นิพนธ์ต้นฉบับ Original Article

Determining the accuracy of malaria RDTs in Thailand

Sujariyakul A*
Bualombai P**
Ruangsirarak P**
*Office of Disease Prevention and Control region 5, Ratchaburi
**Bureau of Vector Borne Disease, Department of Disease Control, Ministry of Public Health,

Abstract

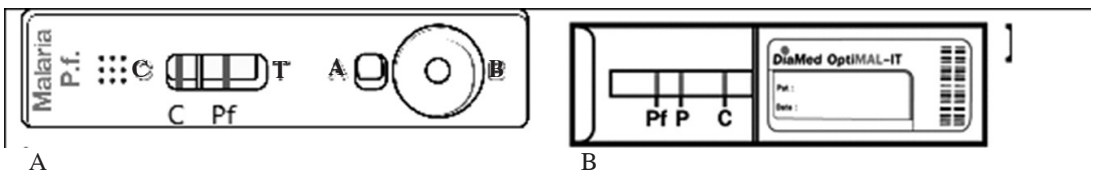
This study aimed to determine the accuracy of malaria rapid diagnostic tests (RDT) used in Thailand Malaria Control Program. Three brands of RDTs: Paracheck P.F.™ (detecting single species), OptiMAL IT™ and SD MRDT (detecting pan or multi-species), were field assessed and compared to Giemsa-Stained Thick Blood Film (GS-TBF). The assessment was conducted during April to July 2013 in the malaria clinics of three provinces, consisting a northern province; Tak, a western province; Kanchanaburi, and an eastern province; Chanthaburi. Totally 899 suspected malaria cases visited to these malaria clinics. Of these cases; 84, 157, and 658 found *Plasmodium falciparum*-positive, *Plasmodium vivax*-positive and malaria-negative, respectively. All cases were diagnosed by four tests; GS-TBF and the three RDT tests, consisting of Paracheck P.F.™, OptiMAL IT™ and SD MRDT. These RDTs revealed the sensitivity for *P. falciparum*, 98.81%, 91.67%, and 94.05% respectively. Whereas the sensitivity for *P. vivax* of OptiMAL IT™ and SD MRDT were 94.27% and 95.54% respectively. The specificity of the three RDTs were higher than 99.00% which was proved that these RDTs were well discriminated for positive cases. This study revealed the superiority of Paracheck P.F.™ in detecting *P. falciparum* to two other RDTs. Regarding *P. vivax*, both OptiMAL IT™ and SD MRDT revealed their accuracy in bottom line of acceptance. In conclusion, this study gave evident to malaria policy maker to determine which kind of tests could be used in remote malaria endemic areas.

Key words
Rapid Diagnostic Test, RDT, accuracy, malaria

Introduction

Malaria is a public health problem in Thailand, especially among the border areas adjacent to Myanmar and Cambodia[1]. Microscopy is the time-honored method to detect malaria parasites, but it is not always immediately accessible in remote areas. A delay in detection and treatment was evident[2]. Therefore, alternative methods are needed to complement or even to replace microscopy. Rapid diagnostic test (RDT) is considered, because it is simple, quick, minimal training and does not require electricity[3]. It also provides prompt diagnosis which is the key factor of any country malaria control

program for promptly detect and treat malaria patient. RDTs are normally produced in dipstick or cassette format. Basically it capture parasitic antigen in a few drop of patient blood and tap it on the bands of specific antibodies which are fixed on the strip. Then, visible color line is occurred as well as the control line is pop-up. The productions of RDT is based on immunochromatographic assays, which can be divided into two groups according to *Plasmodium*'s antigens; histidine-rich protein 2 (HRP2) and *Plasmodium* glycolytic enzyme; lactate dehydrogenase (pLDH)[3]. Most RDTs are produced in the multi-species or Pan species detection because *P. falciparum* (*Pf*) and *P. vivax* (*Pv*) are frequently co-circulated in malaria endemic countries[4]. However, some RDTs are produced to detect single species like Paracheck P.F.™ for *Pf* only. Figure 1 A shows the single species RDT, detecting only Pf. species, the test is valid when the C band (control line) is visible and the appearance of the second band which is embedded monoclonal antibodies produced against Pf. Figure 1 B shows the multi-species RDT, detecting Pf and Pan malaria species, the test is valid when the C band is visible and the appearance of the second band which is embedded monoclonal antibodies produced against Pan malaria species (P band) and the third band which is embedded monoclonal antibodies produced against Pf (Pf band).



A B

C = Control, P = Pan species, Pf = *Plasmodium falciparum*

Figure 1 RDT in a cassette format

Regarding the field studies of Pan species MRDTs[5-10], the Pan species revealed the sensitivity and specificity between 75.00-98.00% and 87.00-100% respectively. However, most of the RDTs' sensitivity decreases when the level of parasitemia is low. According to previous studies[5-8], the sensitivity of Pan species RDT were 69.68+21.01%, where malaria parasitemia less than 100 parasites/μl. Various malaria RDTs heave been tested for their performance at the World Health Organizatio (WHO) and the Foundation for Innovative New Diagnostics (FIND)[11]From 2002 till now, more than 100,000 RDTs have been used in Thailand under national malaria control program (NMCP). By 2004, World Health Organization (WHO) launched the quality assurance guideline for quality control of RDT[17] and it has been applied in Thailand NMCP since 2007.

Materials and methods

Study areas and population

The assessment was conducted during April to July 2013. Three provinces were purposively selected, consisting of two Thailand-Myanmar border provinces, Tak and Kanchanaburi and one endemic Thailand-Cambodia border province, Chanthaburi. The first two are about 426 and 121 kilometers to the west of Bangkok, respectively. The third one is 254 kilometers to the east of Bangkok. Their total

populations in 2013 are 526,045, 838,269 and 521,812, respectively. All are known to be highly endemic malarious areas with Annual Parasite Incidence (API) of 20.58, 1.43 and 1.48 respectively. The malaria transmission period in these provinces is normally throughout the year. People working and living in these areas are therefore at high risk malaria infection. Agriculture remains the majority of the people's livelihood. The pf to pv ratio among these three provinces in 2013 are 46.44/53.56, 9.70/90.30; and 10.46/89.54 respectively[1]. One malaria clinic (MC) with highest malaria cases in the previous year is chosen from each province. These three MCs are managed by well-trained microscopists.

Study design

Three brands of RDTs were validated; namely, Paracheck P.F.™ (detecting only *Pf* HRP2) batch number 31677; expired date 07/13 (Orchid Biomedical Systems, Verna Industrial Estate, Verna, Goa 403 722, India), OptiMAL IT™ (Pan species, detecting *Plasmodium* LDH) batch number 46110.13.01; expired date 10/13 (DiaMed AG, 1785 Cressiers/Morat, Switzerland), and SD BIO-LINE Malaria Antigen Pf/Pan, (detecting *Pf* HRP2 and *Plasmodium* LDH) batch number 71103, expired date: 22/05/2014 (Standard Diagnostics Inc.). The RDTs were quality control by either short or long term quality assurance under WHO guideline[13]. For short term, quality control at perusing phase at Headquarter, RDTs were randomly picked up to check with standard panel of malaria parasitic specimens in either real or heat accelerated temperatures. Then, RDTs were randomly picked up as well for long term quality control every three months.

The experiment was performed to the patients visiting at the three MCs. Sixty microliters (60 µl) of blood samples was collected from the patients by finger-pricking. Twenty microliters (20 µl) of blood was used to make blood film and examined under microscope[14]. Then, 20 µl of the blood was kept in filter paper and sent to Bureau of Vector Borne Diseases (BVBD) for confirmation by PCR according to Muhamad's technique 2011[15]. Another 20 µl was examined by three brands of RDTs according to the manufacturer's instructions. The microscopy was used as gold standard method when the RDT's accuracy was calculated.

Ethical issue of this study

All activities correlated with the patients were done strictly under good clinical practice (GCP). Before collecting the blood samples, the patients were informed consent regarding the procedures and details of this study. All participants agreed and were willing to be part of this study and signed the consent forms[16].

Inclusion and exclusion criteria regarding either patient or RDTs[17] were done strictly to prevent false negative or false positive results.

Quality control of this study

Internal quality control according to WHO guideline[12] was done throughout this experiment for preventing either false positive or false negative result.

Data analysis

Data was cleaned and analyzed by using Microsoft Excel 2007 software. For sensitivity and specificity calculations, the test kits were compared with Giemsa-Stained thick blood films (GS-TBF).

However, the numbers of positive case by GS-TBF were counted only in the asexual stage as of their clinical importance.

Results

A total of 899 suspected malaria subjects were recruited from the three MCs during April to July 2013, in which 12.57% (113 cases) of the cases were under 14 years old and their gender distribution was 54.30% male and 45.70% female respectively. Leading occupations were farmer, agricultural employee, forestry worker and logger. Of the 899 subjects, the numbers of individuals that provided positive results by microscopy for *P. falciparum* with or without *P. vivax*, and *P. vivax* only were 84 (34.85%) and 157 (65.15%) respectively. All 899 blood samples were confirmed by Polymerase Chain Reaction (PCR) and showed the identical results to those examined by GS-TBF. It was proved that no misdiagnosis among the study subjects.

Meanwhile, the numbers of individuals found positive for *P. falciparum* by Paracheck P.F.™, OptiMAL IT™, and SD MRDT test, were 83, 80 and 81 respectively. Whereas, the numbers of individuals found positive for *P. vivax* by OptiMAL IT™, and SD MRDT test, were 155 and 153, respectively. The accuracy of those RDTs was calculated by comparing with standard microscopy results. The analysis revealed that the Paracheck P.F.™ possessed sensitivity to *P. falciparum*, specificity and accuracy, at 98.81%, 99.63% and 99.56% respectively (Table 1). OptiMAL IT™ possessed sensitivity to *P. falciparum*, non-*P. falciparum*, specificity and accuracy, at 91.67%, 94.26%, 99.25%, and 97.66% respectively (Table 2). Whereas the SD MRDT test possessed 94.05%, 95.54%, 99.39% and 98.22% respectively (Table 3).

Table 1. Cross tabulation of Paracheck P.F.™ against GS-TBF.

Expected test kit resulta	GS-TBF		
	Pf.	**Negative***	**Total**
Pf.	83	3	86
Negative	1	812	813
Total	84	815	899

**Negative here consists of Non-falciparum and negative because the Paracheck P.F.™ can detect only Pf. Effectiveness of Paracheck P.F.™, sensitivity for Pf. 98.81%, specificity 99.63%, accuracy 99.56%, PPV[fd]. 96.51% , and NPV[e] 99.88%.*

[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

Table 2. Cross tabulation of OptiMAL IT™ against GS-TBF.

Expected test kit result[a]	GS-TBF			
	Pf.	**Non-Pf.**	**Negative**	**Total**
Pf.	77	2	1	80
Non Pf.	3	148	4	155
Negative	4	7	653	664
Total	84	157	658	899

Effectiveness of OptiMAL IT™, sensitivity for Pf[b]. 91.67%, sensitivity for non-Pf[c]. 94.26%, specificity 99.25%, accuracy 97.66%, PPV for Pf[d]. 96.25%, PPV for non-Pf[d]. 95.48% and NPV[e] 98.34%.

Table 3. Cross tabulation of SD MRDT against GS-TBF.

Expected test kit result[a]	GS-TBF			
	Pf.	**Non-Pf.**	**Negative**	**Total**
Pf.	79	0	2	81
Non Pf.	1	150	2	153
Negative	4	7	654	665
Total	84	157	658	899

Effectiveness of SD MRDT, sensitivity for Pf[b]. 94.05%, sensitivity for non-Pf[c] 95.54%, specificity 99.39% , accuracy 98.22%, PPV for Pf[d].97.53% , PPV for non-Pf[d]. 98.04% and NPV[e] 98.35%.

On the other hand, when the child group was separated to calculate for diagnostic value by using SD MRDT. The sensitivity to *P. falciparum*, non-*P. falciparum*, specificity and accuracy, were at 95.88%, 95.00%, 100%, and 96.46% respectively (Table 4). There were not much different from that in Table 3

Table 4. Cross tabulation of SD MRDT against 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 MRDT, sensitivity for Pf[b]. 95.88%, sensitivity for non-Pf. 95.00%, specificity 100% , accuracy 96.46%, PPV for Pf[d]. 100%, PPV for non-Pf[d]. 98.28% and NPV[e] 87.50%.

The assessment of RDTs against different levels of parasitemia revealed the sensitivity of three RDTs to either *P. falciparum* or Non-*P. falciparum*. They were the fluctuation of the sensitivity of the devices in reverse fashion to the level of parasitemia (Table 5, 6, 7), except OptiMAL IT™, showed decreasing sensitivity to 88.89 percent at >50,000 Parasitemia/µl among Non-*P. falciparum* group (Table 6). Paracheck P.F.™ showed 3 false positive cases to *P. falciparum* (Table 5), whereas OptiMAL IT™ and SD MRDT showed 1 and 2 false positive cases to *P. falciparum* respectively, but showed 4 and 5 false negative cases to *P. falciparum* respectively (Table 6, 7). High false negative to Non-*P. falciparum* found by OptiMAL IT™ and SD MRDT (9 and 7 cases respectively), as well as 4 and 4 false positive to Non-*P. falciparum* found by these RDTs respectively.

Table 5. The sensitivity of Paracheck P.F.™ at different levels of parasitemia.

Parasitemia/µl	*P. falciparum*				
	No. of patient	TP	FN	FP	Sensitivity (%)
<500	3	2	0	0	66.67
501-1,000	4	4	1	1	100
1,001-5,000	12	12	0	1	100
5,001-50,000	39	39	0	0	100
>50,000	26	265	0	1	100
Total	**84**	**83**	**1**	**3**	**98.81**

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

Table 6. The sensitivity of OptiMAL IT™ 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	3	0	0	0	0	11	7	4	2	63.63
501-1,000	4	2	2	0	50	7	5	2	1	71.42
1,001-5,000	12	11	1	1	91.67	71	70	1	0	98.59
5,001-50,000	39	38	1	0	97.44	59	58	1	1	98.31
>50,000	26	26	0	0	100	9	8	1	0	88.89
Total	**84**	**77**	**4**	**1**	**91.67**	**157**	**148**	**9**	**4**	**94.26**

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

Table 7. The sensitivity of SD MRDT 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	3	1	2	0	33.33	11	7	4	2	63.63
501-1,000	4	3	1	0	75	7	5	2	1	71.43
1,001-5,000	12	11	1	1	91.67	71	71	0	0	100
5,001-50,000	39	38	1	1	97.44	59	58	1	1	98.31
>50,000	26	26	0	0	100	9	9	0	0	100
Total	**84**	**79**	**5**	**2**	**94.05**	**157**	**150**	**7**	**4**	**95.54**

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

Discussion

The assessment of the three brands of RDTs, Paracheck P.F.™, OptiMAL IT ᴹ, and SD MRDT showed their diagnostic values higher than 90.00% in terms of sensitivity, specificity, accuracy, positive predictive value and negative predictive value. Even though, OptiMAL IT™ revealed the lowest sensitivity to *P. falciparum* (91.67%, Table 2), but all RDTs revealed higher to average diagnostic values to the previous studies regarding Malaria RDTs[5-10].

In this study, all RDTs were quality control following WHO guideline[12], the test results revealed their genuine attribution. Although it appeared that all performed reproducibility to be more reliable, but the devices misdiagnosed either false negative or false positive which we found to be very interesting. Three false positive to P. falciparum by Paracheck P.F.™ (Table 1) might be due to the persistence of Pf HRP2 antigen in blood circulation after parasite clearance which it can persist more than two weeks[3]. Likewise, SD MRDT detected Pf HRP2 antigen, and revealed two false positive (Table 3). The misdiagnosis might have been affected by either the RDT's attribute factors or by human error that the technician could not see the third band appearing on the device, even though internal quality control was carried out strictly (Figure 1). Similarly, the devices misdiagnosed seven of the non-*P. falciparum* as false negative (Table 2, Table 3), which likely occurred in 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 GS-TBF analysis.

Regarding children being high risk in most malarious countries, SD MRDT was chosen to analyze and compare test results between children group and overall. It was shown no difference in terms of the diagnostic patterns (Table 4). Thus, RDT revealed non-differential among age groups.

The numbers of *Pf* positive by Paracheck P.F.™ were not higher than those detected by microscopy (83 and 84 cases respectively). It might be due to the fact that Pf cases were mostly early infected cases; therefore, these patients have no *Pf* HRP2 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 OptiMAL IT™ and SD MRDT 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 6, 7). 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.

Several factors effect to the quality of RDTs which are rather difficult to prevent. These factors can be categorized into two types, controllable and uncontrollable. Those controllable factors are mainly strictly on the short and long term quality control recommended by WHO[13] as described previously. Whilst uncontrollable factors, firstly, we could not control all experiment atmosphere to control all day temperature in between 2°C–30°C at all day moisture or relative humidity below 60.00%; even though, the WHO recommended that the RDTs should be avoided from excessing to heat and moisture[20]. In most malaria-endemic countries, temperatures frequently exceed the recommended storage temperatures especially during the transportation of RDTs[20]. Secondly, RDTs' sensitivity and reliability depend on parasitemia level. Several field studies of malaria RDTs initially indicated the tests had good sensitivity ranges, particularly for density of *P. falciparum* greater than 500 Parasitemia/µl[19-21]. These finding are correlated with this study when RDTs were tested against <500 Parasitemia/µl level (Table 5, 6, 7). Thirdly, the Pf HRP2 persists after parasite clearance[3], three and four false positive by Paracheck P.F.™ and SD MRDT (Table 5, 7) might be due to this persistency. In this regard, many commercial RDT produced to detect Pf HRP2 antigen based on the reason of the superiority of *P. falciparum*'s sensitivity to other antigens[3] must adopt this phenomenon. Lastly, the Pf HRP2 antigenic variation in sensitivity of Pf HRP2 may be affected by genetic heterogeneity of the Pf HRP2[3]. This heterogeneity is important if a proportion of the parasites produce variant alleles of Pf HRP2 that lack the epitope or have fewer epitopes recognized by monoclonal antibodies. Patients infected with these parasites may be misdiagnosed as malaria-negative without concurrent microscopic examination. Therefore, one and five false negative by Paracheck P.F.™ and SD MRDT (Table 5, 7) might be related to phenomenon as well.

In conclusion, the three RDTs showed their diagnostic values in the bottom line of acceptance (higher than 90.00%)[4]; therefore, these devices might be a useful adjuvant diagnostic tool with the ability to detect malaria in children and suitable to be used in all malaria endemic areas.

Acknowledgements

This study was a part of work granted by the Global Fund to fight Malaria (GFATM) project in Thailand for the containment of artemisinin tolerant malaria parasites in South-East Asia.[22] The authors would like to thank the staffs of the Office of Disease Prevention and Control number 2 Phitsanulok, number 5 Ratchaburi, and Number 6 Chonburi for their kind cooperation to collect samples.

References

1. Bureau of Vector Borne Disease. Vector Borne Diseases: Section 2, Malaria situation in Thailand - 2012 Country Report. Bangkok: Department of Disease Control; 2012.
2. Indaratna K, Kidson C. Changing economic challenges in malaria control. Southern Asian J Trop Med Public Health 1995;26:388-96.

3. Wongsrichanalai C. Rapid diagnostic techniques for malaria control. Trends Parasitol 2001;17 :307-9.
4. World Health Organization. Interim notes on selection of type of malaria rapid diagnostic test in relation to the occurrence of different parasite species, Guidance for national malaria control programmes, Prepared by Roll Back Malaria Department with the collaboration of the Regional Offices for Africa and the Western Pacific. Geneva: World Health Organization; 2005.
5. Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, Ager AL. Evaluation of the Opti-MAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. J Clin Microbiol 1998;36:203-6.
6. Piper R, Lebras J, Wentworth L, Hunt-Cooke A, Houz S, Chiodini P, et al. Immunocapture diagnostic assays for malaria using *Plasmodium* lactate dehydrogenase (pLDH). Am J Trop Med Hyg 1999;60:109-18.
7. Iqbal J, Hira PR, Sher A, Al-Enezi AA. Diagnosis of imported malaria by *Plasmodium* lactate dehydrogenase (pLDH) and histidine-rich protein 2 (Pf HRP-2) based immunocapture assays. Am J Trop Med Hyg 2001;64:20-3.
8. Cho-Min-Naing, Gatton ML. Performance appraisal of rapid on-site malaria diagnosis (ICT malaria *Pf-Pv* test) in relation to human resources at village level in Myanmar. Acta Tropica 2002; 81:13-9.
9. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A Review of Malaria Diagnostic Tools: Microscopy and Rapid Diagnostic Test (RDT). Am J Trop Med Hyg 2007;77:119-27.
10. Murray CK, Gasser RA Jr, Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. Clin Microbiol Rev 2008;21:97-110.
11. World Health Organization. Malaria rapid diagnostic test performance, results of WHO product testing of malaria RDTs: Round 2 (2008-2009). Geneva: World Health Organization; 2009.
12. World Health Organization. Basic malaria microscopy, part I: Learner's guide, Second edition. Geneva: World Health Organization; 2010.
13. World Health Organization. Rapid Diagnostic Tests for Malaria, Document I, Method Manual for Laboratory Quality Control Testing, Regional Office for the Western Pacific, Version II, Geneva: World Health Organization; 2004.
14. World Health Organization. Malaria Microscopy Quality Assurance Manual Version I, Geneva: World Health Organization; 2009.
15. Muhamad P, Chaijaroenkul W, Congpuong K, Na-Bangchang K. SYBR Green I and TaqMan quantitative real-time polymerase chain reaction methods for the determination of amplification of *Plasmodium falciparum* multidrug resistance-1 gene (pfmdr1). J Parasitol 2011;97:939-42.
16. Food and Drug Administration. ICH, good clinical practice, standard operating procedures (SOPs). Bangkok: Drug Control Division, Food and Drug Administratio; 2000.
17. Ratsimbasoa A, Randriamanantena A, Raherinjafy R, Rasoarilalao, Menard D. Which malaria rapid test for Madagascar? Field and laboratory evaluation of three tests and expert microscopy of samples from suspected malaria patients in Madagascar. Am J Trop Med Hyg 2007;76:481-5.

18. USAID, Foundation for Innovative New Diagnostics (FIND), World Health Organization Western Pacific Regional Office (WHO WPRO), Roll Back Malaria Partnership, UNICEF. Transporting, Storing, and Handling Malaria Rapid Diagnostic Tests at Central and Peripheral Storage Facilities. Manila: WHO-WPRO; 2009.
19. Beadle C, Long GW, Weiss WR, McElroy PD, Maret SM, Oloo AJ, et al. Diagnosis of malaria by detection of *Plasmodium falciparum* HRP-2 antigen with a rapid dipstick antigen capture assay. Lancet 1994;343:564-8.
20. Garcia M, Kirimoama S, Marlborough D, Rieckmann KH. Immunochromatographic test for malaria diagnosis. Lancet 1996;347:1549.
21. Verle P, Binh LN, Lieu TT, Yen PT, Coosemano M. ParaSight-F test to diagnose malaria in hypo-endemic and epidemic prone regions of Vietnam. Trop Med Int Health 1996; 1:794-6.
22. Bureau of Vector Borne Diseases. The Global Fund to fight malaria round 7. Nonthaburi: Department of Disease Control, Ministry of Public Health Thailand; 2010.