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ORIGINAL ARTICLE

Evaluation of quantitative point-of-care test for measurement of glucose-6-phosphate dehydrogenase enzyme activity in Malaysia

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Abstract

Introduction: The treatment of *Plasmodium vivax* malaria with 8-aminoquinolines is contraindicated in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals due to the risk of acute haemolytic anaemia. Effective G6PD screening is paramount to avoid adverse drug reactions. This study aimed to evaluate the performance of novel quantitative point-of-care (POC) tests as a new screening method for G6PD deficiency in Malaysia. *Materials and Methods:* A total of 153 neonatal cord blood, 99 peripheral blood of older children aged between 1 month to 12-years old, and 62 peripheral adult blood were screened for G6PD deficiency using two quantitative POC tests, CareStart™ biosensor (Carestart) and CareStart™ Biosensor 1 (S1). The results were compared with OSMMR2000D kit as a reference assay. Two statistical analyses were performed in this study to evaluate the POC test performances, the Spearman's correlation test and the Cohen's kappa method. *Results:* Both Carestart and S1 tests showed significant positive correlations to OSMMRS000D with r^2 = 0.7916 and r^2 = 0.7467. Their measurement of agreement showed a kappa (κ) value of 0.805 ($p<0.001$, 95% CI), and 0.795 ($p<0.001$, 95% CI), respectively. Analysis of the area under the Receiver Operating Curve (ROC) at 60% cut-off illustrated that the Carestart had 90.2% sensitivity, 98.9% specificity, 98.3% positive predictive value (PPV), and 93.8% negative predictive value (NPV). The corresponding values for the S1 were 95.2%, 100%, 100%, and 96.8%, respectively. *Conclusion:* This study showed that the Carestart and S1 biosensors have high-performance reliability for screening of G6PD deficiency, which can guide safe prescriptions of anti-malaria medications and hence, eradication of *Plasmodium vivax* malaria.

Keywords: G6PD, point-of-care, OSMMR2000D, Carestart, S1, Biosensor

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the commonest causes of neonatal jaundice in Malaysia with an incidence rate of 5.1% and 5.5% among male neonates of Malay and Chinese origins, respectively.[1,2] The prevalence of G6PD deficiency among Orang Asli (the indigenous people in Malaysia) who live in the malarial endemic area has been shown to be as high as 9%.[3] Since 1986, Malaysia has implemented a fluorescent spot test (FST) for screening of cord blood for G6PD deficiency, as part of government strategy for prevention of severe neonatal jaundice and kernicterus. The screening program has successfully covered more than 95% of the population.[4] However, the aforementioned method was proven to be lacking sensitivity to detect cases with mild to moderate G6PD activity, particularly when the enzyme level was more than 20% of the mean normal activity.[5] These cases include male neonates of class III variants that have mild to moderate G6PD activity (10 – 60% of

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normal activity), and female heterozygotes with moderate enzyme deficiency who are also at risk to develop neonatal jaundice.[6]

A more accurate diagnosis of G6PD deficiency relies on the quantitation of the enzyme level by spectrophotometric assay. Some laboratories and health facilities such as Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Cheras, Kuala Lumpur used another method which is a combination of FST and quantification of G6PD activity using OSMMR2000D G6PD assay method with haemoglobin normalisation.[5,7] However, the usage of this quantitative spectrophotometry assay was currently limited and not readily available at fields or small laboratories in rural areas.

In areas where malaria is prevalent, health care providers need to confirm the patient's G6PD status before the initiation of treatment with antimalarial drugs, particularly primaquine since the drug may cause haemolysis in G6PD-deficient individuals.[8] The unavailability of tests for G6PD deficiency becomes a great concern, especially in remote areas where the prevalence of malaria cases is high and uncomplicated malaria was treated as an outpatient.[9] Development of a rapid and reliable quantitative tool would be ideal and effective tool for such practice.

A newer method has been developed by researchers to detect the G6PD deficiency among malaria patients who require screening for primaquine therapy using the point-of-care (POC) test kit.[10] It would be useful in resource-limited centres including rural clinics and district hospitals where the FST is the only method currently being used to detect the G6PD deficiency. The tests will also be advantageous for patients in malaria-endemic areas prior to a primaquine prescription, neonatal screening, and any indicated patients during the weekends or public holidays in which the service would be unavailable. This recently developed method was based on the detection of reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH), a membrane-bound enzyme complex by biosensors of the device. It was reported that the POC test was capable to quantify the level of G6PD enzyme activity within 4 minutes using a whole blood specimen from a finger-prick blood sample.[11]

The objective of this study was to evaluate the performance of two quantitative POC tests, the Carestart™ G6PD Biosensor (Carestart; Access Bio, Somerset, New Jersey) and Carestart™ G6PD Biosensor 1 (S1; Wells Bio, Seoul, Republic of Korea) for detection of G6PD deficiency in Malaysia and to compare their results and performance with an established spectrophotometric G6PD assay kit, OSMMR2000D (R&D Diagnostics Ltd., Holargos, Greece) as a reference method.

METHODS

Study Design

This research was a cross-sectional study conducted in the Hematology Unit, Department of Laboratory Diagnostics Service, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur, Malaysia. A total of 153 neonatal cord blood, 99 peripheral blood of older children between 1 month to 12 years old, and 62 peripheral adult blood samples were used in this study. This study was approved by the Institutional Human Research Committees of UKMMC (Ethics Approval Numbers: UKM PPI/111/8/JEP-2016-141 and UKM PPI/111/8/JEP-2016-719) and was carried out according to Helsinki's Declaration.

Sample collection

Informed consent was obtained from each of the adult subjects and the parents of neonates and children before sample collection. The neonatal cord blood and peripheral blood samples of older children were obtained using a consecutive sampling method from samples received by the Hematology Unit for routine neonatal screening for G6PD and full blood count (FBC). Inclusion criteria for the selected neonatal blood samples were normal paediatric values of FBC range of the laboratory setting including the red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, and reticulocyte count. The adult peripheral venous blood samples were obtained from laboratory staff through a convenience sampling method. For each adult subject, additional finger-prick blood samples were also collected using sterile techniques.

Sample Analysis

The analysis of the G6PD enzyme activity of each blood samples was performed using the following methods:

1. OSMMR2000D kit with haemoglobin (Hb) normalisation (OSMMR; R&D Diagnostics Ltd., Holargos, Greece)

OSMMR is an enzymatic colourimetric assay method. The blood samples were collected in ethylenediamine tetraacetic acid (EDTA) tubes sized 2.0 ml and were analysed within 24 hours of samples collection. The measurement of enzyme activity and Hb level were done using a spectrophotometer (Ultramicroplate Reader EL808, Bio Tek, Instruments). Briefly, each blood sample was mixed with 75 μL of elution buffer in a well of microplate and was slowly warmed to 37°C. After 8 minutes of incubation, the microplate was read for the first time at 405 nm for Hb measurement. A colour reagent mixture provided in the OSMMR kit was added and the microplate was read for the second and third times at 550 nm at 0 min, then at 10 min. The results were directly expressed in U/g Hb. This method was used as the reference assay for analysis of all groups of blood sample.

2. Carestart™ G6PD biosensor (Carestart; Access Bio, Somerset, New Jersey)

   Carestart is a POC test kit that is consisted of a handheld analyser device and uniquely designed strips used for the G6PD enzyme activity measurement. This first-generation biosensor does not have a Hb measurement incorporated in the device and therefore necessitates a separate equipment to determine the Hb level. In this study, the Hb measurements and reticulocyte count were determined using an automated blood cell counter; Sysmex XN3000 Hematology Analyzer (Sysmex Asia Pacific Pte Ltd, Singapore). Then, the G6PD enzyme activity was calculated manually and expressed in U/gHb. In this current study, this POC test kit was only used for neonatal cord blood samples.

3. Carestart™ G6PD Biosensor 1 (S1; Wells Bio, Seoul, Republic of Korea)

   S1 is a newer generation of the Carestart test kit. The size of S1 analyser device is smaller compared to the Carestart with a 4-inch touch screen panel. This method is able to simultaneously measure G6PD, Hb and bilirubin concentration. S1 utilised an electrochemical method that relies on the quantification of NADPH for G6PD measurement and a photochemical method for Hb measurement. After the strips were inserted into the strip injector of the device, a total of five microlitres (μl) of whole blood sample was applied to the G6PD strip and seven μl of whole blood sample was applied to the Hb strip, as recommended by the manufacturer. Then, the G6PD enzyme level and Hb measurements were rapidly determined within 4 minutes, and the G6PD/Hb ratio was automatically calculated. The final results were expressed in Units/gram of Haemoglobin (U/gHb). This POC test was used to analyse all group of blood samples.

Determination of G6PD cut-off values

The assay kit's predefined reference was used to compare the blood samples with normal G6PD enzyme activity as determined by the OSMMR. The cut-off level of the reagent with lot normal (Lot no. B1657C) was used for the determination of a normal mean G6PD activity and the cut-off points for G6PD deficiency. For severe and moderate enzyme deficiency, the cut-off values were determined based on a classification recommended by WHO Working Group (1989). The lower limit of G6PD activity which defined as "severe" deficiency was less than 10% of normal mean G6PD activity while the upper limit which defined as "moderate" or "intermediate" deficiency was less than 60%. In this present study, all samples with a severe and moderate deficiency were collectively categorised as G6PD-deficient samples, as the classification of G6PD deficiency into severe and moderate deficiency was not clinically significant for patient's management.[12]

Genotyping of G6PD-deficient neonates

The G6PD-deficient neonatal cord blood samples were subjected to reverse dot blot flow-through hybridisation (RDB-FTH) assay using GenoArray Diagnostic Kit (Chaoxhou Hybribio Biochemistry Ltd., Sheung Wan, Hong Kong.). This method was used to determine the G6PD genotype of neonatal samples and confirm their G6PD status. RDB-FTH is a rapid molecular method that was designed to detect 14 common G6PD mutations in Malaysia, namely Viangchan (871 G>A), Kaiping (1388 G>T), Mediterranean (563 C>T), Mahidol (487 G>A), Canton (1376 G>T), Union (1360 C>T), Coimbra (592 C>T), Vanua Lava (383 T>C), Gaohe (95 (A>G), Chinese 5 (1024 C>T), Orissa (131 C>G), Chatham (1003 G>A), Andalus (1361 G>A) and Quing Yang (392 G>T). Samples were firstly amplified using a polymerase chain reaction (PCR) technique and were subjected to

TABLE 1: Means and ranges of G6PD enzyme activity of neonatal cord blood samples measured by Carestart, S1, and OSMMR (n=90)

	Carestart (U/gHb)	S1 (U/gHb)	OSMMR (U/gHb)
Mean of G6PD activity	9.4	9.3	13.42
Severe deficiency (<10% of mean)	< 0.9	<0.9	< 1.342
Moderate deficiency (10-60% of mean)	0.9 – 5.6	0.9 - 5.6	1.342 - 8.052
Normal G6PD (>60% of mean)	> 5.6	> 5.6	> 8.052

a flow-through hybridisation technique using a HybriMax® instrument.[13]

Statistical Analysis

Two statistical approaches were performed in this study to evaluate the performance of POC tests against the OSMMR reference assay: Spearman's rank correlation test and Cohen's kappa method. The Spearman's rank correlation test was conducted for all neonatal cord blood sample results using the IBM SPSS Statistic Version 26 software. The Cohen's kappa was used to calculate and assess the agreement between the two raters, followed by the calculation of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for both POC tests.

RESULTS

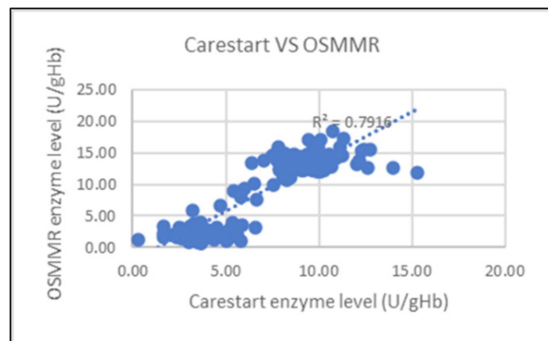
Newborn

The analysis of neonatal cord blood samples using the OSMMR assay showed that 91 neonates (59.0%) have a normal G6PD enzyme activity, whilst 62 neonates (41.0%) were G6PD deficient The G6PD deficient neonates consisted of 55 males and 7 females. According to ethnicity, Malay neonates showed the highest number with G6PD deficient which were 50 (80.6%) followed by 7 Chinese neonates (11.3%), 3 Myanmar neonates (4.8%), 1 Dusun neonate (1.6%), and 1 Iban neonate (1.6%). The means and ranges of G6PD enzyme activity for normal, moderate, and severe deficiency calculated using 90 out of 91 cord blood samples were presented in Table 1, as measured by both POC tests and OSMMR assay. One neonatal cord blood sample labelled as Case 20 with a normal G6PD activity was excluded in this calculation due to a discrepancy in the G6PD activity result shown when measured by the Carestart.

A scatterplot correlation study of all 153 neonatal cord blood samples to compare the performance of both POC tests to the OSMMR assay showed a strong positive correlation (Figure 1). A statistical analysis performed using Spearman's rank correlation coefficient test calculated by the IBM SPSS software (version 20) showed positive correlations, which were ϱ=0.890 and ϱ=0.864 by the Carestart and the S1, respectively.

For Cohen's kappa coefficient analysis, the measurement of agreement between the Carestart and OSMMR was κ=0.805 (p<0.001, 95% CI),

(A)



(B)

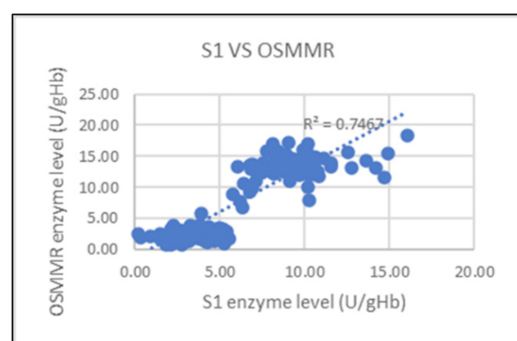


FIG. 1: (A) Scatterplot of enzyme activity measured by CareStart against OSMMR, and (B) enzyme activity measured by S1 against OSMMR (n=90)

TABLE 2: The performance of Carestart versus S1 tests

	Carestart (95% CI)	S1 (95% CI)
Sensitivity	90.2%	95.2%
Specificity	98.9%	100%
Positive Predictive Value (PPV)	98.3%	100%
Negative Predictive Value (NPV)	93.8%	96.8%

and between the S1 and OSMMR, $\kappa=0.795$ ($p<0.001$, 95% CI). Both POC tests showed high sensitivity and specificity of more than 90% with no significant difference. The Carestart and S1 had positive predictive values (PPV) of 98.3% and 100%, respectively, while the negative predictive value (NPV), or likelihood that the participants had a normal G6PD enzyme level when the test results were within the normal range, was 93.8% and 96.8%, respectively (Table 2).

In the current study, data were classified as normal (mean G6PD activity > 60%) or G6PD deficient (mean G6PD activity 60%). Among 62 neonates that were identified as G6PD deficient by the OSMMR, Carestart detected only 57 (91.9%) neonates as G6PD deficient, while the remaining 5 (8%) were identified as normal with G6PD activity ranged from 5.81 to 6.67 U/gHb (61 to 70% residual activity). For the 91 neonates that were identified as normal by the OSMMR, Carestart showed only one subject (Case 20) to be deficient with a residual G6PD activity of 5.4 U/gm Hb (57.4%). Results by the S1 showed that 58 (93.5%) neonates were identified as G6PD deficient, while the remaining 4 (6.4%) neonates showed normal G6PD activity. All 91 neonates with normal G6PD activity detected by OSMMR showed normal activity when tested with the S1. Table 3 summarised the discrepancy of these results with their type of mutations.

We performed a haematological analysis on 9 neonates who were classified as normal by the POC tests but deficient by OSSMR (5 neonates by the Carestart and 4 neonates by the S1). Our results revealed that 8 (89%) neonates had increased reticulocyte counts of more than 5% and only 1 neonate showed a normal result. Molecular analysis was performed on 57 out of 62 neonatal cord blood samples (classified as G6PD deficient by OSSMR) and showed multiple variants of G6PD mutations. The commonest mutations found were G6PD Viangchan (871 G>A) (38.6%), followed by Mediterranean (563 C>T) (17.5%), Vanua Lava (383 T>C)(14%) and Mahidol (487 G>A)(10.5%). Six other G6PD mutations were identified in the remaining neonates: Kaiping (1388 G>T), Union (1360 C>T), Canton (1376 G>T), Chatham (1003 G>A), Coimbra (592 C>T), and Orissa (131 C>G), which accounted for about 2-5% of the studied population for each variant (Table 4).

A molecular analysis conducted for neonates with moderate deficiency as identified by the OSMMR showed that the group had a higher proportion for G6PD Viangchan (871 G>A) and

TABLE 3: Identified cases with G6PD activity discrepancies measured by Carestart and S1 with OSMMR (n=8)

Case Number	Gender	Carestart (U/gHb) (%)	S1 (U/gmHb) (%)	OSMMR2000D (U/gHb) (%)	Type of Mutation
1	Male	6.58 (70%)	5.18 (55%)	3.23 (24%)	Mahidol
8	Female	5.86 (62%)	5.00 (54%)	3.54 (26%)	Het Viangchan
11	Male	5.81 (61%)	5.28 (57%)	0.96 (7%)	Orisa
13	Female	6.67 (70%)	6.18 (66%)	7.66 (57%)	Het Viangchan
14	Female	5.82 (62%)	10.27 (110%)	7.92 (59%)	Het Viangchan
20	Male	5.40 (58%)	5.75 (62%)	8.97(67%)	Normal
57	Female	4.73 (50%)	6.30 (68%)	6.72(50%)	Het Mediterranean
128	Male	5.60 (59%)	3.7 (40%)	2.042(15%)	Viangchan

TABLE 4: Types of G6PD mutations detected among the G6PD deficient newborn group (n=57)

	Type of mutation	Male	Female	Prevalence (%)
1	Viangchan	19	3	38.6
2	Kaiping	3	0	5.3
3	Mediterranean	7	3	17.5
4	Union	1	0	1.8
5	Mahidol	5	1	10.5
6	Canton	2	0	3.5
7	Vanua Lava	8	0	14.0
8	Chatham	1	0	1.8
9	Coimbra	3	0	5.3
10	Orissa	1	0	1.8
	Total	50	7	100

Vanua Lava (383 T>C), while Mediterranean (563 C>T) and Coimbra (592 C>T) constituted a higher proportion among the neonates with severe deficiency (Figure 2). Among the female neonates with moderate deficiency, 4 out of 7 were heterozygous for the G6PD-deficient allele and thus confirming their carrier status. A total of 3 carriers were heterozygous for Viangchan (871 G>A) and 1 was for the Mediterranean (563 C>T).

Older children

We only contrasted the results of the S1 with OSMMR assay for samples from this group. The result of the OSMMR showed that 82 (82.8%) out of 99 older children aged between 1 month to 12 years old had normal G6PD enzyme activity, while the remaining 17 (17.2%) children were G6PD deficient. A lower mean of 7.27 U/gHb and deficient cut-off values of 0.72 U/gHb and 4.32 U/gHb were discovered for this group samples (Table 5). A scatterplot correlation study performed for this group showed a strong positive correlation between both methods (Figure 3). There was no discrepancy in the results of G6PD enzyme activity between both methods.

Adults

All peripheral blood samples from the adult group showed normal G6PD enzyme activity when analysed using OSMMR and S1. The samples were not analysed by Carestart test for

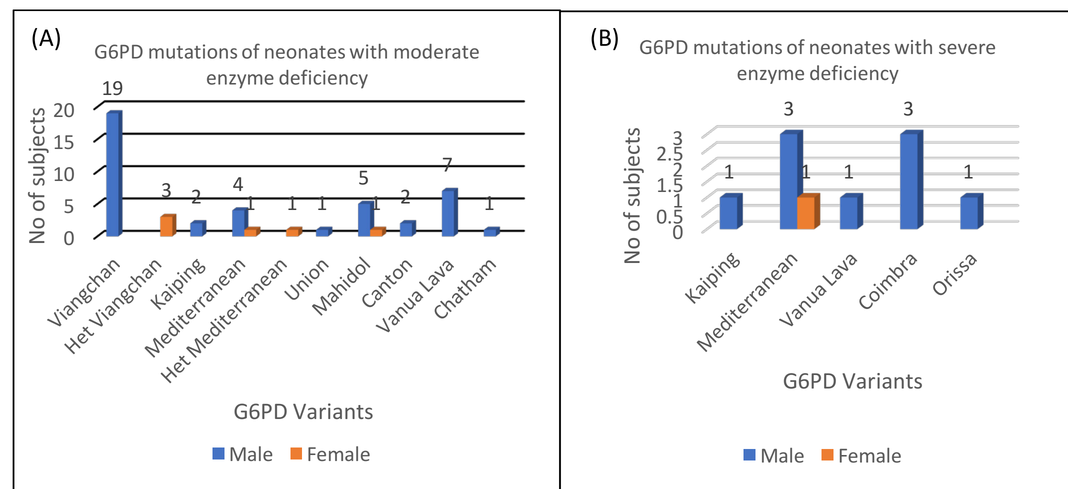


FIG. 2: (A) Variants of G6PD mutations identified in neonates with moderate deficiency (n=47), and (B) Variants of G6PD mutations identified in neonates with severe deficiency (n=10).

TABLE 5: Mean and range of G6PD enzyme activity for G6PD deficient older children (n=99)

	S1 (U/gHb)	OSMMR (U/gHb)
Mean of G6PD activity	7.27	11.04
Severe deficiency (<10% of mean)	< 0.72	< 1.10
Moderate deficiency (10%-60% of mean)	0.72 – 4.32	1.10 – 6.63
Normal G6PD (>60% of mean)	> 4.32	> 6.63

this group. Our findings revealed that the normal mean G6PD enzyme activity of this group of blood samples was 10.73 ± 1.56 U/gHb and 6.20 ± 1.0 U/gHb by OSMMR and S1, respectively. Apart from the peripheral blood (PB) samples, the adult group were also analysed using a finger prick (FP) method for the S1. The result of the FP method showed a mean G6PD enzyme activity of 6.40 ± 1.0 U/gHb, which was not significantly different from that of peripheral blood. We summarised and compared the means and cut-off values of G6PD activity as measured by OSMMR and S1 in Table 6.

A scatterplot correlation study between the FP and PB samples of adults analysed by S1 showed a strong correlation (Figure 4). There was no discrepancy in the G6PD enzyme activity results when measured by S1 and OSMMR in the adult group.

DISCUSSION

Importance of quantitative POC test for G6PD

Mandatory screening of neonatal cord blood using FST as recommended by the WHO has been implemented in Malaysia since 1986 as part of the national strategy for prevention of severe neonatal jaundice and kernicterus. However, the test was found to miss a substantial proportion of cases with mild to moderate G6PD enzyme activity (10 – 60% of normal mean activity) which included the female heterozygotes (where subjects with enzyme activity of more than 20% might be missed) and male neonates of class III variants.[6] Accurate diagnosis of G6PD deficiency in heterozygote females with moderate enzyme deficiency is crucial due to the risk of developing severe neonatal jaundice[7], and prior to the commencement of an antimalarial drug such as Primaquine in which have a high risk of developing acute haemolysis in G6PD-deficient individuals treated with the drugs.[14,15]

Currently, the standard reference method for the determination of G6PD activity was quantitative assay. Since 2007, our

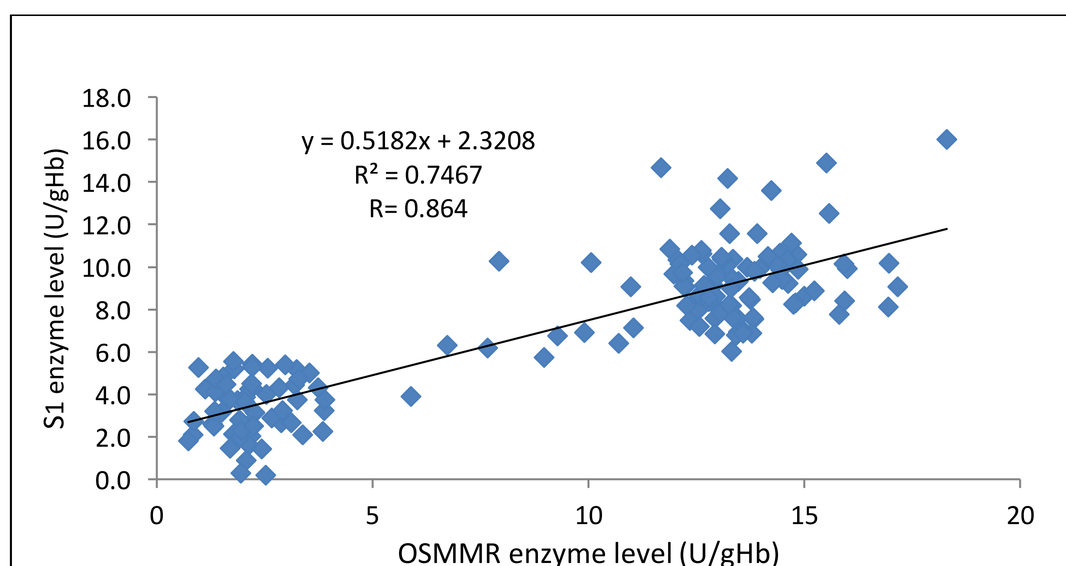


FIG. 3: Scatterplot of G6PD enzyme activity measured by S1 compared to OSMMR in older children group samples (n=99)

TABLE 6: Mean and range of G6PD enzyme activities for G6PD deficient adults

	S1 (FP)* (U/gHb)	S1 (PB)* (U/gHb)	OSMMR (U/gHb)
Mean of G6PD activity	6.38	6.23	10.79
Severe deficiency (<10% of mean)	<0.64	<0.62	< 1.10
Moderate deficiency (10%-60% of mean)	0.64 – 3.83	0.62 – 3.74	1.10 – 6.63
Normal G6PD (>60% of mean)	> 3.83	> 3.74	> 6.63

Note: *FP=finger prick sample, PB=peripheral blood samples

laboratory has practiced a combination of the OSMMR and FST.[8,9] This assay was based on spectrophotometry and has included a haemoglobin normalisation. Albeit being fully automated, there are several disadvantages including requirement of multiple expensive laboratory equipments, costly reagents and a competent and skilled operator. This method is also time-consuming and is not readily available to be used in the fields and small laboratories in rural areas, where resources are limited.

In tropical countries such as Malaysia, there is a necessity for rapid and accurate testing of G6PD deficiency in field applications, particularly for the indigenous (Orang Asli) ethnic communities who often reside in remote areas and deep forests with high malaria endemicity. These communities are also known to have high prevalence of G6PD deficiency. Patients that originated from these communities with uncomplicated malaria are often being treated as outpatients. Therefore, there is a high need for a POC test for screening for G6PD deficiency. At the moment, G6PD assays by spectrophotometry are only available in large laboratories of the referral centres which located in urban areas. Referred samples from these remote communities, especially from states in East Malaysia (Sabah and Sarawak), would take multiple days to arrive at the centre. The delay consequently would affect the integrity of blood samples and accuracy of G6PD activity results. Our previous study found that the stability of G6PD enzyme in an anticoagulant EDTA tube could drop significantly within 3 to 4 days post venepuncture, especially during transportation which required a cold chain maintenance of 2 to 8 °C.[16]

Diagnostic performance of Carestart and S1 tests

POC devices are sensitive to variations in

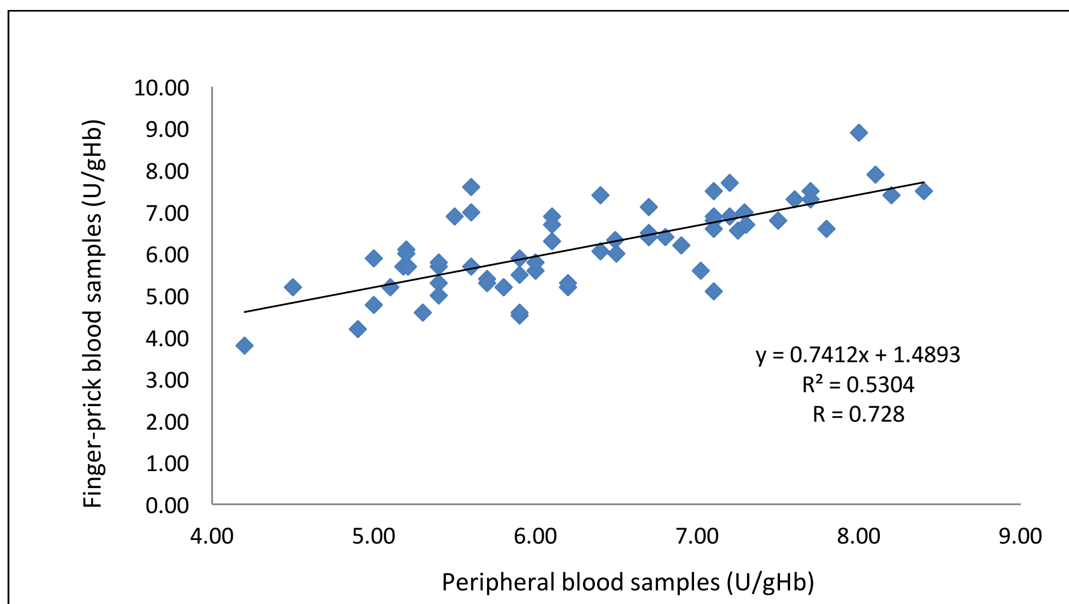


FIG. 4: Scatterplot of G6PD enzyme activity of finger-prick versus peripheral blood samples in adults analysed by the S1 test.

environmental temperature and humidity. Therefore, the novel POC tests need to be evaluated under different real-life conditions and provide evidence of their performance in multiple ethnogeographic variability.[13,17] In this present study, we evaluated two novel quantitative POC tests, Carestart and S1 against a reference spectrophotometric assay, OSSMR in Kuala Lumpur, Malaysia. The different feature between both methods lies in the measurement of Hb level in which it was in-built in the S1 but was on a separate cell counter for Carestart. The evaluation of POC tests were challenging since there were poor harmonisation criteria.[17] This includes defining a normal G6PD activity, which most researchers define it either as mean or median activity for a given population. Several researchers excluded outliers to avoid skewing of the population's normal value.[6] In our study, the mean G6PD enzyme activity for neonatal group of both POC tests was significantly lower than the mean activity and cut-off values for G6PD deficiency measured by OSMMR (<60% normal mean).

A comparison of both POC tests showed that there was no significant difference in the mean activity and cut-off points for severe and moderate deficiency (Table 1). All mean values of G6PD activity measured by the S1 for newborns, older children and adults groups were lower as compared to the mean values of G6PD activity measured by OSMMR, even though both tests had a moderate Pearson's correlation. A study by LaRue *et al*. (2014) proved similar results when comparing G6PD activities from two quantitative G6PD assays, Trinity Biotech and OSMMR.[18]

There was no comparison of mean G6PD activity according to gender and races in this current research as studies by Azma *et al*. 2010 and 2014 have shown no significant difference in the mean G6PD activity between gender and races.[5,8] The mean values of normal G6PD activity measured by the S1 in the newborns group had a higher value, followed by older children and then the adults, which were similar to the findings in previous studies.[5] However, the reason for increased G6PD activity in neonatal samples was not clear. It was postulated that the high reticulocyte counts as were found in 89% of the samples might have consequently caused false higher level of enzyme activity.[19]

The sensitivity and specificity of the POC tests were based on 60% cut-off point where both kits showed more than 90% sensitivity and specificity. The Carestart showed a rate of 1% false positive and 9.7% false negative, whereas S1 showed no false positive and a lower false negative of 4.8%. The higher specificity compared to sensitivity, with fewer false positive in the POC tests reflected their usefulness for the diagnosis of G6PD deficiency and screening patients before offering a Primaquine treatment. Aside from the remarkable performance, the handheld size of the tests made them more convenient to be stored, carried, and used anywhere by clinicians. Thus, their usage for field applications could circumvent the problem of sample quality deterioration during transport, particularly when the sampling procedures were conducted in remote areas.[18] Testing procedures using the S1 were uncomplicated and required only minimal user training without any other additional laboratory equipment.

We performed a correlation study between the FB and PB samples in adults which showed a strong correlation in G6PD activity and good performance of the POC tests. Only a small amount of blood sample (5 – 7 μl) was required for these methods. It would be highly advantageous for patients living in remote areas that requires a definitive determination of G6PD status before commencement of anti-malarial drugs. Automatic measurement of both enzymatic activity and Hb level can be done immediately using the S1. Following this, treatment of malaria could be given immediately to those without requiring a second visit.

The G6PD mutation analysis performed in our study on 57 neonatal cord blood samples that were diagnosed as deficient by OSMMR confirmed that the samples carried abnormal G6PD-deficient alleles. However, a total of seven neonates were misclassified as normal by both Carestart and S1. Among them, six neonates had moderate enzyme deficiency and one neonate had a severe enzyme deficiency, which was shown to be G6PD Orissa (131 C>G). Our finding suggested that G6PD Orissa is a type II variant as shown in a previous study by Sarker et al. (2016) [20]

To assure the safety of those heterozygotes' individuals with G6PD activity between 60 and 80% who may experience side effects from Primaquine and other anti-malarial medications, recent recommendations for the treatment of malaria proposed to redefine a "normal" G6PD activity to either >70% or >80% of normal G6PD enzyme activity[21]. Therefore, based on our findings, both POC tests would be able to satisfy this requirement and be regarded as a

good diagnostic assay if one uses this percentage (either > 70% or > 80%) as a cut-off point to start an antimalarial medication. Thus, this study affirmed the positive diagnostic performance of Carestart and S1 and quantitative POC assay in measuring G6PD enzyme activity.

CONCLUSION

In summary, the present study revealed that the Carestart and S1 performed well as a POC test for G6PD deficiency in comparison to the spectrophotometric reference assay, OSMMR. The tests were promising and showed high reliability to aid in the identification of G6PD deficiency in Malaysia. The usability of this test would help to improve the management of *Plasmodium vivax* malaria cases particularly in resource-limited settings, where reliable alternatives to current quantitative spectrophotometry assay are much needed.

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