Validation of a diagnostic test for glucose-6-phosphate dehydrogenase deficiency: diagnostic accuracy and repeatability in capillary samples

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Location of Research:
Biological Specialty Company d/b/a BioIVT, LLC

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Study title	Validation of a diagnostic test for glucose-6-phosphate dehydrogenase deficiency: diagnostic accuracy and repeatability in capillary samples
Précis	Cross-sectional diagnostic accuracy study with up to 300 volunteer participants. The clinic will recruit and consent 250 adult study participants (all comers, both male and female) and up to an additional 50 women. Clinic staff will draw 3 ethylenediaminetetraacetic acid (EDTA) tubes, 1 heparin tube, and 1 acid citrate dextrose (ACD) tube from venous blood and obtain finger stick capillary blood. Clinic staff will perform the investigational SD Biosensor STANDARD™ G6PD Test for glucose-6-phosphate dehydrogenase (G6PD) deficiency and a HemoCue® hemoglobin test on finger stick capillary blood and EDTA venous blood. Two EDTA anti-coagulated venous blood samples will be sent to a Clinical Laboratory Improvement Amendments (CLIA) certified lab for G6PD reference testing by the gold standard assay: G6PD measurement by spectrophotometry; one sample will also have a hemoglobin measurement by a hematology analyzer. Deidentified blood samples collected in tubes with other anti-coagulants will be sent to PATH laboratories for evaluating equivalency across anti-coagulant types. Individuals identified as G6PD deficient or intermediate by the reference test will be notified of their results by the clinic and referred to their physician for follow-up. This study includes a nested repeatability study. Up to 20 consented participants will provide 4 additional finger stick samples. Two clinic staff using 2 instruments will perform the STANDARD™ G6PD Test to assess the repeatability of the test in capillary blood over 8 G6PD and hemoglobin measurements. This study also includes a nested sample stability study. Up to 8 consented participants will provide an additional venous blood draw sample to be tested at additional time points to document stability of the samples over time when tested by the STANDARD™ G6PD Test.
Objective	To assess the accuracy of a point-of-care (POC) G6PD test in measuring G6PD activity and classifying results compared to a reference assay and across repeated measurements in capillary samples.
Endpoints	Sensitivity and specificity of SD Biosensor STANDARD G6PD Test compared to the Pointe Scientific G6PD Reference Assay for identifying G6PD deficient individuals and women with intermediate G6PD activity • Accuracy between the SD Biosensor STANDARD G6PD Test and the Pointe Scientific G6PD reference assay for the measurement of G6PD activity • Accuracy between the SD Biosensor STANDARD G6PD Test measure of hemoglobin (Hb) and HemoCue® Hb 201+ System, a POC reference hemoglobin assay Nested repeatability study: • % coefficient of variation for each instrument, and for the instruments combined • % coefficient of variation for each operator, and for the operator combined Nested sample stability study • Change over time in SD Biosensor STANDARD G6PD Test result for each specimen, stored at one of two temperatures, by anti-coagulant, analyte, and level.
Population	250 African American male and female participants and up to 50 additional African American female participants 18-65 years of age presenting at the clinic for care. Individuals who have received a blood transfusion within the last 3 months, according to self-report, will be excluded.
Study sites	Clinical procedures: Biological Specialty Company d/b/a BioIVT, LLC Reference testing: University of Washington Department of Laboratory Medicine Sample storage and deidentified testing: PATH
Study duration	6 months (estimated).

Contents

Abbreviations

ACD	acid citrate dextrose
AE	Adverse Event
BSC	Biological Specialty Company
CAP	College of American Pathologists
CE mark	European Conformity certification
CI	confidence interval
CLIA	Clinical Laboratory Improvement Amendments
CRF	case report form
EC	Ethics Committee
EDTA	ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
FN	false negative
FP	false positive
G6PD	glucose-6-phosphate dehydrogenase
GCP	Good Clinical Practice
Hb	hemoglobin
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IRB	institutional review board
POC	point-of-care
PQ	prequalification
P.	*Plasmodium*
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SOP	standard operation procedure
TN	true negative
TP	true positive
U	unit
UADE	Unanticipated Adverse Device Effect
US FDA	United States Food and Drug Administration
UW	University of Washington
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 when possible, newborns should be screened for G6PD deficiency where G6PD deficiency is common [4]. As a genetic disorder, the prevalence of G6PD deficiency varies among different racial and ethnic groups. In U.S. populations, an overall prevalence of G6PD deficiency of 2.5% among males and 1.6% among females has been observed [5]. The highest prevalence of G6PD deficiency was among African American males (12.2%), Asian males (4.3%), and African American females (4.1%).

G6PD status is particularly relevant for the treatment and prevention of malaria. Malaria is prevented and treated using a variety of treatments, some of which pose a high risk to those with G6PD deficiency. The 8-aminoquinoline–based malaria drugs for treatment and prophylaxis such as primaquine and tafenoquine are the only ones with the capacity to prevent relapse and eliminate the liver stage parasites in *Plasmodium* (*P.*) *vivax* infections. Due to the risks associated to G6PD deficiency for primaquine, WHO recommends that “the G6PD status of patients should be used to guide administration of primaquine for preventing relapse” [6].

Current tests for measuring G6PD activity are complex and require sophisticated laboratory facilities to execute [7-10]. Quantitative enzyme activity-based tests are considered by WHO and the US Food and Drug Administration (FDA) as the true gold standard for classifying G6PD status in individuals. The Pointe Scientific test (Cat No. G7583) is one of the few FDA-cleared products which use the accepted gold standard assay conditions for evaluation of novel G6PD tests. The Pointe Scientific test (Cat No. G7583) run in a College of American Pathologists (CAP) certified laboratory will be used as the reference standard for the evaluation.

PATH is working with SD Biosensor to advance an *in vitro* diagnostic test for G6PD deficiency that meets the target product profile [11]. Recent studies performed in the US, Thailand, and Bangladesh confirm the performance of this test on venous potassium EDTA anti-coagulated blood samples [12]. This study aims to confirm the performance of this test across capillary and venous samples and across repeated measurements of G6PD activity and hemoglobin in capillary samples.

2. Study objectives

The goal of this study is to contribute to a body of evidence that will support the submission of the point of care G6PD test to the US FDA, WHO prequalification (PQ) process, and for product registration in target countries.

The primary objective is to assess the accuracy of the SD Biosensor STANDARD G6PD Test in measuring G6PD activity and classifying results when used by trained health care workers. This study aims to establish performance characteristics for SD Biosensor STANDARD G6PD Test. Results from the SD Biosensor STANDARD G6PD Test will be compared to results from an FDA-cleared quantitative G6PD assay and FDA-cleared hemoglobin assay to assess the accuracy of a point-of-care (POC) G6PD test compared to a reference assay and across repeated measurements in capillary samples.

3. Study design

This is a prospective cross-sectional diagnostic accuracy study with a nested repeatability study and a nested sample stability study. Study endpoints include:

- Sensitivity and specificity of SD Biosensor STANDARD G6PD Test compared to the Pointe Scientific G6PD Reference Assay for identifying G6PD deficient individuals and women with intermediate G6PD activity
- Accuracy between the SD Biosensor STANDARD G6PD Test in different whole blood specimen types and the Pointe Scientific G6PD reference assay for the measurement of G6PD activity
- Accuracy between the SD Biosensor STANDARD G6PD Test measure of Hb and HemoCue® Hb 201+ System, a POC reference hemoglobin assay

Repeatability study:

- Mean value for each instrument, and for the instruments combined
- SD value for each instrument, and for the instruments combined
- %CV value for each instrument, and for the instruments combined
- Mean value for each operator, and for the operators combined
- SD value for each operator, and for the operator combined
- %CV value for each operator, and for the operator combined

Sample stability study

- Change over time in SD Biosensor STANDARD G6PD Test result for each specimen, stored at one of two temperatures, by anti-coagulant, analyte, and level.

3.1 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 [13]. This requires obtaining samples from participants with a range of G6PD activity levels. The WHO PQ process defines these levels as shown in Table 1.

Table 1. 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 and 99% sensitive for detecting individuals with G6PD activity < 30% activity of normal enzyme activity. Assuming a sensitivity of 99%, with a confidence interval of 95%, and a 1.5% maximum margin of error, a minimum of 169 participants with deficient and intermediate G6PD activity will be needed for the analysis. 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%.

The estimated prevalence of G6PD activity at less than 30% of normal enzyme activity can be up to 12% in certain subpopulations [14,5]. In this study, testing a minimum of 250 and maximum of 300 African American individuals is expected to generate approximately 10-25 deficient and 15-20 intermediate individuals. Targeted enrollment of up to 50 African American women after the minimum of 250 participants is reached is intended to help yield intermediate individuals. These data will be combined with data generated in other clinical evaluations in the US, Ethiopia, Brazil and one other international site. The additional evaluations will include approximately 3,000 total participants with 200 expected deficient and 200 intermediate individuals.

The nested repeatability study will be conducted with up to 20 participants with G6PD activity levels across the analytical measuring range. 15 participants with complete analyzable data from the repeatability measurements are needed for the analysis.

The nested sample stability study will be conducted with up to 8 participants with G6PD and/or Hb levels across the analytical measuring range. 5 different levels of G6PD and Hb from participants with complete analyzable data from the sample stability measurements are needed for the analysis. Depending on an individual's values, this could be accomplished in as few as 3 participants.

3.2 Study sites

All clinical procedures including enrollment, sample collection and other site-specific procedures will be conducted at Biological Specialty Company (BSC) in Reading and/or Allentown, Pennsylvania. The BSC study sites are donation centers with expertise in Anatomic and Blood Banking/Transfusion Medicine as well as previous studies regarding the specificity for point of care devices used to detect infectious diseases such as hepatitis and HIV. The Reading center sees approximately 30-40 walk-in donors per day and the Allentown center sees approximately 15 walk-in donors per day. The study site serves an urban blood donor population that is primarily a mixture of students, blue-collar workers, and currently unemployed/underemployed individuals. Approximately 15-20% of the donation centers' volunteers are African American. Roughly a quarter to a third of the typical donor population at these sites is female.

The University of Washington Department of Laboratory Medicine will provide reference laboratory services. G6PD reference testing will be performed at the Department's laboratories at the University of Washington Medical Center and/or Northwest Hospital. Both laboratories are CLIA/CAP certified.

PATH will store deidentified samples and conduct additional analytical testing according to analytical testing protocols. Deidentified frozen blood samples not used in analytical testing will be archived within the PATH freezers located at the PATH laboratories (Seattle, WA, USA)

4. Research participants

4.1 Characteristics of research participants

This study will involve healthy adult volunteers aged 18-65 years old capable of providing informed consent. Both men and women will be recruited for the first 250 enrollments. Up to an additional 50 women will be enrolled.

4.2 Inclusion and exclusion criteria

Criteria for inclusion of volunteers:

- Age 18-65.
- Must communicate an understanding of the study procedures.
- Must be able to provide written consent to undergo screening and provide medical history.
- Participants must be afebrile and in general good health in the opinion of the investigator as determined by vital signs, medical history, and targeted physical examination for blood donation eligibility.
- Black/African-American, by self-report.

Criteria for exclusion of volunteers:

- Blood transfusion in the past 3 months by self-report.

5. Study procedures

Recruitment and screening at BioIVT

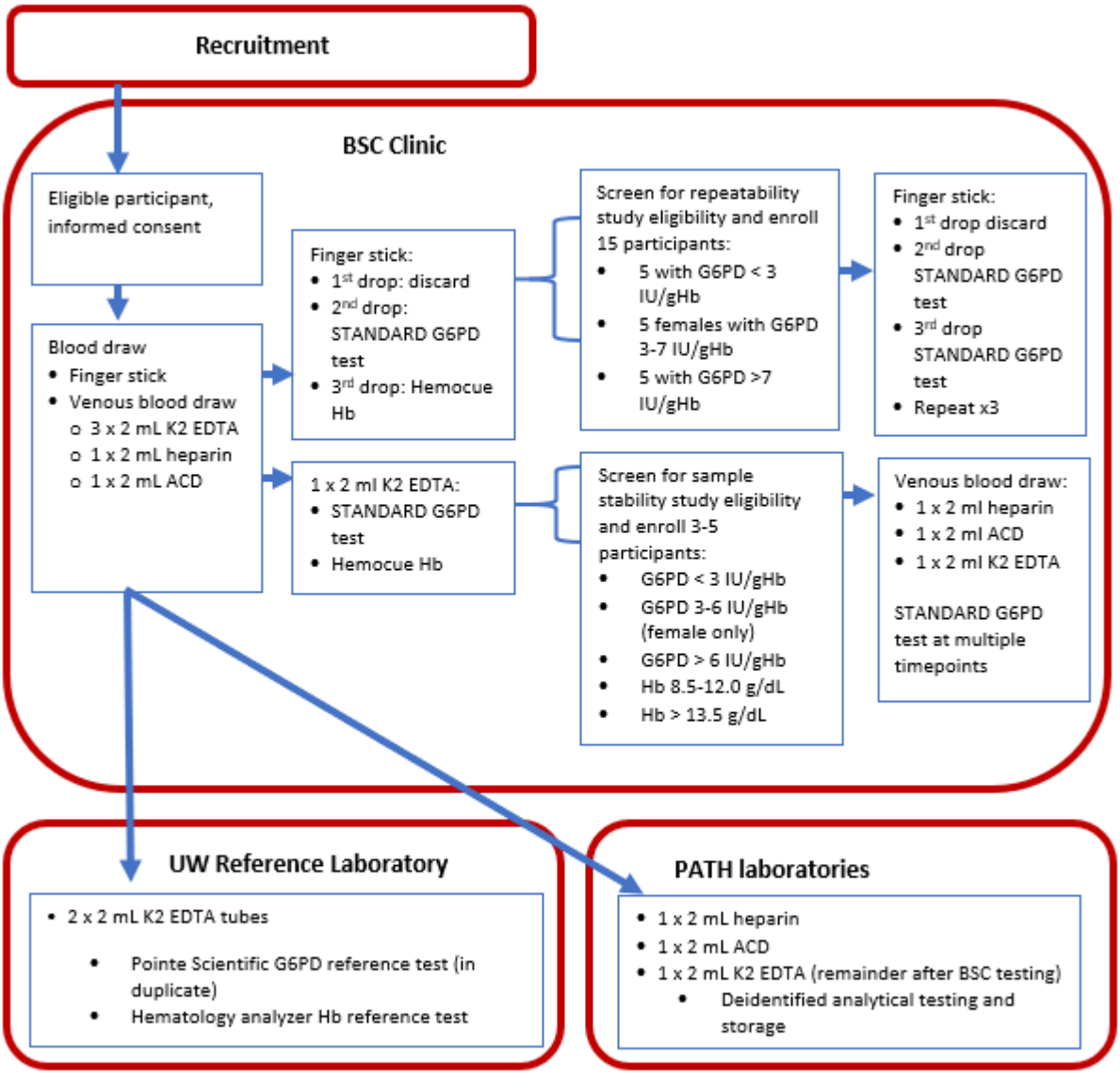
BSC will perform recruitment and screening of participants for the study among donors who present at the blood donation center. The approach to recruitment will take place in two phases. For the diagnostic accuracy study, BSC will to offer information about this study to blood donors as they present for blood donation via a recruitment script. This recruitment script may also be used to call donors who are listed in the donor database. A newspaper advertisement and online advertisements specific to this study may also be used for recruitment. All recruitment materials (i.e. the recruitment script used in-person or over the phone and the advertisement text) will be approved by the IRB. Participants will be recruited into the study based on the eligibility criteria above.

For the nested repeatability study, participants from the diagnostic accuracy study will be recruited based on their G6DP test results. See section 5.1.2 for more information. For the nested sample stability study, participants from the diagnostic accuracy study will be recruited based on their G6PD and/or Hb test results. See section 5.1.3 for more information. Participants who may have completed the diagnostic accuracy study previously and present at the BSC donation center for a subsequent blood donation, but who had not been previously presented with the option of enrolling in either the nested repeatability or sample stability studies, may be recruited for the sub-studies. The informed consent form will also authorize study staff to call back eligible participants for recruitment into the sub-studies if they had not been presented with the option of enrolling in the sub-studies previously. IRB-approved recruitment scripts would be used for any recruitment into a sub-study of a participant previously enrolled in the diagnostic accuracy parent study.

Sample testing at all sites

See Figure 1 for a summary of tests to be performed on the samples.

Figure 1. Diagnostic performance assessment process



Abbreviations: EDTA, ethylenediaminetetraacetic acid; G6PD, glucose-6-phosphate dehydrogenase; Hb, hemoglobin; mL, milliliter.

As noted in Table 2 below and Figure 1, volunteers will come to the clinic site, undergo the informed consent process, provide written informed consent, and undergo blood draw and finger stick procedures.

Table 2. Summary of study procedures.

Participant group	List of study procedures	Estimated time	Data collection forms used
Adults	• Written informed consent • Blood draw • POC tests	30-45 min	Case Report Form

5.1 Diagnostic performance assessment

5.1.1 Blood collection and testing procedures at the point of care

Following completion of the informed consent process, the study staff, who are trained in phlebotomy, will draw the following blood volumes:

- a finger stick sample of approximately 50 µl obtained with lancet
- three 2-mL of ethylenediaminetetraacetic acid (EDTA)-anti-coagulated blood
- one 2-mL tube of heparin-anti-coagulated blood
- one 2-mL tube of acid citrate dextrose (ACD)-anti-coagulated blood

The tubes will be identified using a study participant number. At the clinic site lab, study staff will conduct the SD Biosensor STANDARD G6PD Test and the POC HemoCue Hb test on the finger stick blood and 1 tube of EDTA venous blood. Results of the in-clinic performed POC tests will be recorded on data collection forms. If an invalid test result is obtained from the finger stick sample, a second finger stick sample will be obtained, and a second test will be run. If an invalid test result is obtained a second finger stick, the results will be recorded as invalid and no additional finger sticks will be done. The STANDARD G6PD and HemoCue Hb testing at the clinic site lab may be batched and performed at the end of each day.

The venous blood samples will be processed as follows:

- Two 2-mL EDTA venous blood sample will be transported refrigerated to the CLIA/CAP certified laboratory at the University of Washington where the reference G6PD and hemoglobin tests will be run,
- Remaining blood samples, including any remaining blood from the EDTA sample used for in-clinic testing, will be transferred to PATH laboratories for deidentified analytical testing.

The blood draw and use of the POC tests will take up to 30 minutes.

5.1.2 Nested repeatability study

Recruitment for this study will be nested within recruitment for the matrix equivalency study. BSC will consent all participants for both the accuracy study and the repeatability study. Participants will be recruited based on the result of the STANDARD G6PD test. The study will recruit:

- 5 individuals with G6PD value less than 3 IU/gHb;
- 5 females (intermediates) with G6PD values between 3 IU/gHb and 7 IU/gHb;
- 5 individuals with G6PD values > 7 IU/gHb;

for a total of 15 study participants with complete data. Up to a total of 20 participants may be enrolled if any individual's data is missing or unanalyzable.

BSC clinic staff will use the result of the STANDARD G6PD test to screen for eligibility in the nested repeatability study. BSC will maintain a record of the number of participants who test in the categories listed above and recruit participants into the sub-study until the quotas above have been filled. If a participant is eligible, he or she will be informed of eligibility but not informed of the results of the investigational test.

If the participant agrees to enroll in the nested repeatability study, clinic staff will perform STANDARD G6PD test across four fingers, two specimens per finger, on two instruments per participant on the same day as the diagnostic accuracy study procedures. See table 3 for an outline of blood draws, study procedures, and alignment with the diagnostic accuracy study.

Table 3: Repeatability study

	Operator	Drop*			G6PD value (U/g Hb)	Hemoglobin (g/dL)
Finger 1 (Hand 1)	Operator 1	2	Specimen 1.1	Instrument A	G6PD1.1A	Hb1.1A
	Operator 2	3	Specimen 1.2	Instrument B	G6PD1.2B	Hb1.2B
Finger 2 (Hand 1)	Operator 1	2	Specimen 2.1	Instrument A	G6PD2.1A	Hb2.1A
	Operator 2	3	Specimen 2.2	Instrument B	G6PD2.2B	Hb2.2B

Finger 3 (Hand 2)	Operator 1	2	Specimen 3.1	Instrument A	G6PD3.1A	Hb3.1A
	Operator 2	3	Specimen 3.2	Instrument B	G6PD3.2B	Hb3.2B
Finger 4 (Hand 2)	Operator 1	2	Specimen 4.1	Instrument A	G6PD4.1A	Hb4.1A
	Operator 2	3	Specimen 4.2	Instrument B	G6PD4.2B	Hb4.2B

*drop 1 is wiped clean

5.1.3 Nested sample stability study

Recruitment for this study nested within recruitment for the matrix equivalency study. BSC will consent all participants for both the accuracy study and the sample stability study. Participants will be recruited based on the result of the STANDARD G6PD test. The study will recruit participants to cover the following G6PD and/or Hb levels with at least 1 participant each:

- G6PD value less than 3 IU/gHb
- G6PD value between 3 IU/gHb and 6 IU/gHb (female only)
- G6PD value > 6 IU/gHb
- Hb value between 8.5 g/dL and 12.0 g/dL
- Hb value greater than 13.5 g/dL

for a maximum of 5 study participants and a minimum of 3 study participants with complete data. Up to a total of 8 participants may be enrolled if any individual's data is missing or unanalyzable. See table 4 for an outline of the specimens to be tested for the sample stability study.

Table 4. Sample stability study

No.	Anti-coagulant	Analyte	Level	Specification	Description
1	K2 EDTA, ACD, Heparin	G6PD	Level 1	< 3.0 IU/gHb	Deficient
2			Level 2	3.0 – 6.0 IU/gHb	Intermediate female
3			Level 3	> 6.0 IU/gHb	Normal
4		T-Hb	Level 1	8.5 – 12.0 g/dL	Low Hb
5			Level 2	> 13.5 g/dL	High Hb

BSC clinic staff will use the result of the STANDARD G6PD test to screen for eligibility in the nested sample stability study. BSC will maintain a record of the number of participants who test in the categories listed above and recruit participants into the sub-study until the quotas above have been filled. If a participant is eligible, he or she will be informed of eligibility but not informed of the results of the investigational test.

If the participant agrees to enroll in the nested sample stability study, clinic staff will perform an additional blood draw in different anti-coagulants for that participant and will run a series of additional STANDARD G6PD tests on the samples at different time points.

- one 2-mL of EDTA-anti-coagulated blood
- one 2-mL tube of heparin-anti-coagulated blood
- one 2-mL tube of ACD-anti-coagulated blood

The freshly collected venous whole blood samples will be tested within 1 hour of collection for baseline (0 hour) and then aliquoted into two containers – one of which will be stored at room temperature (15-30^{o}C/59-86^{o}F) and one of which will be stored in a refrigerator (2-8^{o}C/25-46^{o}F).The refrigerated samples will be tested in triplicate using 3 different analyzers at 5 additional timepoints, up to 7 days (168 hours) after collection. The samples stored at room temperature will be tested in triplicate using 3 different analyzers at 4 additional timepoints, up to 2 days (48 hours) after collection. A single operator will perform each triplicate testing sequence. Any residual blood sample after the sample stability testing is complete will be destroyed.

The blood draw for the nested sample stability study may be done on the same day as the procedures for the diagnostic accuracy study or the participant may be scheduled to return to the clinic for the sample stability blood draw on a different date.

5.1.4 Testing procedures at the reference laboratory

At the reference laboratory, deidentified samples will be tested. G6PD activity and hemoglobin will be measured within 7 days of collection using the Pointe Scientific G6PD reference assay and a hematology analyzer, respectively.

Test operators in the reference laboratory will be blinded to the results of the POC tests obtained and recorded at the clinic site. The G6PD reference assay will be run in duplicate. If there is a discrepancy between the replicates of the reference assay greater than allowed by the target product profile, duplicate testing with the reference test will be repeated. Performance characteristics and the results of additional testing of samples with discrepant results will be reported as per WHO guidelines [13].

5.1.5 Deidentified analytical testing at PATH laboratory

Deidentified sample will be transferred to PATH for applicable analytical testing. Leftover samples will be aliquoted, temporarily stored refrigerated, and stored at -80° C for longer term at the PATH for possible confirmatory or additional testing.

5.2 Specimen collection, transport, and storage

As noted in Section 5.1.2 above, the clinic site staff will collect three EDTA tubes, one heparin tube, and one ACD tube of venous whole blood as well as finger stick capillary blood samples. To ensure the integrity of the G6PD enzyme activity measurement, venous blood samples should be stored at 2-8 degrees Celsius and tested within 7 days of collection. Clinic staff will test the blood as in Section 5.1.2. Immediately after collection, whole blood will be stored in a refrigerator at the clinic. At the site, refrigerator temperatures are maintained and continuously monitored. Site staff will notify PATH of any temperatures recorded out of range. As early as convenient and always within 24 hours, the samples will be transported to the reference and PATH laboratories for testing. The samples will be transported by an overnight courier in ice pack. UW reference laboratory staff will receive samples in ice pack and store in a refrigerator (2-8 degrees Celsius) until testing. At the PATH laboratory, study staff will review specimen quality, including for issues of clotting and cold storage conditions, and accept or reject specimen according to study-specific SOPs. The reference laboratory will follow its own SOPs for specimen quality to accept or reject each specimen.

Frozen aliquots will be stored for up to 10 years at PATH laboratories for any confirmatory testing or specimen investigation if required to resolve discrepant values between the investigational tests and the reference tests.

5.3 Test result return

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. 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. Up to three attempts will be made to reach these participants by phone. Additionally, study staff will also mail all participants found to be G6PD intermediate or deficient their result in a written letter for their records.

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 recommend to participants how to get their immediate family members referred to primary care physicians for follow-up testing. All staff involved in returning these results are medical professionals and 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 site staff involved in study procedures and will be 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 User proficiency

Study staff will be trained in the use of the assays. A proficiency panel of a characterized set of samples representative of varying levels of G6PD activity and Hb will be provided to ensure study personnel are able to perform the HemoCue hemoglobin assay, and the SD Biosensor correctly. Competency testing will occur prior to the start of participant enrollment and specimen testing. Users must pass proficiency before study testing can begin. Proficiency test results will be forwarded to PATH for review.

5.5 Quality Control Testing

Quality Control (QC) results must be within specifications for any test method result to be reported. QC testing on the Pointe Scientific G6PD assay, the HemoCue Hb assay, and the SD Biosensor will be performed each day of testing using high and low control reagents.

6. Consent process

The consent process will be conducted at the BSC clinic. Informed consent encompasses all written or verbal study information the BSC study staff provides to the participant, before and during the trial. All informed consent discussions will be documented by the study staff in the participant's source documentation. Consent discussions include, but are not limited to, background on G6PD and rationale for this study, an overview of the study design, study procedures, requirements for participation in the trial, and risks and benefits to the participant. Prior to signing the informed consent document, an Assessment of Understanding will be administered to confirm comprehension of the study procedures. Confirmation of the assessment of understanding will be recorded in study enrollment logs.

Written informed consent will be obtained only by the BSC study staff trained in the protocol and designated on the study signature log, using the protocol-specific Informed Consent Form approved by the local IRB/Independent Ethics Committee (IEC) and developed and administered in accordance with local IRB/IEC requirements, federal guidelines 21 CFR 50.20, 45 CFR 46.116 and the ICH E6 Guidance Section 4.8.10.

Participants will be provided with a copy of all consent forms that they sign. The original signed and dated copy will be kept on file in their study binder and stored in locked, limited-access cabinets within BSC.

The participant will be consented into the diagnostic accuracy study, the capillary repeatability study, and the sample stability study, eligibility pending. Compensation will be given separately for each study, see section 11.8 and participants will be informed of the levels of compensation provided for participation in each study. Compensation will be provided when participants are enrolled into each study. Participants are able to consent to participate in only the diagnostic accuracy study and not the repeatability study or sample stability study. As the diagnostic accuracy study serves to screen participants for eligibility for the nested repeatability and samples stability studies, participants are not able to consent to participate in only the repeatability or sample stability study.

7. Study products

7.1 SD Biosensor STANDARD G6PD Test (investigational product)

The STANDARD G6PD Analyzer (Figure 2 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 in the US and is considered an investigational product. System components shall be labeled in accordance with regulatory requirements, including the following statement, "For Investigational Use Only. The performance characteristics of this product have not been established.".

Figure 2. SD Biosensor STANDARD G6PD Test.

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

Previously, the STANDARD G6PD Test was evaluated against the Pointe Scientific quantitative G6PD reference assay and another reference assay: Trinity Biotech quantitative assay [12]. Receiver operating characteristic analysis was performed to generate the optimal thresholds for the STANDARD G6PD Test (Table 5).

Table 5. STANDARD G6PD Test performance data.

Summary data for the performance of the Trinity Biotech quantitative G6PD assay and the SD Biosensor STANDARD G6PD test compared with the Pointe Scientific quantitative G6PD assay

G6PD diagnostic test	Trinity Biotech quantitative reagent kit	SD Biosensor STANDARD G6PD test		
Study description	Fresh venous blood (K_2EDTA), PATH, USA	Fresh venous blood (K_2EDTA), PATH, USA	Fresh venous and capillary blood (both K_2EDTA), PATH, USA	Frozen venous blood (K_2EDTA), SMRU, Thailand
No clinical sample (male/female)	183 Male: 128 Female: 55	210 Male: 137 Female: 73	100 Male: 60 Female: 40	150 Male: 42 Female: 108
No deficients	21	25	10	54
No intermediates	7	13	8	53
Adjusted PS median (U/g Hb)	9.6	8.89	8.97	6.84
Normal* PS (SD) (U/g Hb)	10.4 (2.4)	9.7 (2.1)	9.3 (1.8)	6.7 (1.1)
Normal* device (SD) (U/g Hb)	11.4 (3.0)‡	10.9 (2.9)	11.4 (2.4)	6.7 (1.2)
Optimal threshold (U/g Hb) equivalent to 30% normal	2.9	2.7	2.7	2.1
% Sensitivity (95% CI)	100.0 (82.4–100.0)	100.0 (95.7–100.0)	100.0 (83.2–100.0)	100.0 (93.4–100.0)
% Specificity (95% CI)	98.2 (94.7–99.6)	97.0 (94.5–98.5)	100 (98.0–100.0)	94.8 (88.3–98.3)
AUC	> 0.99	> 0.99	1	0.99
Optimal threshold (U/g Hb) equivalent to 70% normal	6.7	6.2	6.3	4.8
% Sensitivity (95% CI)	100.0 (86.8–100.0)	95.5 (89.7–98.5)	97.2 (85.5–99.9)	95.0 (88.8–98.4)
% Specificity (95% CI)	100.0 (97.7–100.0)	97.0 (94.5–98.6)	80.5 (73.6–86.3)	81.6 (68.0–91.2)
AUC	1	> 0.99	0.98	0.97
Optimal threshold (U/g Hb) equivalent to 80% normal	7.7	7.1	7.2	5.5
% Sensitivity (95% CI)	89.7 (75.8–97.1)	95.0 (89.5–98.2)	97.8 (88.5–99.9)	96.3 (90.7–99.0)
% Specificity (95% CI)	95.1 (90.2–98.0)	86.3 (81.9–90.1)	63.6 (55.5–71.2)	74.4 (58.8–86.5)
AUC	0.96	0.98	0.95	0.97

AUC = area under the curve; CI = confidence interval; G6PD = glucose-6-phosphate dehydrogenase; K_2EDTA = dipotassium ethylenediaminetetraacetic acid; PS = Pointe Scientific; SD = standard deviation; SMRU = Shoklo Malaria Research Unit. Confidence intervals are provided in brackets under each sensitivity and specificity value.
* Normal by PS.
† Normal by SD Biosensor STANDARD G6PD test.
‡ Estimates for the Trinity Biotech assay.

7.2 Pointe Scientific test kit (reference assay)

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.
- Performed at University of Washington (UW) Medicine Northwest Hospital Laboratory (UW Department of Laboratory Medicine) with optional backup send out to Mayo Medical Laboratories

7.4 HemoCue system

The HemoCue Hb 201 system is designed for quantitative POC whole blood Hb determination in primary care using a specially designed analyzer, the HemoCue Hb 201 Analyzer, and specially designed microcuvettes, the HemoCue Hb 201 Microcuvettes. The HemoCue Hb 201 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 201 Analyzer. The measurement takes place in the analyzer, which measures the absorbance of whole blood at an Hb/HbO_2 isobestic point. The system is factory-calibrated and needs no further calibration. The HemoCue is available and registered for use at the study site.

7.5 Maintenance and storage of study products

Commercial assays shall be stored at recommended storage conditions as provided in the product labeling. The STANDARD G6PD Test shall be stored at ambient temperature. Control material for all assays shall be stored at recommended storage conditions as provided in the product labeling. See Table 6 below for a summary of storage conditions.

Table 6. Recommended Storage Conditions for Study Products

Study Product Description	Storage Conditions per Product Labeling	
	Temperature Range	Humidity Range
STANDARD G6PD test device	-20°C to 50°C	10%-93% RH
STANDARD G6PD controls	2°C to 30°C	N/A
HemoCue device	0°C to 50°C	"Should not be operated at high (i.e. > 90 % non-condensing) humidity"
HemoCue controls	2°C to 8°C	N/A

Temperature and humidity will be recorded by the study team at least once a day during business hours. In the event that any study product is observed to be stored out of range, PATH will be notified immediately. That study product is not to be used by the site until written confirmation from PATH to proceed is received. Quality control testing with control reagents will be conducted each day of data collection to ensure that the study tests are functioning.

8. Data and data management

8.1 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, will be used to summarize and characterize the study population.

8.1.1 Diagnostic performance

The performance of the POC G6PD test against the spectrophotometric gold standard test will be determined by calculating the sensitivity and specificity [15]. For purposes of the analysis of the performance of the investigational G6PD test, normal (100%) will be defined by the adjusted male median, which will be calculated as the median of males with >30% activity of the total male population median G6PD activity. This threshold was chosen in order to align with WHO guidance and to ensure that all male deficients are excluded from this population.

In order to investigate the performance of the assay to distinguish females with intermediate activity from female with normal activity, the sensitivity and specificity will be determined at G6PD activity thresholds of 70% and 80%. 70% refers to the threshold used in the clinical trials for tafenoquine as a cure to *P. vivax*, and 80% refers to the most recent WHO G6PD phenotype classification.

For the purposes of this study, an individual will be considered G6PD deficient (case) if they test positive by the Pointe Scientific spectrophotometric gold standard assay. The primary success criterion will be focused on the ability to identify G6PD deficient samples correctly, such that the SD Biosensor STANDARD G6PD Test on finger stick blood and the spectrophotometric gold standard test on venous blood should both accurately identify all severely G6PD deficient specimens (with <30% normal) as deficient.

An adjusted male median will be calculated for the spectrophotometric gold standard test; from this median, the 30%, 70%, and 80% cutoff levels will be used to categorically define G6PD deficient cases.

To quantify the accuracy between the POC G6PD test and the spectrophotometric gold standard test, receiver operating characteristic (ROC) curves will be plotted for each G6PD threshold activity (30%, 70% and 80%). The area under the curve (AUC) will be used to evaluate the clinical performance of the POC G6PD test.

Sensitivity will be determined using the following method:

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

Specificity will be determined by the following method:

- FP = false positive (negative by reference assays according to case definition and positive by POC G6PD test).
- TN = true negative (negative by reference assays according to case definition and negative by POC G6PD test).
- Specificity = TN/(TN+FP)

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

In addition, Youden's index (the point on the ROC curve where both sensitivity and specificity are maximized) will also be estimated to determine the optimal cutoff point of the POC G6PD test at 30%, and sensitivity and specificity will be calculated for the entire population and separately for males and females. As the 70% and 80% threshold levels are only applicable to females, Youden's index to determine the optimal cutoff point of the POC G6PD test at 70% and 80%, will be restricted to the female population. Hence, the sensitivity and specificity and corresponding using 95% confidence intervals at 70% and 80% activity, will be estimated only for females.

8.1.2 Accuracy between G6PD activity and hemoglobin methods

Quantitative agreement for both G6PD activity and Hb values between the SD Biosensor STANDARD 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. The following R-squared values will be considered acceptable:

1. STANDARD G6PD Test G6PD activity on capillary vs reference assay on venous blood > 0.8
2. STANDARD G6PD Test G6PD activity on venous vs reference assay on venous blood > 0.85
3. STANDARD G6PD Test hemoglobin concentration on capillary vs reference assay on capillary > 0.75
4. STANDARD G6PD Test hemoglobin concentration on venous vs reference assay on capillary > 0.90

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). The number, percent (95% confidence intervals) of samples within and outside these limits will be assessed.

No more than 15% of all samples should fall outside of these limits. Due to the importance of correctly classifying intermediate and deficient patients, 99% of all deficient samples and 95% of all intermediate samples are expected to be within these limits. No more than 5% of female normal samples should be misclassified as intermediates.

All statistical analyses will be performed using Stata 13.0.

The data comparison for the analyses is outlined in Table 7 below.

Table 7. Comparison methods.

Index test by sample type	Reference method	
	G6PD normalized for Hb (U/g Hb)	Hemoglobin concentration (g/dL Hb)
Venous	Pointe Scientific G6PD normalized for Hb from venous specimen	Hb from venous specimen
Capillary		HemoCue® Hb from finger stick

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

8.1.3 Repeatability of the STANDARD G6PD test

Analysis of variance will be used to determine the repeatability of the STANDARD G6PD test. Acceptable percentage coefficient of variation is as follows:

- G6PD
  - STD ≤ 0.5 I U/g Hb
  - CV ≤ 15%
- Hemoglobin
  - CV ≤ 10%

8.2 Data management

8.2.1 Data entry

The investigator is required to maintain original case report forms (CRFs)/source documents at the site. Participant data and POC test results will be entered on paper forms at the time the sample is taken. Additional paper forms will

be completed in the laboratory with results from the POC hemoglobin and G6PD tests. Pointe Scientific test results from the reference laboratory will be provided as lab-generated source documents.

Each week, upon completion of all testing, data from the paper forms for the primary study will be entered into a Research Electronic Data Capture (REDCap) electronic database system that is 21 CFR Part 11, FISMA, and HIPAA-compliant. Data from the paper forms for the nested repeatability study will be recorded into an Excel template. Specific procedures for transferring electronic instrument data will be described in study-specific Data Management Plan and training materials. Electronic study records will be deidentified upon completion of data collection.

8.2.2 Data access

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

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

8.2.3 Data storage

The study team will maintain, and store securely, complete, accurate and current study records throughout the study. In accordance with regulations, study staff will retain all study records on site for at least 10 years after study closure. Study records will not be destroyed prior to receiving approval for record destruction from PATH. No study records will be destroyed while study specimens are still being stored. Applicable records include source documents, site registration documents and reports, informed consent forms, and notations of all contacts with participants. The electronic records will be maintained for at least 10 years in the databases and remain password protected.

8.2.4 Quality control and quality assurance

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

Regular monitoring will be performed according to ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Study data will be aggregated into a database and PATH will generate a monitoring report every two weeks summarizing key indicators for data quality and study compliance. These indicators include but are not limited to the number of participants consented, the number of samples acquired, any deviations from study procedures, and corrective actions taken. Following written standard operating procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. In addition, a PATH representative will conduct site monitoring visits as needed to ensure compliance with the protocol and relevant SOPs.

9. Benefit and risk considerations

9.1 Benefit to study participants

Participants in this study will have convenient access to a hemoglobin test. Participants who are found to be anemic will be counseled and referred to a health care provider for follow-up. Participants in this study will have the opportunity to have their G6PD status tested by a reference assay. This information may inform the health care they receive 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.

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.

9.2 Risk and risk-mitigation considerations

The proposed study involves the use of an investigational product that has European Conformity certification (CE mark) regulatory approval and likely to receive an exemption from the Investigational Device Exemption.

The study will involve collection of venipuncture and capillary finger stick blood. Only commercially available specimen collection products will be utilized, and the specimen collection methods will be those normally employed by a physician, clinic, or hospital. 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 using 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 adults [16]. All decisions regarding clinical care will be made through referral to the local health care facilities.

There is a risk that the confidentiality of participant data will be compromised. The specimens will be inventoried and stored by participant codes established by the clinical site and will only be linked to participant identifiers and source documents through a study key. Individually identifiable protected health information or data will not be shared, expect when required during audits by institutional and ethical review boards and regulatory agencies. All testing results will be filed and transferred to the study database as deidentified. Results from the investigational test, may not be used as a diagnostic procedure without confirmation of the diagnosis by another, medically established diagnostic product or procedure.

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.

10. Study safety and 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. The study team 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.

10.1 Adverse Events

10.1.1 Adverse Event Definitions

Adverse Event (AE): Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease in a participant that is temporally associated with the use of an investigational product or procedures, even if the event is not considered to be related to the study product or procedures.

Serious Adverse Event (SAE): An SAE is any AE occurring during study participation that results in any of the following outcomes:

- Death
- Life Threatening (refers to any event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Hospitalization or prolongation of a hospital stay
- Persistent or significant disability or incapacitation (refers to any event which results in a substantial and/or permanent disruption of the participant's ability to carry out normal life functions)

- Required intervention to prevent permanent impairment/damage
- Congenital anomaly/birth defect
- Important medical event that may require intervention to prevent one of the preceding conditions.

Unanticipated Adverse Device Effect (UADE): Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of participants. Refer to Protocol Section 8.2 (for a list of anticipated adverse events, signs or symptoms. (21CFR-812.3(s))

10.1.2 Adverse Event management

Some reported or observed signs and symptoms are inherent to blood specimen collection and are likely to occur transiently for nearly all participants in this study. Such signs or symptoms will not be considered AEs as long as they are mild (transient, easily tolerated, no interference with daily activities). The following will not be considered AEs:

- Mild, self-limited pain, swelling, bruising, or brief and minimal bleeding at the collection site
- Lightheadedness or fainting
- Nausea and/or vomiting

However, these signs and symptoms must be considered AEs and documented on the Adverse Event CRF should any of them occur in such a way that the extent or nature of the experience exceeds that normally associated with the procedure, as judged by the PI, or the event meets the criteria for a Serious Adverse Event (SAE). For example, any injury that occurs as a result of fainting would be considered an AE.

10.1.3 Assessment of Adverse Events

All AEs must be assessed for Seriousness, Severity, and Relationship. All AEs, regardless of classification, must be comprehensively documented in the CRF and on the SAE form, if applicable, and reported. This includes AEs related to marketed study products. The following information about the event is to be reported on the AE CRF:

- Seriousness, classified as: Non-Serious or Serious
- Severity, classified as:
    - Mild: Transient symptoms, easily tolerated, no interference with daily activities
    - Moderate: Marked symptoms, moderate interference with daily activities, tolerable
    - Severe: Considerable interference with daily activities, intolerable
- Relationship, to the study product or study procedures:
    - Not Related: Evidence suggests absolutely no possible causal relationship between the event and the investigational study device (or procedures).
    - Unlikely Related: Evidence suggests that other possible causes or contributing etiological factors may have caused the event other than the investigational study device (or procedures).
    - Possibly Related: Evidence suggests a causal relationship between the event and the investigational study device (or procedures) cannot be ruled out
    - Related: Evidence suggests a reasonable causal relationship between the event and the device (or procedures) is likely

In addition, the following should be recorded for each AE:

- Action(s) taken to remedy the AE, including change in study treatment or participation, or medical/surgical treatments
- Duration of the AE from onset through resolution, as applicable
- Cause (including suspected product/procedure and/or other cause)
- Outcome of the event, including resolution and sequelae, as applicable

10.1.4 Additional procedures for Assessing & Reporting Serious Adverse Events (SAE)

SAE criteria are specified in Section 9.1. All SAEs must also be assessed by the Investigator determine whether an SAE is expected or unexpected. An adverse event will be considered unexpected or unanticipated if the nature, severity or frequency of the event is not consistent with the risk information previously described in the protocol, Informed Consent, or Investigator's Brochure (if applicable).

Any adverse event meeting the criteria for 'Serious', regardless of the Investigator's opinion of expectedness or relationship to the study product, must be reported to PATH within 24 hours. The Investigator or designee must report the event by telephone or email to PATH.

10.1.5 Reporting Obligations to IRB/EC and Health Authorities

The Investigator must report any adverse events which are serious, unanticipated/unexpected and probably or possibly related to the study product or procedures to the reviewing IRB/Ethics Committee (EC). This report must be submitted as soon as possible, but in no event later than 72 hours after the Investigator first learns of the event.

PATH will provide results of any evaluation of an unanticipated/unexpected adverse device effect to appropriate IRB/ECs within 72 hours after notification of the event.

10.2 Monitoring

The study team will be supervised by the local study lead. Study data will be entered into a database, and a monitoring report will be generated at least every two weeks, summarizing key indicators for study compliance. PATH and BioIVT will hold 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 procedure (SOPs).

PATH will designate trained and qualified personnel to monitor the progress of this clinical study. Prior to study start, a study initiation visit will be conducted to provide training to site staff with regard to 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 participants' protection and safety, when relevant, as well as the quality, completeness, and integrity of the data.

11. Ethical considerations

11.1 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 ICH GCP regulations and guidelines, whichever affords the greater protection to the participant. 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.

11.2 Informed consent

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

11.3 Ethical review committees

The protocol, informed consent form, and recruitment materials will be submitted to the PATH Research and Ethics Committee for review and approval. BSC conducts blood collection and other clinical procedures under the IRB-

approved protocol (2010-017) reviewed by Western IRB. The procedures outlined in this protocol are in line with other clinical procedure conducted at BSC.

11.4 Amendments

Amendments to the protocol will not be implemented without agreement from PATH and prior submission to and written approval from the governing IRB/EC, except when necessary to eliminate an immediate hazard to the participant. Notice of an emergency modification shall be given to the reviewing IRB/EC as soon as possible, but in no event later than 5 working days after the emergency occurred. Protocol amendments may affect Informed Consent Forms for current and future participants.

11.5 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.

11.6 Deviations

Protocol deviations are not permitted and should be implemented prospectively as a protocol amendment whenever practical or appropriate, unless required to protect the safety and well-being of the participant. Any deviation from the protocol that may have an impact on the safety or rights of the participant, 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. Significant deviations may also need to be reported to the IRB/EC and local health authority. If the Investigator or their staff inadvertently deviates from the study plan, the Investigator should implement appropriate corrective and preventive procedures.

11.7 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.

11.8 Compensation

Participants will be compensated for time and travel to participate in this trial. Participants will receive $25 for participation in the matrix equivalency study. Based on eligibility, participants who participate in the repeatability study will receive an additional $15. Based on eligibility, participants who participate in the sample stability study will receive an additional $15. Information about compensation, including the amount and schedule of payment and applicable reporting to the Internal Revenue Service, will also be described in the informed consent form.

11.9 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.

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. Laboratory documentation indicates that this G6PD reference assay is prone to interlab variation. Given the rates of G6PD prevalence in the

United States and the data requirements in the WHO verification guidelines, purposive sampling is required, and we expect a significant number of the samples tested to be G6PD normal.

13. Investigator responsibilities

The three project partners involved in this evaluation are BioIVT, PATH, and the University of Washington (UW). Roles and responsibilities for each of the partners are listed below (Table 8).

Table 8. Roles and responsibilities for study partners.

Task	*BioIVT*	*PATH*	*UW*
Award oversight		*X*	
Study design and protocol development	*X*	*X*	
Institutional review board submission and approval		*X*	
Study participant recruitment and enrollment	*X*		
Procurement of all study supplies		*X*	
Training on the use of study assays		*X*	
Recruitment, consent, enrollment, and field data collection	*X*		
Reference testing			*X*
Data entry and cleaning	*X*		
Data analysis and reporting		*X*	
Site monitoring		*X*	

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Appendices

A. Data collection forms

B. Consent form and assessment of understanding

C. Recruitment materials

D. Results return materials