

FIELD EVALUATION OF THE ICT MALARIA PF/PV IMMUNOCHROMATOGRAPHIC TEST FOR THE DETECTION OF ASYMPTOMATIC MALARIA IN A *PLASMODIUM FALCIPARUM/VIVAX* ENDEMIC AREA IN THAILAND

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Abstract. Rapid antigen assays provide an effective tool for the detection of malaria in symptomatic patients. However, the efficacy of these devices for detecting asymptomatic malaria, where parasite levels are normally significantly lower than in symptomatic patients, is less well established. We evaluated the efficacy of a new combined *Plasmodium falciparum*-Plasmodim vivax immunochromatographic test (ICT Malaria Pf/Pv) in a cross-sectional malaria survey of the village of Ban Kong Mong Tha, Kanchanaburi Province, Thailand, from August to December 2000. A total of 1,976 bleeds were made from 559 individuals over the course of the study. Blinded microscopy of thick and thin blood films was used as the gold standard; all discordant and 10% of concordant results were cross-checked. Of 1,976 ICT Malaria Pf/Pv dipsticks tested, 98.3% (n = 1,943) performed as expected, as evidenced by the appearance of the control line. The ICT Malaria Pf/Pv test was both sensitive (100.0%) and specific (99.7%) for the diagnosis of falciparum malaria with parasitemias of ≥ 500 trophozoites/ μL ; however, only 15.9% (13/82) of infected individuals had parasitemia rates this high. When *P. falciparum* parasitemia rates were $< 500/\mu\text{L}$, the sensitivity of the diagnosis was only 23.3%, with a positive predictive value (PPV) and a negative predictive value (NPV) of 76.2 and 97.2%, respectively. The ICT Malaria Pf/Pv test was specific, but not sensitive, for the diagnosis of vivax malaria with parasite rates of ≥ 500 trophozoites/ μL , with sensitivity, specificity, PPV, and NPV of 66.7%, 99.9%, 66.7%, and 99.9%, respectively. At parasite rates of $< 500/\mu\text{L}$, corresponding values were 0.0%, 99.9%, 0%, and 95.1%. Because of the relatively high cost of these assays, low parasite rates found in the majority of asymptomatic individuals, and low sensitivity of this assay with rates of $< 500/\mu\text{L}$, use of this assay as a tool for active case detection is of limited value in western Thailand.

INTRODUCTION

The detection of asexual parasites by light microscopy of Giemsa-stained thick and thin films remains the standard laboratory method for the diagnosis of malaria.^{1,2} Although detection of parasites in symptomatic patients reporting to local malaria clinics is the primary means used for malaria diagnosis in Thailand, use of active (cross-sectional) surveillance provides a tool for detecting patients with “asymptomatic” malaria and relatively low parasite rates. In Thailand, active surveillance is used in remote areas where individuals may have difficulty in reaching a malaria clinic; in this situation, malaria clinic personnel make periodic visits to a given village and examine blood smears from all individuals present in the village. In Thailand and many other malaria endemic regions, there are problems and limitations associated with reliance on microscopic diagnosis of malaria for both active and passive case detection,^{3–5} including lack of skilled microscopists, variation in individual training and/or experience, limited supply of microscopes and reagents as well as variation in equipment maintenance, and inadequate quality control. When symptomatic patients with a relatively high parasitemia (> 500 trophozoites/ μL) report to a malaria clinic for treatment, microscopy can provide a “relatively” accurate diagnosis that is used to initiate appropriate chemotherapy. However, accuracy of microscopy can decrease significantly at lower parasitemia levels.⁴ Parasitemia rates in “asymptomatic” patients are often quite low; in a study in western Thailand, the median asexual parasitemia rate in 271 *P. falciparum* (PF) and 347 *P. vivax* (PV) positive individuals was 98 and 70 trophozoites/ μL , respectively (Coleman et al, 2001, unpublished data).

The World Health Organization⁶ has repeatedly emphasized the urgent need for simple and cost-effective diagnostic tests for malaria to overcome the deficiencies of both light microscopy and clinical diagnosis. In recent years, multiple studies have clearly demonstrated that dipstick antigen-capture tests for circulating *Plasmodium falciparum*-specific HRP2 have excellent sensitivity and specificity for the diagnosis of falciparum malaria.^{2,7–12} In this study, we compared the efficacy of the new combined PF/PV immunochromatographic test (ICT Malaria Pf/Pv) with that of gold standard expert microscopy in an active malaria surveillance program.

MATERIALS AND METHODS

Study site. The study was performed from August to December 2000 in the village of Ban Kong Mong Tha, Laivo Tambon (Subdistrict), Sangkhlaburi Amphur (District), Kanchanaburi Province, 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 and Material Command, Fort Detrick, Maryland.

Patients and sample collection. A total of 559 adults and children (1 year of age or older) living in Ban Kong Mong Tha were enrolled in the study. At the start of the study, informed consent and demographic information were obtained from all individuals willing to participate in the study. During the period when the actual study was conducted, three teams of investigators went house to house during a 4-day period each month. Fingerprick blood samples were collected from all individuals present in the village who had consented to participate. In addition, each individual was questioned about

signs and symptoms of malaria, travel history, and medications taken during the prior 2 weeks. 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, four drops of blood were spotted onto filter paper for subsequent molecular diagnosis, and 15 μ L of blood was collected into a microcapillary tube. Blood from the microcapillary tube was immediately tested for PF and PV using the ICT Pf/Pv immunochromatographic assay.

Microscopy and immunochromatographic testing. Thick and thin films were stained with 10% Giemsa solution and examined at $\times 700$ by a microscopist from the local malaria clinic. Procedures used in this initial (field) examination followed the malaria clinic standard operating procedure and consisted of reading approximately 100 oil fields before considering the slide negative. Because there is no electricity in Ban Kong Mong Tha, slides were examined using natural daylight. Each slide took approximately 1–2 minutes to read. On return to the Armed Forces Research Institute of Medical Sciences (AFRIMS) laboratory in Bangkok, Thailand, each slide was re-examined by an expert microscopist (NM) with more than 30 years experience. The microscopist was unaware of the results from the field examination or immunochromatographic test. The parasite density was counted per 500 leukocytes and was then expressed as the number of trophozoites per microliter by assuming a leukocyte count of 7,000/ μ L. Each slide took approximately 5 min to read in the laboratory.

After a period of training at AFRIMS, ICT Pf/Pv immunochromatographic testing was conducted at each house in the village of Ban Kong Mong Tha according to the manufacturer's instructions. The results were initially read at the house where the fingerprick blood sample was collected; a second read was made at the field laboratory by the principle investigator (RC). The MLO2 ICT Pf/Pv test card (AMRAD-ICT, Sydney, Australia) was used. A total of four separate lots (nos. 012379, 011940, 011610, and 011190) were used over the course of the study. The test was considered valid if the control line was visible and positive if the HRP2 line and/or panmalarial antigen line was visible. An immunochromatographic test diagnosis of PV malaria was made if only the panmalarial antigen line was visible. A diagnosis of PF malaria was made if the HRP2 line was visible with or without the panmalarial antigen line. Coinfection with both PF and PV cannot be distinguished from infection with PF alone; therefore, test interpretation when both HRP2 and panmalarial antigen lines are both visible is PF malaria. 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 slides with concordant results were cross-checked by an expert microscopist (NR) at AFRIMS with more than 5 years experience. The microscopist was blinded to the results from previous microscopy and immunochromatographic test results. A thick film was considered negative on cross-checking if no parasites were seen per 500 leukocytes.

Data analysis. Epi-Info version 6¹³ was used to calculate test performance and acceptability evaluation indices; microscopy was used as the gold standard. Performance indices followed those used by Tjitra and others² and were calculated for the following microscopic diagnoses: total malaria (diag-

TABLE 1

Comparison of the ICT Malaria Pf/Pv assay and expert laboratory microscopy for active surveillance for *Plasmodium falciparum* and *Plasmodium vivax*

Expert microscopy result	Number of samples with the following result by ICT Malaria Pf/Pv assay				Total
	<i>P. falciparum</i>	<i>P. vivax</i>	Pf and Pv	Negative	
<i>P. falciparum</i>	29	0	0	52	81
<i>P. vivax</i>	1	2	0	94	97
<i>P. falciparum</i> and <i>P. vivax</i>	1	0	0	0	1
Negative	4	1	0	1,759	1,764
Total	35	3	0	1,905	1,943

nosis of PF and PV), PF malaria (to include mixed infections), and PV malaria. Variables measured included the number of true-positive (TP), true-negative (TN), false-positive (FP), and false-negative (FN) results. Sensitivity was calculated as TP/(TP + FN), specificity as TN/(TN + FP), positive predictive value (PPV) as TP/(TP + FP), and negative predictive value (NPV) as TN/(FN + TN). Test accuracy, the proportion of all tests that gave a correct result, was defined as (TP + TN)/number of all tests. Reliability was expressed as the J index ((TP \times TN) - (FP \times FN))/((TP + FN)(TN + FP)). Results were considered false positive if microscopy detected PF and the immunochromatographic test detected PV, and vice versa. Spearman rank correlations procedure was used to evaluate the relationship between parasite density and the intensity of the line on the ICT Malaria Pf/Pv assays for all samples that were PF positive by microscopy (Analytical Software: Statistix 7, 2000, Tallahassee, FL).

RESULTS

Of the 559 individuals who participated in the study, 305 (54.6%) were males and 254 (45.4%) were females. The age range was 1–90 years: 1–9 years, 31.5% (n = 176); 10–19 years, 23.6% (n = 132); 20–29 years, 13.2% (n = 74); 30 or older, 31.7% (n = 177). The mean and median ages were 22.0 and 16.0 years, respectively. Of the 1,943 blood films collected over the course of the study, 9.2% (n = 178) were found to have malaria parasites by microscopy; 45.5% (81/178) were PF, 54.5% (97/178) were PV, and 0.6% (1/178) were mixed PF/PV (Table 1). Of the PV-positive blood films, 77.6% (76/98) had asexual parasites alone, 21.4% (21/98) had both asexual parasites and gametocytes, and 1.0% (1/98) had gametocytes alone. For PF-positive films, 90.2% (74/82) had asexual parasites alone, whereas 9.8% (8/82) had asexual parasites and gametocytes. The mean density of PF parasites was 848.2/ μ L (SEM = 300.3), with a range from 28–14,000/ μ L. For PV, the mean density was 154.5/ μ L (SEM = 46.3), with a range from 28–3,136/ μ L.

The results of parasite detection by microscopy and immunochromatographic testing are compared in Table 2; performance characteristics of the ICT Malaria Pf/Pv assay are presented in Table 3. At parasite densities of 500/ μ L or greater, the assay was both sensitive and specific for PF; however, only 15.8% (13/82) of positive films had parasite densities this high. Performance of the assay dropped off markedly with decreasing PF densities; sensitivity dropped to 47.8% at densities of 100–499/ μ L and only 10.9% at densities of < 100/ μ L.

TABLE 2

Performance of the ICT Malaria Pf/Pv test relative to expert laboratory microscopy at different *Plasmodium falciparum* and *Plasmodium vivax* trophozoite densities

Trophozoite/ μL	<i>P. falciparum</i>		<i>P. vivax</i>	
	Number positive by expert microscopy	Number correct by ICT pf/Pv test	Number positive by expert microscopy	Number correct by ICT Pf/Pv test
<100	46	5 (10.9%)	76	0 (0.0%)
100–499	23	11 (47.8%)	19	0 (0.0%)
≥ 500	13	13 (100.0%)	3	2 (66.7%)
Total	82	29 (35.4%)	98	2 (2.0%)

(see Table 3). Of all PF-positive blood films, 28% (23/82) had a density of 100–499/ μL , whereas 56% of positive films had a density of < 100/ μL (see Table 2). There was a weak positive correlation (0.70) between PF parasite density and intensity of the line observed on the dipstick. There were significant differences in the performance of the various lots of the ICT Malaria Pf/Pv assay; lot no. 011940 performed better than lot nos. 012379 or 011190 (Table 4). Performance of lot no. 011610 was not evaluated because only 25 assays from this lot were tested.

The ICT Malaria Pf/Pv assay was specific, but not sensitive, for the detection of PV at all parasite densities (see Table 3). Only 3.1% (3/98) of PV-positive blood films had a parasite density of $\geq 500/\mu\text{L}$; the ICT Malaria Pf/Pv assay detected PV parasites in two of these three blood samples; however, both assays were only scored as a +1 (faint line). The assay detected 0% (0/95) of PV-positive blood films with a parasite density of < 500/ μL . When all lots were considered, the sensitivity of the ICT Malaria Pf/Pv assay was only 2.0% for PV.

DISCUSSION

A variety of studies have shown that, for detection of PF, rapid dipstick antigen-capture tests specific for the circulating PF-specific antigen HRP2 have excellent sensitivity and specificity comparable to that achieved by a skilled microscopist.² The three commercially available methods of HRP2 antigen detection have included the ParaSight-F assay,^{7–8,11} the *P. falciparum* immunochromatographic test (ICT Malaria Pf),^{11,14–16} and the ICT Malaria Pf/Pv test.²

Tjitra and others² found that the ICT Malaria Pf/Pv assay had a sensitivity of 96% and specificity of 90% for the detec-

tion of PF. These values are comparable to the high sensitivities (range, 92–100%) and specificities (range, 84–99%) previously reported for the ICT Malaria Pf assay^{14–16} and are not surprising because both assays use the same monoclonal antibody to HRP2. In contrast to Tjitra and others,² who found that the ICT Malaria Pf/Pv assay had a sensitivity of > 85% for PF even with parasite densities as low as < 50/ μL , we found that the sensitivity of this assay dropped markedly with decreasing parasite densities (100% at $\geq 500/\mu\text{L}$, 47.8% at 100–499/ μL , and only 10.9% at < 100/ μL). Even more significantly, the various lots that we evaluated had marked differences in their sensitivity for PF; lot no. 011940 had a sensitivity of 73.7% and lot no. 012379 had a sensitivity of only 21.8% (see Table 4).

Although it is difficult to determine why the sensitivity of the ICT Malaria Pf/Pv assay was so much lower in this study than in previous studies, differences in patient populations may have accounted for the low sensitivity. All patients participating in Tjitra and others' study (even those with PF parasite densities < 50/ μL) were symptomatic individuals attending a primary health center who had a presumptive diagnosis of clinical malaria. In contrast, fewer than 10% of individuals who were PF positive by microscopy in this study had symptoms indicative of malaria (fever, headache, or muscle pain within the last 48 h). It is possible that patients enrolled by Tjitra and others² had decreasing parasite densities that might have been significantly higher a few days or weeks earlier. FP tests for HRP2 were twice as likely in their study of individuals with a history of chloroquine in the preceding 4 weeks compared with those without recent treatment. One half of all patients with FP results in their study had chloroquine within the preceding 4 weeks, whereas one third were infected with gametocytes. These data clearly suggest that HRP2 may persist even after parasites have cleared and that the high sensitivity observed at low parasite densities may actually reflect higher densities than actual parasite numbers at the time of the blood draw suggest.

Alternatively, lot-to-lot variation may have been responsible for the low sensitivity of the ICT Malaria Pf/Pv assay observed in this study. Data from this study (see Table 4) clearly demonstrates that significant lot-to-lot variation was present in at least some of the different lots we tested. Although it is not possible to make direct comparisons with previous studies evaluating either the ICT Malaria Pf/Pv or the ICT Malaria Pf assay, the fact that assay sensitivity in this

TABLE 3

Performance characteristics of the ICT Malaria Pf/Pv assay at different parasite densities relative to those of expert laboratory microscopy for active surveillance for *Plasmodium falciparum* and *Plasmodium vivax*

Trophozoites/ μL (total positive)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Accuracy	J index
<i>P. falciparum</i>						
>500/ μL (13)	100.0% (71.9–100.0)	99.7% (99.3–99.9)	72.2% (46.4–89.3)	100.0% (99.7–100.0)	99.7%	0.99
100–499 (23)	47.8% (27.4–68.9)	99.7% (99.3–99.9)	68.8% (41.5–87.9)	99.4% (98.8–99.7)	99.1%	0.48
<100/ μL (46)	10.9% (4.1–24.4)	99.7% (99.7–99.9)	50.0% (20.1–79.9)	97.8% (97.1–98.4)	97.6%	0.11
Total (82)	35.4% (25.3–46.8)	99.7% (99.3–99.9)	85.3% (68.2–94.5)	97.2% (96.4–97.9)	97.0%	0.35
<i>P. vivax</i>						
>500/ μL (3)	66.7% (12.5–98.2)	99.9% (99.6–100.0)	66.7% (12.5–98.2)	99.9% (99.6–100.0)	99.9%	0.67
100–499/ μL (19)	0.0% (0.0–20.9)	99.9% (99.6–100.0)	0.0% (0–94.5)	99.0% (98.4–99.4)	98.9%	0.00
<100/ μL (76)	0.0% (0.0–6.0)	99.9% (99.6–100.0)	0.0% (0–94.5)	96.0% (95.0–96.8)	96.0%	0.00
Total (98)	2.0% (0.4–7.9)	99.9% (99.6–100.0)	66.7% (12.5–98.2)	95.1% (94.0–96.0)	95.0%	0.02

TABLE 4

Performance characteristics of three lots of ICT Malaria Pf/Pv assay relative to those of expert laboratory microscopy for active surveillance for *Plasmodium falciparum* and *Plasmodium vivax*

Lot number (# tested)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Accuracy	J index
<i>P. falciparum</i>						
012379 (973)	21.8% (12.2–35.4)	99.6% (98.8–99.9)	75.0% (47.4–91.7)	95.5% (93.9–96.7)	95.2%	0.21
011940 (688)	73.7% (48.6–89.9)	99.9% (99.0–100.0)	93.3% (66.0–99.7)	99.3% (98.2–99.7)	99.1%	0.74
011190 (257)	42.9% (11.9–79.8)	100.0% (98.1–100.0)	100.0% (31.0–100.0)	98.4% (95.7–99.5)	98.4%	0.43
All lots (1,943)	35.4% (25.3–46.8)	99.7% (99.3–99.9)	85.3% (68.2–94.5)	97.2% (96.4–97.9)	97.0%	0.35
<i>P. vivax</i>						
012379 (973)	1.5% (0.1–9.1)	99.9% (99.3–100.0)	50.0% (2.7–97.3)	93.2% (91.4–94.7)	93.1%	0.01
011940 (688)	4.3% (0.2–24.0)	100.0% (99.3–100.0)	100.0% (5.5–100.0)	96.8% (95.1–97.9)	96.8%	0.04
All lots (1,943)	2.0% (0.4–7.9)	99.9% (99.6–100.0)	66.7% (12.5–98.2)	95.1% (94.0–96.0)	95.0%	0.02

study was much lower than previously demonstrated suggests that quality control may have been an issue. Data from an evaluation of the ICT Malaria Pf/Pv assay against symptomatic patients in western Thailand indicates that sensitivity of the assay has dropped markedly from that observed previously in this area (Miller et al, 2001, unpublished data). This suggests that the poor sensitivity of the assay for PF observed in this study may not be attributed solely to the fact that blood samples were predominantly obtained from asymptomatic individuals but may, in fact, reflect problems inherent to the ICT Malaria Pf/Pv assay itself.

In contrast to the excellent sensitivity of HRP2 based assays for the detection of PF, Tjitra and others² reported that the overall sensitivity (75%) and PPV (50%) of the ICT Malaria Pf/Pv assay for PV was less than the desirable levels, particularly at levels < 500/μL, where the sensitivity was only 29%. Data from this study (Tables 2–4) clearly demonstrate that the ICT Malaria Pf/Pv assay is not sensitive for the detection of PV. Three blood samples contained > 1,000 PV trophozoites/μL; however, the ICT Malaria Pf/Pv assay only detected HRP2 in two of these individuals. The positive line was very faint in the other two individuals, and it was very difficult to differentiate this weak positive from a false positive because of nonspecific binding of the monoclonal antibody to the substrate.

On the basis of results from this study, we believe that the ICT Malaria Pf/Pv assay is not suitable for use in active malaria surveillance programs in Thailand. The combination of poor assay sensitivity (particularly for PV), low (< 10%) parasite prevalence, and high proportion of asymptomatic individuals with low (< 100/μL) parasite densities suggests that the majority of malaria cases will not be detected using the ICT Malaria Pf/Pv assay. The ICT Malaria Pf/Pv assay has a relatively high price (approximately \$2.33 per test for 1,000 tests when purchased in Thailand), and fewer than 2% (38/1,943) of all assays that we tested gave a positive result.

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