

Assessment of CareStart™ G6PD RDT for G6PD deficiency screening in newborns and malaria diagnosed subjects in the Northern Thailand

Nattasit Pienthai¹ Chedtapak Ruengdit¹ Suphara Manowong² Kanyakan Kongthai³ Kritsanee Maneewong⁴
Kankanitta Pongmorn⁵ Maneewan Inta⁶ Nardlada Hhantikul⁷ Aungkana Saejeng⁷ Sakorn Pornprasert¹

¹Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai Province, Thailand

²Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai Province, Thailand

³Laboratory Department, Chiang Mai Health Promoting Hospital, Chiang Mai Province, Thailand

⁴Laboratory Department, Jomthong Hospital, Chiang Mai Province, Thailand

⁵Laboratory Department, Hangdong Hospital, Chiang Mai Province, Thailand

⁶Laboratory Department, Sanpathong Hospital, Chiang Mai Province, Thailand

⁷Laboratory Department, Office of Disease Prevention and Control 1, Chiang Mai, Province, Thailand

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ABSTRACT

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an inherited enzymatic disorder associated with severe neonatal hyperbilirubinemia and acute hemolysis after exposure to certain drugs or infections. The most common test for screening G6PD deficiency is fluorescent spot test which is rapid and convenient. However, fluorescent spot test needs tools and skills in diagnosis and interpretation of the results. CareStart™ G6PD Rapid Diagnosis Test (RDT) is an enzyme chromatographic strip test based on reduction of colorless nitro blue tetrazolium into formazan which gives purple color and the results could be read within 10 minutes.

Objectives: This study aimed to analyze the sensitivity, specificity and accuracy of CareStart™ G6PD RDT for screening of G6PD deficiency in newborn and subjects with malaria diagnosis.

Materials and methods: This study was conducted from December 2017 to February 2018. G6PD diagnostic tests including fluorescent spot test and Carestart™ G6PD RDT were performed in 196 newborns aged varied from 1 day to 12 years and 48 subjects with malaria diagnosis. The efficiency of CareStart™ G6PD RDT was analyzed by comparing with the reference method, fluorescent spot test.

Results: CareStart™ G6PD RDT demonstrated 100% sensitivity, 100% specificity and 100% accuracy for screening of G6PD deficiency in malarial diagnosed samples. However, it presented invalid results up to 27.04% in newborns. Therefore, sensitivity, specificity and accuracy were not applicable in this group.

Conclusion: CareStart™ G6PD RDT could be used for preliminary screening of G6PD deficiency in malarial diagnosed samples. However, it should be improved to reduce the invalid results in newborns.

* Corresponding author.

Author's Address: Department of Medical Technology,
Faculty of Associated Medical Sciences, Chiang Mai
University, Chiang Mai Province, Thailand

** E-mail address: sakornmi001@gmail.com

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme deficiency worldwide and the best described red blood cell (RBC) enzymopathy. It is an X-linked recessive disorder affecting about 400 million people globally with a high prevalence in Sub-Saharan Africa, Asia, Middle East, Latin America, and the Mediterranean.^{1,2} In Thailand, G6PD deficiency can be found in 16.9% of the total population with 11.1% in men and 5.8% in women.³ In addition, the G6PD deficiency occurs most frequently in areas where malaria has been endemic, presumably the result of natural selection due to some degree of protection from malaria infection.^{1,4}

Most of G6PD deficient individuals are asymptomatic throughout their lives until or following triggering by agents such as oxidative drugs, some infections or ingestion of fava beans.^{5,6} G6PD deficiency was discovered half a century ago. Severe hemolytic anemia may occur in some G6PD deficient individuals who are treated with anti-malarial drug in group of 8-aminoquinolines such as primaquine, which is the only available for radical cure of *Plasmodium vivax*.⁷ The risk of primaquine-induced hemolysis in G6PD deficient individuals is an essential public health concern in malaria endemic countries, especially in tropical areas.⁸ Even with treatment, severe malaria has a mortality rate of approximately 10-40% depending upon the time between initial symptoms and effective treatment and hospital facilities for the management of its complications.⁹ In addition, there was a previous report of recurrent parasitemia of *P. vivax* within 6 months in 18.3% patients treated with primaquine (15 mg/day for 14 days).¹⁰ At this dose, malaria-infected patients with G6PD deficiency could not stand the effect of primaquine, which lead to hemolysis on G6PD deficient RBCs and eventually forced the patients to stop taking medicine. Unfortunately, these series of events result in drug resistance of *P. vivax*. Subsequently, World Health Organization (WHO) had suggested 30 mg/day of primaquine treatment for 14 days to gain effective cure against *P. vivax*.¹¹ Thus, the diagnostic test for G6PD deficiency is necessary for a malaria-infected patient to prevent the clinical manifestation and mortality. Moreover, in 2015, WHO recommended screening for G6PD deficiency before primaquine treatment.¹²

The most common screening test for G6PD deficiency is the fluorescent spot test (FST) which had 100% sensitivity and 98% specificity of detection.¹³ It was also recommended by International Committee for Standardization in Hematology (ICSH) as the most acceptable and reliable screening test for G6PD deficiency.¹⁴ This method is easy to perform and gives a rapid result. However, a source of electricity for an ultraviolet (UV) light is required. To increase the efficiency of the G6PD screening test, the Access Bio has developed a Carestart™ G6PD Qualitative Rapid Diagnostic Test (RDT) which is based on the reduction of colorless nitro blue tetrazolium to dark purple color formazan.¹⁵ It has been used for screening G6PD deficiency in malaria endemic areas, including Cambodia,¹⁵ Haiti,¹⁶ Philippines¹⁰ or Southwestern Uganda.¹⁷ Thus, the aim of this study is to analyze the sensitivity, specificity and accuracy of CareStart™ G6PD RDT for preliminary screening of G6PD deficiency in newborn

and malaria diagnosed subjects.

Materials and methods

Study sites and populations

The study was conducted from December 2017 to February 2018 in Chiang Mai and Mae Hong Son Provinces, Thailand. Briefly, the 196 EDTA blood samples of newborn with ages varied from 1 day to 12 years were obtained from five hospitals (Maharaj Nakorn Chiang Mai Hospital, Chiang Mai Health Promoting Hospital, Jomthong Hospital, Hangdong Hospital and Sanpathong Hospital) in Chiang Mai, Thailand. In addition, the 48 EDTA blood samples of subjects with malarial diagnosis were obtained from Pai Hospital and Malaria clinic of Mae Sariang, Mae Hong Son, Thailand. The study protocol was reviewed and approved by Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (AMSEC-60EX-014).

Fluorescent spot test

The test was performed according to the ICSH with a minor modification.¹⁸ Briefly, 5 µL blood samples were transferred into 100 µL of G6PD screening reagent that was prepared immediately before using. The reactions were then incubated at 25°C and spotted onto a Whatman filter paper after incubating for 5, 10 and 15 min. The spots were air-dried and examined under UV light with a wavelength of 340-365 nm. The peripheral bloods from G6PD non-deficient and deficient control samples were included in each assay. Samples were interpreted as G6PD deficiency when opaque blood spots were observed under UV light whereas samples with strong fluorescence on all three spots of 5, 10 and 15 min for incubation periods were interpreted as G6PD normal. In addition, samples with a weak fluorescence on three spots were interpreted as G6PD intermediate.

CareStart™ G6PD RDT

CareStart™ G6PD RDT was provided by the manufacturer (AccessBio, New Jersey, USA). It is a qualitative enzyme chromatographic test, based on the reduction of colorless nitro blue tetrazolium dye to purple colored formazan. Following the manufacturer's instruction, 2 µL of EDTA blood samples were transferred into the sample well and then two drops of provided buffer were immediately added into the buffer well. The results were read visually after 10 min. The purple color appeared on the reading window in samples with G6PD normal while no color change was observed in samples with G6PD deficiency. If red color appeared on the reading window or the blood sample flowed poorly in the lateral flow, it could not be interpreted and was an invalid result. The samples with G6PD deficiency and non-deficiency samples were used as a control when analyzing patient samples.

Statistical analysis

Data were presented as median and interquartile range (IQR). Statistical analyses were performed using SPSS software package (Statistical Package for the Social Sciences 11.0, Chicago, IL, USA). In addition, sensitivity [true positive/(true positive+false negative)], specificity [true negative/(true negative+false positive)], positive predictive value

[true positive/(true positives+false positive)], negative predictive value [true negative/(true negative+false negative)] and accuracy [(true positive+true negative)/n] of CareStart™ G6PD RDT were analyzed by using fluorescence spot tests as the reference method.

Results

Performances of CareStart™ G6PD RDT in newborns

A total of 196 newborns were recruited in this group, 102 males and 94 females. Median age was 4 days (IQR

4-6 days). The test results and efficiency performances of CareStart™ G6PD RDT compared to fluorescent spot test are shown in Table 1. Fluorescent spot test showed 168 of G6PD non-deficiency, 24 deficiency and 4 intermediate samples while Carestart™ G6PD RDT showed 107 of G6PD non-deficiency, 36 deficiency and also presented the invalid results up to 27.04% (53 samples). Therefore, the sensitivity, specificity and accuracy of the CareStart™ G6PD RDT were not applicable. Prevalence of G6PD deficiency analyzed by using fluorescent spot test was 12.24% (24 of 196 subjects; 19 males and 5 females) while 4 samples were diagnosed as G6PD intermediate and all of them were female.

Table 1. Performance of the Carestart™ G6PD RDT compared to fluorescent spot test in newborns.

Carestart™ RDT	Fluorescent spot test				Performances	
	Normal	Deficiency	Intermediate	Total		
Normal	107	0	0	107	Sensitivity	*NA
Deficiency	17	16	3	36	Specificity	*NA
Invalid	44	8	1	53	Accuracy	*NA
Total	168	24	4	196	Positive predictive value	*NA
Prevalence of G6PD deficiency (%)				12.24	Negative predictive value	*NA

*NA: Not applicable

Performances of CareStart™ G6PD RDT in subjects with malaria diagnosis

A total of 48 subjects including 28 males and 20 females were used for analyzing. The median age was 30 years (IQR 13.5-41.0 years). The prevalence of G6PD deficiency in this group was 4.17% (2 of 48 subjects). The results of Carestart™ G6PD RDT was similar to those of fluorescent spot test,

including 46 subjects with G6PD non-deficiency (27 males and 19 females) and 2 subjects with G6PD deficiency (1 male and 1 female). Moreover, the invalid result of Carestart™ G6PD RDT was not found in this group. Thus, sensitivity, specificity and accuracy of the CareStart™ G6PD RDT were 100% (Table 2).

Table 2. Performance of the Carestart™ G6PD RDT compared to fluorescent spot test in Subjects with malaria diagnosis.

Carestart™ RDT	Fluorescent spot test				Performances	
	Normal	Deficiency	Intermediate	Total		
Normal	46	0	0	46	Sensitivity	100%
Deficiency	0	2	0	2	Specificity	100%
Invalid	0	0	0	0	Accuracy	100%
Total	46	2	0	48	Positive predictive value	100%
Prevalence of G6PD deficiency (%)				4.17	Negative predictive value	100%

Discussion

Many malaria-endemic countries around tropical or sub-tropical areas are participating in malaria eradication by 8-aminoquinolines-based drug treatment for transmission blocking or radical cure from the hypnozoite forms that reside in the liver which is the cause of malaria relapse. Therefore, prior to treatment with 8-aminoquinolines-based drugs, the status of the G6PD deficiency should be considered to minimize adverse effects such as drug-induced hemolysis from primaquine in G6PD deficient patients or kernicterus in newborns. Currently, the reliable G6PD assays require laboratory equipment and skilled technicians. Therefore, to assay the G6PD diagnosis of individuals quickly in remote

area without requiring a high level of clinical laboratory equipment, point-of-care assay, Carestart™ G6PD RDT, plays an important role to determine which individual can be treated with 8-aminoquinolines-based drug. Furthermore, the cost per test of Carestart™ G6PD RDT and Fluorescent spot test was comparable (\$1.5 vs \$1.1 USD, respectively).¹⁶ In this study, Carestart™ G6PD RDT had 100% of sensitivity, specificity and accuracy for screening of G6PD deficiency in malaria diagnosed subjects while those in newborn was not applicable because the invalid results were observed in 27.04% of samples. Thus, it is possible that Carestart™ G6PD RDT is an appropriate and effective tool to determine the G6PD status in endemic rural area of malaria but not in

newborns. The current study is consistency with the previous studies showing that Carestart™ G6PD RDT had a high sensitivity in determining G6PD deficiency and that it also could be used for point-of-care testing in malaria endemic areas.^{10,19,20} Nevertheless, the newborns showed invalid results and that might be due to several causes such as hematocrit levels or in cases of heterozygous G6PD deficiency in females. Generally, the hematocrit of newborns is higher than those of adults. Thus, their blood samples could not easily flow through the filter paper and that might be a cause for difficulty in differentiating between the red color of hemoglobin and the purple color of reduced formazan. In addition, a relatively poor diagnostic performance among females due to mosaic G6PD phenotype had been also reported previously by Satyagraha et al.²¹

The present study has some limitations. First the hematological parameters including hematocrit especially in newborns were not measured thus a correlation between hematocrit and invalid results cannot be verified. Second, the assessment of G6PD enzyme activity which is the gold standard for identified G6PD deficiency is not performed. Therefore, G6PD enzyme concentration which is sufficient to generate purple color of reduced formazan cannot be analyzed especially in mild forms of G6PD deficiency such as in heterozygous females and also in invalid samples.

In conclusion, CareStart™ G6PD RDT is relatively inexpensive, easy to use and requires significantly less-time. Thus, it can be used in malaria endemic areas before administering 8-aminoquinoline-based drug to substantially reduce the risk of adverse treatment outcomes. However, it should be improved to reduce the invalid results in newborns.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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