A field trial to evaluate the performance of a point-of-care diagnostic for screening G6PD deficiency in a *falciparum* and *vivax* malaria endemic area of Western Cambodia

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Introduction

- Cambodia is moving towards malaria elimination
- Primaquine remains the only available drug capable of killing *P. falciparum* mature gametocytes and *P. vivax* hypnozoites
- Screening patients for Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency in the community remains critical for a safe and successful roll-out of primaquine
- G6PD rapid diagnostic tests (RDTs) are needed alongside malaria RDTs
- Comprehensive assessments to test their feasibility and performances under field conditions are still required prior to their wide-scale implementation

Aim

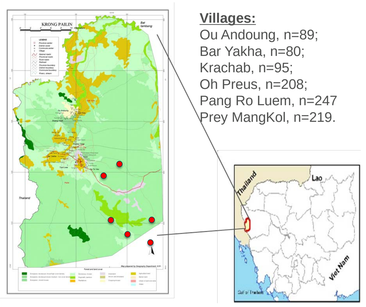
Evaluating the third generation of a new inexpensive and heat-stable point-of-care (POC) diagnostic for screening G6PD deficiency (CareStart G6PD kit), developed by AccessBio CEO (New Jersey, USA)

Objective

To assess the sensitivity and specificity of this new G6PD screening tests under field conditions when compared to the gold standard (quantitative assay) and the fluorescent spot test (qualitative assay);

Methods

Cross-sectional survey conducted in six malaria endemic villages in Pailin Province in Cambodia (see map) in May-June 2013.



Villages:
Ou Andoung, n=89;
Bar Yakha, n=80;
Krachab, n=95;
Oh Preus, n=208;
Pang Ro Luem, n=247
Prey MangKol, n=219.

A total of 938 individuals were enrolled in the study.

Sample size

Calculation was based on the primary objective but was dependent on the number of RDTs (n = 1000) available for testing and assuming an overall G6PD prevalence of 15%. Despite this limiting factor, the relative precision that could be achieved assuming a minimum sensitivity and specificity of 90% was considered to be acceptable (2%).

Data Analysis

Data entered and verified using Microsoft Excel® software, and was analyzed using EpiInfo 6.04® software (CDC, Atlanta, USA), Stata® version 12, and MedCalc® version 11.6.1 software (MedCalc, Mariakerke, Belgium). *P*-values < 0.05 were used to indicate statistically significant differences.

Laboratory Testing

The validity of the tests used in the study were monitored by the use of at least three levels of G-6-PDH controls (Deficient, Intermediate and Normal) for each run. To minimize the impact of heterozygosity on the definition of G6PD activity, and following the recent guidelines proposed by the G6PD working group[1], the adjusted median value of G6PD activity for the entire male population was calculated for which males with severe G6PD deficiency (activity less than 10% normal) were excluded.

Ethical review

Cambodian National Ethics Committee for Health Research (NEHCR) and Centers for Disease Control and Prevention. There was no conflict of interest.

Results

1. Demographic characteristics (Table 1):

938 venous blood samples collected from individuals living in six villages in Pailin province. All were Khmer ethnic group. The male/female ratio was 452/486 (0.93) and age ranged from 18 to 75 years old (median=35 year). The detection of malaria parasite carriers was positive for 28/750 (3.7%). *P. vivax* was the most prevalent species (24/750, 3.2%).

Table 1. Demographical and hematological characteristics

Patients' characteristics		Total
N		938
Median age in years (IQR)		35 (24-48)
Sex ratio (% female)		52
No. Individuals with T°> 37.5°C (%)		19 (2.0)
Malaria: No. of individuals found positive by	RDT	1 Pf/non-Pf & 2 Non-Pf
	PCR	2 Pf & 12 Pv
Median WBC - x10 ^{3}/mm^3 (IQR)		7.1 (5.7-8.2)
Median RBC - x10 ^{6}/mm^3 (IQR)		4.2 (3.9-4.6)
Median haemoglobin in g/dL (95% CI)		11.5 (10.6-12.3)
Haemoglobin electrophoresis profile (%)	Normal	64
	Hb E - homozygous	1
	Hb E - heterozygous	18
	Heterozygous E - α-thalassaemia	10
	Heterozygous Hb E - β-thalassaemia	4
	α-thalassaemia	2
	β-thalassaemia	1
Median G6PD activity in UI/g Hb (IQR)		11.7 (10.0-13.6)
G6PD Classes (%)	II (0.1-10% of normal)	6.0
	III (10.1-60% of normal)	8.2
	IV (60.1-150% of normal)	80.6
	V (>150% of normal)	5.2

Figure 1a

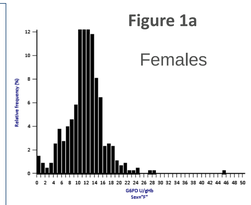
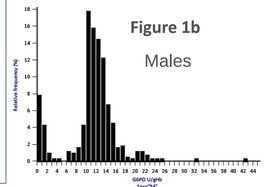


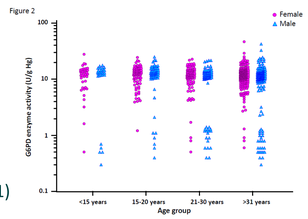
Figure 1b



G6PD enzymatic activity

- Ranged from 0.3 to 45.9 UI/g (Figure 1a and 1b)
- No significant difference was observed between gender (P=0.19), but trend for decrease was observed from the youngest to the oldest: <15 years, median=12.9 UI/g; 15-20 years, 12.6 UI/g; 21-30 years, 11.6 UI/g and >31 years, 11.4 UI/g (P=0.001) (Figure 2)

Figure 2



Reference range of G6PD enzymatic activity by gender:

Reference values	Total	Female	Male	Adjusted male
Number of cases	938	486	452	410
Mean (95% CI) U/g Hb	11.48 (11.17-11.79)	11.78 (11.39-12.18)	11.15 (10.68-11.62)	12.23
SD	4.76	4.42	5.08	4.00
Median (95% CI) U/g Hb	11.8 (11.5-12.0)	11.9 (11.5-12.3)	11.65 (11.4-12.0)	12.0 (11.7-12.4)
IQR	10.0-13.7	9.8-14.0	10.1-13.4	10.6-13.6
Range	0.3-45.9	0.5-49.5	0.3-42.5	1.2-42.5

Based on the adjusted median 100% G6PD activity was estimated to 12.0 UI/g and cut-off values according to the WHO classification were defined as followed: 10%, 1.2 UI/g; 20%, 2.4 UI/g; 30%, 3.6 UI/g and 60%, 7.2 UI/g

Table 2

		Cutoffs						
Cutoff value (% and UI/g)		<10%	<20%	<30%	<40%	<50%	<60%	≥60%
		<1.2	<2.4	<3.6	<4.8	<6.0	<7.2	≥7.2
No. of samples with G6PD activity < to the cutoff value (%)		56	12	6	15	18	26	805
Fluorescent spot test	No. of individuals classified as deficient/intermediate	56	12	6	10	5	3	2
	No. of individuals classified as normal	0	0	0	5	13	23	803
	Sensitivity (95% CI)	100% (93.6-100%)	100% (94.7-100%)	100% (95.1-100%)	94.4% (87.4-98.1%)	83.2% (74.7-89.7%)	59.9% (51.8-67.6%)	59.9% (51.8-67.6%)
	Specificity (95% CI)	95.7% (94.1-96.9%)	97.0% (95.6-98.0%)	97.7% (96.5-98.6%)	98.8% (97.8-99.4%)	99.4% (98.6-99.8%)	100% (99.5-100%)	100% (99.5-100%)
	Positive Predictive Value (95% CI)	59.6% (49.0-69.6%)	72.3% (62.1-81.1%)	78.7% (69.1-86.5%)	89.4% (81.3-94.8%)	94.7% (88.0-98.2%)	100% (96.1-100%)	100% (96.1-100%)
	Negative Predictive Value (95% CI)	100% (99.6-100%)	100% (99.6-100%)	100% (99.6-100%)	99.4% (98.6-99.8%)	97.9% (96.6-98.7%)	92.6% (90.6-94.2%)	92.6% (90.6-94.2%)
	Positive Likehood Ratio	23 (17-32)	33 (23-49)	43 (28-67)	80 (43-149)	138 (57-333)	-	-
	Negative Likehood Ratio	0	0	0	0.06 (0.02-0.13)	0.17 (0.11-0.26)	0.4 (0.33-0.49)	0.4 (0.33-0.49)
CareStart G6PD RDT	No. of individuals classified as deficient/intermediate	56	12	6	9	5	3	9
	No. of individuals classified as normal	0	0	0	6	13	23	796
	Sensitivity (95% CI)	100% (93.6-100%)	100% (94.7-100%)	100% (95.1-100%)	93.3% (85.9-97.5%)	82.2% (73.6-88.9%)	59.9% (51.7-67.6%)	59.9% (51.7-67.6%)
	Specificity (95% CI)	95.0% (93.4-96.3%)	96.3% (94.8-97.5%)	97.0% (95.6-98.0%)	98.0% (96.8-98.8%)	98.6% (97.5-99.2%)	99.2% (98.3-99.7%)	99.2% (98.3-99.7%)
	Positive Predictive Value (95% CI)	56.0% (45.7-65.9%)	68.0% (57.9-77.0%)	74.0% (64.3-82.3%)	83.0% (74.2-89.8%)	88.0% (80.0-93.6%)	94.0% (87.4-97.8%)	94.0% (87.4-97.8%)
	Negative Predictive Value (95% CI)	100% (99.6-100%)	100% (99.6-100%)	100% (99.6-100%)	99.3% (98.5-99.7%)	97.7% (96.5-98.6%)	92.5% (90.5-94.2%)	92.5% (90.5-94.2%)

Prevalence of G6PD deficiency:

Severe deficiency (Classes I and II, enzyme activity <10%): 56/938 (6.0%)
Moderate to mild deficiency (Class III, enzyme activity: 10%-60%): 77/938 (8.2%)
Very mild to no enzyme deficiency (enzyme activity: 60%-100%): 370/938 (39.4%)
Class V (enzyme activity > 150%): 49/938 (5.2%)
Males were more frequently severely deficient (10.6% vs. 1.6%, OR=7.1, P<10-6) while females were more frequently moderate-mildly deficient (12.1% vs. 4.0%, OR=3.3, P<10-4).

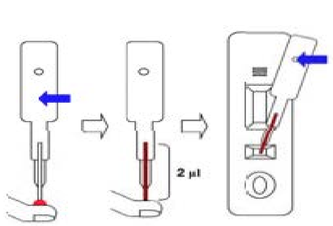
Performance of the CareStart™ G6PD deficiency screening test:

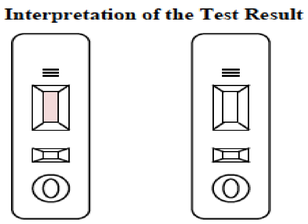
The distributions of G6PD enzymatic activity of our sampling population according to the results of the CareStart™ G6PD deficiency RDT test and the fluorescence spot test are given in Table 2.

The sensitivity *(probability that the qualitative test classifies individuals as G6PD-deficient [white background color/non-fluorescent spot] in individuals defined as G6PD-deficient by the quantitative assay and the selected cut off value)* of the CareStart™ G6PD deficiency RDT test and the fluorescent spot test to detect <30% enzyme activity (equivalent to <3.6 U/g) were found to be 100%.

The specificity *(probability that the qualitative test classifies individuals as G6PD-normal [purple background color/fluorescent spot] in individuals defined as G6PD-normal by the quantitative assay and the selected cut off value)* of the CareStart™ G6PD deficiency RDT test was found to be 97%.

The performances of the CareStart™ G6PD deficiency RDT test and the fluorescent spot test were not significantly different between gender (data not shown).





Discussion and conclusions

Better performance compared to the previous generation of this RDT where sensitivity was low (68%) and therefore the risk of false "normal" status unacceptably high. [2]
The evaluation of this test "outside of the laboratory" showed its capability of detecting reliably G6PD deficient individuals with enzyme activity levels <30% UI/g comparable to the fluorescent spot test.
To accelerate the roll out of G6PD RDT use and primaquine in Cambodia, further evaluations are needed to assess the operational challenges and programmatic usefulness of the tests when implemented by health workers in the field.
In parallel, more clinical data on the optimal and safe primaquine doses for malaria elimination are needed urgently in order to improve the development of the next generation of tests which will aim at providing a reliable and safe "go vs. no-go" answer to using primaquine in routine practice.

References

[1] G6PD testing in support of treatment and elimination of malaria: guidelines for evaluation of G6PD tests. A report by the G6PD Diagnostics for Radical Cure Working Group. Bangkok Meeting Report October 4th and 5th 2012.
[2] Kim S., *et al*. (2011). "Performance of the CareStart G6PD deficiency screening test, a point-of-care diagnostic for primaquine therapy screening." PLoS One 6(12): e28357.

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