Rapid Immunochromatographic OptiMAL Assay for Detection of *Plasmodium Vivax* and *Plasmodium Falciparum* Malaria from Two Endemic Districts of Nepal

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Abstract

Introduction	The study was conducted in two malaria endemic districts of Nepal from August, 2000 to October, 2001 to compare the performance of OptiMAL, an immunochromatographic antigen detection assay for the diagnosis of malaria using parasite lactate dehydrogenase (pLDH), against standard *microscopy* in patients with suspected malaria.
Objective	This study compares the performance of OptiMAL, an immunochromatographic antigen detection assay using parasite lactate dehydrogenase (pLDH), with standard *microscopy* in patients with suspected malaria.
Methods	OptiMAL Assay, ICT and Paracheck methods were used for the detection of *Plasmodium vivax* and *Plasmodim falciparum*. Altogether 180 suspected patients more than one year of age attending the outpatients clinic with fever or history of fever were involved in the study.
Results	The results from the OptiMAL test were compared with those obtained by reading 100 fields of traditional Giemsa-stained thick smear blood films. Whole blood samples as well as thick film of 180 patients suspected of having malaria was examined. A total of 66 (36.7%) were blood film positive microscopically, while 64 (35.6%) samples were positive with OptiMAL test. The blood films indicated that 80.3% (53 of 66) of the patients were positive for *P. vivax* and 19.7% (13 of 66) were infected with *P. falciparum*. The results demonstrated that the OptiMAL test had sensitivity of 97% and specificity 98%, respectively.
Conclusion	The OptiMAL assay can be used to diagnose and evaluate the effectiveness of anti-malarial chemotherapy.
Keywords	Optimal, Immunochromatographic antigen detection, Malaria

Introduction

Malaria, being primarily a vector-borne infection, can also be transmitted through blood transfusion. Over 2 billion people live in malarious areas of the world and 200-300 million are infected, with an estimated 3-4 million deaths each year[1]. Political and environmental factors, coupled with drug and insecticide resistance, have exacerbated the situation and today malaria is on the increase once more. There are four species causes human malaria: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovalae*, 95% of all human malaria is caused by *P. vivax* and *P. falciparum*.

Traditional diagnosis of malaria by microscopical examination of blood smears has its own limitation. In endemic areas where adult populations have some level of immunity, parasite density may be lower and microscopic diagnosis may not be accurate and reliable. For such reason, alternative assays, such as the OptiMAL assay, have been developed for field and laboratory use[3]. OptiMAL is a high-throughout antigen detection assay, which may be used with or without instrumentation, requires only finger prick blood, thus offering distinct advantages in the diagnosis and causes of major form of human malaria, *P. falciparum* and *P. vivax*.

The OptiMAL assay is based on the detection of an abundant intracellular metabolic enzyme (pLDH) produced by viable malarial parasites. The pLDH is present in, and released from, parasite-infected red blood cells. The OptiMAL assay detects the pLDH enzyme with a series of monoclonal proteins. Differentiation of malaria species is based on antigens differences between the pLDH isoforms. Because the presence of pLDH in the blood reflects the presence of viable malaria parasites, the OptiMAL assay can be used

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to evaluate the effectiveness of anti-malarial chemotherapy. It is a simple way for health care personnel to check for anti-malaria susceptibility or resistance.

The current study was conducted in two Terai districts Dhanusha and Kanchanpur of Nepal to compare the performance of OptiMAL as an immunochromato-graphic antigen detection assay for the diagnosis of malaria using parasite lactate dehydrogenase (pLDH), with standard *microscopy* in patients with suspected malaria.

Materials and Methods

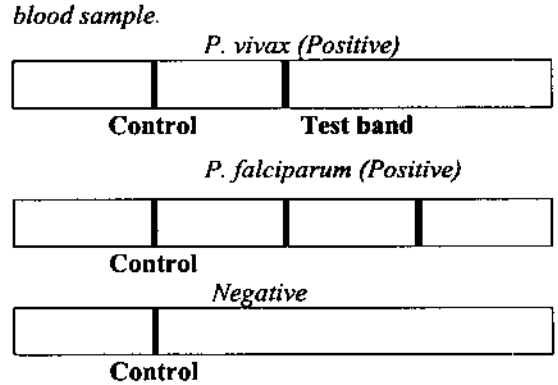
The study was a part of "The value of malaria diagnosis: a preliminary study in the two terai districts of Nepal". Patients more than one year of age and attending the outpatients clinic with fever or history of fever and a suspected diagnosis of malaria from August, 2000 to October, 2001 were involved in the study. Blood samples were collected from persons solely as required to confirm or exclude the presence of malaria parasites. Verbal consent was obtained to use the sample for further testing. Finger prick blood samples (maximum volume of 200µl) were collected from 180 patients. This sample was used to prepare two thick and thin films on two separate slides, which were then labeled with the laboratory number. One of these thick film was then stained with Giemsa's stain and examined using oil-immersion by trained malaria technician. The result of the blood film was reported to the clinician. These results were used for comparison with the OptiMAL test after each day's tests. The second blood film was left unstained and returned to the main laboratory (Tribhuvan University Institute of Medicine Teaching Hospital, Health Research Laboratory Kathmandu) for possible later comparison by the second local expert (Medical Parasitologist) if necessary. After the blood films were made, the remainder of the blood sample was collected into a tube containing potassium EDTA and labeled with the study number. This was matched with the laboratory number only after both the blood film examination and the OptiMAL test had been completed. The EDTA blood taken to the University Research Lab was used for the OptiMAL assay (10 µl blood) and prepares additional thick and thin film for re-examination. Parasite level was calculated by counting the number of parasites against 200 leucocytes and multiplied by 40 as early-described[5].

OptiMAL assay

EDTA blood from each patient was used with the OptiMAL assay. Test strips and reagents were obtained from Flow Inc. 6127 SW Corbett Portland, OR 97201 USA.

30µl (one drop) of colloid/buffer solution was added to a test well on a configured well plate. Four drops of reagents B (80µl of clearing solution) were added to a second test well. 10 µl of blood was then placed into the first test well with gentle mixing. The assay test strip was then placed into the first test well with its wick at the top and the assay test strip was moved to the second test well, which contained the clearing buffer, for an additional 5-10 minutes. Interpretation of the assay results was performed immediately after completion of the clearing step; approximately 15-20 min after the test wsas initiated. A negative control sample, taken from an individual who had not been exposed to malaria for three years, was also test with same method. In the OptiMAL assay, there are two diagnostic zones of reaction containing different antibodies. A monospecific antibody that recognizes only *P. falciparum* is present in the bottom reaction zone. A second pan-specific antibody is present immediately above this zone. This monoclonal antibody recognizes the pLDH isoform of *P. vivax*. A third reaction zone is present at the top of the immunochromatographic test strip where there is an antibody that captures the excess colloid conjugate and serves as a positive control for the assay. The colloid conjugate is coupled with a third monoclonal antibody that is pan-specific. The interpretation of the assay test strip results is as follows: 1) Positive- *P. falciparum*: one control band plus two test bands; 2) Positive- *P. vivax*: one control band plus one test band; 3) Negative- one control band at the top of the test strip, (*e.g.*, shown in Figure 1).

Figure 1. OptiMAL test strips showing detection of *P. vivax, P. falciparum and negative from blood sample.*



ICT and Paracheck Methods

Paracheck and ICT malaria *P. falciparum* were used according to the manufacturer's instruction. However, one such test, the Paracheck test

(manufactured by Orchid Biomedical system, Goa India) detects *P. falciparum* histidine-rich protein 2 (PfHRP-2) in the blood[6,7]. The other test ICT Malaria P.f. (ICT Diagnostics, Sydney, Australia), is an alternative card based test for the immunochromatographic detection of PfHRP-2[8], but both of which detect only *P. falciparum*.

Results

One hundred eighty suspected malaria patients were examined (Table 1). Of the total 66 (36.7%) were positive by blood films examination, while 64 (35.6%) samples were positive with OptiMAL test. However, there was no significant difference between malaria positivity by thick blood film (TBF) and OptiMAL assay (P>0.05), but higher positivity was found from the thick blood film study (Table 1). The blood films indicated that 80.3% (53 of 66) of the patients were positive for *P. vivax* and 19.7% (13 of 66) were infected with *P. falciparum*. The results demonstrated that the OptiMAL test had sensitivity of 97% and specificity 98%, respectively, when compared with thick blood films for the detection of malarial infection. Blood samples not identified by OptiMAL as malaria positive normally contained parasites at concentrations less than 100 parasites/microlitre (less than 0.001% parasitemia) of blood.

Table 1 Distribution of malaria suspected cases, confirm infection and comparison of the OptiMAL test with thick blood film (TBF).

Age group (years)	Suspected cases	Blood film TBF-Positive		Total malaria TBF-Positive*	OptiMAL Positive*
		PV+ve	PF+ve		
1-9	28 (15.6)	14 (26.4)	4 (30.7)	18 (27.3)	17 (26.5)
10-18	31 (17.2)	11 (20.7)	3 (23.1)	14 (21.2)	14 (21.9)
19-27	44 (24.4)	13 (24.5)	2 (15.4)	15 (22.7)	15 (23.4)
28-36	30 (16.7)	4 (7.5)	1 (7.7)	5 (7.6)	5 (7.8)
37-45	26 (14.4)	2 (3.8)	1 (7.7)	3 (4.5)	3 (4.7)
46-54	9 (5.0)	4 (7.6)	0 (0.0)	4 (6.1)	3 (4.7)
55 +	12 (6.7)	5 (9.5)	2 (15.4)	7 (10.6)	7 (11.0)
Total	180	53	13	66	64

Note: Figures in parentheses indicate percentages.
P >0.05

Parasitemia level

The thick blood film identified 2 positive samples of *P vivax* with parasitaemia rate less than 100 (0.001%) were not identified by OptiMAL assay. However, there was 100% agreement between thick blood film results and OptiMAL results for the other 51 samples containing *P. vivax* with parasite level above 100 (Table 2). All patients with *P. falciparum* present in TBF were found positive by OptiMAL test, including the parasitaemia level less than 100 as shown in table 2. The OptiMAL test in the study proved that 100% sensitivity for the detection of *P. falciparum* malaria.

Table 2: Parasite levels detected by thick blood films and by OptiMAL assay

Number of parasites/µl of blood	Malaria parasites species and number of TBF Positive	No of OptiMAL Test Positive
1-100	P. vivax: 3 P. falciparum: 1	1 (2 negative) 1
101-200	P. vivax: 10 P. falciparum: 3	10 3
201-400	P. vivax: 11 P. falciparum: 0	11 0
401-800	P. vivax: 14 P. falciparum: 4	14 4
801-1600	P. vivax: 6 P. falciparum: 2	6 2
> 1601	P. vivax: 9 P. falciparum: 3	9 3

Samples found to *have P. falciparum* positive were further tested with two other commercially available rapid malaria diagnostic tests, Paracheck (manufactured by Orchid Biomedical system, Goa India) and ICT Malaria P.f. (ICT Diagnostics, Sydney, Australia), both of which detect only *P. falciparum*. Only 10 of the 13 (77%) *P. falciparum*-positive blood samples were identified by ICT and 11 of the 13 (84.6%) with Paracheck tests. Thus, OptiMAL accurately identified *P. falciparum* malaria parasites in patient blood samples more often than did the other two commercially available diagnostic tests and established better correlation with traditional blood films in the identification of both *P. vivax* malaria and *P. falciparum* malaria.

Discussion

Traditional diagnosis of malaria using thick blood film examination is most widely used routine method in Nepal[9]. However, microscopic examination is labor-intensive and individuals that examine slides need to be skilled and experienced to differentiate parasites from artifact, but it still remain the gold standard for malaria diagnosis. The current study was subjected to compare the performance of OptiMAL, an immunochromatographic antigen detection assay for the diagnosis of malaria using parasite lactate dehydrogenase (pLDH) with standard microscopy in patients with suspected malaria. The OptiMAL test was designed to diagnose *P. falciparum* and *P. vivax* malaria and to differentiate between them. This differentiation is clinically relevant since the salient feature of malaria diagnosis is to determine whether a malarial infection is positive or negative. A total of 66 were malaria positive by blood film examination, 64 were positive with OptiMAL test. The blood film indicated that 53 patients were infected with *P. vivax* and 13 patients with *P. falciparum*. Overall sensitivity and specificity of OptiMAL test for the diagnosis was better. Similar result from the evaluation of the OptiMAL test samples by Palmer[10] had sensitivities of 94 and 88% and specificity of 100 and 99%, respectively, when compare with thick blood smear for the detection of *P. vivax* and *P. falciparum*. Thus, the OptiMAL test is capable of diagnosing a high proportion of the two malaria parasites that are numerically most important.

Recently developed two immunochromatographic based tests are commercially available for the diagnosis of *P. falciparum* malaria: (1) PfHRP-2 antigen based: the Paracheck (manufactured by Orchid Biomedical system, Goa India)/ the ParaSight-F test (manufactured by Becton Dickinson, Meylan, France)[11,7], and (2) ICT Malaria P.f. (ICT Diagnostics, Sydney, Australia)[8]. In a United Kingdom based study, the Parasight-F test detected *P. falcipaurm* with a sensitivity of 92% and a specificity of 98% [12]. In Nepal, the overall sensitivity was 85% and specificity 60%[7]. *P. vivax* is the predominant species in most of the malarious areas of Nepal (by a factor of 10:1) and a test mainly targets *P. falciparum* but has a degree of cross reactivity with *P. vivax*, will of necessity appear to have an inadequate degree of specificity. However, ParaSight-F test is a very promising test, which certain circumstances would be helpful for ruling out the most dangerous malaria species at the first patient encounter and prevent the development of severe *Falciparum* malaria; in such a situation even a 50% false positivity would be acceptable and would ensure that a majority of cases of *P. falciparum* are given appropriate treatment.

In a field evaluation in the Solomon Islands[8], the ICT test had a sensitivity of 100% and a specificity of 96.2%, compared to thick blood film examination, for the diagnosis of *P. falciparum* malaria. The OptiMAL test was compared with the ParaSight-F test and the ICT malaria P.f test for the diagnosis of malaria in samples from Honduras (Palmer *et al.* 1998). The OptiMAL test had a sensitivity of 94% for the detection of *P. vivax*. The sensitivities for diagnosis of *P. falciparum* were OptiMAL test= 88%, ParaSight-F test= 65%, and the ICT malaria P.f test= 65%. In the present study OptiMAL test had sensitivity of 95% for malaria diagnosis. Blood samples tested by ICT malaria P.f and Paracheck test-HRP-2 based test, both of which detect only *P. falciparum*. Only 10 of the 13 (77%) *P. falciparum* positive blood sample were identified by ICT and 84.6% with Paracheck tests, where as 100% positive was found by OptiMAL. Thus, OptiMAL accurately identified *P. falciparum* malaria parasites in patient blood samples more often than did the other two commercially available diagnostic tests and established better correlation with traditional blood films in the identification of both *P. vivax* malaria and *P. falciparum* malaria.

The advantage of OptiMAL assay over currently available rapid test based on the detection of HRP-2, is that the test follows the course of *P. falciparum* infection, since the data[13] show that a profound decrease in circulating pLDH activity occurs immediately after parasites are cleared from the peripheral blood. Secondly, samples found with *P. vivax* are clearly and easily distinguished from those infected with *P. falciparum*.

There is some justification where the rapid test is necessary in the context of Nepal. Most of the rural clinics (Health Post, Sub-Health Post and Primary Health Care Centre) are unable to

diagnose malaria due to lack of microscopes, microscopic slides, stain and trained technicians. Many people can not afford transportation or do not have transportation facility due to not having motor able road, so they have to walk several hours, some carrying small children, to reach a local clinic; once they have been seen, they do not return for follow-up examinations. Diagnosis must therefore be immediate in order to provide proper treatment. This is of particular importance in areas where viral infection such as Japanese encephalitis, dengue fever and other tropical diseases mimic each other's symptoms (such as fever, chills, headache, convulsion, and coma). In our study with clinical symptom of suspected malaria were actually infected with malaria parasites (37%). In addition, the presence of pLDH in the blood reflects the presence of viable malaria parasites; the OptiMAL assay can be used to evaluate the effectiveness of anti-malarial chemotherapy. It is a simple way for health care personnel to check for anti-malaria susceptibility or resistance in hospital settings or in the field. However, further study are necessary to evaluate the use of OptiMAL assay in monitoring drug therapy and in the detection of drug resistance malarial strains in Nepal.

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