Diagnostic Assay–
Target Product Profile

Diagnostic Assay: Quantitative glucose-6-phosphate dehydrogenase (G6PD) activity
Product: Point-of-care test for G6PD deficiency

Version: 8.5

Completed: 4/22/2021

PATH

Abbreviations

CLIA	Clinical Laboratory Improvement Amendments
EDTA	ethylene diamine tetra acetic acid
FDA	US Food and Drug Administration
G6PD	glucose-6-phosphate dehydrogenase
GSK	GlaxoSmithKline
Hb	hemoglobin
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IFU	instructions for use
NADPH	nicotinamide adenine dinucleotide phosphate
P. falciparum	*Plasmodium falciparum*
P. vivax	*Plasmodium vivax*
QC	quality control
RDT	rapid diagnostic test
TPP	target product profile
WHO PQ	World Health Program prequalification

Context

Defining the need for a quantitative point-of-care G6PD diagnostic test for safe treatment of Plasmodium vivax infection with an 8-aminoquinoline

Clinical need: Tafenoquine and primaquine are 8-aminoquinoline–based drugs that completely clear the human body of *Plasmodium (P.) vivax* parasites (radical cure). As 8-aminoquinolines, primaquine, and tafenoquine represent a risk to subjects with a common genetic trait manifested as deficiency in glucose-6-phosphate dehydrogenase (G6PD) activity. A major barrier to wide-scale adoption of radical cure is toxicity in people with G6PD deficiency. Whereas all people exposed to 8-aminoquinolines experience some drop in hemoglobin concentrations, people with G6PD deficiency are more likely to experience severe hemolysis, leading to severe hemolytic anemia and, potentially, death. As such, tafenoquine and primaquine should be administered only to subjects who are not G6PD deficient or who have sufficient G6PD activity in their red blood cells that exposure to the drug does not represent a risk.

Test description: A diagnostic test that determines whether a patient presenting with *P. vivax* infection has sufficient G6PD activity in their red blood cells to be treated with primaquine or tafenoquine is required to make the drug widely and safely available. Currently, the only reliable tests for G6PD deficiency are laboratory based and require significant expertise to run, as well as laboratory resources. Point-of-care tests for G6PD deficiency would permit treatment decision-making to happen where people seek health care—at primary health care facilities, dispensaries, and even through trained health care workers. The test should have similar workflow and resource requirements as malaria rapid diagnostic tests (RDTs).

Executive Summary Table

Variable	Targeted requirement	Optimistic specification
1. Product use summary/medical need/differentiation strategy		
1.1 Intended use(s)	An *in vitro* enzyme test for the semi-quantitative determination of glucose-6-phosphate dehydrogenase (G6PD) enzyme activity in finger-stick blood and ethylene diamine tetra acetic acid (EDTA) anticoagulated blood. For *in vitro* diagnostic use only. The test will provide results expressed as the ratio of units per deciliter of G6PD activity per gram of hemoglobin per deciliter (G6PD U/g Hb) to normalize G6PD activity for hemoglobin level. The "G6PD plus Hb" system is intended for differentiating normal from deficient G6PD activity levels in whole blood in order to identify individuals with G6PD deficiency.	The rapid diagnostic test is intended for the simultaneous quantitative measurement of red blood cell G6PD activity and hemoglobin (Hb) in finger-stick (capillary) or venous (EDTA-anticoagulated) whole blood. The test will provide results expressed as the ratio of units per deciliter of G6PD activity per gram of hemoglobin per deciliter (G6PD U/g Hb) to normalize G6PD activity for hemoglobin level. The "G6PD plus Hb" system is intended for differentiating normal from deficient G6PD activity levels in whole blood in order to identify individuals with G6PD deficiency. The test will also provide a stand-alone quantitative hemoglobin measurement in g/dL from fingerstick (capillary) or venous (EDTA-anticoagulated) whole blood.
1.2 Examples of use	Determination of G6PD enzymatic activities in whole blood to inform radical cure treatment with tafenoquine of symptomatic patients infected with *P. vivax.* Test will be able to discriminate normal G6PD levels (>70%) from deficient (≤30%) and borderline (>30%–70%). Performance will be assessed for >30-70%.	Quantitative determination of G6PD enzymatic activity and hemoglobin concentration in whole blood to inform treatment with tafenoquine and primaquine of symptomatic patients infected with *P. vivax.* Also, in support of *P. vivax* elimination, can be used in mass screening and treatment of asymptomatic individuals. The test supports other national G6PD testing requirements.
1.3 Target population	Symptomatic *P. vivax*–infected children ≥6 months of age and adults of both genders.	Symptomatic *P. vivax*–infected children and adults. Symptomatic and asymptomatic individuals in *P. vivax* malaria elimination setting.
1.4 Infrastructure level	Primary health care facilities and mobile clinics with trained staff.	All health care settings where people access malaria care, including village health care workers.

Variable	Targeted requirement	Optimistic specification
1.5 End user	Medical or laboratory qualified worker (e.g., primary care level/laboratory technician/ health care worker).	Community health care worker.
2. Design		
2.1 Format/ instrumentation requirements	Possible battery-operated reading system, temperature monitor, hemoglobin concentration measuring capability.	No instrumentation required.
2.2 Target analyte	Erythrocytic or red blood cell G6PD activity. Nicotinamide adenine dinucleotide phosphate (NADPH) concentration or other validated analytes that indicate G6PD enzyme activity.	Erythrocytic or red blood cell G6PD activity. NADPH concentration or other validated analytes that indicate G6PD enzyme activity and hemoglobin concentration. Analytes indicative of risk of hemolysis on exposure to 8-aminoquinolines.
2.3 Additional analyte	If the output of the test is hemoglobin concentration corrected, the additional analyte is either hemoglobin concentration or hematocrit.	Same.
2.4 Sample type	Venous whole blood collected in anticoagulant EDTA and capillary blood from finger-stick.	Venous whole blood collected in anticoagulant EDTA/heparin/citrate and capillary blood from finger-stick.
2.5 Sample collection and processing	Finger-stick, prepared in one or two steps (buffer and lysis). Venous blood prepared in one or two steps (buffer and lysis).	Finger-stick and venous blood with no preparation steps.
2.6 Sample volume	≤30 microliters.	≤10 microliters; volume is self-metered in the test.
2.7 Quality control	Demonstrated compatibility with commercially available external controls. Instructions for use (IFU) must contain quality control (QC) section with clear language about when and how to perform QC of the test. The suggested approach must be practical for the intended use of this TPP. See section 4.16.	Diagnostic test manufacturer provides controls configured appropriately for intended use.
2.8 Supplies needed	Lancet and alcohol swabs not included in the kit.	None.

Variable	Targeted requirement	Optimistic specification
2.9 Portability	Highly portable.	Same.
2.10 Safety	Universal blood safety precautions to be observed by the user.	Same.
3. Performance		
3.1 Assay output	The test will provide results expressed as the ratio of units per deciliter of G6PD activity per gram of hemoglobin per deciliter (G6PD U/g Hb) to normalize G6PD activity for hemoglobin level. The "G6PD plus Hb" system is intended for differentiating normal from deficient G6PD activity levels in whole blood to identify individuals with G6PD deficiency. The test will also provide a stand-alone quantitative hemoglobin measurement in g/dL from finger-stick (capillary) and venous whole blood collected in anticoagulant EDTA.	The test will provide results expressed as the ratio of units per deciliter of G6PD activity per gram of hemoglobin per deciliter (G6PD U/g Hb) to normalize G6PD activity for hemoglobin level. The "G6PD plus Hb" system is intended for differentiating normal from deficient G6PD activity levels in whole blood to identify individuals with G6PD deficiency. The test will also provide a stand-alone quantitative hemoglobin measurement in g/dL from finger-stick (capillary) and venous whole blood collected in anticoagulant EDTA/heparin/ citrate.
3.2 Test limit of detection	G6PD: <1.2 U/g Hb. 7g/dL hemoglobin.	G6PD: 0.4 U/g Hb (i.e., equivalent to Trinity reference method or Pointe Scientific method); 1 g/dL hemoglobin (equivalent to HemoCue reference method).
3.3 Test linearity	G6PD: Reference assay equivalent to Trinity or Pointe Scientific method approximately 1 to 20 U/g Hb. In analytical testing, R^2 should be ≥ 0.9 in clinically relevant range of 0-100% G6PD activity. Any other reference assay will be calibrated by PATH. Hb: Equivalent to HemoCue reference method for the clinically relevant range Hb: 7 to 15 g/dL. In analytical testing, R^2 ≥ 0.9 in clinically relevant range.	G6PD: Equivalent to Trinity reference method or Pointe Scientific method approximately 1 to 20 U/g Hb. Equivalent to HemoCue reference method for Hb: 2 to 25 g/dL.

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3.4 Precision	Total imprecision ≤8%. Display of G6PD and Hb results will be rounded to first decimal place. CV≤10% for G6PD enzyme activity >7 U/g Hb; SD ≤ ±1.0 U/g Hb for G6PD enzyme activity ≤7 U/g Hb. Variation of Hb within the medically allowable error for Hb (<6%) across entire dynamic range of test.	Same.
3.5 Test analytical accuracy relative to reference method	Accuracy (test result bias relative to reference) of the **G6PD method** must be bias ≤15% for G6PD enzyme activity >7 U/g Hb; SD ≤ ±1.0 U/g Hb for G6PD enzyme activity ≤7 U/g Hb. **HB method** must be within the medically allowable error for Hb (<6%) across entire dynamic range of test.	Same.
3.6 Diagnostic sensitivity/specificity (percent positive agreement; negative percent agreement relative to reference method) for final kit embodiments	G6PD (U/g Hb): The device will correctly identify >99% of severe deficient cases (<30% G6PD activity – male hemizygotes) and >95% of cases with <70% of normal enzyme activity. Hemoglobin (g/dL): The test must clearly discriminate severe anemic (<7 g/dL) and normal Hb levels at greater than 95% agreement with reference method.	Same.
3.7 Time to result	10 minutes or similar to times of current malaria rapid diagnostic tests (RDTs) that are successfully deployed.	5 minutes or less.

Variable	Targeted requirement	Optimistic specification
3.8 Throughput	6 results per hour (10 minutes to results).	12 results per hour (5 minutes to results).
3.9 Target shelf life/ stability	Assay: 18 months at temperatures between 2°C and 40°C and relative humidity of 75% ± 5% (as per test operating range).	Assay: 36 months at temperatures between 2°C and 40°C; stable for 2 weeks at 50°C; time-temperature monitors included on each kit as per International Conference on Harmonisation (ICH), and relative humidity of 75% ± 5% (as per test operating range).
3.10 Complexity: number of steps required, excluding sample collection	No more than one timed step; total less than five steps.	No more than three steps, with no timed steps. Complexity compatible for Clinical Laboratory Improvement Amendments (CLIA) waiver.
3.11 Operating temperature	20°C–37°C, 30%–75% non-condensing humidity.	18°C–40°C, 20%–90% non-condensing humidity.
4. Validation/configuration/format/other		
4.1 Reference methods	G6PD: Trinity Quantitative G-6-PDH Kit 345-A or Pointe Scientific Kit (CATALOG #G7583) since both are FDA-510(k)-cleared test methods. (Any other method needs validation by PATH). Hb: HemoCue is the reference test.	Same.
4.2 Data storage	1,000 test results minimum will be stored in the battery-operated device.	Same.
4.3 Data transfer/access	Stored test results can be accessed from the device by USB cable or flash drive.	Stored test results can be accessed from the device by USB cable or flash drive and secure wireless data exchange option.
4.4 Data communication/ standards	XML/JSON data structure used for results.	Leverages appropriate HL7 FHIR resources and adhere to appropriate IHE profiles.
4.5 Recorded data/metadata	G6PD measurement is recorded on the device with sample ID and/or hemoglobin measurement where applicable.	All test result measurements associated with a test run are recorded on the device.
4.6 Firmware updates	Ability to update firmware in the field by authorized user.	Same.

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4.7 Shipping conditions	Same as 3.9: Shipping simulation testing to conform to applicable requirements of ASTM D4169-05.	World Health Organization Prequalification (WHO PQ) recommends the product to endure simulated extreme stress conditions, ensuring that application of those conditions is consistent and controlled as per ISO 23640:2011, CLSI EP25-A, WHO TGS-2, and ASTM D4169-14.
4.8 Training requirements	One or less days for any level of provider to achieve proficiency. Language-appropriate training materials, results guide, and job aids should be made available. Users score a minimum of 85% according to a standard proficiency assessment of product label comprehension and results interpretation.	Same, plus one hour for health care workers familiar with RDTs.
4.9 Instrumentation requirements	Possible battery-operated G6PD reading system, temperature correction, Hb measuring device.	No instrumentation required.
4.10 Instrumentation weight and size	Small equipment close to palm size weighing less than one pound.	Comparable to handheld glucose monitor.
4.11 Instrument calibration	Manufacturer provides all required calibration biologics/materials.	Internal factory calibration.
4.12 Instrument service and support	None required for a period of 2 years.	None required for a period of 3 years.
4.13 Waste disposal	Does not include material that cannot be disposed of in the normal laboratory waste streams.	Same.
4.14 Power requirements	Possible battery with a battery life of 10 hours.	None.
4.15 Water requirements	Self-contained kit operates independent of water.	None.
4.16 Quality controls	Demonstrated compatibility with commercially available controls. IFU must contain QC section with clear language of when and how to perform QC of the test. The suggested approach must be practical for the intended use of this TPP.	Diagnostic test manufacturer provides controls configured appropriately for intended use.

Variable	Targeted requirement	Optimistic specification
5. Product costs and channels to market		
5.1 Target price ex works	**Disposable:** ≤US$ 3.00 ≤US$2.50 at scale **Instrumentation:** A reader cost of ≤US$ 380 ≤US$250 at scale	**Disposable:** ≤US$ 1.00 **Instrumentation:** A reader cost of ≤US$ 200
5.2 Target launch countries	All countries with P. vivax radical cure in national malaria policy. Prioritization to be determined.	All countries with *P. vivax* radical cure in national malaria policy.
5.3 Product registration path	CE-IVDR and WHO prequalification. For US manufacturer, FDA-510(k). ISO 13485 certified Quality Management System.	Same.
5.4 Channels to market	To be determined. National health care system supply chain or supply chain of Global Fund to Fight AIDS, Tuberculosis and Malaria.	Same.

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