RESEARCH BRIEF

Evaluation of a point-of-care test for G6PD deficiency in Brazil

The SD Biosensor STANDARD G6PD Test

Background

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common enzyme deficiency that is prevalent in many malaria-endemic countries. G6PD-deficient individuals are susceptible to hemolysis when exposed to certain medications, including 8-aminoquinolines (primaquine and tafenoquine), which are used to treat *Plasmodium vivax* malaria.

The reference standard for measurement of G6PD activity is quantitative spectrophotometry. However, this is a complex test requiring substantial laboratory infrastructure that is not often readily available in many near-patient settings in malaria-endemic areas. Qualitative tests are widely available but are not able to reliably identify G6PD-intermediate women. Importantly, single-dose tafenoquine (known under the brand name Kozenis® by GlaxoSmithKline) is only indicated for patients with G6PD activity >70%—necessitating the use of quantitative or semi-quantitative tests prior to prescription. Access to simple, appropriate, point-of-care tests for G6PD deficiency is critical for the safe treatment of *P. vivax* malaria in support of national malaria control and elimination efforts.

The SD Biosensor STANDARD G6PD Test. Photo: PATH/ Patrick McKern.

SD Biosensor (Republic of Korea) has developed the STANDARD™ G6PD Test, a novel, semi-quantitative test for G6PD deficiency that is intended for use at the point of care. This test uses a handheld, battery-operated analyzer that provides a numeric measurement of G6PD activity normalized by hemoglobin (U/g Hb). This value can then be used in a semi-quantitative manner to classify individuals as G6PD deficient, intermediate, or normal according to thresholds provided by the manufacturer.

Address
2201 Westlake Avenue
Suite 200
Seattle, WA 98121 USA

Date
September 2021

Objectives and methods

PATH, the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT/HVD), and the Centro de Pesquisa em Medicina Tropical de Rondônia (CEPEM) conducted a study in Brazil to evaluate the diagnostic accuracy of the STANDARD G6PD Test when used by trained health care workers.

Between July and December of 2019, this study took place at two sites in Brazil: FMT/HVD in Manaus, Amazonas, and CEPEM in Porto Velho, Rondônia. Participants were recruited at clinics, as well as through an enriched sample of individuals with known G6PD status.

The performance of the test was measured on capillary samples at the point of care and on venous K_2EDTA specimens in the laboratory. The performance of the STANDARD G6PD Test in the measurement of G6PD activity was compared against a quantitative spectrophotometric reference standard (Pointe Scientific, United States) normalized by hemoglobin. The test's performance in measuring hemoglobin was compared against a reference complete blood count in Manaus. At the point of care, G6PD and hemoglobin measurements were obtained from capillary samples using the STANDARD and HemoCue®201+ (HemoCue AB, Sweden) tests. A thick blood slide was prepared for malaria microscopy. Venous blood was collected and transferred to the laboratories, where the STANDARD and HemoCue tests were repeated and the quantitative spectrophotometric G6PD reference assay was performed. G6PD was also assessed in the laboratory by fluorescent spot test. In Manaus, a complete blood count was performed with a hematology analyzer.

The following manufacturer-established thresholds for the STANDARD G6PD Test were used during the study: ≤4.0 U/g Hb for G6PD-deficient males and females (≤30% activity); 4.1 to 6.0 U/g Hb for G6PD-intermediate females (>30% to ≤70% activity); >4.0 U/g Hb for G6PD-normal males (>30% activity); and >6.0 U/g Hb for G6PD-normal females (>70% activity).

Results

In total, 1,736 individuals were included in the study: 924 from Manaus and 812 from Porto Velho. Venous samples were available for analysis from 1,662 participants, and capillary samples were available from 1,663 participants.

Within the study sample, there were 59 G6PD-deficient participants (men and women with G6PD activity levels <30% of normal) and 35 G6PD-intermediate women (30% to 70% of normal) according to the reference test, calculated based on each site's adjusted male median. There were 251 confirmed cases of malaria, the majority of which (94%) were from Porto Velho.

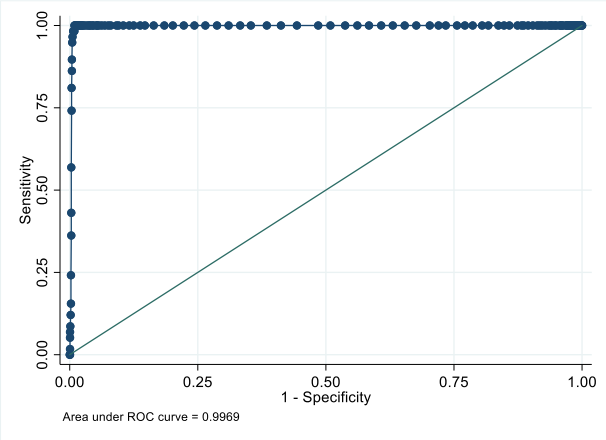
During the study, the STANDARD G6PD Test was run at a broad range of operating temperatures, from 18ºC to 44ºC, which is reflective of real-world use of the device.

Clinical performance of the test for G6PD activity

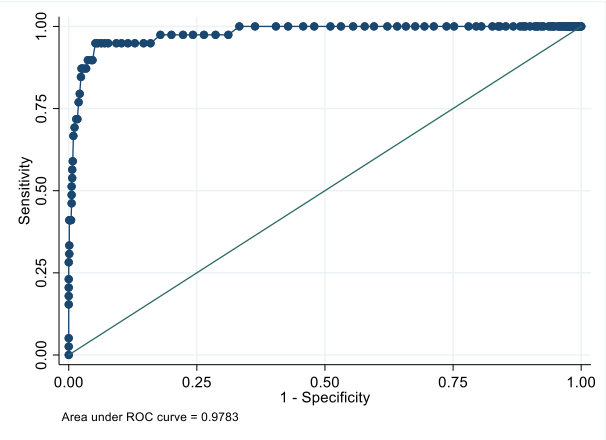
The STANDARD G6PD Test showed good diagnostic capacity to differentiate G6PD-deficient, -intermediate, and -normal cases (Figure 1). The test was able to diagnose G6PD-deficient males and females with a high area under the curve (AUC) of 1.0 on capillary specimens. At the 70% threshold for intermediate females, the test also showed a high AUC of 0.98 for capillary specimens.

Figure 1. Receiver operating curves (ROCs) at 30% and 70% activity thresholds on capillary specimens.

30% G6PD-deficient males and females



70% G6PD-intermediate females



The STANDARD G6PD Test performed well in comparison to the reference standard (spectrophotometry) (Table 1). Using the manufacturer-recommended reference value thresholds, the test's sensitivity at the <30% threshold on both specimen types was very good at 100%. At the 70% threshold—which is particularly relevant for Kozenis administration—the test's sensitivity was 97% on venous specimens and 94% on capillary. Specificity at this threshold was 97% and 92% on venous and capillary specimens, respectively. All specimens with less than 65% G6PD activity had a result on the STANDARD G6PD Test of ≤6.0 U/g Hb. Only two females with G6PD activity levels between 65% and 70% had a normal capillary STANDARD G6PD Test result (>6.0 U/g Hb). The malaria status of participants did not appear to significantly influence the performance of the test.

Table 1. Performance of the STANDARD G6PD Test using the manufacturer's thresholds, compared with reference spectrophotometry, by sample type. Two definitions of intermediates are provided.

	Venous	Capillary
30% G6PD-deficient males and females		
Sensitivity (95% CI)	100.0 (93.6–100.0)	100.0 (93.8–100.0)
Specificity (95% CI)	100.0 (93.6–100.0)	100.0 (93.8–100.0)
70% G6PD-intermedate females		
Sensitivity (95% CI)	96.9 (83.8–99.9)	94.3 (80.8–99.3)
Specificity (95% CI)	96.5 (95.0–97.6)	92.3 (90.3–94.0)

Abbreviation: CI, confidence interval.

Clinical performance of the test for hemoglobin

The correlation between the STANDARD G6PD Test and the complete blood count in the measurement of hemoglobin was low, with R squared values of 0.67 for both capillary and venous specimens. However, the STANDARD G6PD Test showed good categorical agreement with the complete blood count in its ability to identify moderate and severe anemia at >93%. Although this level of agreement was

predominantly driven by the large proportion of non- and mild anemia cases within the study population, the HemoCue showed similar patterns of misclassification when compared to the complete blood count. For the STANDARD G6PD Test, confirmatory hemoglobin testing is recommended when monitoring disease progression or making treatment decisions related to hemoglobin concentration.

Conclusions

The STANDARD G6PD Test is a promising tool to aid in the identification of individuals with G6PD deficiency. Its availability represents an important opportunity to improve *P. vivax* case management in malaria-endemic settings like Brazil, where reliable alternatives to quantitative spectrophotometry are needed to expand access to radical cure. In particular, the results of this study are relevant for the introduction of single-dose Kozenis in Brazil, which was approved in 2019.

For more information

Please see the full publication from this study:

> Zobrist S, Brito M, Garbin E, et al. Evaluation of a point-of-care diagnostic to identify glucose-6-phosphate dehydrogenase deficiency in Brazil. *PLOS Neglected Tropical Diseases*. 2021;15(8): e0009649. https://doi.org/10.1371/journal.pntd.0009649.

For questions, please contact Dr. Marcus Lacerda (FMT/HVD), Dr. Dhélio Pereira (CEPEM), or Dr. Gonzalo Domingo (PATH).

Funding

This work was funded by the United Kingdom's Foreign, Commonwealth & Development Office, grant number 204139, and the Bill & Melinda Gates Foundation, grant number OPP1107113. The findings and conclusions contained within are those of the authors and do not necessarily reflect the positions of the Foreign, Commonwealth & Development Office or the Gates Foundation.