

[Title]

Version [XX]

[Date: Month, DD, YYYY]

Investigators:

[XX]

[XX]

Location of Research:

[XX]

Proposed Project Dates:

[XX]

 OPTIONAL  
PARTNER LOGO

<b>Study title</b>	Validation of diagnostics to identify glucose-6-phosphate dehydrogenase (G6PD) activity
<b>Précis</b>	Cross-sectional diagnostic accuracy study with N patient participants and N health worker participants. The patient population will be recruited from (household surveys/clinics/other). The health worker participants will include trained intended users of the G6PD tests. Health workers will take capillary blood samples and conduct three point-of-care (POC) tests: (1) malaria test; (2) hemoglobin test; and (3) investigational G6PD test. Venous blood samples will be collected and transferred to a laboratory where reference assays will be performed using the Pointe Scientific G6PD Analyzer. Trained health workers will also be surveyed to assess product usability through a questionnaire to assess comprehension of labels and packaging as well as interpretation of results for simulated G6PD tests.
<b>Objectives</b>	<p><i>Primary objective:</i> Assess the accuracy and reliability of G6PD tests in detecting G6PD activity and classifying results when used by trained health care workers.</p> <p><i>Component objectives:</i></p> <ul style="list-style-type: none"> <li>• Determine the performance of point-of-care G6PD tests in detecting G6PD activity and hemoglobin compared to a reference assay.</li> <li>• Assess the comprehension of the G6PD test labels and packaging among intended users.</li> <li>• Assess the usability of G6PD test result outputs among intended users.</li> </ul>
<b>Endpoints</b>	<ul style="list-style-type: none"> <li>• Sensitivity and specificity of G6PD tests compared to the Pointe Scientific G6PD Analyzer: <ul style="list-style-type: none"> <li>○ Accuracy between the POC G6PD test measure of G6PD activity and a reference assay.</li> <li>○ Accuracy between the POC G6PD test measure of hemoglobin and a reference assay.</li> </ul> </li> <li>• Comparison of the POC G6PD test results for capillary and venous samples.</li> <li>• Percentage of trained health workers who can accurately comprehend key messaging included in the test packaging and labels.</li> <li>• Percentage of trained health workers who can accurately interpret the result output and classify results as either normal, invalid, deficient, or intermediate.</li> </ul>
<b>Population</b>	Based on expected G6PD prevalence, we expect to recruit approximately: <ul style="list-style-type: none"> <li>• XX people with normal G6PD activity levels.</li> <li>• XX people with intermediate G6PD activity levels.</li> <li>• XX people with deficient G6PD activity levels.</li> <li>• 15 trained health workers.</li> </ul>
<b>Study sites</b>	XXX
<b>Study duration</b>	9 months (estimated).

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## Abbreviations

CE mark	European Conformity certification
CI	confidence interval
EDTA	ethylenediaminetetraacetic acid
FNTF	false negative true positive
FST	fluorescent spot test
G6PD	glucose-6-phosphate dehydrogenase
G6PDd	glucose-6-phosphate dehydrogenase deficiency
Hb	hemoglobin
HRP2	histidine-rich protein 2
IRB	institutional review board
NA	not applicable
PAN	all species
<i>P. falciparum</i> / <i>Pf</i>	<i>Plasmodium falciparum</i>
pLDH	Plasmodium lactate dehydrogenase
POC	point-of-care
PQ	prequalification
<i>P. vivax</i>	<i>Plasmodium vivax</i>
RDT	rapid diagnostic test
TPTN	test positive true negative
TTN	test and true negative
TTP	test and true positive
U	unit
US FDA	United States Food and Drug Administration
WHO	World Health Organization

## 1. Background and rationale for the study

Glucose-6-phosphate dehydrogenase (G6PD) is a critical housekeeping enzyme in red blood cells that supports protective systems against oxidative challenge by producing the reduced form of nicotinamide adenine dinucleotide phosphate [1, 2]. The most common human enzyme defect is G6PD deficiency, which affects more than 400 million people worldwide [3]. Red blood cells are especially vulnerable to the effects of these mutations because they cannot replenish their supplies of the enzyme once they mature and enter the bloodstream. As a result, these cells are susceptible to hemolysis when subjected to oxidative stress, which can occur after therapy with the anti-malarial 8-aminoquinolines such as primaquine, a few antibiotics, and some anti-inflammatories. Hemolysis can also be activated by other exogenous agents, including foods (e.g., fava beans), henna, and some infections (e.g., hepatitis A or B, pneumonia, and typhoid fever). In newborns, G6PD deficiency is often first manifested as jaundice resulting from hyperbilirubinemia, which, if unchecked, can lead to kernicterus, a form of brain damage. In 1989, the World Health Organization (WHO) working group on G6PD deficiency recommended that “whenever possible, neonatal screening should be performed...in populations where G6PD deficiency is common (i.e., where it affects more than three to five percent of males)” [4]. While knowing the G6PD status of a patient is useful clinical information, access to testing for G6PD deficiency is very limited due to the price and complexity of the diagnostic products available for this condition, especially in malaria-endemic populations and low-resource settings.

Uncomplicated malaria is typically treated by eliminating the asexual stage parasites that circulate in the blood and are the cause of symptoms. These are typically treated with artemisinin-based combination therapy for either all species of malaria or in some countries for *Plasmodium (P.) falciparum* malaria and chloroquine for *P. vivax*, depending on the country policy. Although patients with *P. falciparum* infections are cured with this treatment, in the case of *P. vivax* infection patients are cured of their asexual parasites but some parasites remain sequestered in the liver, which later release into the blood and cause a relapse. The 8-aminoquinoline-based malaria drugs such as primaquine and (potentially, in the future) tafenoquine are the only ones with the capacity to prevent relapse and eliminate the liver stage parasites in *P. vivax* infections. Tafenoquine requires only a single dose in comparison to a multi-day (7 to 14 days) regimen for primaquine.

Because of the risks associated to G6PD deficiency for primaquine, WHO recommends as good practice in the current malaria treatment guidelines that “the G6PD status of patients should be used to guide administration of primaquine for preventing relapse.” In recognition of (1) the diversity in prevalence of G6PD deficiency in malaria-exposed populations and (2) the operational challenges in testing for G6PD deficiency in the context of malaria case management, WHO also recommends that “when G6PD status is unknown and G6PD testing is not available, a decision to prescribe primaquine must be based on an assessment of the risks and benefits of adding primaquine.” [5].

In line with this, some country malaria guidelines only treat *P. vivax* blood stage infections and do not provide primaquine, others provide primaquine without testing for G6PD deficiency, and others provide primaquine only if testing for G6PD deficiency is available.

*[Add country-specific background information.]*

Currently, the G6PD status of a patient is most often defined by the patient’s G6PD phenotype, characterized by analysis of total activity in blood lysate [3]. This method, considered the gold standard, is a costly and complex quantitative laboratory-based spectrophotometric test. The quantitative laboratory test can clearly identify subjects with all ranges of G6PD activities (including those with intermediate levels who may also be at risk of severe hemolysis) but these methods require an equipped laboratory and skilled personnel [6,7].

In field settings, the most commonly performed tests are qualitative devices such as the fluorescent spot test or the AccessBio, Inc. CareStart™ test [8, 9] which can only discriminate gross deficiencies from all the other phenotypes. These are adequate for males who are either deficient or normal in G6PD status. Females, however, who carry two alleles of the G6PD gene, can present as deficient, intermediate, or normal for G6PD activity. Qualitative tests cannot discriminate an intermediate from a normal G6PD status. Quantitative tests are required to provide better case management for women, especially in anticipation of the availability of tafenoquine, which is indicated only for women and men with normal G6PD activity.

Given the target populations where anti-malarial drugs are required, very often in remote rural settings, point-of-care (POC) G6PD tests are required to support broader availability of primaquine and, in the future, tafenoquine. For treatment of women whose enzymatic activity is estimated to be normal by qualitative testing but is still too low for treatment of high-dose primaquine or tafenoquine, a quantitative portable device will be needed to obtain more accurate levels of G6PD activity to ensure appropriate and safe treatment.

PATH and the product developers have assessed the POC test performance through laboratory evaluations using frozen specimens. Next steps in the validation of the POC tests include an assessment of diagnostic accuracy using clinical samples in target geographies. In addition to diagnostic accuracy, there is a need to assess the POC test to ensure its suitability for use among target end users. Data on both diagnostic performance and test usability will be required to support the registration of POC G6PD tests with key regulatory authorities in target countries.

## **2. Study objectives**

The goal of this study is to contribute to a body of evidence that will support the submission of G6PD tests to the WHO prequalification (PQ) process as well as for product registration in target countries.

The primary objective is to assess the accuracy and reliability of G6PD tests in detecting G6PD activity and classifying results when used by trained health care workers in areas of relatively high *P. vivax* malaria prevalence.

Component objectives include:

- Determine the performance of point-of-care G6PD tests in detecting G6PD activity and hemoglobin compared to a reference assay.
- Assess the comprehension of the G6PD test labels and packaging among intended users.
- Assess the usability of G6PD test result outputs among intended users, primarily laboratory technicians working at health centers in the study area.

## **3. Study design**

This is a cross-sectional diagnostic accuracy study that includes both patient and health worker participants and involves recruitment at facilities and within communities at XXX sites. Health workers will include trained intended users of the G6PD tests. Health workers will take blood samples and conduct malaria rapid diagnostic tests and POC G6PD testing on participant samples. G6PD results will be compared to a reference assay. Trained health workers will also be surveyed to assess product usability through a questionnaire to assess label and packaging comprehension as well as results interpretation for simulated G6PD tests.

Study endpoints include:

- Diagnostic performance (sensitivity and specificity) of the POC G6PD test compared to reference assay:
  - Accuracy between the POC G6PD test measure of G6PD activity and a reference assay.
  - Accuracy between the POC G6PD test measure of hemoglobin (Hb) and a reference assay.
- Comparison of the POC G6PD test results for capillary and venous samples.
- Percentage of trained health workers who can accurately comprehend key messaging included in the test packaging and labels.
- Percentage of trained health workers who can accurately interpret the result output and classify results as either normal, invalid, deficient, or intermediate.

### 3.1 Statistical analyses

Data will be entered into a database with built-in validation rules to minimize data entry errors. Descriptive statistical analysis, including calculating point estimates, distribution, and frequencies of responses, will be used to summarize and characterize the study population.

#### 3.1.1 Diagnostic accuracy and performance

For the purposes of this study, an individual will be considered G6PD deficient (case) if they test positive by the spectrophotometric gold standard. The primary success criterion will be focused on the ability to identify G6PD-deficient patients correctly, such that the POC G6PD test and the spectrophotometric gold standard test should both accurately identify all severely G6PD-deficient specimens (with <30% normal) as deficient.

The performance of the POC G6PD test against the spectrophotometric gold standard test will be determined by calculating the sensitivity and specificity. Sensitivity and specificity of the POC G6PD assay will be calculated as per Domingo et al. [10]. In summary, an adjusted male median will be calculated for both the POC G6PD and spectrophotometric gold standard tests; from this median, the 30%, 40%, 70%, and 80% cutoff levels for the two tests will be used to categorically define G6PD-deficient cases.

Sensitivity will be determined using the following method:

- TTP = test and true positive (positive by reference assays according to case definition and positive by the POC G6PD test).
- FNTP = false negative true positive (positive by reference assays according to case definition and negative by the POC G6PD test).
- Sensitivity =  $TTP / (TTP + FNTP)$ .

Specificity will be determined by the following method:

- TPTN = test positive true negative (negative by reference assays according to case definition and positive by POC G6PD test).
- TTN = test and true negative (negative by reference assays according to case definition and negative by POC G6PD test).
- Specificity =  $TTN / (TTN + TPTN)$ .

Sensitivity and specificity results will be reported using 95% confidence intervals.

#### 3.1.2 Accuracy between G6PD activity and hemoglobin methods

Quantitative agreement for both G6PD activity and Hb values between the POC G6PD test and the spectrophotometric gold standard test will be graphically analyzed. Correlation graphs between the POC G6PD test and the gold standard test will be plotted and an R squared value will be determined. An R squared value of greater than 0.9 for both G6PD activity and Hb will be considered acceptable.

Bland Altman plots, where differences between the G6PD POC test and the gold standard test are plotted against the gold standard value, will be used together with the 95% limits of agreement. Acceptable limits of agreement for Hb should be within +/-1.0 g/dL (based on a 6% estimate for allowable method bias) and for G6PD activity should be within +/-2.0 U G6PD/g Hb (based on a 15% estimate for allowable method bias).

All statistical analyses will be performed using Stata 13.0.

The data comparison for the analyses is outlined in Table 1 below.



Table 1. Comparison methods.

Index test by sample type	Reference method	
	G6PD normalized for Hb	Hb
Venous	Pointe Scientific G6PD from normalized for Hb from venous specimen	Hb from venous specimen
Capillary		HemoCue® Hb from finger stick

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; Hb, hemoglobin.

*HemoCue is a registered trademark of HemoCue AB.*

### 3.1.3 Diagnostic usability

Response choices to usability assessment questionnaires will include both multiple-choice and open-ended responses. The usability questionnaire will include a brief assessment of health literacy using a validated health literacy instrument, the Rapid Estimate of Adult Literacy in Medicine – Short Form score sheet. Participants will be encouraged to comment on any aspects of the label or results they find confusing or inadequate. Success criteria are defined as 85% correct participant responses to questions that assess key messages and results interpretation. Any participant who obtains 85% or above correct responses to the usability questionnaire will be considered to accurately comprehend the product labels and instructions for use. Analyses will include descriptive statistics and a tabular presentation of findings.

## 3.2 Sample size

The sample size for this study is based on the expected prevalence of G6PD deficiency and on data requirements set by WHO through their process of prequalification of in vitro diagnostics [11]. This requires obtaining samples from participants with a range of G6PD activity levels. The WHO PQ process defines these levels as shown in Table 2.

Table 2. G6PD activity thresholds.

Sex	Level	Threshold
Female	Deficient	G6PD activity <30% of the adjusted male median
Female	Intermediate	G6PD activity 30% to 80% of the adjusted male median
Female	Normal	G6PD activity >80% of the adjusted male median
Male	Deficient	G6PD activity <30% of the adjusted male median
Male	Normal	G6PD activity >30% of the adjusted male median

Abbreviation: G6PD, glucose-6-phosphate dehydrogenase.

According to the target product profile, the novel POC G6PD will need to be at least 95% sensitive for detecting G6PD activity levels at 30% to 80% of normal enzyme activity. Assuming a sensitivity of 95%, with a confidence interval of 95%, and a 1% to 2% maximum marginal error, a minimum of 162 participants with deficient and intermediate G6PD activity will be needed for the study. To account for any possible device/diagnostic failures or compromised blood samples due to insufficient blood, signs of blood degradation, or contamination, the sample size is increased by 20%. In addition, to ensure enough deficient and intermediate participants if a lower-than-expected enrollment rate of these two groups occurs, at least 200 participants of each group are expected to be included in the study. This enriched sample will also serve to meet the requirements of WHO PQ. Eligible adults and children aged 2 and above will be recruited for potential participation, although diagnostic performance analysis will not be segmented based on age.

*[Add detail regarding the sample size calculations based on estimated prevalence of G6PD activity in study sites.]*  
See reference 12 - Howes RE, Piel FB, Patil AP, et al. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. *PLOS Medicine*. 2012;9(11):e1001339. doi:10.1371/journal.pmed.1001339.

Per guidance from WHO PQ and United States Food and Drug Administration (US FDA) guidelines for usability testing, 15 purposively selected intended users of POC G6PD tests will be sampled for the usability assessment across study sites [11, 13]. The data from this usability assessment may be combined with data from a similar sample of health workers in other evaluations.

### 3.3 Study sites

*[Add detailed information about the geographic location of the study and the facilities the study will be implemented through; highlight key features of sites and why they were selected.]*

## 4. Research participants

### 4.1 Characteristics of research participants

Patients:

*[Add detailed information about the participants that will be recruited for the study; highlight key features of participants and why they will be selected.]*

Health workers:

*[Add detailed information about the participants that will be recruited for the study; highlight key features of participants and why they will be selected.]*

### 4.2 Inclusion and exclusion criteria

#### 4.2.1 Patients

Criteria for inclusion of patients:

- *Age*
- *Willingness to provide consent*
- *Health status*

Criteria for exclusion of patients:

- *Age*
- *Willingness to provide consent*
- *Health status*

#### 4.2.2 Health workers

Criteria for inclusion of health workers:

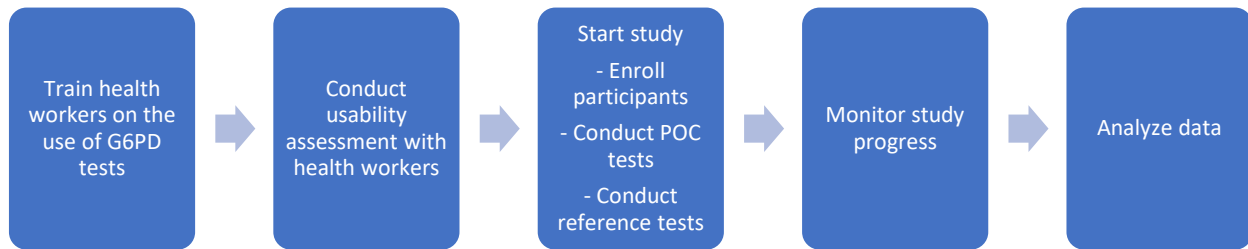
- *Age*
- *Willingness to provide consent*
- *Health status*

Criteria for exclusion of health workers:

- Age
- Willingness to provide consent
- Health status

## 5. Study procedures

Figure 1. Study process.



Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; POC, point-of-care.

### 5.1 Usability assessment

#### 5.1.1 Screening and recruitment

*[Describe the screening and recruitment processes in detail. Clearly indicate how this process may or may not impact the health care worker's job, maintain privacy, etc.]*

#### 5.1.2 Training and data collection

*[Describe how the health care workers will be trained and by whom. Clearly indicate what the training will include and therefore what the health care worker is responsible for doing.]*

### 5.2 Diagnostic performance assessment

#### 5.2.1 Screening and recruitment at [study site name]

*[Describe the screening and recruitment processes in detail. Clearly indicate how this process may or may not impact the patient's access to care, maintain patient privacy, etc.]*

#### 5.2.2 Testing procedures at the point of care

*[Describe in detail the testing procedures done at the point of care involving patients including what information will be shared with patients based on consent.]*

*Following completion of the informed consent process, the study staff, who are trained in phlebotomy, will use the lancet in the POC kit to obtain a finger stick sample of no more than 50 µl from the participant and conduct a malaria test, the G6PD POC test, and the POC Hb test. Results of the POC tests will be recorded on data collection forms. If an invalid test result is obtained, a second finger stick sample will be obtained and a second test will be run. If an invalid test result is obtained a second time, the results will be recorded as invalid. If the participant tests positive for malaria or is found to be anemic based on Hb thresholds from WHO, the participant will be referred to a local health worker or clinic for follow-up and case management.*

*The study staff will also draw a 4-ml tube of venous blood from the participant. The venous blood specimen will be taken using a standard venipuncture kit, and blood will be collected in an ethylenediaminetetraacetic acid (EDTA)–*

treated tube. The tube will be labeled with the study ID number. The samples will be transported refrigerated to the central hematology lab according to standard operating procedures of the study.

The blood draw and use of the POC test will take up to 30 minutes. G6PD testing is not routinely done at the point of care as part of standard care. Participants will be told the results of the malaria rapid test and Hb test immediately at the point of care. In the event of a positive malaria rapid test or an Hb test indicating possible anemia, the participant will be counseled and referred to the health facility for follow-up and any necessary treatment. In the event of a clinically relevant outcome from the laboratory reference test, results will be reported to the participant within one week. At that time, the appropriate counseling for the given conditions will be provided. For more detailed information on results return, see section 5.4.

The study staff will explain the G6PD results to the participant and explain why they are clinically relevant. If the participant has been categorized as G6PD deficient or intermediate by the reference assay, the study staff will inform that participant that G6PD deficiency is a genetic condition and recommend that they may want to encourage their family to be tested as well. Study staff will inform participants where and how their family members can be tested. G6PD testing with the fluorescent spot test (a standard qualitative test) will be offered free of cost to the immediate family members of participants who are found to be G6PD intermediate or deficient.

### **5.2.3 Testing procedures in the laboratory**

*[Describe in detail the testing procedures done in the laboratory with patient samples, including which operators will be responsible for which tests and how operators should be blinded to certain results. Indicate what will be done with leftover samples.]*

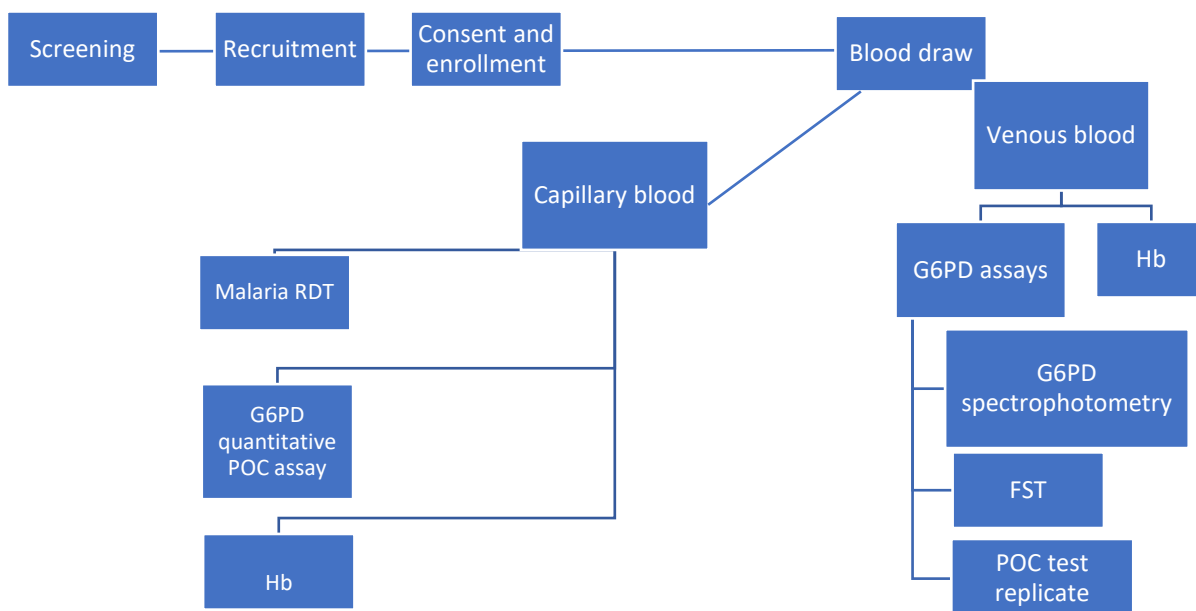
At the laboratory, aliquots of the venous blood sample will be used to run an additional POC test and reference tests. Depending on availability, additional POC G6PD tests that are both commercially available and currently under development may be run as well. Hemoglobin (Hb value [g/dl]) will be determined within 24 hours using a hematology analyzer and HemoCue for each sample to estimate the G6PD activity. Aliquots of the venous blood will be used for running the spectrophotometric assay (reference or gold standard) and the fluorescent spot test. The reference assay, Hb test, and replicate of the G6PD quantitative test will be conducted at the same time. A thermometer and a hygrometer will be placed at the study site near the assays. Temperature and humidity will be observed at the time the tests are run and recorded in the study database.

Test operators in the laboratory will be blinded to the results of the POC tests obtained and recorded in the field, and operators of the reference assay will be blinded to the results of the replicate POC test run on venous samples. If there is a discrepancy between any of the results of the POC test and the reference assay greater than allowed by the target product profile, testing with the reference test will be repeated using stored venous samples when possible. Performance characteristics and the results of additional testing of samples with discrepant results will be reported as per WHO guidelines [11].

Leftover samples will be stored for possible confirmatory or additional testing. Depending on availability of additional novel G6PD tests at the time of study start, additional assays may be run on remaining samples. The protocol will be amended to include these novel tests if they are involved in the study. For any novel assay to be included in the study, appropriate regulatory procedures will be followed to include the assay for research purposes only. This includes products that are not yet commercially available and products that are commercially available but are not part of the standard of care at the clinic site. In the event that stored samples are used, XXX will be responsible for obtaining the necessary ethical approvals.

See Figure 2 on the following page for a summary of tests to be performed on the samples.

Figure 2. [E.g., Diagnostic performance assessment process.]



Abbreviations: FST, fluorescent spot test; G6PD, glucose-6-phosphate dehydrogenase; Hb, hemoglobin; POC, point-of-care; RDT, rapid diagnostic test.

### 5.3 Specimen collection, transport, and storage

*Immediately after collection, whole blood in EDTA will be stored in a refrigerator or a cooler box and transported to the lab.*

*[Add detailed information regarding how samples will be collected, transported, and stored based on study site information.]*

*In the lab, each specimen will be immediately aliquoted as below.*

- *Aliquot 1x 2 ml of whole blood in a falcon tube or conical screw cap tube. Store at 4°C in the refrigerator. This will be used for all assays. Specimens should be kept refrigerated (2°C–8°C) prior to testing for up to 72 hours. Specimens will be discarded 3 weeks after collection.*
- *Aliquot 4x 0.5 ml of whole blood in cryogenic tubes. Store at –80°C in the freezer. Maximum storage time is 10 years after study end. This leftover sample will be stored for possible additional testing. Appropriate permission for additional testing relating to malaria will be obtained through the consent process.*

### 5.4 Test result return

*[Describe in detail what information will be shared with the patient, when the information will be shared, and how the information will be shared. Explain how this may or may not impact the patient's standard care.]*

#### 5.4.1 Test result return when no primaquine is available

*Results of POC malaria testing and Hb testing will be returned immediately to participants. Study staff will relay results and counsel participants who test positive for malaria or anemia according to the results return script.*

*Participants will be notified at recruitment that the study team will tell them the results of the G6PD test after confirmation by the reference assay only if they are found to be G6PD intermediate or deficient. Confirmatory testing will be done prior to informing the participant of this result. Participants who are found to be G6PD normal by the reference assay will not be notified of their result. Any participants found to be G6PD intermediate or deficient will be*

contacted via phone to provide counseling; they will be asked to pick up a card with further information for patients and providers or will be visited by study staff in person (Appendix XX).

The study team will inform the participant that G6PD is a genetic condition and recommend that they may want to encourage their family to be tested as well. The study team will inform participants where and how their immediate family members can be tested free of cost. The card describes G6PD deficiency as it relates to contraindicated medications and other risk factors such as foods. The researchers involved in the return of G6PD test results have significant experience working in G6PD research and counseling study participants as to the results. All staff involved in returning these results will receive dedicated training aimed at providing the necessary information and delivering it in a way that facilitates comprehension.

The study will make use of a study key that links participant ID, name, and contact information. This key will be populated at the time of consent and enrollment. This key will be accessible only by the field team lead and kept in a secure location. This key will enable the study team to follow up with participants who are found to be G6PD deficient or intermediate by laboratory reference assay. Once all laboratory testing and confirmatory testing is complete and all appropriate participants have been notified of their test results, the study key will be destroyed.

#### **5.4.2 Test result return and follow-up when primaquine is available**

Results of POC malaria tests will be returned to participants as per standard care. Results of POC Hb tests will be returned to participants immediately after testing. Study staff will relay results and counsel participants who test positive for malaria or anemia according to the results return script. Any participants identified as malaria positive or anemic will be counseled and referred for follow-up. Participants in the clinic will receive standard care at the clinic and participants recruited through household surveys will be referred for follow-up to the local health agent as per routine practice.

In XXX, no routine screening to identify individuals with G6PD deficiency is implemented in the public health system and treatment with primaquine is recommended for all people infected with *P. vivax* with the exception of pregnant women and children under six months of age. However, information regarding the G6PD status of a *P. vivax* patient is critical for informing safe and effective treatment recommendations. As per WHO guidelines for the treatment of malaria, standard treatment of primaquine should not be given to patients who are G6PD deficient to avoid primaquine-induced hemolysis, and patients who are G6PD intermediate should be monitored closely for signs of hemolysis [15].

The POC G6PD test is registered for use in Thailand and India, and it has a European Conformity certification (CE mark). In addition, initial evaluations by PATH indicate that the POC G6PD test is able to identify G6PD deficiency (see section 7.1). As such, participants who test positive for *P. vivax* malaria and are found to be G6PD deficient or intermediate will be notified of their POC test result in order to make that information available to their health provider when determining their course of treatment.

#### **5.4.3 Participants found to be *P. vivax* positive and G6PD deficient**

For *P. vivax*-positive participants found to be G6PD deficient with the POC test (G6PD test result of below 30% of normal enzymatic activity), the study team will provide referral with the recommendation that primaquine treatment be withheld until confirmatory testing can be completed. Confirmatory testing will take no more than one week to complete. At that time, through referral to the health agent, *P. vivax* patients who are identified as G6PD deficient can receive treatment for the blood stage disease (treatment with chloroquine) immediately.

If the results of the reference assay confirm the results of the POC G6PD test (enzymatic activity less than 30%), the participant will be contacted and informed of the result and their G6PD status by validated reference assay. If the results of the reference assay indicate that the participant is G6PD normal, they will be contacted, informed of the result, and referred to a health agent to receive standard treatment for liver stage disease, as per national guidelines.

#### 5.4.4 Participants found to be P. vivax positive and G6PD intermediate

For P. vivax–positive participants found to be G6PD intermediate with the POC test (G6PD test result between 30% and 80% of normal enzymatic activity), the study team will provide referral with the recommendation that primaquine treatment be closely monitored for signs of hemolysis, given the increased risk of hemolysis associated with moderate G6PD deficiency.

If the results of the reference assay confirm the results of the POC G6PD test (enzymatic activity between 30% and 80%), the participant will be contacted and informed of the result and their G6PD status by validated reference assay. If the results of the reference assay indicate that the participant is G6PD normal, they will be contacted, informed of the result, and referred to a health agent to receive standard treatment for liver stage disease, as per national guidelines.

Among any P. vivax–positive participants, those known to be G6PD intermediate or deficient based on previous studies may receive a false negative result on the POC investigational test in this study and found to be G6PD normal. In the event that this occurs, the study team will proceed in the same manner as for participants found to be P. vivax positive and POC G6PD deficient or intermediate. See Figures 3 and 4.

Participants will be notified at recruitment that the study team will tell them the results of the G6PD test only if they are found to be G6PD intermediate or deficient. For participants who do not have P. vivax malaria, confirmatory testing will be done prior to informing the participant of this result. Participants who are found to be G6PD normal by the reference assay will not be notified of their result unless they were notified of a G6PD deficient and/or intermediate result by the POC test that needs to be corrected. If they were reported a false positive result from the POC test and found to be G6PD normal by the reference assay, the study team will follow up and provide the results of the reference assay and necessary counseling. Any participants found to be G6PD intermediate or deficient will be contacted by the study team to provide counseling and given a card with further information for patients and providers.

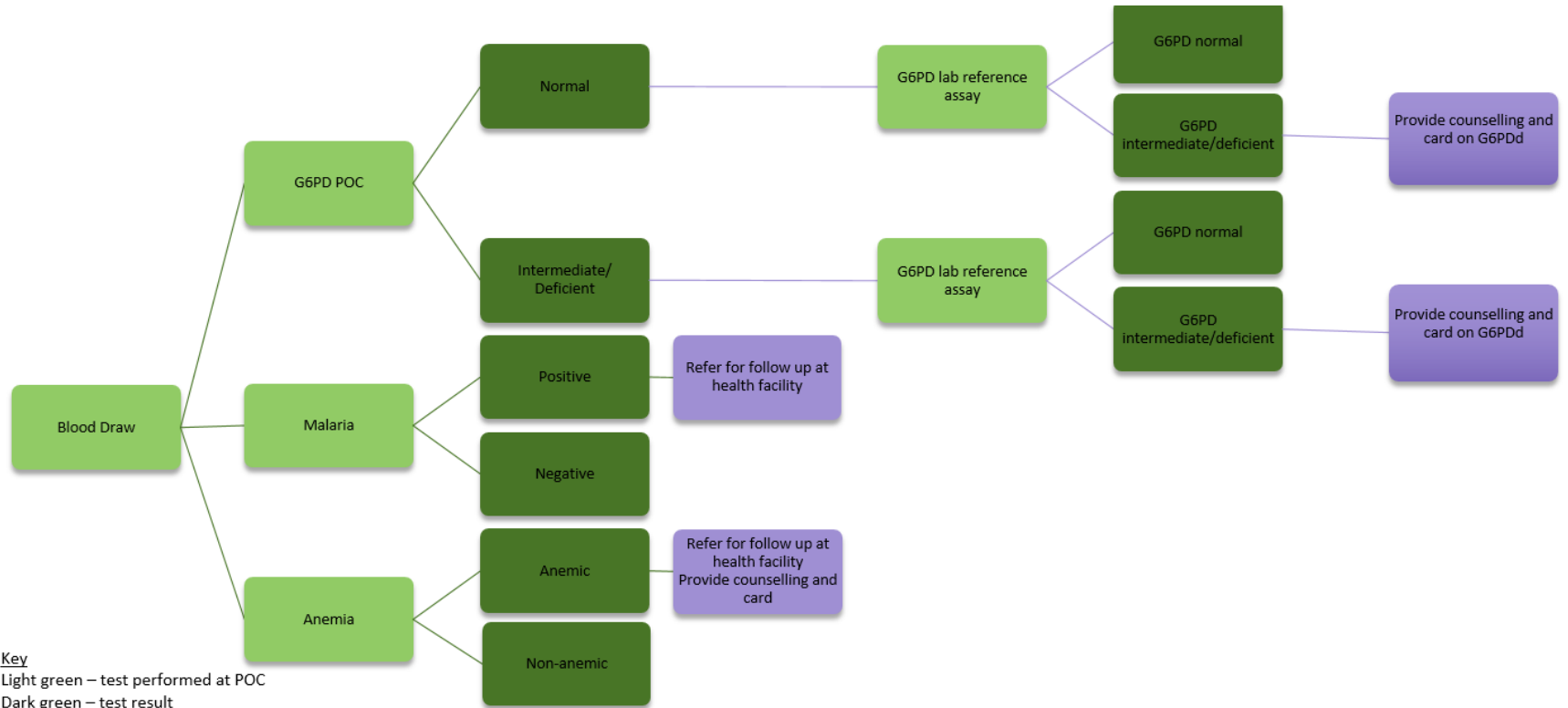
The study team will inform the participant that G6PD is a genetic condition and recommend that they may want to encourage their family to be tested as well. The study team will inform participants where and how their immediate family members can be tested free of cost. The card describes G6PD deficiency as it relates to contraindicated medications and other risk factors such as foods. The researchers involved in the return of G6PD test results have significant experience working in G6PD research and counseling study participants as to the results. All staff involved in returning these results will receive dedicated training aimed at providing the necessary information and delivering it in a way that facilitates comprehension.

#### 5.5 Summary of study procedures

Table 3. Summary of study procedures.

Participant group	List of study procedures	Estimated time	Data collection forms used
Health workers	•	•	•
Children aged 2–18	•	•	•
Adults	•	•	•

Figure 3. [E.g., Patient flow and follow-up procedures] with no primaquine.

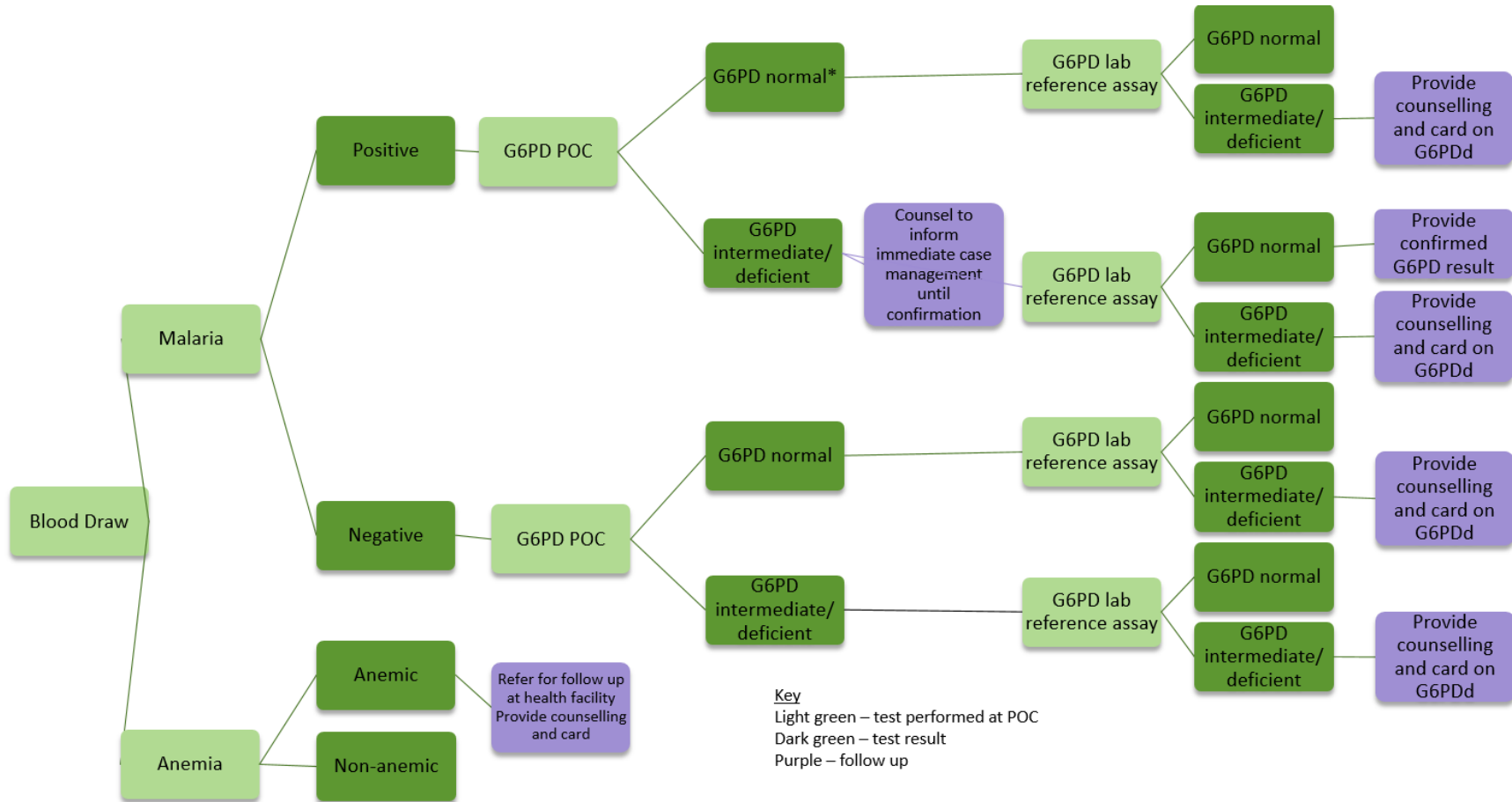


Key  
 Light green – test performed at POC  
 Dark green – test result  
 Purple – follow up

Abbreviations: G6PDd, glucose-6-phosphate dehydrogenase deficiency; POC, point-of-care.



Figure 4. [E.g., Patient flow and follow-up procedures] with primaquine.



Abbreviations: G6PDd, glucose-6-phosphate dehydrogenase deficiency; POC, point-of-care.

\**P. vivax*-positive patients, recruited from the enriched sample of participants known to be G6PD deficient or intermediate, may receive a false negative (G6PD normal) result from the investigational POC test. In the event this occurs, this subpopulation will be considered G6PD intermediate/deficient and provided counseling and a counseling card as described in section 5.4.

## 5.6 Standard care and study procedures

*[Clearly describe the relevant local standard care procedures and how the study procedures relate to them.]*

Table 4 summarizes how the test results from the study protocol will impact the standard of care for the study volunteers.

Table 4. *[Example case management.]*

Study test	Result	Action
Malaria	Negative test result (no malaria)	No action.
	Positive test result (confirmed malaria)	Counseling about the results of the malaria rapid diagnostic test and referral to health system to receive standard care. Malaria treatment is free.
Hemoglobin	Normal hemoglobin levels (by Ethiopia national guidelines)	No action.
	Mild moderate or severe anemia (by Ethiopia national guidelines)	Counseling about the results of the hemoglobin test and referral to the health system for follow-up case management.
G6PD deficiency	Negative test result on investigational device (G6PD normal)	No immediate action. If reference assay is discrepant and identifies G6PD intermediate or deficient status, the study team will conduct follow-up counseling (Appendix XX).
	Positive test result on investigational device (G6PD deficient or intermediate)	Result will be confirmed at the laboratory with the reference assay. If confirmed as G6PD deficient or intermediate with the reference assay, the study team will conduct follow-up counseling (Appendix XX).

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase.

## 6. Consent process

### 6.1 Usability study

*[Describe in detail the consent process for the health care workers participating in the usability study. Clearly indicate how this process may or may not impact the health care worker's job, maintain privacy, etc. Add information that states the health care worker's ability to withdraw from the study at any time.]*

### 6.2 Diagnostic performance assessment

*[Describe in detail the consent process for the patients participating in the performance assessment. Clearly indicate how this process may or may not impact the patient's access to care, maintain patient privacy, etc. Add information that states the patient's ability to withdraw from the study at any time.]*

## 7. Study products

### 7.1 SD Biosensor STANDARD G6PD Analyzer

The STANDARD G6PD Analyzer (Figure 5 on the following page) is designed to measure the quantitative determination of total Hb concentration and G6PD enzymatic activity in fresh human whole blood specimens based on reflectometry assays. The test is intended to aid in the identification of people with G6PD deficiency. **The test is currently [NOT] licensed for use at the study site and is [NOT] considered an investigational product.**

*The STANDARD G6PD Analyzer is currently registered for use in Thailand.*

Figure 5. SD Biosensor STANDARD G6PD Analyzer.



### 7.1.1 Performance of STANDARD G6PD test when stress-tested under multiple temperature and humidity conditions

The STANDARD G6PD values were compared to the Pointe Scientific–generated G6PD values for data collected by PATH across the following ambient conditions: 22°C, 32°C 50% humidity, 37°C 50% humidity, and 37°C 75% humidity. Receiver operating characteristic analysis was performed to generate the optimal thresholds for the STANDARD G6PD test (Table 5).

Table 5. STANDARD G6PD test data.

Threshold (Pointe Scientific value U/g Hb)	Optimal STANDARD G6PD (U/g Hb)	Sensitivity (95% CI)	Specificity (95% CI)
30% (2.8)	3.2	100 (95.1–100.0)	96 (93.0–98.3)
70% (6.5)	6.0	98 (92.4–99.7)	99 (96.8–99.9)
80% (7.4)	6.2	86 (80.7–93.9)	99 (95.9–99.7)
100% (9.3)	NA	NA	NA

Abbreviations: CI, confidence interval; Hb, hemoglobin; NA, not applicable; U, unit.

## 7.2 Pointe Scientific test kit

The Pointe Scientific test kit will serve as the reference assay to assess G6PD activity. Its intended use is for the quantitative, kinetic determination of G6PD in blood at 340 nm. It is designed for in vitro diagnostic use only.

- A spectrophotometer capable of measuring at 340 nm with a temperature-controlled cuvette compartment is required to perform the assay.
- To determine G6PD activity, which is reported in terms of grams of Hb or the number of red blood cells, the Hb or red blood cell count must be determined separately from performing the G6PD assay. Calculations are then performed to obtain the G6PD activity. For purposes of this study, the G6PD activity from the Pointe Scientific kit will be calculated in terms of grams of Hb.

- US FDA cleared: k024006, Regulatory Class II, Product Code JBF.
- Approved as the predicate device for POC G6PD test evaluation by the US FDA.

### 7.3 Fluorescent spot test

The fluorescent spot test or Trinity Biotech G-6-PDH Screen Kit is widely used for in vitro diagnosis of G6PD deficiency using whole blood or dried blood spots. The fluorescent spot test is a qualitative test performed by incubating a small amount of blood with glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate. Drops of the mixture are removed at 5-minute intervals, spotted on filter paper, and then viewed under long-wave ultraviolet light. Fluorescence is clearly evident in mixtures prepared from normal blood, whereas deficient samples yield little or no fluorescence. The test is affordable and produces qualitative, visual results in minutes. This test will represent a qualitative standard care test that is currently available and cleared by the US FDA. The test will be performed according to PATH's guide to fluorescent spot testing for G6PD deficiency [14].

### 7.4 Malaria rapid diagnostic test

CareStart Malaria rapid diagnostic tests can diagnose malaria infection from whole blood of patients in 20 minutes. The CareStart Malaria HRP2/pLDH(Pf/PAN) Combo detects and distinguishes between *P. falciparum* and *P. vivax* infections.

### 7.5 HemoCue system

The HemoCue Hb 301 system is designed for quantitative POC whole blood Hb determination in primary care using a specially designed analyzer, the HemoCue Hb 301 Analyzer, and specially designed microcuvettes, the HemoCue Hb 301 Microcuvettes. The HemoCue Hb 301 system is for in vitro diagnostic use only. It consists of a small portable analyzer (photometer) and plastic microcuvettes. The microcuvette serves both as a pipette and as a measuring cuvette. A blood sample is drawn into the cavity by the capillary action. The filled microcuvette is inserted into the HemoCue Hb 301 Analyzer. The measurement takes place in the analyzer, which measures the absorbance of whole blood at an Hb/HbO<sub>2</sub> isobestic point. The system is factory-calibrated and needs no further calibration. The HemoCue is available and registered for use at the study site.

## 8. Benefit and risk considerations

### 8.1 Benefit to study participants

*Participants in this study will have convenient access to a malaria rapid test and an Hb test. Malaria testing is available for free through the public health system. Hemoglobin testing and G6PD testing are not routinely offered as part of standard care. Participants who test positive for malaria or are found to be anemic will be counseled and referred to a local health worker or clinic for follow-up and case management. Participants in this study will have the opportunity to have their G6PD status tested by a reference assay. This information may inform the care they receive for malaria in the future.*

*G6PD deficiency is a genetic condition that provides valuable clinical information for multiple clinical conditions beyond malaria treatment. If clinically relevant results are determined by the reference assay—that is, if G6PD activity is determined to be deficient or intermediate—participants will receive counseling regarding this information. G6PD testing with the fluorescent spot test (a standard qualitative test) will be offered free of cost to immediate family members of participants who are found to be G6PD deficient or intermediate.*

*This research will also be advantageous for academic study and in the future for other people who will benefit from better G6PD tests and malaria treatment. Health workers will have the opportunity to receive training in the use of POC G6PD tests.*

## 8.2 Risk and risk-mitigation considerations

*The proposed study involves the use of an investigational product that has CE mark regulatory approval and [is or is not registered for use in (add study site)]. As such, study procedures do not represent significant risks to the participants beyond those that are associated with normal blood draws, such as pain, discomfort, feeling light-headed, fainting, and infection at the site of finger stick or venipuncture. The risks associated with blood draws will be mitigated through adherence to standard clinic procedures for infection control and through the use of research staff who have been trained in best practices for blood collection. The volume of blood drawn as part of the study procedures is within the safety limits recommended by WHO and other organizations for both adults and children under 18 [15]. In the unlikely event of a research-related injury, cost of treatment will be covered by [XXX]. All decisions regarding clinical care or malaria case management will be made through referral to the local health care facilities.*

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

*There is a minimal risk that health workers recruited for the usability study may feel compelled to participate in the study if their supervisor has recruited them. They may feel as though the usability study is intended to assess their performance rather than the usability of the test. We will mitigate these risks through the following measures:*

- Study staff will ask supervisors to explain that participation in the study is voluntary and will not affect employment in any way.*
- Consent procedures will be conducted in private to ensure confidentiality. During the consent procedure, participants will be informed that the aim of the study is to understand the user experience and the data will be used for purposes of product development only. The data will not be used to assess their competency or linked in any way to their job performance.*

## 9. Study and safety monitoring

*We anticipate that this evaluation poses minimal risk to participants, as it does not involve any medical intervention and blood draw volumes are within acceptable ranges. No data safety monitoring board will be used. [Study partners] will conduct necessary staff training on study procedures prior to initiating the evaluation. Only trained users who have been certified as proficient in the use of the test will be involved in blood collection. The information participants will provide in the context of this evaluation is not considered sensitive, and sharing it will not pose any significant risk to them personally or professionally.*

*The study team will be supervised by the local study lead. Study data will be aggregated into a database, and a monitoring report will be generated every week, summarizing key indicators for study compliance. [Study partners] will hold weekly data review calls to discuss data collection and data quality to date. These indicators include but are not limited to the number of participants consented, the number of samples acquired, any deviations from study procedures, and corrective actions taken. In addition, a member of the study team will conduct site-monitoring visits as needed to ensure compliance with the protocol and relevant standard operating procedures.*

## 10. Ethical considerations

### 10.1 Study conduct

The investigators will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the International Conference on Harmonisation Good Clinical Practice regulations and guidelines, whichever affords the greater protection to the subject. Additionally, the investigator assures that all activities of this protocol will be guided by the ethical principles of the Belmont Report and by US Department of Health and Human Services 45 US Code of Federal Regulations 46 and all of its subparts (A, B, C, and D). Investigators and study staff

are trained in the protection of human subjects. Training in the principles of informed consent and in the study procedures for obtaining informed consent will be conducted before study initiation.

## 10.2 Informed consent

Study team members trained in the principles of informed consent and human subjects protection will obtain written informed consent from all participants of both the usability and diagnostic performance assessment.

## 10.3 Ethical review committees

The protocol, informed consent form, and recruitment materials will be submitted to *[add all ethical review committees]*.

## 10.4 Amendments

All amendments and modifications will be submitted to the above institutional review boards (IRBs) for review and approval. No changes in protocol conduct will be implemented until approvals by all IRBs are obtained.

## 10.5 Continuing review reports

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

## 10.6 Deviations

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

## 10.7 Unanticipated events

Any adverse events that are unanticipated, serious, and related or possibly related to participation in the research, any serious adverse events, or any incidents that suggest that the research places participants or others at risk, including breach of confidentiality, will be promptly reported to the appropriate IRBs within 72 hours. A complete written report will follow the initial notification. Other incidents will be reported in the annual continuing review report.

## 10.8 Compensation

*[Add details regarding compensation, if any, including reimbursement for travel, etc.]*

## 10.9 Genetic testing

G6PD is a genetic condition. The diagnostics used at the point of care and in the laboratory diagnose G6PD deficiency through a measurement of G6PD enzyme activity in the blood, not full genome sequencing.

# 11. Confidentiality and data management

## 11.1 Participant confidentiality

The investigator will ensure that participant confidentiality is maintained. Participants will not be identified in any publicly released reports of this study. All records will be kept confidential by *[study partner]*. *[Study partner]* will not have access to records that identify the subjects. Results of the usability assessment will be deidentified prior to sharing with anyone outside of the study team, including supervisors or other clinic staff.

## 11.2 Data entry

Participant data are entered on paper forms at the time the sample is taken and included with the samples sent back to the lab. All paper forms will be tracked by study ID number. Paper forms will be stored for 1 year after the study ends in locked cabinets, after which time they will be destroyed following standard site procedures.

All laboratory results will be entered into an electronic, password-protected database. Electronic study records will be deidentified upon completion of data collection. The electronic records will be maintained indefinitely in the databases and remain password-protected.

## 11.3 Data monitoring

The study team will enroll and conduct sample collection at each site and will be supervised by the local study lead. Study data will be aggregated into a database, and a monitoring report will be generated every week, summarizing key indicators for study compliance. *[Study partners]* will hold weekly data review calls to discuss data collection and data quality to date. These indicators include but are not limited to the number of participants consented, the number of samples acquired, any deviations from study procedures, and corrective actions taken. In addition, a member of the study team will conduct site-monitoring visits as needed to ensure compliance with the protocol and relevant standard operating procedures.

## 11.4 Access to data and data dissemination

Information concerning the study, processes, scientific data, and other pertinent information is confidential. Any clinically relevant information will be shared with the participant in a confidential setting. The data generated by this study will inform the commercialization of G6PD tests and may inform programmatic decisions around testing for G6PD deficiency. Data may be reviewed by the sponsor and ethics committees. Only deidentified data will be shared with study partners, test developers, and regulatory bodies that oversee product registration. All data will be published in the open medical literature with the identity of the subjects protected.

Deidentified data and samples will be stored in a secure location in *[study location]*. If the data or specimen stored is planned to be used for other purposes than this study, *[study partners]* will seek approval from the appropriate ethical review committees.

## 11.5 Quality control and quality assurance

The study will be conducted in accordance with the current approved protocol, International Conference on Harmonisation guidelines for Good Clinical Practice (relevant regulations, and standard operating procedures).

## 12. Study limitations

There are some limitations to this diagnostic evaluation. With regard to any diagnostic accuracy evaluation, there are opportunities for bias. This study will rely on the quantitative spectrophotometer assay as the reference test rather than genetic sequencing, and an imperfect reference test may lead to classification bias. Given the rates of G6PD prevalence in *[study location]* and the data requirements in the WHO verification guidelines, some purposive sampling will be required, and we expect a significant number of the samples tested in *[study location]* to be G6PD normal. Finally, given the wide range of potential end users of POC G6PD tests globally and across different segments of the health system, the results of the usability assessment may not be generalizable outside of the study sites.

### 13. Investigator responsibilities

The two project partners involved in this evaluation are [study partners]. Roles and responsibilities for each of the partners are listed below. L = lead; A = assist.

Task	[Study partner 1]	[Study partner 2]
Award oversight		
Study design and protocol development		
Institutional review board submission and approval		
Evaluation logistics arrangements		
Procurement of all study supplies		
Training on the use of study assays		
Recruitment, consent, enrollment, and field data collection		
Laboratory-based data collection		
Data entry and cleaning		
Data analysis and reporting		

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## Appendices

- A. Data collection forms
- B. Consent form
- C. Recruitment materials

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