THE ICT MALARIA® PF : A SIMPLE, RAPID DIPSTICK TEST FOR THE DIAGNOSIS OF *PLASMODIUM FALCIPARUM* MALARIA AT THE THAI-MYANMAR BORDER

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Abstract. The ICT Malaria® Pf test for the detection of *Plasmodium falciparum* infection was evaluated in the diagnosis of 305 patients with fever who were admitted to a hospital located on the Thai-Myammar border. All patients were admitted for at least one week to exclude reinfection. The test was performed using admission blood samples collected into ethylenediaminetetraacetic acid. The sensitivity, specificity and accuracy of the test were 92.7%, 95.1% and 94.7% respectively, compared to standard microscopic diagnosis. The ICT Malaria® Pf test is an accurate method for the diagnosis of *P. falciparum* infection. Its simplicity and rapidity make it particularly appropriate for use in remote areas where microscopic examination of blood films is unavailable.

INTRODUCTION

Malaria is the most important parasitic infection in the world (White, 1996). In patients with severe disease, the mortality rate is between 10% and 50% (Molyneux *et al,* 1986; Wilairatana and Looareesuwan, 1995). Early diagnosis of *P. falciparum* malaria enables treatment to be instituted early, thereby reducing morbidity and mortality (Looareesuwan and Wilairatana, 1997). The definitive diagnosis is currently based on microscopic examination of blood films, a technique which is time consuming, labor intensive and requires technical skill. Recently, a rapid nonmicroscopic test, the ICT Malaria ® Pf test, has been introduced. The test is based on immunochromatographic technology (ICT) for the detection of *P. falciparum* trophozoite-derived histidine-rich protein-2 (PfHRP-2) (Parra *et al,* 1991). The test kit is simple to use, cheap, and requires less than eight minutes to perform. Here, we evaluated the accuracy of this test in the diagnosis of acute, uncomplicated falciparum malaria on the Thai-Myanmar border.

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MATERIALS AND METHODS

The study was carried out at Somdej Prachao Taksin Hospital, Tak, Thailand on the Thai-Myanmar border; an area endemic for highly multidrug resistant falciparum malaria. Patients admitted to the hospital with asexual forms of *P. falciparum* in thick or thin blood films and symptoms of acute falciparum malaria were studied; patients were excluded if they showed parasitemia of greater than 3% or gave a history of receiving malaria treatment within the previous week. All patients were admitted for at least 7 days to prevent re-infection and were treated with artesunate and mefloquine.

The ICT Malaria® Pf test was used to detect circulating PfHRP-2 in samples of whole blood collected into ethylenediaminetetraacetic acid on admission. The test uses two antibodies specific for PfHRP-2 antigen. One of the antibodies is attached to visible colloidal gold and impregnated into a sample pad, while the second antibody is immobilized in a line on a membrane test strip. 10 µl of whole blood is added to the sample pad where lysis occurs and any PfHRP-2 antigen present binds to the colloidal gold-labeled antibody. On adding the running buffer to the sample pad, the blood and

labeled antibody migrate up the test strip to cross the second antibody line. In a positive sample, PfHRP-2 complexed with the gold-labeled antibody is captured by the antibody on the membrane and a pink line is formed. In a negative sample, no pink line is formed.

Parasite counts were also performed on admission. Thick and thin blood films were Giemsa-stained and examined by light microscopy. Parasitemia was counted against 1,000 red blood cells in thin films, or against 200 white blood cells in the thick film. ICT testing and examination of Giemsa- stained blood films were performed by different technicians. Positive ICT Malaria® Pf test results and reports of *P. falciparum* in the blood were confirmed by an expert (PT). The presence of microscopically detectable parasitemia was taken as the standard to determine sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the ICT Malaria® Pf test.

RESULTS

Three hundred and five patients presenting with fever were recruited into the study. Table 1 shows the results of the ICT Malaria® Pf test compared to blood films. Blood smears on patients with negative blood films on admission were repeated every day for 7 days to exclude the diagnosis of malaria. There were three false negative ICT results, corresponding to parasite counts of 4,420, 4,616 and 6,120 per µl respectively. One patient with *P. vivax* malaria had a positive test. The ICT test was positive in 13 patients with repeatedly undetectable malaria parasitemia on blood films. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the ICT test were 92.7%, 95.1%, 74.5%, 95.1% and 94.7% respectively (Table 2). The lowest parasitemia detectable by the ICT Malaria® Pf test was 25 parasites per µl. Table 3 shows the comparisons between ICT Malaria® Pf and *Para*Sight™ -F tests. While the two tests detect the same antigen (PfHRP-2), their format is different.

DISCUSSION

The results of this study demonstrate that the ICT Malaria® Pf test provides an accurate diagnosis of falciparum malaria in patients presenting with fever. The high sensitivity of 92.7% is in agreement with previous reports using a different dipstick test (Shiff *et al,* 1993; Beadle *et al,* 1994; Premji *et al,* 1994; Karbwang *et al,* 1996). The 3 false negative results could have been due to insufficient antigenemia during early malaria infection; the blood films of these three patients showed nearly all ring forms of the parasite - few late stage parasites were seen. Although PfHRP-2 antigen is derived from trophozoites, it is possible that insufficient PfHRP-2 for detection is produced duing early infection. Karbwang *et al* (1996) found a lack of correlation between the amount of PfHRP-2 antigen test and small ring form parasitemia; the level of circulating PfHRP-2 should correlate better with the trophozoite load.

Diagnosis by examination of blood films has several major disadvantages in endemic areas, since it requires a microscope and staff trained to stain specimens and perform microscopy. There is also an inevitable delay between the taking of blood and the provision of a result (Molyneux, 1995). The ICT Malaria® Pf is quick (taking less than 8 minutes for each test) and easy to perform. It should also permit more precise detection of treatment failures and lead to appropriate changes in therapy and a more confident approach to treatment (Karbwang *et al,* 1996).

However, there are limitations to the test. Since the test indicates the presence or absence of PfHRP-2 antigen in the blood specimen, the antigen may be detectable following drug treatment even when parasites are no longer visible in the blood by microscopy. In our study, the 13 false positive test results could have been due to residual circulating antigenemia (WHO, 1995). Beadle *et al* (1994) showed that PfHRP-2 antigen was not detectable in blood 6 days after starting curative chemoterapy, and suggested that such circulating antigens did not often lead to false positive tests. Gametocytes do not appear to produce false-positive results, but Karbwang *et al* (1996) found detectable PfHRP-2 antigen up to 14 days after instituting antimalarial treatment. Even though we excluded patients who had received treatment in the week prior to admission, false positive results were most likely the result of residual circulating antigen from prior treated episodes of malaria. The alternative explanation for false positive ICT Malaria® Pf test may be that the test results are positive but the slide films

Table 1

Result of the ICT Malaria® Pf test on admission (n=305).

Diagnosis	No. of samples positive by blood film	No. of samples positive by ICT Malaria® Pf
Malaria (n=49)		
P. falciparum	37	34
P. falciparum and *P. malariae*	4	4
P. vivax	8	1
Non-malaria (n=256)	0	12

Table 2

Accuracy of the ICT Malaria® Pf

		Microscopic diagnosis +	–
ICT Malaria® Pf diagnosis	+	38	13
	–	3	251

Sensitivity = 38/(38+3) = 0.927; Specificity = 251/(251+13) = 0.951; Positive predictive value = 38/(38+13) = 0.745; Negative predictive value = 251/(251+3) = 0.988; Accuracy = (38+251) / (38+3+13+251) = 0.947

give the false negative. However, false negative blood films rarely occur because thick films were examined (at least 100 oil immersion microscope fields with extended slide examination in the case of negativity). PCR could resolve this issue.

In Table 1, of 8 patients with positive *P. vivax* on blood films, there was one positive test for the ICT Malaria® Pf test. The positive vivax may be a double infection with microscopy failing to detect *P. falciparum*. However, it is unusual when thick films are carefully examined (at least 100 oil immersion microscope fields). The assay for *P. falciparum* appears to show little cross reaction with other species of human parasites, although the data to support this conclusion are limited (WHO, 1995). Although the ICT Malaria® Pf test detects only *P. falciparum* and lacks a *P. vivax* component phase, the test is still useful because in falciparum malaria management delayed diagnosis is one of the major contributing factors to delayed treatment which may lead to a high mortality rate. Comparing with falciparum malaria, delayed diagnosis of vivax malaria unusually contributes to fatal outcome. However, in the future the test for both *P. falciparum* and *P. vivax* components should be developed in one dipstick.

Although there is not any comparative validity study between the ParaSight™ -F and ICT Malaria® Pf tests, the present data (Table 3) show that there are not great differences in sensitivity and specificity of both tests in diagnosis of *P. falciparum* except specificity was low in the report of Karb-

Table 3

Comparisons between *Para*SightTM -F and ICT Malaria® Pf tests.

	*Para*Sight™ -F			ICT Malaria® Pf
	Beadle *et al*, 1994	Karbwang *et al*, 1996	Shiff *et al*, 1994	
Sensitivity	96.5-100%	98.7%	90.9%	92.7%
Specificity	88-95%	50%	97%	95.1%
Simplicity	simple	simple	simple	simpler
Time for each test	20 minutes	ND*	<10 minutes	<8 minutes

* No data available

wang *et al* (1996). The ICT Malaria® Pf test appears simpler and more practically useful than the *Para*Sight™-F test. Comparing with the ICT Malaria® Pf test, the *Para*Sight™ -F test has (i) more steps in dipstick assay, *eg* tranfering blood sample to lysing fluid and adding the washing fluid to well, (ii) needs more material to be used in each assay, and (iii) takes more time (10 to 20 minutes), possibly because of more diagnostic steps.

Rapid diagnosis of malaria at the village and district levels helps to institute to rapid treatment and reduces morbidity and mortality (Shiff *et al,* 1994). The ICT Malaria® Pf test appears to be a useful tool to implement this strategy. It is practical, does not require electricity or special equipment, requires only a small amount of whole blood and can be taught to village health workers. However, further studies in other malarious areas are warranted to confirm our findings.

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