Non-microscopic method for malaria diagnosis using OptiMAL IT, a second-generation dipstick for malaria pLDH antigen detection

A.H. MOODY and P.L.CHIODINI
Department of Clinical Parasitology, Hospital for Tropical Diseases, University College Hospital, Mortimer Market, Capper Street, London WC1E 3BG, UK

Accepted: 30 September 2002

ABSTRACT

Rapid diagnostic tests for malaria are now a commonly used procedure for malaria diagnosis. New or improved devices need to be evaluated against a recognised gold-standard procedure and subjected to conditions of temperature and humidity that may affect their performance. The OptiMAL 48 RDT has now been available commercially for several years and a second-generation OptiMAL IT test is now coming onto the market. In this study the problems associated with the routine use of OptiMAL 48 is investigated and its performance compared with a second-generation individual test, OptiMAL IT. Sensitivity and specificity for detection of all malaria species for both tests were comparable but loss of sensitivity of the test strips due to humidity or temperature found with the routine use of OptiMAL 48 was not seen with the individual OptiMAL IT. False-positive results for *Plasmodium falciparum*, seen in two negative blood samples, were attributed to the presence of high levels of heterophile antibodies.

KEY WORDS: Malaria. Plasmodium falciparum. Dipsticks.

Introduction

The use of dipsticks for the detection of malaria antigen from peripheral blood is now an accepted method for malaria diagnosis worldwide. Comparative evaluation between microscopic diagnosis as the 'gold standard' and detection of malaria antigen using dipsticks has been performed in many centres that test both immune and non-immune populations. The sensitivity and specificity achievable by these devices is now well documented.[1-7]

OptiMAL 48 has been commercially available since 1998 (Diamed, Cressier, Switzerland). The principle of the test is the detection of pLDH, an enzyme found in the glycolytic pathway of *Plasmodium* spp., using methods that can assay pLDH separately from any human LDH present in the sample.

Immunochromatography, using gold-labelled pan-specific anti-pLDH monoclonal antibodies to capture antigen from blood and separate monoclonal antibodies against *P. falciparum*-specific and pan-species antigens on a cellulose nitrate strip, is the format used for the test device. Several studies of the performance of these first-generation dipstick tests have been performed and a sensitivity of 200-500 parasites/μL is generally accepted.[8,9]

Materials and methods

OptiMAL 48 kit
OptiMAL 48 is supplied in a strip well format with the gold-labelled antibody dried into the wells. Individual test strips are packed into vials of 50 tests and the lid of the vial contains a dessicant. A microtitre plate containing gold-conjugated monoclonal antibody sealed with a plate seal and a vial of buffer solution are included.

This device is both sensitive and specific when compared to microscopy in the majority of reports, but less satisfactory results have been reported from tropical areas.[10-12.] Further investigation and reports from overseas field use noted the possibility that the dessicant lids of the cylinders containing the strips could work loose during transportation or be left off during constant use, allowing moisture to contact the strips and affect the result.

OptiMAL IT
OptiMAL IT (Figure 1) is a reconfiguration of the OptiMAL strip into a cassette format, sealed as individual tests in aluminium-coated packets containing a dessicant. The construction provides two wells at one end of the cassette – one containing the gold-conjugated monoclonal antibody and the second empty for use as a wash well. A small sealed tube provides adequate buffer to allow one drop for the test well and four drops for the wash well.

The test strip is held in a sealed slide, which has two prongs that enable the strip to stand vertically, dipping into the test and wash wells. Using the small tube pipette supplied, 10 μL blood is delivered into the test well containing the solubilised gold-conjugated antibody and mixed using the paddle at the top of the pipette. The strip slide is then placed in the slots positioned either side of the well, which holds it vertical and allows the end to dip into the solution in the well. After 10 min the slide strip is

Correspondence to: Mr A H Moody
Email: anthony.moody@uclh.org

Table 1. Routine microscopic diagnosis of malaria compared with OptiMAL IT

Number tested	Initial diagnosis microscopy result	Microscopy negative	Microscopy positive	OptiMAL IT negative	OptiMAL IT positive
7	*P falciparum* >1.5%	0	7	0	7
4	*P falciparum* 0.5-1.5%	0	4	0	4
4	*P falciparum* 0.1%	0	4	0	4
2	*P falciparum 0.01%*	0	2	0	2
6	*P falciparum 0.001%*	0	6	1	5
6	*P falciparum 0.0001%*	0	6	1	5
1	*P falciparum*	0	1	0	1
	Gametocytes				
20	*P ovale*	0	20	16	4
3	*P malariae*	0	5	0	5
24	*P vivax*	0	24	2	22
34	No malaria parasites	34	0	32	2

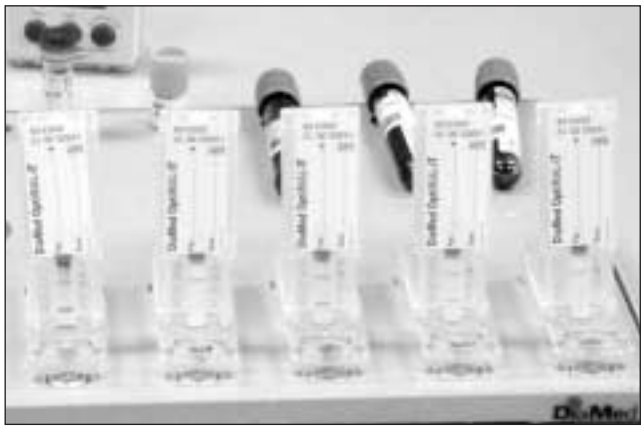

Fig. 1. OptiMAL IT test format. Left to right: *P. falciparum*, negative, *P. vivax*, *P. malariae*, negative.

transferred to the wash well for a further 10 min to clear the strip, following which the result can be assessed.

A cap is provided to seal the used wells, which can then be broken from the cassette and discarded. The exposed strip is slotted into the base of the cassette, rendering it both safe to handle and preserved as a permanent record. The test procedure instructions are stepwise, pictorial and easy to follow.

Methods

OptiMAL 48 first-generation test kit: Investigation into the possible reasons for loss of sensitivity of the device was made in this laboratory. Previous experience with the OptiMAL 48 test suggested that exposure of test strips to raised temperatures and humidity levels for 24 h or more may lead to weaker positive or false-negative results. However, incubating strips in containers with the tops securely sealed at 37˚C and 40˚C for 48 h and at humidity levels >70% did not give weaker or negative results. A safety issue with the completed test was also apparent, with the possibility of direct contact with blood on the exposed strip. In order to investigate the possibility of degradation of the strip by exposure to temperature or humidity variables, OptiMAL 48 test strips were removed from the canister and tested, following the manufacturer's instructions, at room temperature (20˚C) and low humidity (40%), with a sample of blood containing 500 *Plasmodium falciparum* parasites/μL. Further test strips from the same kit were removed from the canisters and exposed in an incubator at 37˚C at variable humidity levels of 40%, 50%, 60% and 70% for 24 and 48 h. Subsequently, they were tested with the same sample of blood used for the initial test.

Routine comparison of OptiMAL IT and microscopic examination of blood films:
113 samples of blood received in the department for routine microscopic testing for malaria parasites were tested in parallel using the OptiMAL IT, following the manufacturer's instructions, and the results are shown in Table 1. The ability of the enzyme-based dipsticks to follow response to chemotherapy is key to the wider use of OptiMAL IT. Previous work has shown that OptiMAL can parallel microscopic monitoring of parasitaemia accurately.[8,9] In this series, OptiMAL IT tests were performed daily, in parallel with microscopy, following the course of therapy in patients on treatment for *P. falciparum* infection (Table 2).

Temperature and humidity: The sealed packaging of the OptiMAL IT is designed to prevent moisture affecting the strip and the single-use format does not allow exposure of other test strips. OptiMAL IT test devices were incubated unopened at temperatures from 40˚C to 60˚C with humidity levels between 40% and 70% for three-hour periods and at 40˚C with >70% humidity for 36 h. The devices were then tested with a single blood sample containing 500 parasites/μL *P. falciparum*. Comparison was made with a similar device held at room temperature (20˚C) and low humidity (40%) for a similar period.

Results

OptiMAL 48 strips demonstrated loss of sensitivity when exposed to humidity levels approaching 70% (Table 3).

Table 2. Comparison of OptiMAL IT with microscopy for following sequential parasitaemia from four patients on treatment with quinine for *P. falciparum* (Pf) malaria

Day of testing	Microscopy result	OptiMAL IT			Comment
		Control	m/c19	m/c17	
Day 1	2% Pf	✓	+++	+++	
Day 2	1.1% Pf	✓	+++	+++	
Day 3	NEG	✓	NEG	NEG	
Day 4	NEG	✓	NEG	NEG	Discharged
Day 1	12% Pf	✓	+++	+++	
Day 2	0.8% Pf	✓	+++	+++	
Day 3	0.01% Pf	✓	++	++	
Day 4	0.05% Pf	✓	+	+	
Day 5	0.0001% Pf	✓	+/--	+/---	
Day 6	0.0001% Pf	✓	NEG	NEG	Discharged
Day 1	0.15% Pf	✓	+++	+++	
Day 2	0.08% Pf	✓	+++	+++	
Day 3	0.0001% Pf	✓	+/--	+/--	
Day 4	0.0001% Pf	✓	NEG	NEG	
Day 5	0.0001% Pf	✓	NEG	NEG	Discharged
Day 1	7.5% Pf	✓	+++	+++	
Day 2	8.5% Pf	✓	+++	+++	
Day 3	5.0% Pf	✓	+++	+++	
Day 4	2.2% Pf	✓	+++	+++	
Day 5	1.6% Pf	✓	+++	+++	
Day 6	0.005% Pf	✓	+	+	
Day 7	NEG	✓	+/--	+/--	Discharged

Table 3. Temperature and humidity stability of exposed OptiMAL 48 strips using a single blood sample of 500 parasites/μL *P. falciparum*

Incubation temperature →°C Humidity ↓ %	25°C	37°C	40°C
40%	+ + +	+ + +	+ + +
50%	+ + +	+ + -	+ + -
60%	+ + -	+ - -	+ - -
70%	+ - -	- - -	- - -

Exposure at 37°C did not have a detrimental affect on the result.

Incubation of the unopened OptiMAL IT test device when subjected to humidity and temperature variables and retested with a single pretested sample containing *P. falciparum*, showed that these conditions had no effect on the test result (Table 4).

The two negative samples, which gave positive results with OptiMAL IT, were centrifuged and the plasma separated from the red cells. Repeated tests using OptiMAL IT were performed on each component and it was found that the red cells tested negative and the plasma remained positive. Previous work on plasma samples from false-positive results in this laboratory and at Flow Inc., Portland, USA, using Heterophilic Blocking Tube reagent (Scantibodies Laboratory Inc., Santee, Ca, USA) suggested that there is cross-reaction of the test system with heterophile antibodies present in the plasma.

Discussion

OptiMAL IT has been evaluated in the Department of Clinical Parasitology, Hospital for Tropical Diseases, London – a clinical laboratory that receives blood specimens for malaria diagnosis from immune and non-immune patients travelling from a wide range of countries.

The format of the OptiMAL IT was found to be compact, easy to handle and the instructions with the device were easy to follow. Safety features included in this format satisfy the concerns expressed with the first-generation format for routine use. Means to seal contaminated fluids with a cap and to render the test strip safe to handle with clearly visible reading have been satisfactorily dealt with. The single-use sealed package prevents moisture affecting the test strip performance.

Table 4. Temperature and humidity stability of unopened OptiMAL IT 48 device using a single blood sample of 500 parasites/μL *P. falciparum*

Incubation temperature → °C Humidity ↓ %	40	45	50	55	60
40%	+ + +	+ + +	+ + +	+ + +	+ + +
50%	+ + +	+ + +	+ + +	+ + +/-	+ + +
60%	+ + +	+ + +	+ + +	+ + +	+ + +
70% for 3 hours	+ + +	+ + +/-	+ + +	+ + +	+ + +/-
and 36 hours	+ + +				

The results of malaria testing on routine samples (Table 1) did not vary greatly from previous trials conducted at this laboratory. Seventeen samples with 200-75000 parasites/μL *P. falciparum* and 10/12 samples with 5-50 parasites/μL *P. falciparum* were positive with OptiMAL IT. Results for Plasmodium species other than P. falciparum showed that 22/24 samples containing *P. vivax*, 4/16 *P. ovale* and 5/5 of those with *P. malariae* were detected.

P. ovale remains the most difficult species to detect by OptiMAL IT, as was the case withy the first-generation test. This may be due to the presence of at least two isomers of this parasite antigen and the failure of the currently selected monoclonals to recognise at least one of these (R Piper, personal communication). The majority of *P. ovale* infections in this study were contracted in tropical Africa.

Daily microscopic examination of blood film parasitaemia during therapy for falciparum malaria is routine practice in this laboratory. OptiMAL IT was performed daily on the same samples and proved to be effective in showing a decline in the activity of pLDH, reflecting the decline in viable parasites (Table 2).

The two false-positive samples were referred for confirmation from other laboratories and our results confirmed earlier work on similar false-positive results found with first-generation OptiMAL. These were the only false-positive samples encountered. No false-negative or weak results were obtained with OptiMAL IT on samples containing >100 parasites/μL, even when strips were stored under high humidity conditions. Nevertheless, further work on test strip stability under field conditions is required.

OptiMAL IT is a second-generation OptiMAL test device for malaria. The performance of the test proved acceptable and can be recommended for use in parallel with microscopy in laboratories where experience with routine microscopic screening for malaria may be limited, and also in areas where microscopic diagnosis is not available. □

References

1 Moody AH, Hunt Cooke A, Gabbett E, Chiodini PL. Performance of the OptiMAL malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital for Tropical Diseases, London. *Br J Haematol* 2000; **109**: 891-4.

2 Iqbal J, Sher A, Hira PR, Al-Owaish R. Comparison of the OptiMAL test with PCR for diagnosis of malaria in immigrants. *J Clin Microbiol* 1999; **39**: 3644–6.

3 Labbe AC, Pillai DR, Hongvangthong B *et al*. The performance and utility of rapid diagnostic assays for *Plasmodium falciparum* malaria in a field setting in the Lao Peoples Democratic Republic. *Ann Trop Med Parasitology* 2001; **95** (7): 671-7.

4 Congpuong K. Bualombai P, Jitchamroen S, Konchom S. Comparison of the OptiMAL rapid test with routine microscopic examination of Giemsa-stained thick blood film for diagnosis of malaria. *J Med Assoc Thai* 2001; **84** (3): 357-63.

5 Asian G, Ulukanligil M, Seyrek A, Erel O. Diagnostic Performance Characteristics of Rapid Dipstick test for *Plasmodium vivax* malaria. *Memorias do Insitituto Oswaldo Cruz* 2001; **96**(5): 683-6.

6 Palmer CJ, Lindo JF, Klaskala WI *et al*. Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. *J Clin.Microbiol* 1998; **36**: 203–6.

7 Quitana, M, Piper R, Boling H *et al*. Malaria diagnosis by dipstick assay in a Honduran population with coendemic *Plasmodium falciparum* and *vivax*. *Am J Trop Med Hyg* 1998; **59**: 868-71.

8 Oduola AM, Omitowoju O, Sowunmi A *et al*. *Plasmodium falciparum*: evaluation of lactate therapeutic response to standard anti-malarial drugs in Nigeria. *Exp Parasitol* 1997; **87**: 283–9.

9 Srinavasan S, Moody AH, Chiodini PL. Comparison of blood-film microscopy, the OptiMAL dipstick, rhodamine 123 and PCR for monitoring anti-malarial treatment. *Ann Trop Med Parasitol* 2000; **94**: 27–32.

10 Mason DP, Kawamoto F, Lin K, Laoboonchai A, Wongsrichanalai C. A comparison of two rapid field immunochromatographic tests to expert microscopy in the diagnosis of malaria. *Acta Tropica* 2002; **82**(1): 51-9.

11 Hernandez E, De Pina JJ, Fabre R, Garrabe E, Raphenon G, Cavallo JD. Evaluation of OptiMAL test in the diagnosis of imported malarial outbreak *Med Trop* 2001; **61**(2): 153-7.

12 Fryauff DJ, Purnomo, Sutamihardja MA *et al*. Performance of the OptiMAL assay for detection and identification of malaria infections in asymptomatic residents of Irian Jaya, Indonesia. *Am J Trop Med Hyg* 2000; **63** (3-4): 139-45.