QUANTITATIVE POINT-OF-CARE G6PD TESTS FOR RADICAL CURE OF *PLASMODIUM VIVAX* MALARIA

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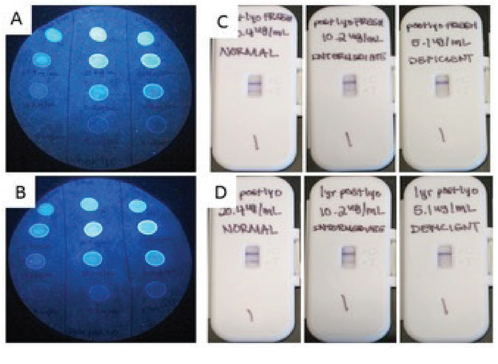
INTRODUCTION

Currently, the only drugs that can completely cure a patient of *Plasmodium vivax* parasites (radical cure), are 8-aminoquinoline–based drugs such as primaquine. The dosage required for these drugs to be effective curative agents for *P. vivax* infection may cause severe hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency is an X-linked genetic disorder that affects more than 400 million people worldwide. Moderate to severe life-threatening hemolytic anemia episodes can develop if G6PD-deficient individuals are treated with 8-aminoquinoline drugs. This risk represents a major barrier to widescale adoption of radical cure. Therefore, determination of a malaria patient's G6PD activity level is critically important before 8-aminoquinoline drug therapy. We are currently evaluating novel G6PD-Hb (hemoglobin) integrated quantitative tests and a G6PD qualitative test using whole blood in a point-of-care setting. Analytical performances of the tests have been evaluated following the criteria of the target product profile. To support the development process of G6PD quantitative tests, we have validated the correlation of two quantitative reference assays.[1] Additionally, to support the development of G6PD qualitative tests, we have established recombinant G6PD controls.[2]

Workflow for the use of the lyophilized control with a G6PD rapid diagnostic test.

1. Dried control
2. Rehydrated with deionized water
3. Ready to use in 10 minutes.
4. Run in the same way as test sample

Performance of human recombinant controls on qualitative tests for G6PD.



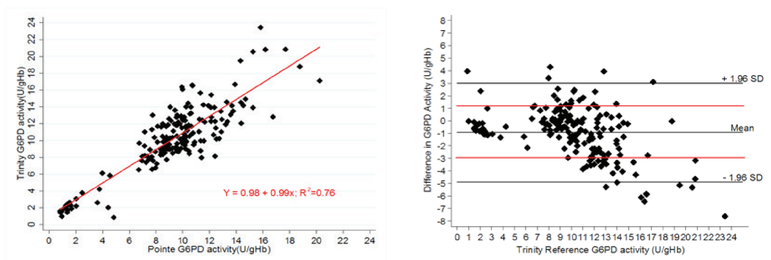
Two lots of three concentrations (normal, intermediate, and deficient) of human recombinant G6PD (r-G6PD) were tested on two qualitative assays for G6PD: the fluorescent spot test (panels A and B) and a novel prototype rapid diagnostic test for G6PD (panels C and D). In panels A and B, the top row represents the Trinity normal controls for reference; the second, third, and fourth rows show the signals for the normal, intermediate, and deficient human r-G6PD controls, respectively. In panels C and D, the line intensity of the test output correlates with expected enzyme activity, referring to normal, intermediate, and deficient activity. The signal for one lot of freshly lyophilized human r-G6PD (A and C) is compared to a second lot that had been stored at 4°C for more than one year (B and D).

EVALUATION OF TWO QUANTITATIVE REFERENCE ASSAYS

Total of 183 fresh venous blood specimens in K_2EDTA were obtained from Bioreclamation, Inc.: 128 male and 55 female; African American population. The blood specimens were tested using the **Pointe Scientific** and **Trinity Biotech** G6PD assays in the PATH laboratory. The Trinity G6PD quantitative assay was considered as the G6PD reference assay. Hemoglobin was determined using the HemoCue method for normalized G6PD activity for both the Pointe and Trinity assays.

Accuracy between the Trinity and Pointe G6PD methods

Dot plot and Bland Altman plots using Pointe G6PD with Trinity G6PD assay.



G6PD activity values for the PATH Trinity and Pointe assays

	40%	70%	100%
Trinity	4.12	7.21	10.3
Pointe	3.84	6.72	9.6

Sensitivity and specificity using 70% as the cutoff[1]

Deficient (70%)

	Pointe ≤6.72	Pointe >6.72	Total
Trinity ≤7.21	26	2	28
Trinity >7.21	0	155	155
Total	26	157	183

Sensitivity % (95% confidence interval): 92.9 (76.5–99.1).
Specificity % (95% confidence interval): 100 (97.6–100).

Sensitivity and specificity using 40% as the cutoff[1]

Severe deficient (40%)

	Pointe ≤3.84	Pointe >3.84	Total
Trinity ≤4.12	20	2	22
Trinity >4.12	1	160	161
Total	21	162	183

Sensitivity % (95% confidence interval): 90.9 (70.8–98.9).
Specificity % (95% confidence interval): 99.4 (96.6–100).

ACKNOWLEDGEMENT

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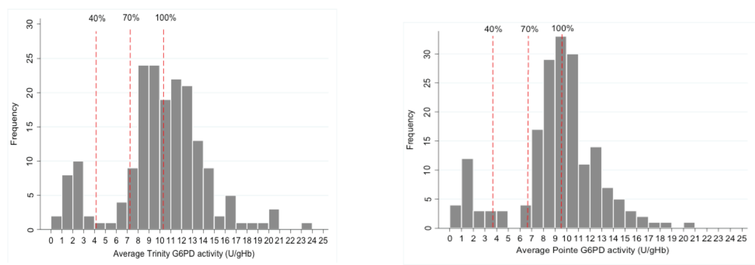
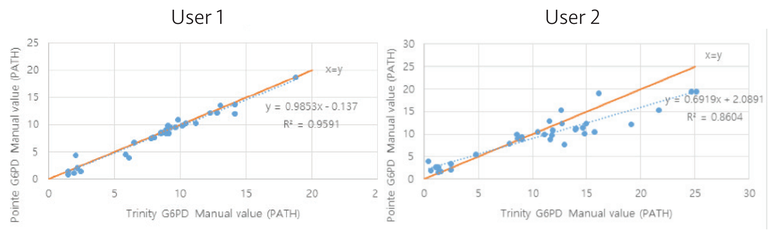
Sensitivity and specificity of the Pointe G6PD assay[1]

Histogram of G6PD activity thresholds with Pointe assay.



Variability within the laboratory: A difference in the Pointe data was observed between users in the same laboratory, following the same standard operating procedure (Trinity was performed by one user).



SUMMARY

- Lyophilized r-G6PD enzyme can be used as a control for point-of-care tests for G6PD deficiency. Additional studies will be needed to explore the preservation of hemoglobin with the r-G6PD enzyme in a lyophilized format. The combination would make an ideal control for quantitative tests that rely on hemoglobin or hematocrit to determine G6PD activity.
- Sensitivity of the Pointe assay was 92.9% and specificity 100% for the 70% cutoff.
- Sensitivity of the Pointe assay was 90.9% and specificity 99.4% for the 40% cutoff.
- Correlation with the Trinity method demonstrated an R2 value of 0.76.
- The overall distribution of Pointe G6PD values indicates that deficients and normals cluster similarly to the Trinity G6PD values.
- Precision was less than 10% for specimen with normal G6PD activity (data not shown).
- Variability between users was observed.

REFERENCES

1. Domingo GJ, Satyagraha AW, Anvikar A, Baird K, Bancone G, et al. G6PD testing in support of treatment and elimination of malaria: recommendations for evaluation of G6PD tests. *Malaria Journal.* 2013;12:391. doi:10.1186/1475-2875-12-391.
2. Kahn M, LaRue N, Zhu C, Pal S, Mo JS, et al. Recombinant human G6PD for quality control and quality assurance of novel point-of-care diagnostics for G6PD deficiency. *PLOS ONE.* 2017;12(5):e0177885. https://doi.org/10.1371/journal.pone.0177885.