

A Comparison of Antigen Dipstick Assays with Polymerase Chain Reaction (PCR) Technique and Blood Film Examination in the Rapid Diagnosis of Malaria

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Abstract

Early diagnosis of malaria for military personnel training in the field is crucial in providing proper treatment for the infected and in taking appropriate preventive measures for the non-infected. Present preliminary diagnosis of malaria in the field depends on the clinical symptoms of the patients and there is a need for rapid diagnosis of malaria in the field. The presence of drug-resistant strains of the *Plasmodium* species in the region also increases the urgency of finding a quick and sensitive way of identifying the different strains. This study evaluated current methods available for the diagnosis of *Plasmodium falciparum* and *Plasmodium vivax*. The dipstick assays, the ParaSight™ F test and the OptiMAL malaria rapid test were compared with the methods of microscopic examination of blood film and polymerase chain reaction (PCR). On comparison to the blood film and PCR methods, the ParaSight™ F test has specificity of 98.6% and sensitivity of 91% for *P. falciparum* detection. The OptiMAL malaria rapid test has a specificity of 100% and 98.4% and sensitivity of 92.8% and 92.6% for *P. vivax* and *P. falciparum* detection respectively. We conclude that both tests are suitable for use for rapid malaria diagnosis in the field but the OptiMAL rapid malaria test, which can detect both *vivax* and *falciparum* malaria, would be more useful.

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Key words: Microscopic examination, *P. falciparum*, *P. vivax*, Polymerase chain reaction

Introduction

According to the 1996 epidemiological studies in Singapore done by the Ministry of the Environment, a total of 364 cases of malaria were reported in 1995.¹ 90.4% of the cases were imported. The surveillance indicates that 80.2% of the cases were caused by *P. vivax*, 19.0% by *P. falciparum* and 0.8% by mixed infections. From 1985 to 1994 in the Singapore Armed Forces (SAF), a total of 104 cases of malaria have been reported, of which 67% were contracted during overseas training. Forty-three cases of malaria were caused by *P. falciparum*.

There is a need to evaluate the methods currently used in the diagnosis of malaria in the field since the current methods used depend heavily on the military doctor's knowledge of the symptoms of malaria, which are often non-specific compared to other infections.

For malaria diagnosis, the primary method used for detection is the microscopic examination of thin or thick blood smear stained with Giemsa. The microscopic method is considered by the World Health Organisation as the gold standard for the diagnosis of malaria. This method requires skilled and experienced personnel to examine at least 100 microscopic fields of a thick film for

each blood sample. The method is time-consuming, laborious and only a limited number of such tests can be done in one day.²

We evaluated two new methods of malaria diagnosis namely, the ParaSight™ F test (Becton Dickinson Tropical Disease Diagnostics, Cockeysville, MD) and the OptiMAL rapid malaria assay (Flow Inc., Portland, Oregon), for suitability for use in the field. Both tests are based on the dipstick antigen capture assay. To assess their suitability for use in the field, we have compared the two tests with the traditional microscopic blood film examination and the polymerase chain reaction (PCR) methods for their sensitivity, specificity and ease of use.

Materials and Methods

Collection of Blood Samples

A total of 103 EDTA-treated blood samples with stained and unstained blood slides of malaria patients sent to the National Malaria Reference Centre (NMRC) at the Department of Microbiology, National University of Singapore, were used in this study. Fifty-eight blood samples from individuals with no malaria symptoms collected from a school, prison and SAF camp after at least one

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case of malaria was found in these areas were used as negative controls.

Microscopic Examination of Blood Film

Thin and thick blood smears were stained with Giemsa and examined under light microscope by various clinical laboratories. Their diagnosis was subsequently confirmed by the NMRC.

Polymerase Chain Reaction

The DNA was isolated from 0.1 to 1.0 ml of EDTA-treated blood using the Qiagen kit (Qiagen GmbH, Germany) for DNA isolation. If there was insufficient blood in the samples, 20 µl of the blood was treated with 5% chelex to obtain DNA for PCR. The DNA was amplified by PCR using primers from Tirasophon et al.³ The primers were derived from a highly repetitive DNA fragment. The PCR was carried out in 1.5 mM MgCl₂, 200 µM dNTPs, 0.2 µM primers and 1.25 U of Taq Polymerase. Primers specific for *P. vivax* (PV-1: 5'-GGTGAAAA TCGAAGGTATCGA-3', PV-2: 5'-TCCCTGCCCGCT GTTGC-3') and *P. falciparum* (PF-1: GCTACATATGC TAGTTGCCAGAC-3', PF-2: 5'-CGTGTACCATACA TCCTACCAAC-3') were added into one reaction tube. The DNA was amplified for 40 cycles at 90°C for 15 sec, 55°C or 60°C for 15 sec and 72°C for 15 sec, the first denaturation and the last extension steps were for 30 sec. After amplification, the 183-bp and 206-bp PCR products were detected for *P. vivax* and *P. falciparum* respectively using a 2% agarose gel.

ParaSight™ F Test

The test is a rapid dipstick antigen capture assay for diagnosis of falciparum malaria developed by Becton Dickinson Tropical Disease Diagnostics. Three drops of Reagent 1 were squeezed into a DispensTube. The tube was placed on a reaction stand. A capillary tube was filled with venous blood in EDTA (about fifty microlitres) from a collection tube or from capillary blood via a finger prick. The blood was mixed with the anticoagulant coating in the capillary tube. The blood sample was drained into the DispensTube containing the lysing fluid. The dispensing tip was placed onto the DispensTube and one drop of lysed blood was squeezed into one disposable well in the reaction stand. The dipstick was placed in the drop of lysed blood until all the blood was absorbed into the dipstick. Two drops of Reagent 2 (detection fluid) was squeezed into the same well. All of Reagent 2 was allowed to absorb into the dipstick. Two drops of Reagent 3 (wash agent) was squeezed into the same well. For a *P. falciparum*-positive blood sample, a solid pink line would appear in addition to the control dotted line. For a negative test, only the dotted control line would appear.

OptiMAL Rapid Malaria Test

Flow Inc. (Oregon, USA) developed the OptiMAL rapid assay for the diagnosis of malaria caused by *P. vivax* and *P. falciparum*. The test uses a series of monoclonal antibodies to detect the parasite antigen, lactate dehydrogenase (pLDH). The OptiMAL rapid malaria test was performed as indicated by the instruction in the kit. Two drops of buffer A was dispensed into a test well. Ten microlitres of blood was added and mixed well. One OptiMAL test strip was added to the test well and all the samples were allowed to wick completely up the test strip. The test strip was moved to a second test well containing four drops of buffer B. The test strip was read after the blood colour has cleared. A single band (top) appearing on the strip would indicate a negative result with the control band appearing. If *P. vivax* was present, two bands (top and middle) would appear on the strip. If *P. falciparum* was present, three bands (top, middle and bottom) would be seen on the strip. In the case of a mixed infection, three bands would be seen with a more intense middle band.

Results

Comparison of ParaSight™ F with Blood Film and PCR Methods

One hundred and three blood samples from hospitals were examined for malaria infections by the microscopic blood film, PCR and ParaSight™ F test. For each blood sample obtained, the results of the microscopic examination of the blood were confirmed by the NMRC. The results were not released to the laboratory performing the PCR, ParaSight™ F and OptiMAL tests until the tests were completed.

When compared with the results from the microscopic and PCR methods, the ParaSight™ F test has specificity of 98.6% (68 of 69) and sensitivity of 91% (31 of 34). Table I shows the results of comparison between the ParaSight™ F tests and the blood film/PCR methods for diagnosis of *P. falciparum* infection. False positives rate occurred at 3.1%. Blood film/PCR results in the tables were obtained by using both or either one of the blood

TABLE I: COMPARISON BETWEEN RESULTS OF THE PARASIGHT F TEST AND THE BLOOD FILM/PCR FOR DIAGNOSIS OF *P. falciparum* INFECTIONS

Blood film/PCR*	ParaSight F		Total
	Positive	Negative	
Positive	31	3	34
Negative	1	68	69
Total	32	71	103

* Blood film/PCR results were obtained by using both or either the blood film microscopic or PCR methods.

film with the PCR method as the reference test for comparison. PCR was mainly done to confirm. ambiguous result obtained from the laboratory doing the blood film examination where specification of the malarial parasites was not conclusive and also when blood film results were not obtained.

Comparison of OptiMAL with Blood Film and PCR Methods

Ninety-eight samples collected from the hospitals were analysed with the OptiMAL malaria rapid test. Table II shows that the specificity of the OptiMAL test compared to the microscopic blood film for *P. vivax* infection was 100% (27 of 27) and the sensitivity was 92.8 % (64 of 69). For *P. falciparum* infection, the specificity was 98.6% (68 of 69) and sensitivity was 92.6% (25 of 27). Two out of two mixed infections were detected by the test. The number of false positives was about 3.6% (1 out of 28, inclusive of the 2 mixed infections) and the number of false negatives was about 2.9% (2 out of 70) for the OptiMAL test for *P. falciparum*.

Table III shows the results of comparison between the OptiMAL malaria rapid test with blood film and PCR methods in 58 blood samples from asymptomatic individuals in a school, prison and SAF camp. For *P. vivax* detection, the OptiMAL test was 100% specific and there was no false positive result. For *P. falciparum* detection, the specificity was 87.9% (51 out of 58) and the number of false positives was 7 out of 58 (12.1%).

Discussion

Both the dipstick assays were found to be rapid tests, taking not more than 15 minutes to complete a single test. Minimal training in the form of a demonstration and hands-on practice for a couple of times would be sufficient. The results can be easily read from the dip-

TABLEIII: COMPARISON BETWEEN RESULTS OF THE OptiMAL RAPID MALARIA TEST AND THE BLOOD FILM/PCR METHODS FOR DIAGNOSIS OF MALARIA IN ASYMPTOMATIC INDIVIDUALS

Species	Blood film/PCR*	OptiMAL		Total
		Positive	Negative	
<i>P. vivax</i>	Positive	0	0	0
	Negative	0	58	58
	Total	0	58	58
<i>P. falciparum</i>	Positive	0	0	0
	Negative	7	51	58
	Total	7	51	58

* Blood film/PCR results were obtained by using both or either the blood film microscopic or PCR methods.

stick. Of the two tests, the OptiMAL test may be more useful since it can be used to detect either *P. vivax* or *P. falciparum* or both in the case of mixed infections.

Many field trials have been conducted by different countries on the sensitivity and specificity of the ParaSight™ F test compared to the microscopic method.⁴ Specificity and sensitivity ranged from 81.1% to 99.5% and 84.2% to 93.9%, respectively. Our results fell within these ranges. It has been reported that lower sensitivity of the tests can result from parasitaemia lower than 60 parasites per microlitre of blood.

A field trial for the ParaSight™ F test has been conducted and the results indicated that the test was 100% specific and sensitive for falciparum malaria compared to the microscopic method. The trial has been conducted on a small scale of 35 samples with 5 falciparum-positive, 8 vivax-positive, one mixed infection and 20 negative blood samples (personal communication-Wong Yue Sie, HQ Medical Corps).

Palmer et al⁵ have also evaluated the OptiMAL test in a recent study. Their results showed sensitivities of 94% and 88% and specificities of 100% and 99%, respectively, when compared to traditional blood films for the detection of *P. vivax* and *P. falciparum* malaria. Our study results indicate sensitivities of both 93%, and specificities of 100% and 98.6% for *P. vivax* and *P. falciparum* malaria, respectively. False negative results can be obtained at parasite concentration of less than 100 per microlitre of blood.⁵

In view of the lack of laboratory facilities, the dipstick assay is still the most convenient method for confirming a malaria diagnosis based on clinical symptoms in the field. Our results showed that the tests are rapid, accurate and sensitive enough to pickup 91% of the falciparum malaria cases using the ParaSight™ F test and 93% of the vivax and 93% of the falciparum malaria cases using the OptiMAL test.

TABLEII: COMPARISON BETWEEN RESULTS OF THE OptiMAL RAPID MALARIA TEST AND THE BLOOD FILM/PCR FOR DIAGNOSIS OF *P. vivax* AND *P. falciparum* INFECTIONS

Species	Blood film/PCR*	OptiMAL		Total
		Positive	Negative	
<i>P. vivax</i>	Positive	64	5	69
	Negative	0	27	27
	Total	64	32	96
<i>P. falciparum</i>	Positive	25	2	27
	Negative	1	68	69
	Total	26	70	96
Mixed infections	Positive	2	0	2
	Negative	0	0	0
	Total	2	0	2

* Blood film/PCR results were obtained by using both or either the blood film microscopic or PCR methods.

Minimal training is required before the person is capable of performing the test. The ParaSight™ F test has a shelf life of at least six months when stored at 2°C to 37°C. The OptiMAL test has a shelf life of 6 months when stored at 4°C to 10°C.

There is a need to emphasise that the tests have to be used in conjunction with the clinical symptoms diagnosed by medical doctors as a higher number of false positives can result from tests done on asymptomatic individuals. A minimum training period of a few hours in the laboratory is essential to ensure that the person performing the test is capable of performing the test and can differentiate a positive from a negative test.

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