Evaluation of a diagnostic to identify G6PD deficiency in Brazil

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Investigators:

[redacted]

Location of Research:
Manaus and Porto Velho, Brazil

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Study Title	Validation of diagnostics to identify G6PD deficiency in Brazil
Précis	Cross-sectional diagnostic accuracy study with 2,000 patient participants and 15 health worker participants. The participant population will be recruited at clinics and through a household survey using an enriched sample of a population with known G6PD status, established through previous epidemiological studies. The health worker participants will include trained intended users of the G6PD tests. Health workers will take capillary blood samples and conduct two point of care tests: 1) hemoglobin test, and 2) investigational point-of-care (POC) G6PD test. A thick blood slide will also be prepared for malaria microscopy. Venous blood will be collected and transferred to a laboratory where reference assays will be performed on venous samples using the Pointe Scientific G6PD Analyzer and hemoglobin tests. Trained health workers will also be surveyed to assess product usability through a questionnaire to assess label and packing comprehension as well as results interpretation.
Objectives	*Primary Objective:* To assess the accuracy and reliability of G6PD tests in detecting G6PD activity and classifying results when used by trained health care workers in Brazil. *Component Objectives:* • To determine the performance of G6PD tests in detecting G6PD activity and hemoglobin (Hb) compared to a reference assay • To assess the comprehension of the G6PD test packaging and labelling among intended users • To assess the usability of G6PD test result outputs among intended users
Endpoints	• Sensitivity and specificity of G6PD tests compared to the Pointe Scientific G6PD Analyzer o accuracy between POC G6PD test measure of G6PD activity and a reference assay o accuracy between POC G6PD test measure of hemoglobin and a reference assay • Comparison of POC G6PD test results using capillary and venous samples • Percent of trained health workers who can accurately comprehend key messaging included in the test packaging and labels • Percent of trained health workers who can accurately interpret the result output and classify results as either normal, invalid, deficient or intermediate
Population	Based on estimated G6PD prevalence, we expect to recruit approximately: • 1,700 people with normal G6PD activity levels • 200 people with intermediate G6PD activity levels • 100 people with deficient G6PD activity levels For the usability portion, we will recruit 15 trained health workers.
Study sites	This study will be conducted in Manaus and Porto Velho.
Study Duration	9 months (estimated)

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1.0 Background and rationale for the study

Glucose-6-phosphate dehydrogenase (G6PD) is a critical housekeeping enzyme in red blood cells (RBC) 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 [3,4]. 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 antimalarial 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 often is first manifested in newborns 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)."[5]. 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 (ACT) for all species of malaria or, in some countries, for *Plasmodium (P.) falciparum* malaria and chloroquine for *P.vivax* malaria depending on the country policy. While patients with *P. falciparum* infections are cured with ACT, 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 relapse. 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 regimen in comparison to a multi-day (7-14 days) regimen for primaquine. Tafenoquine is not currently available on the market.

Because of the risks associated to G6PD deficiency for primaquine, the WHO recommends as good practice in the current malaria treatment guidelines:
"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, the WHO also recommends:
"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."

Considering these recommendations, some country malaria guidelines only treat *P.vivax* blood stage infections and do not provide primaquine. Other countries provide primaquine without testing for G6PD deficiency and others provide primaquine only if testing for G6PD deficiency is available.

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 [6,7]. This method, considered the gold standard, is a costly and complex quantitative laboratory based spectrophotometric test. While 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), these methods require an equipped laboratory and skilled personnel.

In field settings, the most commonly performed tests use qualitative devices such as the fluorescent spot test (FST) or the CareStart test, which can only discriminate gross deficiencies from all the other phenotypes [8-10]. These qualitative tests are adequate for males who are either deficient or normal in G6PD status. Because females carry two alleles of the G6PD gene, they can present as deficient, intermediate, or normal for G6PD activity. Qualitative tests cannot discriminate intermediate from normal G6PD activity. Quantitative tests are required for better case management for women, especially in anticipation of the availability of tafenoquine which is indicated for use only for women and men with normal G6PD activity.

Given the target populations where antimalarial drugs are required, 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.

To date, no routine screening of G6PD deficiency is performed in Brazil before primaquine use for radical cure of *P. vivax* malaria. However, recent data suggest that G6PD screening could be cost-effective and that implementation of this additional step in malaria diagnosis and treatment is being considered by the Ministry of Health.

In order to enable the use of radical cure treatment for *P. vivax* malaria, PATH is working to accelerate the development of G6PD tests that meet the following criteria:

- Quantitative
- Measures G6PD activity normalized by hemoglobin (IU/g Hb)
- Measures hemoglobin (g/dL)
- Corrects for enzyme temperature dependence
- Finger stick (<10 ul)
- Point-of-care
- Easy-to-use
- Affordable
- Reviewed by WHO Prequalification Programme (WHO PQ)

PATH and the product developers have assessed 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.

In Brazil, there is no routine screening to identify individuals with G6PD deficiency in the public health system and treatment with primaquine is recommended for all persons infected with *P.vivax*, with the exception of pregnant women and children less than six months of age [11-12]. In a recent cost study, Peixoto et al. estimate that the use of primaquine among the population with G6PD deficiency represents a great burden for the public health system on Brazil [13].

2.0 Study objectives

The hypothesis of this study is that the rapid test for G6PD and Hemoglobin, SD Biosensor, has equal accuracy by means of a comparison with the gold standard and high level of usability and acceptability among healthcare professionals who will execute it. The goal of this study is to contribute to a body of evidence that will support the submission of G6PD tests to the WHO PQ process and 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. Component objectives include:

- To determine the performance of G6PD tests in detect G6PD activity compared to a reference assay
- To assess the comprehension of the G6PD test packaging and labelling among intended users
- To assess the usability of G6PD test result outputs among intended users

3.0 Study design

This is a cross-sectional diagnostic accuracy study that includes both participants and health worker participants. Health workers will include trained intended users of the G6PD tests. Health workers will take capillary blood samples and conduct POC G6PD testing on participant samples. 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 packing comprehension as well as results interpretation for simulated G6PD tests.

Study endpoints include:

- Diagnostic performance (sensitivity and specificity) of POC G6PD test compared to reference assay
    - Accuracy between POC G6PD test measure of G6PD activity and a reference assay
    - Accuracy between POC G6PD test measure of hemoglobin and a reference assay
- Comparison of POC G6PD test results using capillary and venous samples
- Percent of trained health workers who can accurately comprehend key messaging included in the test packaging and labels
- Percent 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 Microsoft Excel 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 participants correctly, such that both the POC G6PD test and the spectrophotometric gold standard test should both accurately identify all G6PD deficient specimens (with < 20% normal) as deficient.

The performance of the POC G6PD against the spectrophotometric gold standard will be determined by calculating the sensitivity and specificity. Sensitivity and specificity of the SD Biosensor (SDB) G6PD assay were calculated as per Domingo et al. [14]. In summary, an adjusted male median will be calculated for both the POC G6PD and spectrophotometric gold standard test from where the 30%, 40%, 70% and 80% cutoff levels for the two tests will be used to categorically define G6PD deficient cases.

Sensitivity calculations will be determined by the following method: TTP=true and test 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 spectrophotometric gold standard will be graphically analyzed. Correlation graphs between the POC G6PD test and 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 tests and gold standard test are plotted against the gold standard value, together with the 95% limits of agreement. Acceptable limits of agreements 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 below:

Table 1. Comparison methods

Index test by sample type	**Reference method**	
	G6PD normalized for Hb	**Hb**
Venous	Point Scientific G6PD from venous specimen normalized for Hb from venous specimen	Hb from venous specimen
Capillary		Hemocue Hb from finger stick

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. 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 response to questions that assess key messages and results interpretation. Any participant that obtains 85% or above correct responses to the usability questionnaire will be considered to accurately comprehend the product IFU and labelling. 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 at the study site and by data requirements set by WHO PQ [15]. This requires obtaining samples from participants with a range of G6PD activity levels. The WHO PQ process defines these levels as such:

Table 2. G6PD activity thresholds

Females	
Deficient	G6PD activity < 30% of the adjusted male median
Intermediate	G6PD activity 30%-80% of the adjusted male median
Normal	G6PD activity > 80% of the adjusted male median
Males	

Deficient	G6PD activity < 30% of the adjusted male median
Normal	G6PD activity > 30% of the adjusted male median

According to the Target Product Profile (TPP), 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. To account for any possible device/diagnostic failures or compromised blood samples due to insufficient blood, signs of blood degradation, or contamination, to the sample size will need to increase by 20%. In addition, to ensure there are sufficient deficient and intermediate participants in the case of a lower-than-expected enrollment rate of these two groups occurs, at least 200 participants of each group should be included in the study. This additional enriched sample will also serve to meet the requirements of the WHO PQ. Eligible adults and children aged 2 years old and above will be recruited for potential participation though diagnostic performance analysis will not be segmented based on age.

The estimated prevalence of G6PD activity at less than 30% of normal enzyme activity is 5% in Brazil [16]. In Brazil, testing a minimum of 2,000 individuals is expected to generate approximately 100 deficient and 200 intermediate individuals. These data will be combined with data from other clinical evaluation sites in Ethiopia and India. The estimated prevalence of G6PD activity at less than 30% of normal enzyme activity is 2.5% in Ethiopia and 5% in India [16]. In Ethiopia, testing a minimum of 1,500 individuals is expected to generate approximately 38 deficient and 75 intermediate individuals and in India testing a minimum of 1,000 individuals is expected to generate approximately 50 deficient and 100 intermediate individuals. By combining the data from multiple clinical sites, these studies will reach the target number of samples required by WHO PQ.

Per guidance from the WHO PQ and US FDA guidelines for usability testing, 15 purposively selected intended users of POC G6PD tests will be sampled for the usability assessment across both study sites [15] [17]. As in the diagnostic performance assessment, the data from this usability assessment will be combined with data from a similar sample of health workers in other evaluations (a minimum of 15 in each Brazil and India) in order to reach the target number of users required by WHO PQ.

Participant group	Recruitment sample size	Target enrollment sample size
Participants	2,200	2,000
Health workers trained in the use of the G6PD test	18	15

3.3 Study sites

This study will be conducted in Manaus and Porto Velho, reference centers for malaria diagnosis and treatment which already perform gold standard diagnosis of G6PD because of concomitant clinical trials in which this information is key (e.g., tafenoquine trials).

These centers are also responsible for technical supervision and training of other teams working in the primary care in the municipalities. Therefore, these sites have access to lower levels of health care service delivery.

4.0 Research participants

4.1 Characteristics of research participants

Participants:

Participants for this study will include both febrile patients seeking care at the Manaus and Porto Velho clinics and participants recruited via and enriched sample with known G6PD status established through previous epidemiological studies. Approximately half of the total participants will be recruited from each patient population. Participants with known G6PD recruited from the previous epidemiological studies have agreed to have their G6PD status retained and to be contacted again for participation in future studies as part of the informed consent process.

Health workers:

The health workers recruited for participation in this study will be representative of intended end users of point of care G6PD tests, as per requirements of the WHO PQ. This will include both health care providers such as community health workers, and laboratory technicians who perform malaria rapid diagnostic tests and other malaria testing near the point of care.

4.2 Inclusion and exclusion criteria

4.2.1 Participant with unknown G6PD status

Inclusion criteria:

- Seeking care at the Manaus or Porto Velho clinics
- 2 years age or older
- Willing to provide informed consent

Exclusion criteria

- Younger than 2 years of age
- Participants who received a blood transfusion in the last 3 months, self-report
- Unwilling to provide informed consent

4.2.2 Participant with known G6PD status

Inclusion criteria:

- Included in previous G6PD surveys and provided consent to be contacted again
- 2 years of age or older
- Willing to provide informed consent or assent

Exclusion criteria:

- Younger than 2 years of age
- Participants who received a blood transfusion in the last 3 months, self report
- Unwilling to provide informed consent or assent or unavailable during study visit

4.2.3 Health worker:

Inclusion criteria:

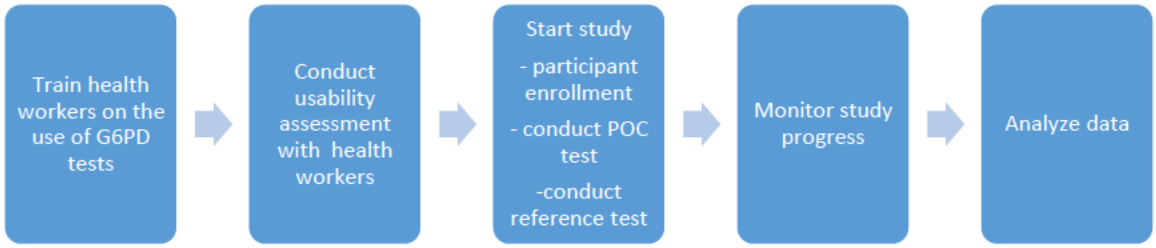
- Provides malaria case management at study facility or study site
- Considered an intended user of quantitative POC G6PD tests
- Trained and proficient in the use of the POC G6PD test
- Willing to provide informed consent

Exclusion criteria:

- Does not provide malaria case management at study facility or study site
- Not considered an intended user of quantitative POC G6PD tests
- Not trained or is not considered proficient in the use of the POC G6PD test
- Unwilling to provide informed consent

5.0 Study procedures

Figure 1. Study process



5.1 Usability assessment

5.1.1 Screening and recruitment

First, study staff will conduct an orientation for laboratory managers and health workers to explain the study, its objectives, and what participation will entail. At the orientation, the study staff will explain that participation is voluntary, that it will not affect their employment, and ask managers and supervisors to emphasize the voluntary nature of participation among their staff. Health workers who meet the above inclusion and exclusion criteria will be identified by study staff through discussions with laboratory managers and invited to participate in a training on G6PD testing. Health workers will be recruited purposively based on whether their role aligns with a use case for the POC G6PD tests and in order to obtain a representative sample of intended users of the POC G6PD test. Once identified, health workers will be study staff will explain the study and invited health workers to participate in a private setting, and if they express interest, they will be consented and enrolled into the study.

5.1.2 Training and data collection

Training content will be developed and delivered in collaboration with the relevant health administrators at the study site and the test developers. The study team will train health workers at the study site in the proper use of all POC tests being evaluated and the reference assay. Health workers will be certified as proficient in the assays as determined by the study team. Any health worker not certified as proficient will have the opportunity

to receive additional training. Records of the user proficiency assessment and certification will be kept as study documents and not shared with the laboratory managers or supervisors. Trained health workers will then be given a questionnaire to assess test label comprehension and results interpretation. At that time, the study staff will emphasize the participants that the questionnaire is being used to assess the effectiveness of the training content and test instructions, and not the skills or performance of the user.

5.2 Diagnostic performance assessment

5.2.1 Recruitment

Screening of participants with unknown G6PD status will occur at the clinics in Manaus and Porto Velho and collaborating health facilities in the clinical catchment area. At clinics, the study team will use the recruitment script to recruit participants in the public areas, such as the waiting areas, of the clinics. Recruitment will be conducted in such a way as to not interfere with the potential participant's care.

Recruitment of participants with known G6PD status will take place at through home visits conducted by the study staff. Participants who were previously involved in epidemiological studies will be contacted in person by the study team and screened against inclusion criteria. Participants with known G6PD recruited from the previous epidemiological studies have agreed to have their G6PD status retained and to be contacted again for participation in future studies as part of the informed consent process.

5.2.2 Screening and consent-clinics

Study staff will screen participants for eligibility. The study team will explain the study and invite the person to participate. If the person expresses interest in participating in the study, she or he will be directed to a private space for consenting. Written consent or assent will be obtained from all participants. See detailed consent procedures and explanation of ethical considerations in sections 6.0 and 10.0.

5.2.3 Screening and consent-household visits

For participants with known G6PD status, the study team will use the recruitment script to recruit participants in their homes. Only members of the household who have a known G6PD status from past epidemiological studies will be eligible for participation. Other household members will not be asked to participate in the study. He or she will screened for inclusion in the study. The study team will explain the study and invite the person to participate. If the person expresses interest in participating in the study, she or he will be directed to a private space for consenting. Written consent or assent will be obtained from all participants. See detailed consent procedures and explanation of ethical considerations in sections 6.0 and 10.0.

5.2.4 Testing procedures at the point of care

All study procedures will be done in concordance with the health system; study procedures done at the clinics will be done in collaboration with clinic staff and the household surveys will be in collaboration by the health system agents and malaria control program.

Following completion of informed consent process, the study staff, who are trained in phlebotomy, will obtain a finger stick sample of no more than 30µl from the participant to perform the G6PD POC test, the POC hemoglobin test, and a thick blood film will be prepared to test for malaria as per the standard of care. 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. 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 the EDTA treated tube.

Slides will be viewed at the nearest health facility by a microscopist. Results will be delivered to patients by the health agents as per standard routine.

If the participants test positive for malaria or is found to be anemic based on hemoglobin thresholds from the WHO, the participant will be referred to a local health worker or the clinic for follow-up and case management (see Appendices C2 and C3).

5.2.5 Testing procedures in the laboratory

At the laboratory, aliquots of the venous blood sample will be used to run an additional replicate of the SBD POC test and reference tests in duplicate. 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 for each sample to estimate the G6PD activity. Aliquots of the venous blood will be used for running the spectrophotometric assay (reference standard) and the florescent spot test (FST). The reference assay, hemoglobin, 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.

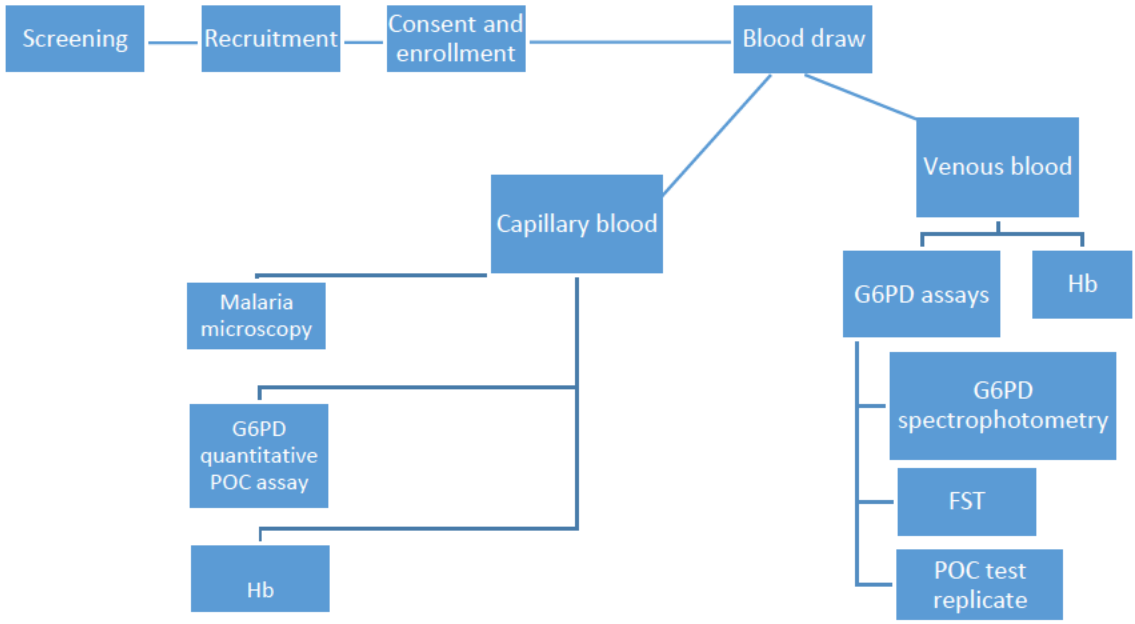
Test operators in the laboratory will be blinded to the results of the POC tests obtained and recorded in the field and operators of the reference assay will be blinded to the results of the replicate POC test run on venous sample. If there is a discrepancy between the results of the reference assay replicates, greater than allowed by the TPP, testing will be repeated. Performance characteristics and the results of additional testing of samples with discrepant results will be reported as per WHO technical specifications [15].

Leftover sample 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. If additional POC G6PD tests are added, in particular tests that are under investigation and not registered for use in Brazil, the study team will amend the protocol and advise the ethical review boards and appropriate regulatory authorities. For all novel assays to be included in the study, appropriate regulatory procedures will be followed in order to include the assays for research purposes only.

This includes products that 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 the stored sample is used, the Fundação de Medicina Tropical Dr Heitor Vieira Dourado (FMT/HVD) and Fiocruz will be responsible for obtaining the necessary ethical approvals.

For a summary of tests to be performed on the samples, see Figure 2.

Figure 2. Diagnostic performance assessment process



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. Samples from each region will be tested at a laboratory that will be established locally to provide reference testing within the given period. In the lab, each specimen will be immediately aliquoted as below.

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

5.4 Test result return and follow up

Results of point of care malaria microscopy will be returned to participants as per standard care. Results of the POC hemoglobin testing will be returned to participants

immediately after testing, as the test is registered for use in Brazil. Study staff will relay results and counsel participants who test positive for malaria or anemia according to the results return script (Appendix C3). Any participants identified as malaria positive or anemic will be counselled 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 Brazil, 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 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 in order to avoid primaquine-induced hemolysis and patients who are G6PD intermediate should be monitored closely for signs of hemolysis [18].

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 for vivax malaria.

Participants found to be vivax positive and G6PD deficient
For 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%) they 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 with standard treatment for liver stage disease, as per national guidelines.

Participants found to be vivax positive and G6PD intermediate
For 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 30-80%) they 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 and informed of the result and referred to a health agent to receive with standard treatment for liver stage disease, as per national guidelines.

Among any 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 vivax positive and POC G6PD deficient or intermediate. See Figure 3.

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 counselling. Any participants found to be G6PD intermediate or deficient will be contacted by the study team to provide counselling and given a card with further information for patients and providers (Appendix C2).

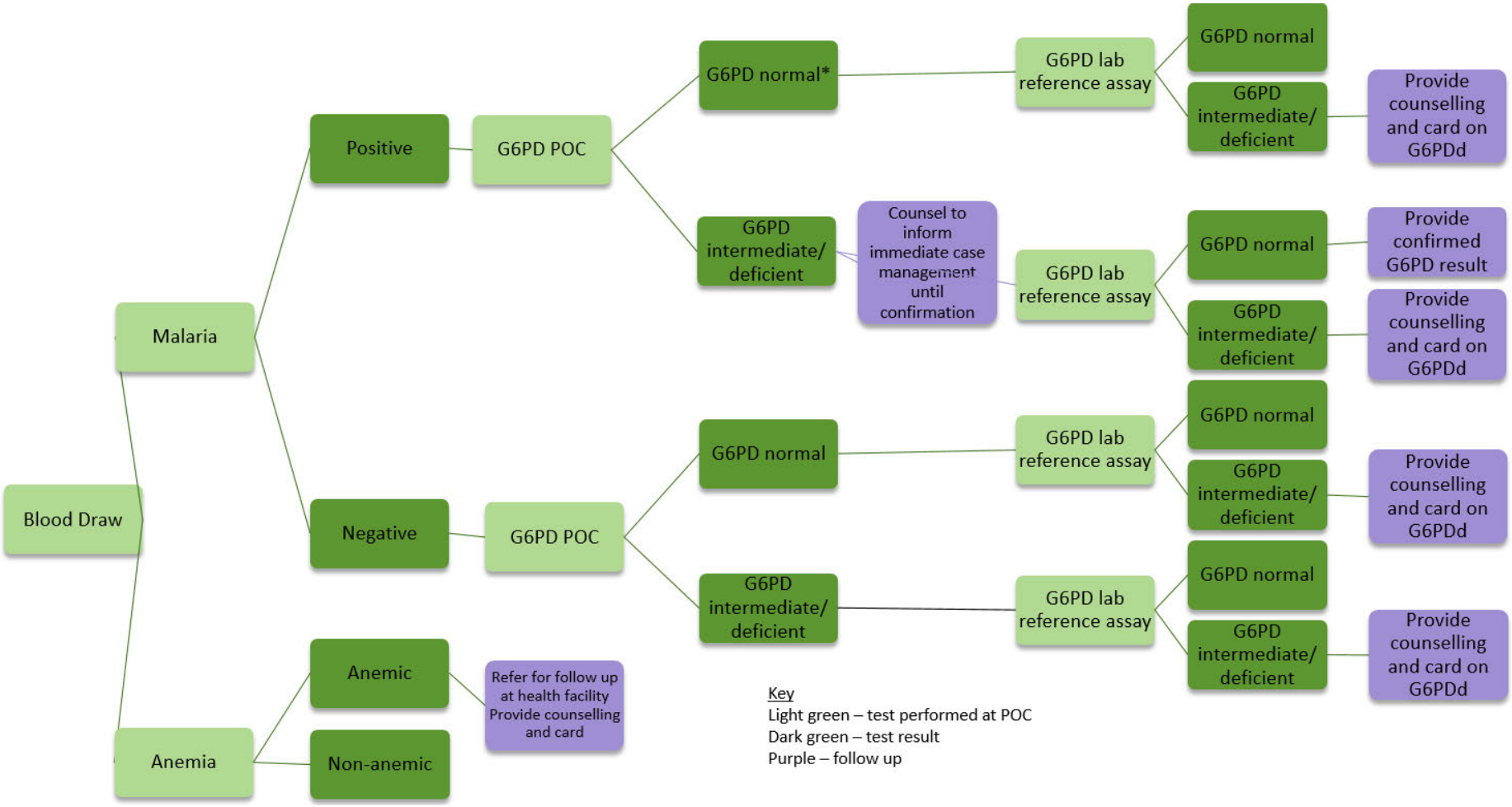
The study team will inform the participant that G6PD is a genetic condition and recommend that they may want to encourage their family to be tested as well. The study team will inform participants where and how their immediate family members can be tested free of cost. The card describes G6PD deficiency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

5.5 Summary of study procedures

Table 6. Summary of study procedures

Participant group	List of study procedures	Estimated time	Data collection forms used
Health workers	• Training on the use of POC G6PD tests • Consent and enrolment • Usability assessment questionnaire	• Up to 5 hours • 20 minutes • 20 minutes	• Appendix A2
Children aged 2-18	• Assent, parental permission, and enrollment • Conduct POC tests and blood draw	• 20 minutes • Up to 30 min	• Appendix A1
Adults	• Consent and enrolment • Conduct POC tests and blood draw	• 20 minutes • Up to 30 min	• Appendix A1

Figure 3. Participant flow and follow-up procedures



*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 counselling card as described in section 5.4.

5.6 Standard care and study procedures

In Brazil, national guidelines for *P. vivax* malaria call for treatment of the blood stage of the parasite. For the prevention of relapse, the guidelines recommend treatment that includes 3 days of chloroquine (CQ) and then 7 days of primaquine (PQ). As part of standard malaria case management, suspected malaria cases are confirmed with microscopy. No G6PD testing is recommended or routinely available prior to treatment with primaquine.

In Brazil, anemia screening is not routinely done at the point of care as part of case management for suspected malaria. The thresholds for anemia are as follows:

- Mild anemia:
  - Hb 10.0 – 11.9 g/dl in women
  - Hb 10.0 – 10.9 in pregnant women and children
  - Hb 10.0 – 12.9 g/dl in men
- Moderate anemia: Hb 7.0 – 9.9 g/dl
- Severe anemia: Hb <7.0 g/dl

There are no national guidelines regarding the treatment of anemia in Brazil. Treatments provided at the discretion of the medical care provider and generally involves iron supplementation, using oral iron tablets or parenteral injections, depending on severity. In this study, all diagnoses and treatment of anemia will be done through referral to a health agent or clinic.

For the research study, in addition to conducting microscopy to screen for malaria, the study will test participants using an investigational POC G6PD test, an approved hemoglobinometer, and laboratory reference assays intended to assess the performance of the POC tests. Participants who test positive for malaria will be referred to a local health agent for further testing and case management. All treatment decisions will be made through referral to a local health worker or clinic.

If any participants are confirmed *P. vivax* malaria positive and G6PD deficient at the point of care, the study team will relay this the information as part of the counselling. The study team will refer the participant to a health for treatment, equipped with counselling information that the participant may be G6PD deficient or intermediate and if given primaquine treatment is at an increased risk of severe hemolysis. This will enable the participant to receive immediate treatment for the blood stage infection and provide better information to the health worker responsible for case management, including any treatment for the liver stage infection. The participant's G6PD status will then be confirmed by a reference assay to inform further case management as necessary. The table below outlines the study procedure and subsequent follow up action.

Table 5. Case management

Study test	Result		Action
Hemoglobin	Normal hemoglobin levels (by national guidelines)		No action
	Mild moderate or severe anemia (by national guidelines)		Counselling re: the results of the hemoglobin test and referral to the health system for follow-up case management. This may involve the provision of iron tablets or other treatment depending on severity.
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 counselling (Appendix C2).
	Positive test result on investigational device (G6PD deficient or intermediate)		Result to be confirmed at the laboratory with the reference assay. If confirmed G6PD deficient or intermediate with the reference assay, the study team will conduct follow-up counselling (Appendix C2).
Malaria	Negative test result (no malaria)		G6PD and hemoglobin managed as above, otherwise no action
	Positive test result (confirmed malaria)	POC G6PD normal	Patient referred to health system for free treatment: 3 days CQ and 7 days PQ. If confirmed deficient or intermediate with reference assay, provide follow up counselling as described above.
		POC G6PD intermediate	Patient referred to health system for free treatment: 3 days CQ and 7 days PQ. Provide counseling re: G6PD intermediate status and recommend additional monitoring and attention to possible signs/symptoms of hemolysis If confirmed deficient or intermediate with reference assay, provide follow up counselling as described above.
		POC G6PD deficient	Patient referred to health system for free 3 days CQ with recommendation for health care provider to withhold PQ until confirmatory G6PD testing. Based on the reference assay, provide appropriate counselling for G6PD status as well as for any future treatment with PQ.

6.0 Consent process

6.1 Usability study

Consent will take place, after health workers have be recruited, in a private setting in the health facility. Members of the study team trained in the protection of human subjects will conduct the consent procedure. During the consent process, the study team will explain the purpose of the study and what involvement will entail. It will be emphasized that participation is voluntary and that their decision will not affect their employment in any way. The potential participant will have the opportunity ask questions. Consent for

participation will be documented on a written informed consent form. The participant's understanding of the study and their potential involvement will be assessed with brief questions on the consent forms. One copy of the signed informed consent form will be provided to the participant and one copy will be kept for study records.

6.2 Diagnostic performance assessment

Consent will occur, after participant recruitment, in a private setting in the health facility or home. Members of the study team trained in the protection of human subjects will conduct the consent procedure. Study staff will review the study details with the volunteer. The potential participant will be given an opportunity to review the informed consent form and ask questions. Written consent or assent will be obtained from all participants. Parent permission will be obtained for all participants under the age of 18. Child assent will be obtained for all participants between the ages of 5 and 11 with one form (Appendix B4.1) and between the ages of 12 and 17 using another form (Appendix B4.2). Where appropriate, a conversational style oral presentation of consent information will be made in the local language to parents and children in order to account for any difficulties understanding written consent forms due to low literacy. Parents will confirm their permission for a child to participate in the study by signing or thumb printing on the consent forms. Children 5-17 will express their assent in writing in the presence of their parents as a witness, to ensure the assent process is without any coercion. The consent process will be conducted in Portuguese and consent forms were written in Portuguese and translated into English.

During the consent process, the study team will explain the purpose of the study and what involvement will entail. It will be emphasized that participation is voluntary and that their decision will not affect the care they receive in any way. The potential participant will have the opportunity ask questions. Consent for participation will be documented on a written informed consent form. The participant's understanding of the study and their potential involvement will be assessed with brief questions after the consent is reviewed. One copy of the signed informed consent form will be provided to the participant and one copy will be kept for study records.

If a potential participant is illiterate, an independent literate witness will be asked to join the consent process. This witness will be a health care worker or other family member uninvolved in the study. The study staff will read the consent form aloud to the potential participant and the witness will verify that the information read aloud matches the information written on the consent form. The witness will affirm that the study participant chose to be in the research study, that he or she was present the whole time the study was being explained, and that the participant had a chance to ask questions. The participant will get a copy of this form to keep. The witness will also sign the consent form.

7.0 Study products

7.1 SDB G6PD Analyzer

The STANDARD G6PD Analyzer is designed to measure the quantitative determination of total-hemoglobin concentration and G6PD enzymatic activity in fresh human whole blood specimen based on reflectometry assays. The test is intended to aid in the identification of people with G6PD deficiency. The test is currently registered in Brazil but not used by the public health system or considered part of routine use in malaria case management. PATH has conducted some initial laboratory evaluations which suggest that the test aligns to performance specifications outlined in the target product profile [19]. The test has CE mark and is registered for use in other malaria endemic countries.

Performance 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 all 314 data points generated across the following ambient conditions: 22 °C, 32 °C 50% humidity, 37 °C 50% humidity, and 37 °C 75% humidity. ROC analysis was performed to generate the optimal thresholds for the STANDARD G6PD test. CI refers to the confidence interval, NA, not applicable.

Table 7. Performance of SD Biosensor STANDARD G6PD test

Threshold (Pointe Scientific value U/gHb)	Optimal STANDARD G6PD (U/gHb)	Sensitivity (95% CI)	Specificity (95% CI)
30% (2.8)	4.2	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

Figure 4. SD Biosensor STANDARD G6PD Test

7.2 Pointe Scientific

The Pointe Scientific 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. For in vitro diagnostic use only.

- A spectrophotometer capable of measuring at 340 nm with 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 RBCs, the Hb or RBC 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 (FST) or TrinityBiotech G-6-PDH Screening test is widely used for in vitro diagnosis of G6PD deficiency using whole blood or dried blood spots. The G-6-PDH Screening test is a qualitative test performed by incubating a small amount of blood with glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate (NADP). 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 FDA. The test will be performed according to PATH's guide to fluorescent spot testing for G6PD deficiency [20].

7.4 HemoCue

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 consists of a small and 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 a Hb/HbO2 isobestic point. The system is factory calibrated and needs no further calibration.

7.5 Sysmex Hematology Analyzer

The Sysmex hematology automated analyzer is used to perform full blood counts and reticulocyte counts. This analyzer is registered and available for use in Brazil. It is used for research purposes routinely at the sites in Manaus and Porto Velho.

8.0 Benefit and risk considerations

8.1 Benefit to study participants

Participants in this study will have convenient access to a malaria test and a hemoglobin 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 counselled and referred to a local health worker or clinic for follow up and case management. Participants who have malaria will benefit from having their treatment informed by G6PD status, measured at the point of care, thus lowering the risk of severe hemolysis associated with standard vivax malaria treatment for G6PD deficient individuals, see Figure 3.

Participants in this study will have the opportunity to have their G6PD status tested by a reference assay. 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 G6PD activity is determined to be deficient or intermediate- participants will receive counselling regarding this information. G6PD testing will be offered to immediate family members of participants who are found to be G6PD deficient or intermediate free of cost.
The study team will test all family members with the FST, which will be more readily accessible both at the study sites and allow for faster results return. For females who test normal on the FST, the study team will follow-up with the gold standard test to determine if they are intermediate or normal, and provide appropriate counselling. This information may inform the care they receive for malaria in the future.

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

Study procedures do not represent significant risks to the participants beyond those that are associated with normal blood draw, 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 safely limits recommended by the WHO and other organizations for both adults and children [21]. In the unlikely event of a research related injury, cost of treatment will be covered by the study. All decisions regarding clinical care or malaria case management will be made through referral to the local health care facilities.

There are no known reports of stigma associated with G6PD deficiency.

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 him or her. They may feel as through 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.

9.0 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. PATH and the FMT/HVD and Fiocruz 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 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. PATH and FMT/HVD and Fiocruz will hold weekly or

bi-weekly study 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. This also includes any adverse effects related to blood sampling. Compliance indicators related to counselling and follow up discussed on this call will include:

- number of participants found to be G6PD deficient or intermediate by laboratory reference assay
- number of counselling cards delivered or picked up by participants found to be G6PD deficient or intermediate by laboratory reference assay
- proportion of participants found to be G6PD deficient or intermediate by laboratory reference assay that received appropriate counselling and counselling cards.

PATH will designate trained and qualified personnel to monitor the progress of this clinical study in accordance with Monitoring SOPs and the study-specific Monitoring Plan. Prior to study start, a study initiation visit will be conducted to provide training to site staff about the protocol, the completion of study documentation and data collection forms, the monitoring schedule, and all regulatory requirements. During the study, routine monitoring visits will be conducted to assure the site continues to adhere to the protocol, the investigator agreement, and regulations regarding conduct of clinical studies. Assessments will be made regarding the subjects' protection and safety, when relevant, as well as the quality, completeness, and integrity of the data.

10.0 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 for Harmonization Good Clinical Practice (ICH-GCP) 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, 45 CFR 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 PATH Research Ethics Committee and the local ethics review boards in Manaus and Porto Velho, and the national IRB of the Ministry of Health in Brazil (CONEP)

10.4 Amendments

All amendments and modifications will be submitted to the above institutional 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 Primary 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

Compensation will be given in order to reimburse any costs related to the participation in the study, such as transportation costs for participants recruited through household survey.

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.

11.0 Confidentiality

11.1 Participant confidentiality

The investigator will ensure that each participant confidentiality is maintained. Any clinically relevant information will be shared with the participant in a confidential setting. Participants will not be identified in any publicly released reports of this study. All samples and records labeled with a participant ID and will be kept confidential by the FMT/HVD and Fiocruz. PATH will not have access to records that identify the subjects.

All health workers will have the opportunity to participate in the POC G6PD test training regardless of their decision to participate in the research activities. Results of the usability assessment will be de-identified 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 de-identified 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 an monitoring report will be generated every week, summarizing key indicators for study compliance. PATH and local investigators 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 PATH study team will conduct site-monitoring visits as needed to ensure compliance with the protocol and relevant SOPs.

11.4 Access to data and data dissemination

The data generated by this study will inform the commercialization of G6PD tests and may inform programmatic decisions around testing for G6PD deficiency. Data will be shared with study partners and the test developers. All data will be published in the open medical literature with the identity of the subjects protected.

Groups that supervise the study may access the results. This includes members of the ethics committees of PATH, FMT / HVD, Ethics Committee Involving Human Beings from the Center for Tropical Medicine Research (CEP / CEPEM), and any other test auditor or regulator such as the US Food and Drugs Administration (FDA). Only de-identified data will be shared with groups outside of the study team.

In addition, a description of this study will be available on the website: http://www.ClinicalTrials.gov, as requested by the US government. On this site, there will be no personal information that identifies individual participation, only the description of the study and the results when available.

De-identified data and samples will be stored in a secure location in FMT/HVD and Fiocruz clinical research unit. If the data or specimen stored is planned to be used for

other purposes than this study, FMT/HVD and Fiocruz and PATH will seek approval from the appropriate ethical committees.

11.5 Quality control and quality assurance

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

12.0 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 Brazil 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 Brazil 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.

13.0 Investigator responsibilities

The two project partners involved in this evaluation are PATH (prime award recipient and evaluation lead), and FMT/HVD and Fiocruz (implementing research partner). Roles and responsibilities for each of the partners are listed below. L= lead; A= assist

Task	**PATH**	**FMT/HVD and Fiocruz**
Award oversight	L	A
Study design and protocol development	L	A
IRB submission--PATH	L	A
IRB submission—Brazil	A	L
Evaluation logistics arrangements	A	L
Procurement of all study supplies	L	A
Training on the use of study assays	L	A
Conduct recruitment, consent, enrollment, and field data collection	A	L
Conduct laboratory based data collection	A	L
Data entry and cleaning	A	L
Data analysis and reporting	L	A

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Appendices:

A. Data collection forms
B. Consent forms and assent forms
C. Recruitment and result return materials
D. Investigator's brochure