

SHORT REPORT: FAILURE OF THE OPTIMAL[®] RAPID MALARIA TEST AS A TOOL FOR THE DETECTION OF ASYMPTOMATIC MALARIA IN AN AREA OF THAILAND ENDEMIC FOR *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

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Abstract. We evaluated the efficacy of the OptiMAL[®] assay in a cross-sectional malaria survey in western Thailand from April to August 2001. Expert microscopy of Giemsa-stained thick and thin blood films was used as the gold standard. Positive control lines were evident in 99% (1,128 of 1,137) of the assays tested. However, 34% (384 of 1,128) of assays produced an aberrant result (a positive *P. falciparum*-specific line and a negative panmalarial line). False-positive panmalarial and *Plasmodium falciparum*-specific lines occurred in 25.9% (270 of 1,042) and 60.3% (628 of 1,042) of microscopy-negative samples, respectively. Due to the preponderance of false-positive test results, it was necessary to develop subjective criteria for test positivity based on line intensity. For determination of assay performance during this study, we therefore considered all test lines that were scored as intermediate or strong as positive and lines that were faint as negative. Using these criteria, we determined that the sensitivity of the OptiMAL[®] assay for *P. falciparum* was 25% with > 500 parasites/ μ L and 10.5% with > 100 parasites/ μ L, while for *P. vivax*, the sensitivity at the same parasite rates was 100% and 41.7%, respectively. Further studies are required to determine whether the problems we identified are limited to this particular lot of OptiMAL[®] assays.

Although the detection of asexual parasites by light microscopy of Giemsa-stained thick and thin blood films remains the standard laboratory method for the diagnosis of malaria,^{1,2} the World Health Organization³ has repeatedly emphasized the urgent need for simple and cost-effective diagnostic tests for malaria that can overcome the deficiencies of light microscopy. Criteria for implementation of these tests in malaria control programs were recently reviewed.⁴ Multiple studies have indicated that the OptiMAL[®] rapid malaria test, based on detection of *Plasmodium* lactose dehydrogenase in whole blood, may be a useful tool for the diagnosis of malaria in areas where experienced microscopists may not be available.^{5–12} In spite of excellent performance of the OptiMAL[®] assay at high (> 500 parasites/ μ L) parasite densities, several studies have reported that the assay is not sufficiently sensitive at low parasite densities.^{10–12} In this study, we compared the efficacy of the OptiMAL[®] rapid malaria test (DiaMed, Morat, Switzerland) with that of expert microscopy in an active malaria surveillance program. The goal was to define performance of the assay at low parasite densities.

The study was performed from April to August 2001 in the village of Ban Kong Mong Tha in Laivo Tambon Sub-District, Sangkhlaburi Amphur District, Kanchanaburi Province, in western Thailand. The study was approved by the Ethics Committee of the Ministry of Public Health (Bangkok, Thailand) and by the Human Subjects Research Review Board of the United States Army Medical Research Institute of Infectious Diseases (Fort Detrick, Frederick, MD). At the start of the study, informed consent was obtained from all individuals willing to participate in the study, along with demographic information. A total of 529 adults and children (\geq 1 year of age) were enrolled in the study. During the course of the study, investigators went house to house and collected fingerprick blood samples from all individuals participating in the study. The fingerprick blood samples were used to prepare thick and thin blood films at each house. At the same time that blood films were prepared, 10 μ L of blood was collected into a microcapillary tube and immediately tested using the OptiMAL[®] assay. Thick and thin blood films were

stained with 10% Giemsa solution and examined microscopically at 1,000 \times by an expert microscopist (N.M.) with more than 30 years of experience. The parasites were counted on thick blood smears until 500 leukocytes were reviewed and the parasite density was then expressed as the number of trophozoites per microliter by assuming a leukocyte count of 7,000/ μ L.

The OptiMAL[®] assay that we tested was produced by DiaMed AG (Morat, Switzerland) under license from Flow, Inc. (Portland, OR) and obtained locally in Thailand. Each package (Lot # 46050.03.06) consisted of 48 tests, with an expiration date of September 2001. Assays were delivered to the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand on March 29, 2001. Assays were stored at room temperature (24–26 $^{\circ}$ C) at AFRIMS prior to being brought to the field, where they were stored at ambient temperature (26–33 $^{\circ}$ C) for 1–2 days before being used. The OptiMAL[®] assays were tested at the site of collection according to the manufacturer's instructions. Results were initially read at the house where the fingerprick blood sample was collected, with a second reading made at the field laboratory by the principle investigator (R.C.). A test result was considered valid if the control line was visible. A diagnosis of *P. falciparum* was made if both the panmalarial (upper) and *P. falciparum*-specific (lower) lines were visible. A diagnosis of *P. vivax*, *P. malariae*, or *P. ovale* (these three species cannot be distinguished from one another by this test) was made if only the panmalarial line was visible. The intensity of each line was graded into five categories: 0 (absent), 1 (faint), 2 (intermediate), 3 (strong, but lighter than the control line), and 4 (strong and darker than the control line).

All slides with discordant results and 10% of the slides with concordant results were cross-checked at AFRIMS by an expert microscopist (N.R.) with more than five years of experience. The microscopist was blinded to the results from initial field microscopy and immunochromatographic test results. A thick blood film was considered negative on cross-checking if no parasites were seen per 500 leukocytes. Epi-Info version 6¹³ was used to calculate test performance and acceptability

evaluation indices, with microscopy used as the gold standard. Variables measured included the number of true positives (TP), number of true negatives (TN), number of false positives (FP), and number of false negatives (FN). Sensitivity was calculated as $TP/(TP + FN)$ and specificity was calculated as $TN/(TN + FP)$. Test accuracy, the proportion of all tests that gave a correct result, was defined as $(TP + TN)/\text{number of all tests}$. Reliability was expressed as the J index $((TP \times TN) - (FP \times FN))/((TP + FN)(TN + FP))$.

Of the 1,137 blood films collected over the course of the study, 7.8% (89) were found to have malaria parasites by microscopy; 39.3% (35 of 89) were *P. falciparum*, 58.4% (52 of 89) were *P. vivax*, and 2.2% (2 of 89) were *P. malariae*. The mean density of *P. falciparum* parasites was $852/\mu\text{L}$ (SEM = 428, mode = 70), with a range of 14–14,000/ μL . For *P. vivax*, the mean density was $943/\mu\text{L}$ (SEM = 454, mode = 70), with a range of 28–3,136/ μL . There were no mixed infections.

The performance characteristics of the OptiMAL[®] assay are shown in Tables 1 and 2. In our initial analysis, all visible lines were considered as indicative of a positive test result. Assay failure (no visible control line) only occurred with 0.8% (9 of 1,137) of the samples. However, aberrant results (presence of the *P. falciparum*-specific test line in the absence of the panmalarial test line) occurred with 34% (384 of 1,128) of the samples. False-positive panmalarial and *P. falciparum*-specific lines occurred in 25.9% (270 of 1,042) and 60.3% (628 of 1,042) of the microscopy-negative samples, respectively. Even when assays with aberrant results (i.e., considering them negative) were discounted, the OptiMAL[®] assay was not sensitive and specific for *P. falciparum* or *P. vivax* at any of the parasite densities encountered (Table 2). Although the assay was sensitive for *P. vivax* at densities > 500 parasites/ μL , the specificity was only 73.9%.

The poor performance of the OptiMAL[®] assay was unexpected. Although the assays were stored according to the manufacturer's instructions while at AFRIMS, they were held at temperatures that exceed 30°C for 1–2 days while in the field. Although these high temperatures may have affected performance of the assay, false-positive results were obtained with approximately 50% (9 of 20) of assays tested at AFRIMS at the start of the study. These assays had not been exposed to high temperatures.

The false-positive results may be due to a mechanical phenomenon such as a visualization of the monoclonal antibody strip applied to the nitrocellulose paper when the paper is

dampened (so-called shadow line). Alternatively, it may reflect non-specific binding of the antibody to the capture lines. However, it is unlikely to be due to antecedent antimalarial drug use prior to our survey since each individual was questioned about drug use during the two weeks prior to the collection of the fingerprick blood sample.

In a post hoc analysis of assay performance, we considered only test lines with a score of 2 (intermediate intensity) or higher as a positive result and those less than 2 as negative. Although use of these subjective criteria significantly enhanced the specificity of the assay, an unacceptable decrease in sensitivity occurred (Table 2). Furthermore, such modifications of test interpretation criteria are not practical for the field use of these tests, especially by inexperienced personnel.

Overall, the performance of this lot of DiaMed OptiMAL[®] Rapid Malaria Test was significantly worse than that achieved using microscopy. The high number of false-positives resulted in low specificity of the assay when compared with microscopy. The cause of the false-positives is not clear, but reflects a marked variance in performance standards reported in other published studies. However, other investigators (Thimasarn K, Wongsrichalai C, unpublished data) also observed poor specificity in another lot of DiaMed OptiMAL[®] tests obtained in Thailand, while Mason and others reported poor sensitivity during testing in Myanmar.¹⁴

Based on the results of this study, we conclude that this lot of the OptiMAL[®] assay does not appear suitable for use in active malaria surveillance programs in Thailand. The combination of poor assay specificity and low sensitivity, particularly in individuals with parasite densities < 500/ μL , suggests that the majority of malaria cases in similar surveillance programs will not be accurately detected using the OptiMAL[®] assay. Further studies are required to determine whether the problems we encountered are restricted to this particular lot of assays or are more widespread.

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Disclaimer: The views of the authors do not purport to represent the position of the Department of the Army or the Department of Defense.

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TABLE 1

Comparison of the OptiMAL[®] assay and expert laboratory microscopy for active surveillance for *Plasmodium falciparum* and *P. vivax* in a malaria-endemic area in Thailand

Microscopy result	No. of samples with the following result by the OptiMAL assay*					Total
	<i>P. falciparum</i> (C + T + B)	<i>P. vivax</i> (C + T)	Aberrant (C + B)	Negative (C)	Assay failure (No C)	
All visible lines indicative of a positive assay result						
<i>P. falciparum</i>	11	2	10	12	0	35
<i>P. vivax</i>	12	3	16	20	1	52
Negative	268	2	358	414	8	1,050
Total	291	7	384	446	9	1,137
Lines graded as a 2 or higher indicative of a positive assay result						
<i>P. falciparum</i>	2	0	0	33	0	35
<i>P. vivax</i>	0	6	1	44	1	52
Negative	1	0	15	1,026	8	1,050
Total	3	6	16	1,103	9	1,137

* C = control line; T = top test line; B = bottom test line.

TABLE 2

Performance characteristics of the OptiMAL® assay at different parasite densities relative to those of expert laboratory microscopy for active surveillance for *Plasmodium falciparum* and *P. vivax**

Parasite	Parasite density	Microscopy positive		Microscopy negative		Sensitivity (95% CI)	Specificity (95% CI)	Accuracy	Reliability
		OptiMAL positive	OptiMAL negative	OptiMAL positive	OptiMAL negative				
All visible lines indicative of a positive assay result									
<i>P. falciparum</i> [†]	≥500/μL	2	2	294	830	50.0 (9.2–90.8)	73.8 (71.2–76.4)	0.74	0.24
	≥100/μL	8	11	288	821	42.1 (21.1–66.0)	74.0 (71.3–76.6)	0.73	0.16
	≥1/μL	11	24	285	808	31.4 (17.4–49.4)	73.9 (71.2–76.5)	0.73	0.05
<i>P. vivax</i> [‡]	≥500/μL	5	0	293	830	100.0 (46.3–100)	73.9 (71.2–76.4)	0.74	0.74
	≥100/μL	7	5	291	825	58.3 (28.6–83.5)	73.0 (71.2–76.3)	0.74	0.32
	≥1/μL	15	36	283	794	29.4 (17.9–44.0)	73.7 (71.0–76.3)	0.72	0.03
Lines graded as a 2 or higher indicative of a positive assay result									
<i>P. falciparum</i> [†]	≥500/μL	1	3	0	1,124	25.0 (1.3–78.1)	100.0 (99.6–100)	1.00	0.25
	≥100/μL	2	17	0	1,109	10.5 (1.8–34.5)	100.0 (99.6–100)	0.98	0.11
	≥1/μL	2	33	0	1,093	5.7 (1.0–20.5)	100.0 (99.6–100)	0.97	0.06
<i>P. vivax</i> [‡]	≥500/μL	5	0	0	1,123	100.0 (46.3–100)	100.0 (99.6–100)	1.00	1.00
	≥100/μL	5	7	0	1,116	41.7 (16.5–71.4)	100.0 (99.6–100)	0.99	0.42
	≥1/μL	6	45	0	1,077	11.8 (4.9–24.0)	100.0 (99.6–100)	0.96	0.12

* CI = confidence interval.

[†] Assays were considered *P. falciparum*-positive only if panmalarial and *P. falciparum*-specific lines were visible.

[‡] Assays were considered *P. vivax*-positive only if the panmalarial line was visible (with or without the *P. falciparum*-specific line being visible).

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