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Evaluation of a rapid whole blood immunochromatographic assay for the diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* malaria

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(Index words: Rapid diagnostic test, sensitivity, specificity)

Abstract

Objective Microscopic examination of blood smears is the 'gold standard' for malaria diagnosis, but is labour intensive and requires skilled operators. *Plasmodium vivax* malaria accounts for up to 70% of infections in Sri Lanka. The objective of this study was to determine the effectiveness of an immunochromatographic test which can detect both the species of *Plasmodium, P. vivax and P. falciparum,* present in Sri Lanka.

Design Prospective study from May 2001 to March 2002.

Setting and methods All persons above 5 years of age who presented to the Malaria Research Station, Kataragama or the Anti-malaria Clinic, Kurunegala, with a history of fever were recruited to the study. Thick and thin blood smears were examined for malarial parasites. The rapid diagnostic test (RDT), ICT Malaria P.f/P.v (AMRAD ICT, Australia) was performed simultaneously by an independent investigator. The severity of clinical disease of all patients was evaluated.

Results The study sample comprised 328 individuals of whom 126 (38%) were infected, 102 with *P. vivax* (31.1%) and 24 with *P. falciparum* (7.3%). The RDT was found to be highly sensitive (100%) and specific (100%) for the diagnosis of *P. falciparum* when compared with field microscopy. The sensitivity for the diagnosis of *P. vivax* malaria was only 70%. When *P. vivax* parasitaemia was greater than 5000 parasites/μL the RDT was 96.2% sensitive. A significant association was noted between the band intensity on the dipstick and both peripheral blood parasitaemia ($p<0.001$) and clinical severity of disease with *P. vivax* ($p=0.011$).

Conclusions The ICT Malaria P.f/P.v test can be used in Sri Lanka in the absence of microscopists.

Introduction

One major contributing factor hindering malaria control is that the laboratory diagnosis of malaria is nearly exclusively dependent on microscopy, a valuable technique when performed correctly but unreliable and wasteful when poorly done. Although microscopic diagnosis may be available at more central levels of the health care system, it is often unreliable or absent in remote areas where health facility coverage is low and the population at the risk of contracting malaria is high [1].

The development of rapid and specific diagnostic tests to identify individuals infected with malaria is of paramount importance to control the disease [2]. Over the past 10 years the world has seen the development of RDTs for malaria using immunochromatographic test strips, which might offer a valid alternative to or complement microscopy [3]. The test kits have been found to be useful in malaria control programmes, as well as in special situations such as emergencies, epidemics and the diagnosis of malaria in returning travellers [1].

Studies have been conducted in Sri Lanka using the Parasight-F test based on the detection of HRP-2 antigen of *P. falciparum* [4]. The major disadvantage with this RDT was that it could not detect *P. vivax* malaria which constitutes 70% of the infections in Sri Lanka. Currently, tests to detect both the species of malaria are being used only by the private hospitals in Sri Lanka.

Materials and methods

A prospective study was conducted in the Kataragama and Buttala areas of the Moneragala district and the Kurunegala district of Sri Lanka during a period of 11 months from May 2001 to March 2002.

All persons above 5 years of age who presented to the Malaria Research Stations in Kataragama and Buttala or the Anti-malaria Clinic, Kurunegala, with a history of fever were recruited into the study after obtaining informed consent. Malaria was diagnosed by detection of parasites in a Giemsa-stained thick blood smear, and species identification and parasite densities (parasitaemia and gametocytaemia) were recorded from thin blood smears. All thick blood films were again examined microscopically at a central laboratory; 400 microscopic fields were examined under the oil immersion to assess sensitivity and specificity of the new RDT.

In all samples the ICT Malaria P.f/P.v assay was performed simultaneously by an independent investigator. It is a rapid in vitro immunodiagnostic test (AMRAD, Australia) for the detection of circulating *P. falciparum* and *P. vivax* antigens in whole blood. Whole blood (15μL) was applied to a sample pad impregnated with colloidal gold-labelled antibodies, which are directed against the

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two malarial antigens. No colour development occurs if the test is negative. The colour changes at different parasitaemias were graded as faint, clear and strong using a pretested colour bar chart.

The severity of clinical disease of all people who presented to the treatment centres with fever, irrespective of being positive or negative for malaria, were evaluated using a clinical evaluation form [5]. The questionnaire contains a series of frequently associated symptoms of malaria which were scored, based on the patients' perception of the symptom, as 0 if the symptom was absent, 1 if the severity of the symptom was mild, 2 if it was of a moderately severe nature and 3 if it was severe. The scores of individual symptoms were summed up to obtain a total clinical score for each participant. The average score for all symptoms was calculated. Individuals positive for malaria were treated with appropriate doses of chloroquine and primaquine in accordance with WHO criteria [6]. All those who were positive for *P. falciparum* were requested to report to the treatment centres on days 7 and 14, and if symptoms persisted, to repeat the blood smear. They were treated with pyrimethamine/sulfadoxine if parasitaemia existed.

Data analysis

The variables measured were the numbers of true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN). Sensitivity was then calculated as TP/(TP+FN), specificity as TN/(TN+FP), the positive predictive value (PPV) as TP/(TP+FP), and the negative predictive value (NPV) as TN/(FN+TN). Data were analysed using two-sample t-tests and analysis of variance (ANOVA) to test for differences in mean between groups. EpiInfo and SPSS statistical software packages were used for data analysis. Ethical approval was obtained from the Ethics committee of the Faculty of Medicine, University of Colombo.

Results

A total of 328 people were included in the study; 272 were from Kataragama and 56 from Kurunegala. The mean age of the group was 28.3 years (range 5.2–72.5); males comprised 64%. By thick and thin blood smear examination 126 (38%) were found to be infected, 102 with *P. vivax* (31.1%) and 24 with *P. falciparum* (7.3%). No mixed infections were detected.

Validation of the immunochromatographic test

The results of parasite detection by microscopic examination of 100 and 400 thin blood film fields and the RDT are compared in Table 1. On examination of 100 microscopic fields on a thin blood smear, 21 individuals tested positive for *P. falciparum,* but 24 tested positive for *P. falciparum* by the RDT. Re-examination of the blood films in a central laboratory (400 microscopic fields) revealed that all 24 individuals who were positive by the RDT had positive blood smears.

Ninety nine individuals were positive for *P. vivax* by blood smear examination, but only 72 gave a positive reaction with the RDT (Table 1). Of the 72 positives, two cases of *P. vivax* were detected only by the RDT.

Table 1. **Comparison of parasite detection by microscopy (for 100 and 400 fields) and immunochromatographic testing**

	ICT Malaria P.f/P.v			
Microscopic result	*P. falciparum*	*P. vivax*	*Negative*	*Total*
100 field examination				
P. falciparum	21	0	0	21
P. vivax	0	70	29	99
Negative	3	2	203	208
Total	24	72	232	328
400 field examination				
P. falciparum	24	0	0	24
P. vivax	0	70	32	102
Negative	0	2	200	202
Total	24	72	232	328

The RDT was found to be highly sensitive (100%) and specific (100%) for diagnosis of *P. falciparum* when compared with field microscopy (100 field examination), with a PPV of 87.5% and NPV of 100% (Table 2). The corresponding sensitivity for the diagnosis of vivax malaria was 70%, significantly lower than for *P. falciparum* ($p<0.001$). However, PPV (97%) and NPV (88.6%) were not significantly different from *P. falciparum*.

Table 2. **Performance of ICT Malaria P.f/P.v relative to those of microscopy for 100 and 400 microscopic fields**

Microscopic result	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*
Field microscopy (100 fields)				
P. falciparum	100.0	100.0	87.5	100.0
P. vivax	70.0	99.0	97.0	88.6
Laboratory microscopy (400 fields)				
P. falciparum	100.0	100.0	100.0	100.0
P. vivax	68.6	99.0	97.0	87.5

NPV = negative predictive value, PPV = positive predictive value

Table 3. **Sensitivity of ICT Malaria P.f/P.v assay by level of *P. vivax* and *P. falciparum* parasitaemia**

Parasites/ìL	*Samples positive by blood film (100 fields)*	*Samples positive by blood film (400 fields)*	*Samples positive by ICT*	*Sensitivity (%) (100 fields)*	*Sensitivity (%) (400 fields)*
P. vivax					
≤500	0	4	0	0	0
501–1000	9	9	1	11.1	11.1
1001–5000	63	63	43	68.1	68.1
>5000	27	27	26	96.2	96.2
P. falciparum					
≤500	1	4	4	100	100
501–1000	9	9	9	100	100
1001–5000	9	9	9	100	100
>5000	2	2	2	100	100

Table 4. **Association between band intensity of ICT Malaria P.f/P.v, mean parasitaemia and mean clinical scores**

Band intensity	n^{α}	*P. vivax*		n^{β}	*P. falciparum*	
		Mean (± SD) parasitaemia/µL	*Mean clinical score*		*Mean(± SD) parasitaemia/µL*	*Mean clinical score*
No band visible	32	1221 (611)	8.47 (4.0)	-		
Faint	45	4266 (3060)	9.38 (4.2)	-		
Clear	25	7296 (4110)	11.68 (3.5)	10	2642 (2702)	9.00 (2.4)
Strong	-			14	1389 (1020)	11.79 (3.7)
F-value		31.143	4.749		2.542	4.323
p-value		<0.001	0.011		0.125	0.049

$^{\alpha}$ Number of cases of *P. vivax,* $^{\beta}$Number of cases of *P. falciparum*

When the RDT sensitivity was determined using 400 microscopic field examination, the test was 100% sensitive and 100% specific for the diagnosis of *P. falciparum* infections (Table 2), with a PPV and NPV of 100% each. In contrast, the corresponding sensitivity for the diagnosis of vivax malaria was 68.6%, significantly lower than *P. falciparum* (p<0.001).

Sensitivity of ICT Malaria P.f/P.v assay by level of parasitaemia

Table 3 shows several strata of *P. vivax* and *P. falciparum* parasite densities, the number of specimens that were positive by dipstick and by blood film within each stratum, and the resulting specificity. The results show that when *P. vivax* parasitaemia is greater than 5000 parasites/µL the RDT is 96.2% sensitive. At lower levels the sensitivity decreases; at 1001–5000 parasites/µL the assay still detects 68% of infections. In *P. falciparum* infections the test could detect <500 parasites/µL with a sensitivity of 100%.

Association between band intensity of ICT Malaria P.f/P.v assay and mean parasitaemia

The band intensity of the RDT of those who tested positive for *P. falciparum* was clear or strong, whereas in *P. vivax* the positive band appeared faint or clear. A significant association was seen between the band intensity on the RDT and peripheral blood parasitaemia

in *P. vivax* infections (p<0.001) with non-appearance of the band with a lower parasitaemia and a clearer band with a mean parasitaemia of 7000 parasites/μL (Table 4). No association was seen between the band intensity and peripheral parasitaemia in *P. falciparum* (p=0.125).

Association between the band intensity of ICT Malaria P.f/P.v assay and mean total clinical score

A significant association was seen between the mean clinical score and band intensity for both *P. falciparum* (p=0.049) and *P. vivax* (p=0.011) infections (Table 4).

Discussion

In Sri Lanka, in remote villages and in the northern and eastern parts where there is a dearth of microscopists, control of malaria is a challenge. Strengthening national capabilities to provide early diagnosis and treatment both within and outside the health services is the highest priority in WHO's action plan for malaria control [2]. If rapid diagnostics are being used in the field, these tests can be used not only for diagnosis of complex emergencies but also for diagnosis and treatment of both *P. vivax* and *P. falciparum* infection by trained staff. In view of this, the recently developed ICT Malaria P.f/P.v has been tested at village level and compared with the results of traditional blood film examination. *P. vivax* is the predominant species in Sri Lanka, accounting for about 70% of all malaria infections, and the Parasight-F test which has been used in Sri Lanka, did not detect *P. vivax* infections [4].

The results indicate that the RDT has a high sensitivity (100%) and specificity (100%) for detecting *P. falciparum* as compared to field microscopy. In the case of *P. vivax* infection the sensitivity of the test was only 70% with a specificity of 99%. These results are similar to previous studies in determining the sensitivity and specificity of ICT Malaria P.f/P.v. [7, 8]

The band intensity of the RDT could be used to give an indication of parasitaemia and clinical severity of the disease in *P. vivax* infections, although limited by its subjective nature. In *P. falciparum* infections although the band intensity did not correlate with parasitaemia, a significant positive correlation was seen with the clinical severity of the disease. Similar findings have been reported for Parasight-F test in Sri Lanka [4].

The mean time required to perform the ICT is 9.6 min as compared to blood film examination which took an average of 26 min a slide. The RDT is faster and requires less training and equipment. The stability of the test cards has been good, as the test strips did not lose their pink colour after one year at an average temperature of 38^{0}C and average humidity of 70%.

Cost is often perceived as being the most important obstacle to the widespread introduction of RDTs. The unit cost is higher for RDTs than for microscopy except at low levels of utilisation. However, this is balanced by the fact that the costs of organisation, supervision, quality control and training of skilled personnel are likely to be lower for RDTs than for microscopy [1].

This study shows that *P. vivax* infections may be underdiagnosed by using rapid diagnostics. Inexpensive, rapid and accurate *P. vivax* specific diagnostic tools that detect low parasitaemia and mixed malaria infections would contribute to both research and efforts to control it. Promoting the use of an "ideal dipstick" for malaria diagnosis and management could lead to a profound improvement in the health of those who live under the threat of malaria. The ideal tool should have a specificity of >90% for 200 parasites/μL. Until such tools are available, ICT Malaria P.f/P.v test should be used with caution for diagnosis of *P. vivax* malaria as an alternative to microscopy in Sri Lanka.

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References

1. World Health Organization. Malaria Diagnosis: New Perspectives. WHO/CDS/RBM/2000. 14. 2000; pp. 1–20.
2. WHO expert committee on malaria. 20th Report. WHO Technical report series 2000; **892**: 1–71.
3. A rapid dipstick antigen capture assay for the diagnosis of falciparum malaria. WHO informal consultation on recent advances in diagnostic techniques and vaccines for Malaria. *Bulletin of the World Health Organization* 1996; **74**: 47–54.
4. Kodisinghe HM, Perera KLRL, Premawansa S, Naotunne T de S, Wickramasinghe AR, et al. The ParaSight-F dipstick test as a routine diagnostic tool for malaria in Sri Lanka. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1997; **91**: 398–402.
5. Karunaweera ND, Grau GE, Gamage P, Carter R, Mendis KN. Dynamics of fever and serum levels of tumour necrosis factors are closely associated during clinical paroxysms in *Plasmodium vivax* malaria. *Proceedings of the National Academy of Science, USA* 1992; **89**: 499–505.
6. WHO Model-prescribing information. Drugs used in parasitic diseases; 2nd edn. Geneva: World Health Organization, 1995; pp. 24–54.
7. Singh N, Saxena A, Valecha N. Field evaluation of the ICT malaria P.f/P.v immunochromatographic test for diagnosis of *Plasmodium falciparum* and *P. vivax* infection

in forest villages of chhindwara, central India. *Tropical Medicine and International Health* 2000; **5**: 765–70.

8. Tjitra E, Suprianto S, Dyer M, Currie BJ, Anstey NM. Field evaluation of the ICT malaria P.f/P.v immunochromatographic test for detection of *Plasmodium falciparum* and *Plasmodium vivax* in patients with presumptive clinical diagnosis of malaria in eastern Indonesia. *Journal of Clinical Microbiology* 1999; **37**: 2412–7.