A COMPARISON OF THREE MALARIA DIAGNOSTIC TESTS, UNDER FIELD CONDITIONS IN NORTH-WEST THAILAND

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Abstract. A hospital-based trial to compare the clinical diagnosis of malaria; microscopy, and a rapid diagnostic antigen capture detection dipstick (*Para*Sight™-F) was conducted in North-west Thailand. 301 people who presented themselves at the hospital were selected. 204 (68%) were presumptively diagnosed as having malaria by the triage nurses; 64 (21.3%) were *P. falciparum* parasite positive, and 94 (32%) tested positive for *P. falciparum* with the *Para*Sight™-F test strips.

There was no association between hemoglobin levels (<10g/dl and ≥ 10g/dl) and malaria, and although there was a good statistical association between temperature and malaria the specificity, sensitivity and positive predictive values were all low, indicating that temperature alone is a poor indicator of the disease.

Based on the microscopy results, we found that a presumptive clinical diagnosis dramatically over-diagnosed malaria, and similarly there were a large number of false positives using the *Para*Sight™-F test. We believe that many of the patients had received some form of malaria treatment prior to presentation at the hospital, and that the high number of false positives are explained by persistent antigenemia and the possibility of there being sequestered parasites following incomplete chemotherapy.

INTRODUCTION

The diagnosis of malaria appears to be in the midst of a transition stage. Since the association between malaria fever and malaria parasites was established, malaria has been diagnosed either clinically or microscopically. As long ago as 1971 it was shown that malaria diagnosed on symptomatic evidence alone frequently results in overdiagnosis, particularly in children (Hendrickse *et al*, 1971). In Malawi it has been demonstrated that using microscopy to confirm malaria, as opposed to using presumptive diagnosis, can save 3% of the annual drugs budget of a hospital (Jonkman *et al*, 1995). Over the years antibody detection tests have been developed in an attempt to 'improve' the diagnosis of malaria, but throughout these developments, microscopy has remained the *gold standard* against which all other tests have been evaluated. The main criticisms against antibody detection tests have been that they are expensive; time consuming, and lack sensitivity because antibodies remain in the system after the parasites have been eliminated, resulting in a high proportion of false positives. Gillespie and Chiodini (1988) summarize the role of serology in the diagnosis of malaria, pointing out that although serology does have a part to play, conventional parasitological techniques remain the investigation of choice.

More recently, antigen tests have been, and continue to be, developed. These have the reputed advantage, over antibody tests, of producing few false positive results because antigens are quickly eliminated from the system following the elimination of the malarial parasites. A further advantage is that antigen detection tests can be incorporated onto disposable 'dip-sticks' and results are available after approximately ten minutes. One such test (*Para*Sigh™-F test strips) has been developed and field-tested in Tanzania and in Thailand (Shiff *et al*, 1993; Premji *et al*, 1994; Banchongsorn *et al*, 1996). As with all previous tests, the antigen detection tests have had to be evaluated against Giemsa stained blood films examined by experienced microscopists.

We report a hospital-based trial in north-west Thailand, to compare the clinical diagnosis of malaria; microscopy and a rapid diagnostic antigen capture detection dip-stick (*Para*Sight™-F) designed to detect trophozoite-derived histidine-rich protein-2 from *Plasmodium falciparum* infections. We also compared hemoglobin levels with parasite counts.

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MATERIALS AND METHODS

The study was conducted in the Srisungval Hospital, Mae Hong Son in North-west Thailand, 20 km from the Myanmar border, in April-May 1996 (the dry season). Mae Hong Son Province is the most sparsely populated in Thailand, with a population of 160,000. Situated at 1,800 m a.s.l., more than 75% of the province consists of mountains and tropical forest. Malaria transmission is perennial, with seasonal peaks.

The subjects were 301 people selected by triage from those presenting themselves at the hospital. They came from a radius of 6 to 67 km from Mae Hong Son town; had an age range of <1 month to 81 years, and the male: female ratio was 6:4. The average daily wage was c.£2.50 (US$4.00).

Four malaria diagnostic methods were evaluated:

1. Microscopy: thick and thin blood films were made from finger-prick blood. Thick films were stained with 10% Giemsa's stain, and the thin films with Leishman's stain. The thick films were examined by one microscopist and the thin by another. The results of their findings were compared at the end of the study to exclude any suggestion of complicity. Parasite density was evaluated using the method of Greenwood and Armstrong (1991).

2. The *Para*Sight™ -F test strips, designed to indicate the presence of *Plasmodium falciparum* Pf HRP-2 antigen, were tested on 296 of the subjects.

3. Clinical malaria was diagnosed by four triage nurses. The diagnosis was based on medical history and symptoms, which included a history of fever, temperature, headache, vomiting, loss of appetite, and general joint and muscle pain.

4. Using the method of Stott and Lewis (1995), hemoglobin estimations were made, and anyone having a Hb level of <10g/dl was considered anemic.

Table 1
Basic results of the diagnostic tests.

	No. positive	n	% positive
P. falciparum (microscopy)*	64	301	21.3
P. vivax (microscopy)*	39	301	13.0
*Para*Sight™-F	94	296	31.8
Clinical	204	301	68.0

* 5 of these infections were mixed *P. falciparum*/*P. vivax*, *ie* 98 malaria infections (32.6%).

Table 2
A: - comparison between microscopy and *Para*Sight™-F test;
B: - comparison between microscopy and presumptive diagnosis;
C: - comparison between *Para*Sight™-F test and presumptive diagnosis, and
D: - comparison between microscopy and the *Para*Sight™-F test excluding all *P. vivax* infections.

A Diagnosis by *Para*Sight™-F	*P. falciparum* by microscopy +	-	
+	55	39	94
-	7	195	202
	62	234	296

Sensitivity: 88.7%; Positive predictive value: 58.5%
Specificity: 83.3%; Negative predictive value: 96.5%

B Presumptive clinical diagnosis	*P. falciparum* by microscopy +	-	
+	62	142	204
-	2	95	97
	64	237	301

Sensitivity: 96.9%; Positive predictive value: 30.4%
Specificity: 40.1%; Negative predictive value: 97.9%

C Presumptive clinical diagnosis	Diagnosis by *Para*Sight™-F +	-	
+	80	124	203
-	14	79	93
	94	202	296

Sensitivity: 85.1%; Positive predictive value: 39.2%
Specificity: 39.1%; Negative predictive value: 84.9%

D Diagnosis by *Para*Sight™-F	*P. falciparum* by microscopy +	-	
+	55	33	88
-	7	168	175
	62	201	263

Sensitivity: 88.7%; Positive predictive value: 62.5%
Specificity: 83.6%; Negative predictive value: 96.0%

Table 3
Summary finding of the *P. falciparum* gametocyte carriers.

Para-F test	Age (years)	Sex	Pf trophs[1]	Pv trophs	Pv gamet	Treat-ment
Positive	35	M	<500	0	0	No
Positive	12	F	12,000	0	0	Yes
Negative	20	F	6,500	+	0	Yes
Negative	65	M	0	0	0	No

[1]Trophozoites per µl blood

Table 4
Association between temperature and malaria.

Temperature	Malaria		
	Yes	No	
≥ 37.5°C	35	38	73
≤ 37.4°C	21	77	98
	56	115	171

χ^2 (Yates correction) = 12.18; p = 0.0005
Sensitivity: 62.5%, specificity: 66.9%, positive predictive value: 47.9%

RESULTS

The basic results are shown in Table 1. 204/301 (68%) subjects were diagnosed as having malaria by the triage nurses (presumptive clinical diagnosis); 98 (32.6%) were parasite positive (64 had *P. falciparum*, 39 had *P. vivax* - of which five had mixed infections) identified by microscopy, and 94 (32%) tested positive for *P. falciparum* with the *Para*Sight™-F test strips. There was no disparity between the results of the thick and thin blood films.

Table 2 shows the comparison between each of the tests. Of the 64 people who had *P. falciparum* infections diagnosed microscopically, four had gametocytes present and three also had trophozoites. The *Para*Sight™-F test was negative for two of the gametocyte carriers, one a woman of 20 years (who also had trophozoites and a *P. vivax* infection) and one a 65-year old man with no trophozoites. Table 3 summarizes the finding for the four *P. falciparum* gametocyte carriers. Two of the subjects, one who was *Para*Sight™-F positive and another who was *Para*Sight™-F negative, stated that they had both received antimalarial treatment prior to visiting the hospital.

Hemoglobin levels below 10g/dl were recorded from 143 subjects (considered anemic), and 151 subjects had higher Hb levels. There was no association between Hb levels (defined as anemic or non-anemic) and malaria, *ie* (χ^2 tests gave p>0.05; sensitivity, specificity and positive predictive values were all below 50%.

171 of the 301 subjects had their temperature recorded (although all subjects had it taken). 56 of the 171 had malaria (either *P. falciparum* or *P. vivax* or both) (Table 4).

Although the χ^2 test shows that there was a good statistical association between temperature and malaria, the specificity, sensitivity and positive predictive values are all low, indicating that temperature alone is not a good diagnostic parameter.

The costs of conducting a clinical examination; a clinical examination plus microscopy and a clinical examination plus *Para*Sight™-F were £10.00 (US $16.00); £11.32 (US$18.00) and £11.29 respectively. These costs included consultation, drug, laboratory and travel time, but excluded hospital overheads and the cost of a microscope. The purchase price of each *Para*Sight™-F test strip was US$1.00, and the cost of a microscopical examination of a thin blood film was also US$1.00 at this specific location.

A comparison of six previously published studies that evaluated the *Para*Sight™-F test (Table 5) shows that the four parameters: sensitivity, specificity, positive and negative predictive value, all fall within similar ranges, generally above 90%. The study involving the Tanzanian Village Health Workers had relatively low values for sensitivity, specificity and negative predictive values, and the Indonesian study had a low positive predictive value. Our study had lower values for all parameters, but the most significant one was that of the positive predictive value, due entirely to the high number of false positives.

A test with a high sensitivity has a low number of false positives. However, it is more important to know the ability of a positive assay to predict the probability of infection, *ie* its positive predictive value.

Table 5
Comparison of sensitivity, specificity, positive and negative predictive values.

Country	Sensitivity	Specificity	Pos predict value	Neg predict value	References
Tanzania	95.9	90.1	88.6	96.4	Shiff *et al* (1993)
Tanzania	89.0	84.0	91.0	82.0	Premji *et al* (1994)
Thailand	93.4	98.2	95.0	97.6	Banchongakorn *et al* (1996)
France	93.9	98.8	88.6	99.4	Uguen *et al* (1995)
Brazil	93.9	89.1	86.8	95.0	Anonymous (1993)
Indonesia	97.4	86.2	80.9	98.2	Anonymous (1993)
Thailand	88.7	83.3	58.5	96.5	This study

DISCUSSION

An interesting observation in this trial, compared to those conducted in Tanzania and previously in Thailand, is that the rapid diagnostic test (*Para*Sight™-F) diagnosed more (94) cases of *P. falciparum* than did microscopy (62), *ie* a high false positive rate. In the other trials microscopy always revealed more cases than the antigen test. We believe that this is because the trial was conducted on self-selected patients presenting at a hospital (*cf* malaria clinics and health centers). Patients who attend a hospital for treatment are generally in a more serious medical condition than those presenting at a clinic. It is therefore highly likely that they have tried some form of treatment before going to a hospital (as a last resort). This treatment is likely to have been inappropriate or an incomplete dose. In the case of malaria, partial treatment suppresses parasitemia, with a high possibility of sequestering or contributing to submicroscopic parasite levels. In all these cases there is still the probability of antigens being present, which were detected by the *Para*Sight™-F test.

Of the 39 subjects who were *Para*Sight™-F positive but *P. falciparum* microscopy negative, six were infected with *P. vivax*. Seventeen of the 39 subjects said that they had received malaria treatment during the preceding 7 days, but only one of these had *P. vivax*. One can only speculate on why there should be so many false positive cases. It is unlikely that any parasites were missed since all the blood slides were examined by two microscopists, and there was complete agreement at the end of the trial. It seems likely that many of the subjects, due to the availability of antimalarial drugs in the area, received some sort of medication prior to their attendance at the hospital, and/or that they obtained their treatment from the private sector and did not consider that it was appropriate to reveal this information. Beadle *et al* (1994) state that PfHRP -2 antigen is not detectable in blood 6 days after starting curative therapy, and suggests that circulating antigens do not often lead to false positive tests. However, Shiff *et al* (1993) state that antigenemia may persist up to two weeks after the clearance of parasites, and further suggest that sequestered parasites at the time of examination could be expected to lead to a number of apparently false positive results. We believe that our findings are in concordance with the latter explanation.

Although there were very few patients with gametocytes, our conclusions tend to support the findings of Beadle *et al* (1994) who found no evidence that the presence of gametocytes were responsible for false positive results.

The clinical diagnosis compared to both microscopy and the *Para*Sight™-F test had good sensitivity, but poor specificity and positive predictive values. The sensitivity must be interpreted with caution, since overall clinical diagnosis gave a prevalence of 68% when microscopy gave 32.5%. The low specificity demonstrated that the diagnostic criteria were not specific enough to differentiate between illness due to malaria and illness due to other causes. Similarly, the positive predictive values were low, indicating that the clinical criteria were not precise enough to accurately predict malaria illness. In our study, 73/171 (43%) patients presented with a fever, although only 35 of the fever cases had *P. falciparum* parasites detected (21 parasite carriers had no fever). Since fever is normally considered one of the diagnostic features of clinical malaria, it is probably this symptom more than any other that contributes to the over-diagnosis of malaria by peripheral health staff. Thus, presumptive diagnosis of malaria will result in over-treatment, whereas in this area, microscopy-diagnosed malaria probably results in under-treatment

because of the high rate of drug use.

We were concerned that the low positive predictive value resulting from our comparison of the *Para*Sight™-F test and microscopy (Table 2A) was because the test strip was cross reacting with *P. vivax* antigens. We therefore excluded the mono-*P. vivax* infections from the data and repeated the statistical tests (Table 2D). The results were virtually unchanged from the overall results, lending emphasis to our conclusion that, as an antigen detection test, the *Para*Sight™-F test correctly identified Pf HRP-2 antigen when parasites were absent, sequestered or at sub-microscopic levels.

However, one could argue that 6 *P. vivax* infections/39 *Para*Sight™-F positive cases (15.4%) indicate a reasonably high cross-reactivity with the test. This suggests that the test would be inappropriate in an area of high *P. vivax* endemicity, *eg* Nepal (Sherchand, 1996).

We did not determine the origin of the patients presenting for this study, but in the Thai/Myanmar border area people do cross into Thailand for malaria treatment. Often they have taken quinine and/or artesunate which is readily available from the private sector at 2-3 bhat (5-8 pence) per tablet - sufficient to suppress parasitemia, but not to effect a cure.

The question of possible cross-reactivity raises other points:

1. The argument that a patient has taken a drug may not always be applicable as *P. falciparum* parasites may become sequestered without drug inducement, leaving the *P. vivax* parasites visible;
2. After treating a *P. falciparum* case, *P. vivax* enters the circulation from the liver whilst HRP-2 antigen is still present, and
3. With a heavy *P. vivax* infection, it may not be possible to detect *P. falciparum* parasites (particularly free-floating merozoites from ruptured schizonts) on a thick blood film.

We understand that the price of the *Para*Sight™-F test strips varies from country to country (in the UK a 20-test *Para*Sight™-F kit costs £78+VAT), but whether this variation is due to internal affordability (what the market can bear) or because the test is still in the developmental/experimental stage is unknown. The cost of microscopy does vary from country to country, and is dependent on salary variations and the cost of consumables. The question therefore arises whether public sector finances can afford to purchase rapid antigen detection tests for future routine malaria diagnosis. There is little doubt that these tests, and also microscopy, are more accurate than presumptive clinical diagnosis at most health provider centers in endemic areas, but do not differentiate between malaria parasitosis and malaria illness. It is also true to say that many, if not most, of the rural health providers (both public and private) in endemic areas do not use microscopy for the diagnosis of malaria. The future of accurate malaria diagnosis would therefore seem to lie in whether it can be shown that there is a cost benefit to both the provider and the recipient (Shiff *et al*, 1994; Jonkman *et al*, 1995). If, for example, the saving on drugs is greater than the cost of diagnosis by either microscopy or antigen detection, then the provider would be well advised to invest in either test. However, it is becoming increasingly common for the patient to have to bear the cost of both diagnosis and treatment at public sector health facilities (and always with the private sector). In these situations the recipient has little power to influence decisions, other than to refuse expensive medication. It therefore becomes a moral obligation for the provider to implement the most appropriate strategy.

Additional emphasis for the introduction of accurate malaria diagnostic tools is given by Phillips and Phillips-Howard (1996). They consider the growth in drug resistance and the need to use more expensive drugs. Thus, the use of expensive drugs on suspected cases who do not have malaria becomes a major factor in the cost of malaria control. Consequently, with the increased frequency of drug resistance and the use of more expensive drugs; the use of cheap, specific diagnostic tools becomes increasingly important.

ACKNOWLEDGEMENTS

We would like to thank the staff of the Srisungval Hospital, Mae Hong Son for their willingness and help during the course of this trial, and the people of the area without whose co-operation this study could not have been conducted. This study was supported by Dr Surang Tanpradist, Director, Malaria Division, Ministry of Public Health, Nonthaburi and Dr Somsak Prajakwongse, Director, Malaria Region 2, Chiang Mai, Thailand, and the DFID-funded Malaria Work Programme of the Liverpool School of Tropical Medicine To them all we extend our appreciation. Dr B Dando, WHO Representative for Ghana, supported JS's overseas visit; we gratefully acknowledge his contribution. Financial support for JS was provided by WHO AFRO.

Prof M Hommel kindly commented on the manuscript.

This study was partly financed by the DFID-funded Malaria Work Programme of the Liverpool School of Tropical Medicine. However, the Department for International Development can accept no responsibility for any information provided or views expressed.

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