Comparison of the OptiMAL® Rapid Test with Routine Microscopic Examination of Giemsa-Stained Thick Blood Film for Diagnosis of Malaria†

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Abstract

The OptiMAL® is a rapid immunodiagnostic test developed by Flow Inc., Portland, Oreg. for diagnosis and differentiation of *P. falciparum* and non *P. falciparum* malaria infection. It has been based on detection of circulating parasite lactate dehydrogenase enzyme (pLDH), produced by live Plasmodium parasites. The purpose of this study was to compare the efficacy of the OptiMAL® test with routine microscopic examination of Giemsa-Stained Thick Blood Film (routine GS-TBF) for the diagnosis of malaria at a local malaria clinic in a hyperendemic area of Thailand by using a standard GS-TBF (standard GS-TBF) as reference. One hundred and seventy five patients attending the clinic were recruited; 50, 42 and 83 were falciparum malaria, vivax malaria and non-malaria patients, respectively.

Compared with the reference, the OptiMAL® test had sensitivities of 92 per cent and 97.6 per cent, whereas, the routine GS-TBF had sensitivities of 81.3 per cent and 81 per cent for the detection of *P. falciparum* and *P. vivax*, respectively. Both tests showed no false positive resulting in 100 per cent specificities. However, the OptiMAL® test was able to detect only 20 per cent of infection with less than 200 parasitaemia /microlitre. It was also shown in our study that the OptiMAL® test was advantageous in follow-up of the treatment outcome. No false positive occurred among 40 follow-up cases.

The OptiMAL® test detected malaria infection more accurately than the routine GS-TBF ($p < 0.05$) and was simple, easy to perform and rapid. It is an alternative tool for the diagnosis of malaria in a hyperendemic area where experienced microscopists are not available.

Key word : Malaria, OptiMAL®, Rapid Diagnosis

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Thong Pha Phum, Kanchanaburi Province, located west of Thailand is a malaria hyperendemic area of both *Plasmodium falciparum* and *P. vivax*. Routine diagnosis of malaria in this area relies on microscopic examination of Giemsa-Stained Thick Blood Film (GS-TBF). At least 20,000 slides are examined yearly. Processing and reading such a large number of blood smear results in some misdiagnosis. Thus alternative techniques which are simple, able to be performed rapidly, easy to interpret, discriminate between *P. falciparum* and *P. vivax* are required. The OptiMAL® test developed by Flow Inc., Portland, Oreg. is the newest rapid malaria detection test which complies with these criteria. The assay has been tested in several countries and found to perform well in routine diagnosis of malaria[1-3]. This test is based on detection of circulating parasite lactate dehydrogenase (pLDH), produced by live Plasmodium parasites. It is capable of detecting 100-200 parasites/μl and producing a result rapidly (10-15 min)[1,4]. It is commercially available as kits, which include all the necessary reagents, and does not require extensive training or equipment to perform or to interpret the results. It has been reported to have the ability to distinguish *P. falciparum* from *P. vivax* and differentiate between viable and non-viable parasites essential for monitoring of antimalarial treatment[5]. The purpose of this study was to compare the efficacy of the OptiMAL® test with the routine microscopic examination of Giemsa-Stained Thick Blood Film (routine GS-TBF) for the diagnosis of malaria at a local malaria clinic in a hyperendemic area of Thailand by using a standard GS-TBF as reference.

MATERIAL AND METHOD

This study was conducted at a malaria clinic in Amphoe Thong Pha Phum, Kanchanaburi Province, Thailand from August to November 1998. A total of 175 patients whose GS-TBF examined by the clinic microscopists (routine GS-TBF) showed positive results for *P. falciparum*, *P. vivax* or negative results were recruited in the study. The proportion of the samples was 1:1:2. Verbal consents were obtained from the patients.

Blood for processing of the OptiMAL® test and thick film smear was obtained by finger prick before patients received treatment in some cases at the time of follow-up of treatment. Thick blood film was stained with Giemsa Stain and kept for further examination by the standard GS-TBF method at Malaria Division. The OptiMAL® test was proceeded by a technician in the clinic who had no knowledge of the routine GS-TBF results.

The OptiMAL® test.

The OptiMAL® test was performed in strict accordance with the manufacturer's instructions. Briefly, 10 μl of finger-prick whole blood (1 drop) was mixed with 30 μl (2 drops) of Buffer A in a microtitre test well. Buffer A is lysing buffer containing a colored bead conjugated to the pan-specific anti-pLDH antibody 6C9[2]. The test strip was then placed into the well and the entire sample was allowed to wick up the strip. After 8-15 min, the strip was moved to the second test well containing 80 μl (4 drops) of Buffer B to clear the hemoglobin color (10 min) for proper viewing of the test result. Complete test took a total of 10-20 minutes. Interpretation of the test results was performed immediately after completion of the clearing step as follows: 1) a control band presented at the top of the test strip showed whether the test had been conducted properly and the reagents were functional (Fig. 1). 2) the appearance of a second dark band on the strip indicated a positive reaction for any of the four major malaria species infecting humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*. The monoclonal antibody attached at this area was a pan-specific antibody 19G7[2]. 3) a positive *P. falciparum* was evident when a third band appeared on the bottom of the strip. A monospecific antibody that recognized only *P. falciparum* (17E4) was present here[2].

Microscopy and parasite density determination.

The routine GS-TBF was examined by local microscopists at the malaria clinic. Each slide was inspected for 100 oil-immersion fields (100 high power field) 1000x magnification, while searching for malaria parasites. Standard GS-TBF was examined by a qualified microscopist at the Malaria Division who had no knowledge of the routine GS-TBF or the OptiMAL® test results. The standard GS-TBF result was used as reference. Each slide was inspected as the routine GS-TBF. In addition, parasites and white blood cells (WBCs) were counted and calculated parasitaemia per 200 WBCs. The number was multiplied by 40 to estimate the parasite density as number/

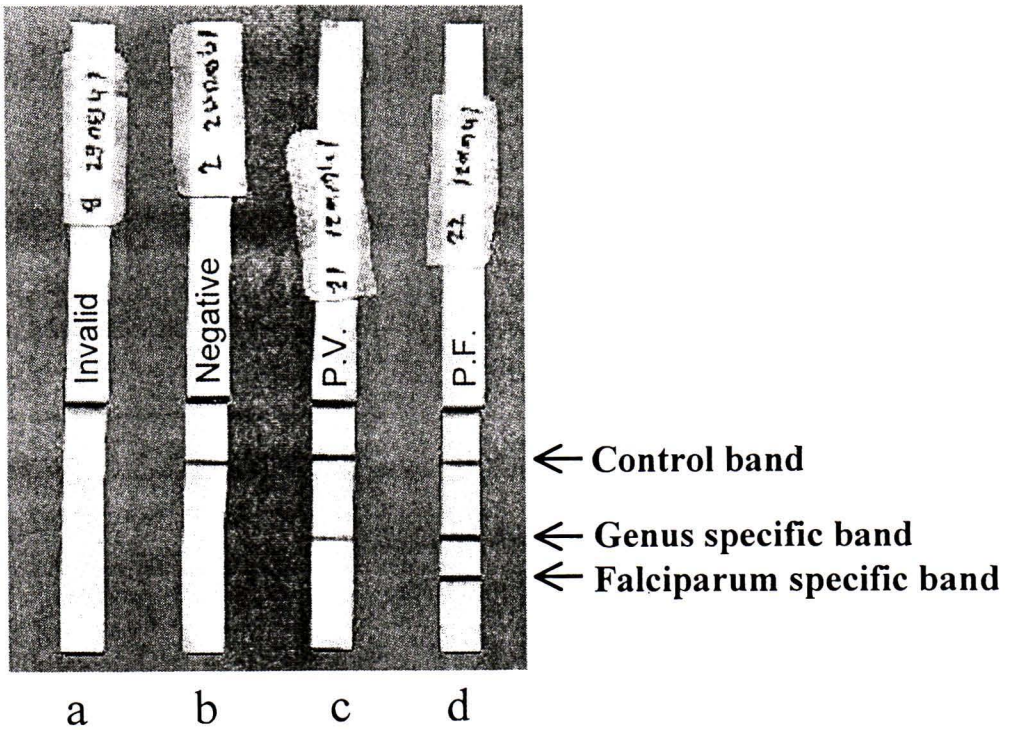

Fig. 1. Representative examples indicating (a) invalid test result (b) negative result (c) positive *P. vivax* infection (d) positive *P. falciparum* infection.

μl blood(6). The slide was reexamined by inspecting 200 fields if the corresponding OptiMAL® result had been different.

RESULTS

One hundred and seventy five patients were recruited, of which 50 blood films were positive for *P. falciparum* (including two mixed infection with *P. falciparum* and *P. vivax*), 42 positive for *P. vivax*, and 83 negative for malaria. Of these, 46 and 41 samples had matching OptiMAL® test and standard GS-TBF results for *P. falciparum* and *P. vivax*, respectively (92% and 97.6% sensitivities; Table 1). Concordance between the OptiMAL® test and the standard GS-TBF for malaria infection was 94.6 per cent (87 of 92). Among 83 samples negative for malaria, none was positive by the OptiMAL® test resulting in 100 per cent specificity.

Of the 50 *P. falciparum* cases positive by the standard TBF, 3 were negative by the OptiMAL® test and one was misidentified as *P. vivax*. These three false negative individuals showed the presence of only gametocytes in their blood films. Both individuals having mixed infections, had positive *P. falciparum* dipstick results. Among 42 *P. vivax* cases, one was misidentified as *P. falciparum*. Both of the species misidentified cases had parasitaemia as high as 3,448 and 9,501 parasites/μl, respectively.

Table 2 shows the comparison of the routine GS-TBF with the standard GS-TBF. Thirty-nine and thirty-four samples had matching results for *P. falciparum* and *P. vivax*, respectively (81.3% and 81% sensitivities). Concordance between two microscopic examinations was 81.1 per cent (73 of 90). The routine GS-TBF was not able to identify mixed infection correctly. Two patients having mixed infection were diagnosed of having infected by only *P. falciparum* and two vivax patients were misidentified as mixed infection. Error was not likely caused by low parasitaemia. Of the thirteen false negative slides, only two had parasitemia less than 100 parasites/μl. When compa-

Table 1. Sensitivity and specificity of the OptiMAL® test for *P. falciparum* and *P. vivax*[a].

	Standard GS-TBF		
		Positive for	
OptiMAL® test	Negative	*P. falciparum*	*P. vivax*
Negative	83	3[b]	0
Positive for *P. falciparum*	0	46	1
Positive for *P. vivax*	0	1	41
Total	83	50[c]	42

(a) Sensitivity for detection of *P. falciparum* = 46/50; sensitivity for detection of *P. vivax* = 41/42; specificity for detection of *P. falciparum* and *P. vivax* = 83/83
(b) Of the three negative OptiMAL® results, only gametocytes were found in their corresponding standard GS-TBFs
(c) Two patients infected with mixed infection of *P. falciparum* and *P. vivax* were included.

Table 2. Sensitivity and specificity of the routine GS-TBF for detection of *P. falciparum* and *P. vivax*[a].

	Standard GS-TBF			
		Positive for		
Routine GS-TBF	Negative	*P. falciparum*	*P. vivax*	Mixed[b]
Negative	83	9	4	0
Positive for *P. falciparum*	0	39	2	2
Positive for *P. vivax*	0	0	34	0
Positive for Mixed	0	0	2	0
Total	83	48	42	2

(a) Sensitivity for detection of *P. falciparum* = 39/48; sensitivity for detection of *P. vivax* = 34/42; specificity for detection of *P. falciparum* and *P.vivax* = 83/83
(b) Mixed infection of *P. falciparum* and *P. vivax*

Table 3. Agreement of the diagnosis outcome between the routine GS-TBF and the OptiMAL® test[a].

	OptiMAL® test		
Routine GS-TBF	Correct	Wrong	Total
Correct	156	1	157
Wrong	11	7	18
Total	167	8	175

(a) Chi-square test (X^2) = 6.75, $p < 0.05$

rison was made between the routine GS-TBF and the OptiMAL® test as shown in Table 3, it was found that the OptiMAL® test detected malaria infection more correctly ($p < 0.05$). However, it is shown in Table 4 that where parasitaemia was less than 200 parasites/μl, the OptiMAL® test could detect only 20 per cent of the infections.

The absence of false-positive results in this study may prove to be advantageous of the OptiMAL® test for use in monitoring of treatment outcome. We further examined our data and found that 40 of 175 cases were follow-up patients (Table 5). All had matching OptiMAL® test and standard GS-TBF results except one negative OptiMAL® case whose corresponding standard GS-TBF presented only gametocyte of *P. falciparum*.

DISCUSSION

Rapid diagnostic test kits will play an important role in malaria diagnosis in the near

Table 4. Sensitivity of the OptiMAL® test at different levels of parasitaemia.

	Standard GS-TBF	OptiMAL®		Sensitivity (%)
		Positive	Negative	
Positive samples	92	88	4	95.7
Parasitaemia				
>200/µl	85(a)	85	0	100
200/µl	5	1	4	20
Negative samples	83	0	83	NA(b)

(a) Excluded two slides that had only thin blood film
(b) Not applicable

Table 5. Comparison of the OptiMAL® test with the standard GS-TBF in follow-up of malaria treatment.

Day of follow-up	No. of cases	GS-TBF		OptiMAL® test	
		Positive	Negative	Positive	Negative
2	1	1	0	1	0
7	11	4	7	3	8
14	13	0	13	0	13
21	3	0	3	0	3
28	6	2	4	2	4
42	6	0	6	0	6
Total	40	7	33	6	34

future. At present, widespread use of these test kits is obstructed by their high cost. Appropriate implementation, e.g., supplementing the routine GS-TBF should be studied in order that patients suffering from malaria will have access to early diagnosis and prompt treatment.

The present study and several published results[1-3] indicate that the OptiMAL® test was nearly as sensitive as the standard microscopic examination. Our study also showed the superiority of the test to the routine GS-TBF in a malaria hyperendemic area. The sensitivities in diagnosis of *P. falciparum* and *P. vivax* were higher (92% and 97.6% compared to 81.3% and 81% of the routine GS-TBF). Thus, by using the OptiMAL® test, approximately 10 per cent of the false negative routine GS-TBF cases could be detected. False negative results and misidentification of Plasmodium species by the routine GS-TBF were likely caused because the microscopists in this studied area had to examine a large number of slides daily--up to 100 slides in the morning during the malaria peak season. Variability in slide staining and long-term use of the microscope increased the chance for error dramatically.

The performance of the OptiMAL® test was easier and less time consuming. Many tests can be done simultaneously, saving in the technician's time-including time to prepare stained blood smears and microscopic examination of the slides. The test can be performed in remote areas where electricity is lacking, and the test does not require highly skilled personnel to perform or to interpret the results. Thus, malaria could be diagnosed without delay on the spot by nonmedical staff.

The need for differential diagnosis of *P. falciparum* is essential because of the severe nature of this infection and the drug-resistant *P. falciparum*. The OptiMAL® test has the ability to discriminate *P. falciparum* from *P. vivax*. Furthermore, the test is based on detection of enzyme pLDH that is produced by only live organisms

so it is beneficial in monitoring the success of chemotherapy and early detection of drug-resistant malaria.

A *P. vivax* case was misidentified as *P. falciparum*. This event is possible in patients who have heavy infection of *P. vivax* but a small amount of *P. falciparum*. Microscopists may not be able to detect this small amount of *P. falciparum* due in part to the similar ring form morphology of these two species.

Gametocytaemia is an indicator of delayed treatment and having parasite transmission in the area. This indicator is highly concerned by the malaria control programme. It has been reported that pLDH enzyme is presented in all stages of Plasmodium sp. including gametocyte[1]. Nevertheless, in the present study, three individuals, who presented with only gametocyte of *P. falciparum* in their blood, were negative by the OptiMAL® test. This may be due to the low gametocytemia of less than 200 parasites/μl. It was also shown in the present study that the sensitivity of the OptiMAL® test was only 20 per cent in patients with less than 200 parasites/μl.

Other limitations of the OptiMAL® test included the inability to distinguish *P. falciparum* from mixed infection; both yielded the same pattern of results. Two cases infected with mixed infection of *P. falciparum* and *P. vivax* in our study showed positive results for *P. falciparum* by the OptiMAL® test.

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การวินิจฉัยมาลาเรียอย่างรวดเร็วโดยวิธี OptiMAL® เปรียบเทียบกับวิธีตรวจหาเชื้อมาลาเรียในฟิล์มเลือดชนิดหนาที่ย้อมด้วยสียิมซ่าโดยใช้กล้องจุลทรรศน์ในงานปกติ†

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OptiMAL® เป็นชุดทดสอบสำเร็จรูป ใช้ตรวจวินิจฉัยโรคมาลาเรีย สามารถแยกมาลาเรียชนิดที่เกิดจากเชื้อมาลาเรียฟัลซิปารัม จากชนิดที่ไม่ใช่ฟัลซิปารัม การทดสอบอาศัยหลักการตรวจหาเอ็นซัยม์ parasite lactate dehydrogenase (pLDH) ซึ่งผลิตโดยเชื้อมาลาเรียที่ยังมีชีวิตอยู่ การศึกษานี้มุ่งหมายที่จะเปรียบเทียบประสิทธิภาพของวิธี OptiMAL® กับวิธีตรวจหาเชื้อมาลาเรียจากฟิล์มเลือดแบบหนาที่ย้อมสียิมซ่าด้วยกล้องจุลทรรศน์ในงานปกติ โดยใช้วิธีตรวจหาเชื้อมาลาเรียจากฟิล์มเลือดแบบหนาที่ย้อมสียิมซ่า และตรวจโดยเจ้าหน้าที่ที่มีความเชี่ยวชาญจากกองมาลาเรีย เป็นวิธีอ้างอิง

ตัวอย่างเป็นผู้ป่วยมาลาเรียชนิดฟัลซิปารัม 50 คน ชนิดไวแวกซ์ 42 คน และผู้ป่วยที่มีอาการแต่ไม่พบเชื้อมาลาเรีย 83 คน รวมทั้งสิ้น 175 คน ผลการเปรียบเทียบกับวิธีอ้างอิงพบว่า วิธี OptiMAL® มีความไวเท่ากับร้อยละ 92 และ 97.6 ในการตรวจวินิจฉัยเชื้อมาลาเรียชนิด ฟัลซิปารัม และชนิดไวแวกซ์ตามลำดับ ในขณะที่การตรวจด้วยวิธีปกติมีความไวเท่ากับร้อยละ 81.3 และ 81 ตามลำดับ ทั้งสองวิธีมีความจำเพาะเท่ากับร้อยละ 100 อย่างไรก็ตามวิธี OptiMAL® มีความไวเพียงร้อยละ 20 เมื่อใช้ตรวจผู้ป่วยที่มีจำนวนเชื้อมาลาเรียในกระแสเลือดน้อยกว่า 200 ตัวต่อไมโครลิตร การศึกษานี้ยังพบว่าวิธี OptiMAL® มีประโยชน์ใช้ในการติดตามผลการรักษาผู้ป่วยได้ดี จากจำนวนผู้ป่วยที่มารับการติดตามผลการรักษา ไม่พบว่ามีผลบวกปลอม

วิธี OptiMAL® มีความไวมากกว่าวิธีตรวจหาเชื้อมาลาเรียจากฟิล์มเลือดแบบหนาที่ย้อมด้วยสียิมซ่าในงานปกติอย่างมีนัยสำคัญทางสถิติ รวมทั้งเป็นวิธีที่ง่าย ใช้เวลาในการทดสอบไม่นาน ดังนั้นจึงเป็นทางเลือกที่ดีสำหรับการวินิจฉัยโรคมาลาเรียในที่ที่ขาดแคลนจุลทัศนากรที่มีความชำนาญ หรือในมาลาเรียคลินิกที่มีผู้ป่วยมารับการตรวจรักษาต่อวันจำนวนมาก

คำสำคัญ : มาลาเรีย, การตรวจวินิจฉัย, เอ็นซัยม์ pLDH

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