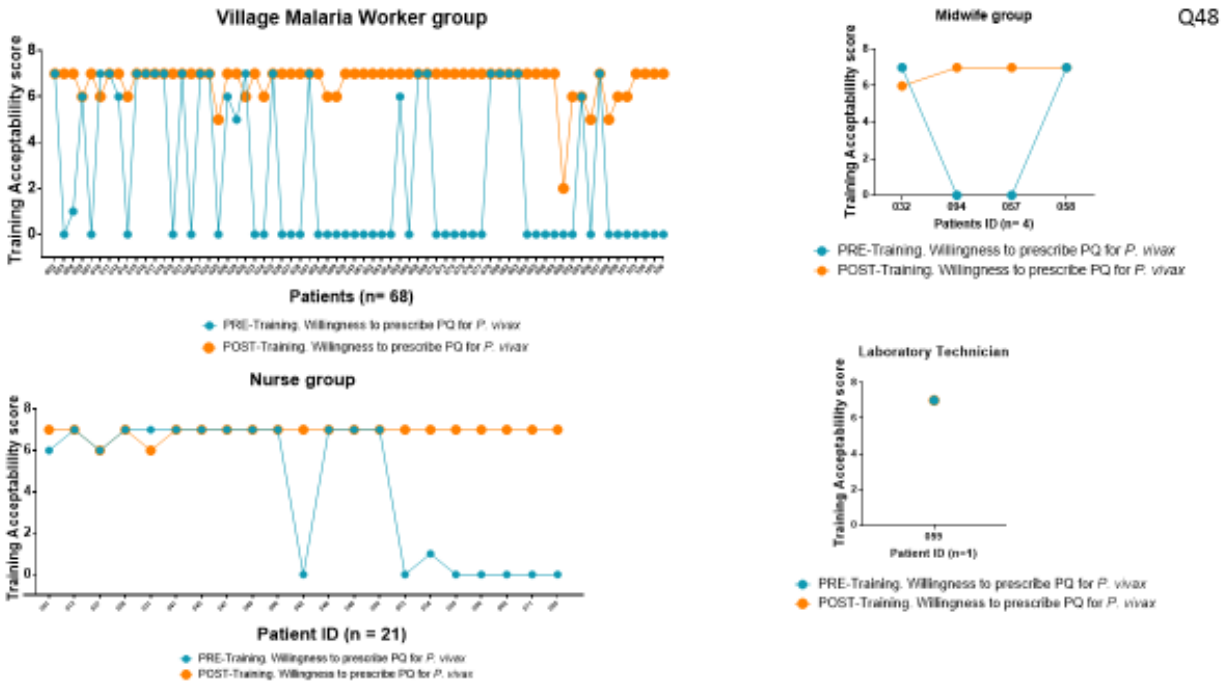
Knowledge Score Assessment Among 1st group only



Acceptability to Test for G6PD Pre and Post Training



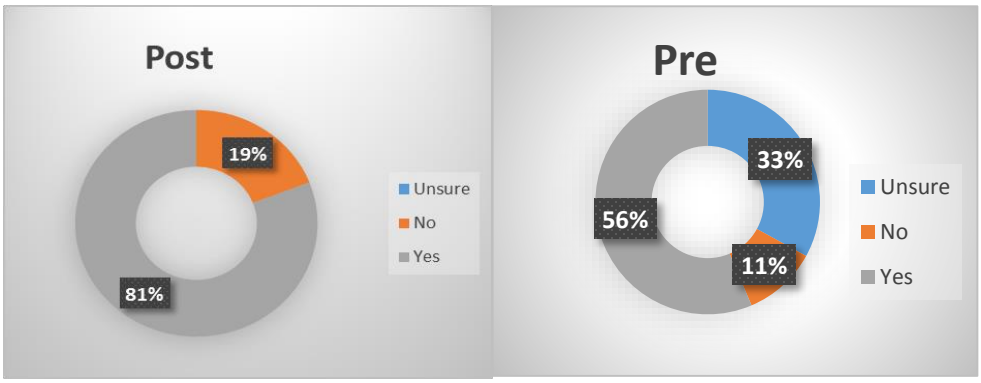
Willingness to Prescribe PQ for *P.v*



VII (Willingness and Acceptance)

Q: Are you willing to test patients for G6PD deficiency every time they have malaria?

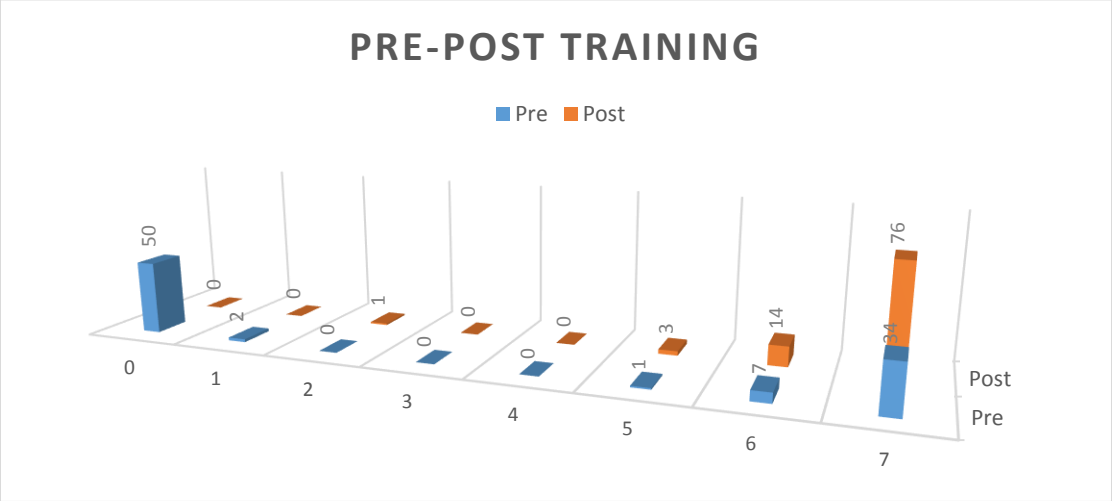
☐ Yes ☐ No ☐ I do NOT know



Q: How willing are you to give Primaquine to a patient with **P. vivax malaria** and **normal G6PD** test?

Not willing						**Willing**
1	**2**	**3**	**4**	**5**	**6**	**7**

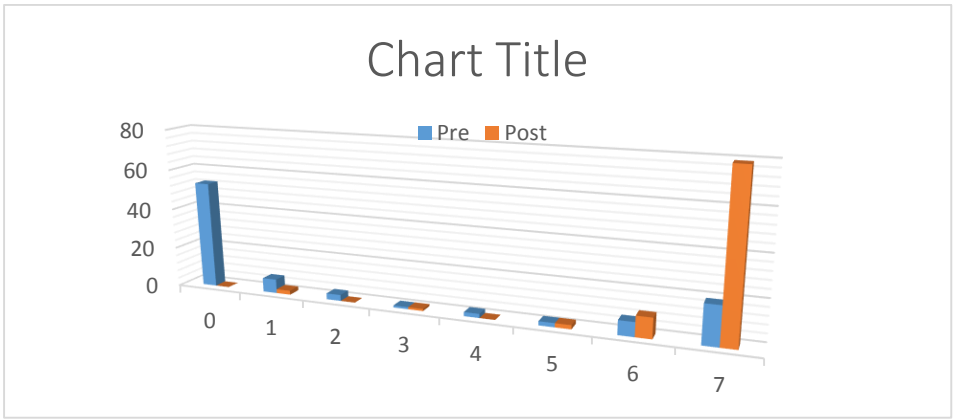
☐ I don't know



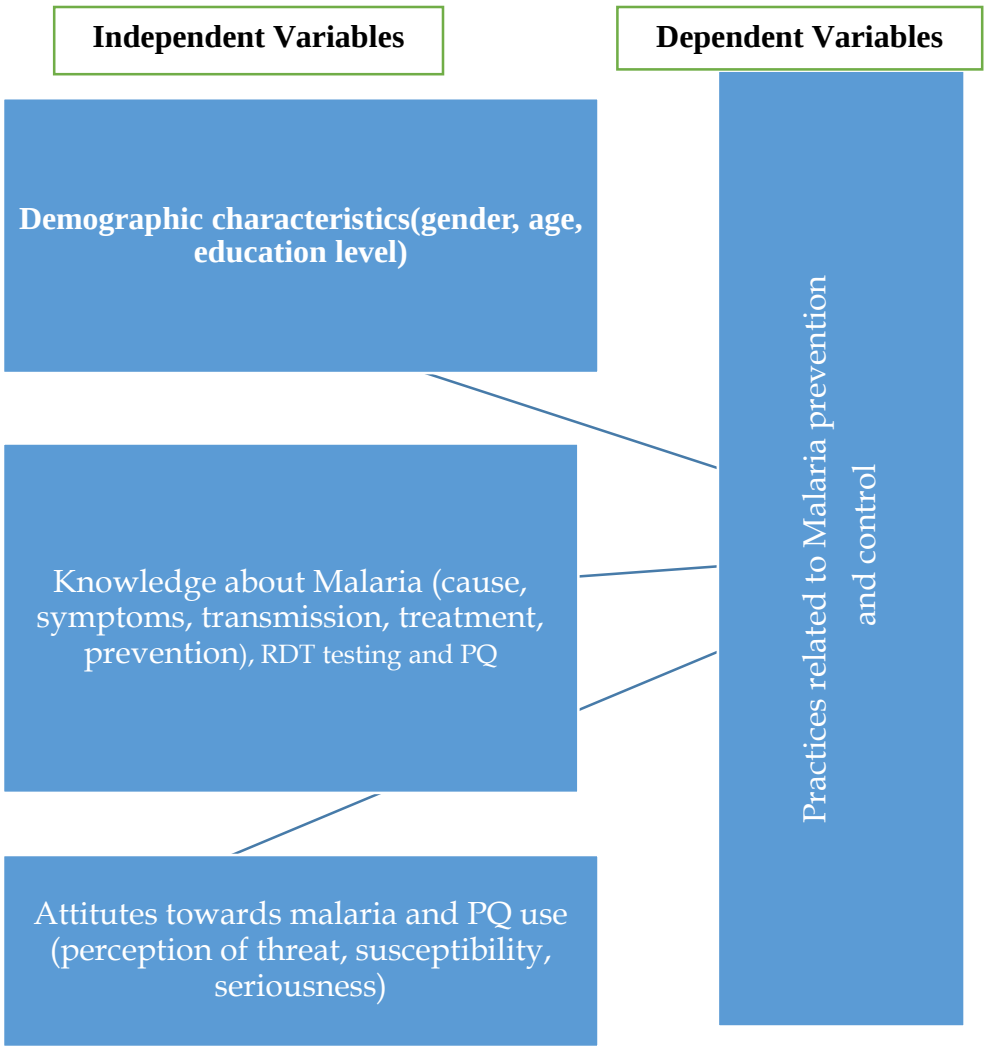
Q: How willing are you to give Primaquine single dose treatment for *P. falciparum* for a patient NOT screened for G6PD deficiency?

Not willing						**Willing**
1	**2**	**3**	**4**	**5**	**6**	**7**

☐ I don't know



Appendix Figure 1: Variables



Appendix Figure 2: G6PD color changes