DETECTING MALARIA USING SD BIOLINE MALARIA Pf/PAN (HRP2, pLDH)

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ABSTRACT: Malaria caused by the *Plasmodium* species has resulted in significant health problems among most tropical countries. The *Plasmodium falciparum* is especially a major cause of global morbidity and, in rare cases, mortality, particularly in the infection of children. Rapid detection and prompt treatment is a promising model accepted among malarious countries by using Malaria Rapid Diagnostic Tests (MRDT). We launched Standard Diagnostics (SD) Point of Care Test (POCT) for Malaria detecting either *P. falciparum* or *non-P. falciparum* in the malaria control of Thailand. Two hundred and ninety-nine suspected cases of malaria, who attended three malaria clinics in Kanchanaburi, Ranong and Tak provinces, were recruited. Of those cases, 37.8% (113) were children under 15 years old. The results showed that the MRDT revealed sensitivity to *P. falciparum*, *non-P. falciparum* and specificity at levels of 95.29, 97.57 and 98.21 respectively. Moreover, it represented in similar trend when the children were separated, this test revealed 95.88, 95.00 and 100 sensitivity to *P. falciparum* and *non-P. falciparum* specificity respectively. This MRDT showed high diagnostic values and could be used in any malaria endemic areas. In addition, it provides great benefit in detecting malaria in children.

Keywords: Rapid diagnostic test, Diagnostic values, Malaria parasite

INTRODUCTION

Malaria is recognized as a serious health problem in Thailand, especially among the border areas adjacent to Myanmar, Cambodia and Malaysia [1]. Microscopy is the time-honored method of laboratory test for this parasite. However, it is not always immediately accessible in those areas therefore causing delay in detection and treatment [2]. The productions of dipsticks are based on immunochromatographic assays, which can be divided into two groups according to the type of *P. falciparum's* antigens, histridine-rich protein 2 (PfHRP2) and *Plasmodium* glycolytic enzyme; lactate dehydrogenase (pLDH) [3]. However, the multi-species detected Malaria Rapid Diagnostic Test (MRDT) should be used in the areas where *P. falciparum* and *P. vivax* co-circulate [4]. Regarding the field studies of Pan species MRDTs [5-10], the Pan species MRDT revealed the sensitivity and specificity between 75-98% and 87-100% respectively. However, most of the MRDTs sensitivity was decreased when the level of parasitemia is low. Previous studies [5-8] revealed the sensitivity of Pan-species MRDT to be 69.68±21.01%, where malaria parasitemia less than 100 parasites/µl. Therefore, MRDTs that can provide higher sensitivity against low parasitemia level are needed. Presently, various MRDTs have been tested for their performance at the World Health Organization (WHO) and the Foundation for Innovative New Diagnostics (FIND) [11]. Standard diagnostics (SD) MRDT test, SD Bioline Malaria antigen Pf/pan (Standard Diagnostics lnc.), which is produced to detect P.f.HRP2 and PAN pLDH, was tested for its performance and revealed the amount of malaria panel detection at 200 parasites/µl for over 90 % of the results. Currently, this MRDT has been used in Thailand's malaria control program since 2010. Therefore, it is necessary to assess its diagnostic values along with its capabilities and provide this information to the users.

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MATERIALS AND METHODS

Study areas and population

The assessment was conducted between April - June 2011 in the three field malaria clinics in a province of Thailand, included with Tak, Kachanaburi and Ranong. Two hundred and ninety nine suspected cases of malaria, who attended those malaria clinics, were recruited for the study.

Table 1 Cross tabulation of SD MRDT against Giemsa-thick blood film (GS-TBF)

Expected test kit result[a]		GS-TBF			
		Pf.	**Non-Pf.**	**Negative**	**Total**
	Pf.	81	0	0	81
	Non Pf.	3	151	1	155
	Negative	1	7	55	63
	Total	85	158	56	299

Effectiveness of SD POCT, sensitivity for Pf[b] 95.29%, sensitivity for non-Pf[c] 95.57%, specificity 98.21%, accuracy 96.00%, PPV for Pf[d] 100%, PPV for non-Pf[d] 97.42% and NPV[e] 87.30%.

[a]'Expected test kit result' means P. falciparum if microscopy detected P. falciparum alone or mix infection with other Plasmodium parasites; and non-P. falciparum if microscopy detected Plasmodium parasites but no P. falciparum. Only asexual parasites are included

[b]'Pf.', Plasmodium falciparum

[c]'Non-Pf.', other Plasmodium parasites including P.vivax, P. malariae and P.ovale.

[d]'PPV', positive predictive value [e]'NPV', negative predictive value

The malaria diagnostic techniques

Three malaria diagnostics methods were utilized in this study, microscopic blood film examination, Polymerase Chain Reaction (PCR) and MRDT. They were used as the gold standard method to confirm malaria parasites species and for validation. Forty microlitres of blood samples were collected from the patients by finger-pricking. Twenty microlitres of blood was used to make blood film and examined under microscope [12]. Then, 20 µl of the blood samples was kept in filter paper and sent for confirmation by PCR according to Muhamad's technique 2011 [13] to Bureau of Vector Borne Diseases. Another 10 µl was examined by SD Malaria Ag Pf/Pan, Standard Diagnostics, Suwon City, South Korea, batch number 080078, expired date: 10/11/2011. This MRDT was tested for its threshold detection limit [14] and it was observed that the test could detect the parasitemia level as low as 150 parasitemia/µl for *P. falciparum* and 500 parasitemia/µl for *P. Vivax*. The SD MRDT was used according to the manufacturer's instructions. This test consists of a membrane strip embedded in a flat plastic housing. The strip is pre-coated with two monoclonal antibodies, one specific to PfHRP2 of *P. falciparum* and the other to pan-specific pLDH of *Plasmodium* species.

Ethical issue of this study

This study was a part of the Thailand Global Fund Project on malaria round 7 [15]. The SD MDRT was used to detect malaria cases in this project and it was evaluated as described in this article. All activities correlated with the patients were done strictly under good clinical practice (GCP). Before collecting the blood samples, the patients were informed about the procedures and details of this study. All participated patients agreed and were willing to be part of this study and signed the consent forms.

Quality control of this study

Internal quality control [16] was undertaken throughout the project. Inclusion and exclusion criteria regarding either patient or MRDT [14] were done strictly to prevent false negative or false positive results.

Data analysis

Data was collected for analysis by using Microsoft Excel 2007 software. For sensitivity and specificity calculations, the test kits were compared with Giemsa-thick blood films (GS-TBF). However, the numbers of positives by GS-TBF were counted only in the asexual stage as of their clinical importance.

RESULTS

Two hundred and ninety-nine suspected malaria subjects were recruited from the three study sites as described previously, in which 37.8% (113 cases) of the cases were under 14 years old and their gender distribution was 56.3% male and 43.7 % female respectively. Leading occupations were farmer, agricultural employee, forestry worker and logger. Of the 299 subjects, the numbers of individuals that provided positive results by microscopy for *P. falciparum* with or without *P. vivax*, and *P. vivax* only were 85 and 158 respectively. Meanwhile, the numbers of individuals found positive by SD MRDT were 81 and 151 respectively (Table 1). The accuracy of SD POCT was calculated by comparing with standard microscopy results. The analysis revealed that the test possesses sensitivity to *P. falciparum*, *non-P. falciparum* and specificity, at 95.29%, 95.57% and 98.21% respectively (Table 1).

On the other hand, children were separated from the

Table 2 Cross tabulation of SD POCT against Giemsa-thick blood film (GS-TBF) in children's group (113)

Expected test kit result[a]		GS-TBF			
		Pf.	**Non-Pf.**	**Negative**	**Total**
	Pf.	31	0	0	31
	Non Pf.	1	57	0	58
	Negative	0	3	21	24
	Total	32	60	21	113

Effectiveness of SD POCT, sensitivity for Pf[b]. 95.88%, sensitivity for non-Pf[c]. 95.00%, specificity 100%, accuracy 96.46%, PPV for Pf[d]. 100%, PPV for non-Pf[d]. 98.28% and NPV[e] 87.50%.

Table 3 The sensitivity of SD Malaria POCT at different levels of parasitemia

Parasitemia/µl	*P. falciparum.*					*Non-P. falciparum.*				
	No. of patient	**TP**	**FN**	**FP**	**Sensitivity (%)**	**No. of patient**	**TP**	**FN**	**FP**	**Sensitivity (%)**
< 500	4	4	0	0	100	12	8	2	2	66.67
501 - 1,000	4	4	0	0	100	7	4	2	1	57.14
1,001 - 5,000	12	11	1	0	91.67	71	70	1	-	98.59
5,001 - 50,000	39	38	1	0	97.44	59	58	0	1	98.30
> 50,000	26	25	0	1	-	9	8	1	-	88.89
Total	85	82	2	1	-	158	148	6	4	

TP= True positive, FN = False negative, FP = False positive
Sensitivity = TP/TP+ FN

main study group, and their results revealed sensitivity to *P. falciparum*, *non-P. falciparum* and specificity at 95.02%, 98.37% and 98.56% respectively (Table 2).

In addition, all 299 blood samples were confirmed by Polymerase Chain Reaction (PCR) and all blood samples, examined by Giemsa thick blood film (GS-TBF), showed the identical results to those examined by PCR.

Lastly, this assessment revealed the sensitivity of SD MRDT at different levels of parasitemia of either *P. falciparum* or *Non- P. falciparum*. Table 3 represented the fluctuation of the sensitivity of the device to *P. falciparum* with reverse fashion to the level of parasitemia, but those of non-*P. falciparum* revealed same trend except for those with the level of parasitemia exceeding 50,000 parasites/µl.

DISCUSSION

The assessment of another rapid device, SD Malaria POCT showed higher diagnostic values or in accordance with previous studies regarding Pan Malaria Rapid Diagnostic Tests [5-10]. Although it appeared that SD MRDT performed reproducibility to be more reliable, the device misdiagnosed three *P. falciparum* cases as non-*P. falciparum*, which we found to be very interesting as the technician could not see the third band appearing on the device. The misdiagnosis might have been affected by either the rapid test's attributable factors or by human error, even though internal quality control was carried out strictly (Table 1). Similarly, the device misdiagnosed seven of the non- *P. falciparum* as false negative (Table 1), which likely occurred for the same reasons mentioned above. Nevertheless, the numbers of false negatives were too few to provide an interpretation on the quality of device, as the rest of results showed concordance with the gold standard method by Giemsa-thick blood film analysis. Results from children revealed no significant differences in terms of the diagnostic patterns (Table 2). In this study, even though the tests detected *P. falciparum* histridine-rich protein 2, the numbers of *P. falciparum* cases were not higher than those detected by microscopy (81 and 85 cases respectively). It might be due to the fact that the *P. falciparum* cases were mostly early infected cases; therefore, some of these patients have no *P. falciparum* histridine-rich protein 2 in their blood circulation. Additionally, we used PCR method to confirm results obtained by microscopy and found identical results. Furthermore, the increasing sensitivity of the device along with increasing parasite densities in non-*P.falciparum* suggests that the problem is due to the ability of the device to detect parasites at low parasitemia level, rather than human error (Table 3). On the other hand, the device was able to detect *P. falciparum* at low level of parasitemia with only a few cases where the parasite could not be detected. In conclusion, this device is a very useful adjuvant diagnostic tool with the ability to detect malaria in

children and suitable to be used in all malaria endemic areas.

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