ORIGINAL ARTICLE 10.1111/j.1469-0691.2005.01253.x

Performance of the Now Malaria rapid diagnostic test with returned travellers: a 2-year retrospective study in a French teaching hospital

F. Durand[1], B. Crassous[1], H. Fricker-Hidalgo[1], F. Carpentier[2], J.-P. Brion[3], R. Grillot[1] and H. Pelloux[1]

[1]Service de Parasitologie-Mycologie Médicale, [2]Service d'Accueil et d'Urgences de Médecine and [3]Service de Maladies Infectieuses et Tropicales, Centre Hospitalier Universitaire de Grenoble, Grenoble, France

ABSTRACT

Malaria caused by *Plasmodium falciparum* remains the major life-threatening parasitic infection in the world. The number of cases in non-endemic countries continues to increase, and it is important that misdiagnosis of malaria should not occur, especially in non-immune travellers, because of the high risk of a fatal outcome. In a retrospective study of 399 sera, the Now Malaria rapid test was compared with the quantitative buffy coat (QBC) test and microbiological examination of thin blood films. Compared with the QBC test and thin blood films, the Now Malaria test had sensitivity and specificity values of 96.4% and 97%, respectively, for the detection of pure *P. falciparum* infection. A negative predictive value of 99.4% allows this test to be included in diagnostic strategies for patients presenting with clinical suspicion of malaria. Two false-negative results were associated with low levels of parasitaemia in the specimens. Thus, use of the Now Malaria test alone to detect *P. falciparum* infection in non-endemic countries could lead to misdiagnosis of malaria. This rapid diagnostic test should therefore be performed in association with another prompt traditional method such as examination of thin blood films.

Keywords Detection, malaria, Now Malaria test, *Plasmodium falciparum*, rapid diagnosis

Original Submission: 18 August 2004; **Revised Submission**: 23 March 2005; **Accepted**: 3 May 2005

Clin Microbiol Infect 2005; 11: 903–907

INTRODUCTION

Malaria is endemic in 91 countries around the world and is responsible for approximately two million deaths annually [1]. Every year, nearly 50 million people travel from the industrialised world to malaria-endemic areas, and these numbers have increased annually in recent years [2]. Among three million people travelling annually from France to malaria-endemic areas, 5000–8000 will catch malaria, and up to 20 may die because of *Plasmodium falciparum* infection [3]. The high risk of death in cases of untreated malaria requires that diagnosis is prompt and accurate, and available 24 h a day for patients admitted to the emergency unit [4]. A laboratory can use one or several techniques in order to reach a positive diagnosis of imported malaria, identify the parasite species, and estimate the degree of parasitaemia [5]. Traditional methods use stained blood films (i.e., thick or thin blood films) [6], with many hospital laboratories using a thin blood smear stained with Giemsa, combined with microscopic detection of parasites using fluorescent dye (acridine orange) in the centrifugal quantitative buffy coat test (QBC test; Becton Dickinson, Le Pont de Claix, France) [7,8]. These tests are not easy to perform for an inexperienced microscopist [6]. Similarly, although PCR-based assays are the most sensitive and specific methods for detecting malarial parasites, these assays cannot be performed routinely to diagnose malaria because of the cost and the time required [9].

Several new rapid diagnostic tests have been developed, including immunochromatographic dipstick assays, which detect malaria antigens in

Corresponding author and reprint requests: F. Durand, Centre Hospitalier Universitaire de Grenoble, Service de Parasitologie-Mycologie Médicale, Grenoble, France
E-mail: frdurand@chu-grenoble.fr

whole blood, and which have been evaluated in both endemic and non-endemic areas [10]. The antigens detected include those for histidine-rich protein-2 (HRP-2), which is a *P. falciparum*-specific antigen (ParaSight F (no longer marketed); Becton Dickinson), and for *Plasmodium* aldolase, which is a pan-specific antigen, the detection of which is combined with detection of the HRP-2 *P. falciparum*-specific antigen in the Now Malaria dipstick test (Binax, Portland, ME, USA) [11–13]. A third antigen, that for *Plasmodium* lactate dehydrogenase, has been used in an immunochromatographic test (OptiMAL; Flow Inc., Portland, OR, USA) to detect *Plasmodium* in blood specimens [6]. In the present study, all results concerning travellers returning from malaria-endemic areas with suspected malaria were analysed retrospectively for a 2-year period. To the best of our knowledge, this study is the first comparison in non-endemic countries of results obtained by the Now Malaria test with those obtained by examination of a thin blood film and the QBC test [10].

MATERIALS AND METHODS

Patients

The study analysed 413 patients presenting to the Grenoble teaching hospital between April 2002 and April 2004 with a clinical history compatible with a diagnosis of malaria. Specimens from other laboratories or hospitals were excluded from this retrospective study because of the heterogeneity of the samples submitted and the lack of clinical history concerning each patient.

Microscopic analysis

Thin blood smears were prepared from 5 mL of venous whole blood collected in an EDTA tube and then stained with Giemsa 10% w/v solution. In daily practice, as patients presented, three expert microscopists (a medical biologist, a technician and an intern) examined each specimen systematically at ×1000 magnification for 20 min, with *c.* 10 000 red blood cells (RBCs) being examined before the test was considered to be negative. The density of parasites was determined by calculating the percentage of parasitised erythrocytes over 50 consecutive fields, representing *c.* 10 000 RBCs [10]. Parasitaemia may be expressed as a percentage of RBCs infected, or as the number of parasites present in 1 μL of blood. Since 1 μL of blood contains 5×10^6 RBCs, a 1% parasitaemia represents 50 000 parasites/μL [6].

QBC test

The centrifugal QBC assay is a fluorescent microscopic method that uses acridine orange to stain the nucleic acid of cells and parasite nuclei [6]. In brief, a whole blood sample is mixed with acridine orange, and used to coat a capillary tube, centrifugation of which allows the parasite to be viewed inside RBCs with a fluorescence microscope [8].

Now Malaria assay

The same sample of whole blood that was used to prepare thin blood films was also used for the Now Malaria assay. The test is an immunochromatographic assay that uses two antibodies which have been immobilised across a test strip [10]; one antibody is specific for the HRP-2 antigen of *P. falciparum*, while the other is specific for an antigen that is common to all species of *Plasmodium*. The QBC method and thin blood films are used systematically in the Grenoble teaching hospital to diagnose malaria, and these two methods were used as the reference standard for comparative purposes.

Statistical analysis

Samples were classified as true-positive (TP), true-negative (TN), false-positive (FP) or false-negative (FN) by comparison with a reference standard. Sensitivity (TP/TP + FN) and specificity (TN/TN + FP), as well as positive (TP/TP + FP) and negative (TN/TN + FN) predictive values, for the Now Malaria test were then calculated.

RESULTS

During the study period, 413 patients presented with a clinical suspicion of malaria and a history of travel to a malaria-endemic country. Of those who were infected with malaria, the ratio of male to female patients was 1.6, and there was a mean age of 32.2 (± 16.5) years (range, 3–66 years). Areas visited were mostly sub-Saharan Africa (95.4%), but Asia (1.5%) and Latin America (3.1%) were also visited. More than half (61.5%) of the cases were diagnosed in the emergency department, often during the night.

In total, 535 specimens were collected, of which 399 (75%) were examined by thin blood films and QBC tests (forming the reference standard) and Now Malaria tests. Of the 399 specimens examined, 311 were negative by all three methods, and 65 were positive by all three methods (53 for *P. falciparum*, eight for *Plasmodium ovale*, three for *Plasmodium vivax* and one for *Plasmodium malariae*). However, 18 samples gave discrepant results, comprising ten samples that were positive for *P. falciparum* by the Now Malaria assay, but negative by thin blood films and the QBC test, and eight samples that were negative by the Now Malaria assay, but positive (two for *P. falciparum*, five for *P. ovale* and one for *P. vivax*) by thin blood films and the QBC test (Table 1).

Table 1. Comparison of the results of the Now Malaria assay with the reference standard of combined thin blood film and quantitative buffy coat (QBC) assay

		Reference standard (thin blood film and QBC test)					
Now Malaria assay results	Total	*P. f.*	*P. o.*	*P. v.*	*P. m.*	Mixed *P. f.* + *P. o.*	Negative
T_1 line + T_2 line +	36	32	1	0	0	2	1
T_1 line + T_2 line 0	30	21	0	0	0	1	8
T_1 line 0 T_2 line +	12	0	8	3	1	0	0
T_1 line 0 T_2 line 0	319	2	5	1	0	0	311
Total	397[a]	55	14	4	1	3	320

P. f., *P. falciparum*; *P. o.*, *P. ovale*; *P. v.*, *P. vivax*; *P. m.*, *P. malariae*.
T_1, line where specific antibodies for histidine-rich protein II (HRP-2) antigen of *P. f.* are immobilised; T_2, line where specific antibodies for a common antigen of *P. f.*, *P. o.*, *P. v.* and *P. m.* are immobilised.
[a]There were two additional samples, one of which haemolysed and could not be examined by microscopy, and one for which the Now Malaria assay could not be interpreted (see main text).

Among the five remaining samples, there were three *P. falciparum–P. ovale* mixed infections, two with no distinction between the species by the Now Malaria assay (both lines were positive), and one that was positive only for the HRP-2 *P. falciparum*-specific line. One sample was haemolysed and was negative by the Now Malaria assay; this sample could not be examined by microscopy. The final sample could not be interpreted with the Now Malaria assay, as the dipstick control line was not present, even after multiple assays performed by several experienced technicians with different batches of Now Malaria kits.

When compared with the results of QBC tests and thin blood films, the Now Malaria assay had a sensitivity of 96.4% and a specificity of 97% for the detection of pure *P. falciparum* malaria (Table 2). The test also had a sensitivity of 66.7% and a specificity of 100% for non-*P. falciparum* malaria. Moreover, the detection sensitivity for *P. falciparum* was 100% if only samples containing >500 parasites/μL (0.01% parasitaemia) were considered. Positive and negative predictive values for the diagnosis of *P. falciparum* malaria were 84% and 99.4%, respectively, and those for diagnosis of non-*P. falciparum* malaria were 100% and 98.2%, respectively. These predictive values should be interpreted in terms of the prevalence of *P. falciparum* and non-*P. falciparum* malaria in the patients studied (12.3% and 4.1%, respectively).

Table 2. Performance characteristics of the Now Malaria assay compared to the reference standard of combined thin blood film and quantitative buffy coat (QBC) assay results

Rapid diagnostic test	Result by reference standard	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Now Malaria assay	*P.f.*	96.4	97	84	99.4
	Non-*P.f.*	66.7	100	100	98.2

PPV, positive predictive value; NPV, negative predictive value; *P. f.*, *Plasmodium falciparum*.

DISCUSSION

This report describes the usefulness of the Now Malaria assay for the diagnosis of malaria in returned travellers. Each year, the teaching hospital in Grenoble receives samples from *c.* 200 patients with a history of recent travel to malaria-endemic areas and clinical signs compatible with *Plasmodium* infection, with a mean of 35 cases of malaria diagnosed annually. The number of people visiting malaria-endemic countries is increasing on an annual basis, so the number of cases of malaria diagnosed upon their return should also be expected to increase [2]. In non-endemic countries, lack of experience could lead to missed diagnoses of malaria [4], thereby increasing the risk of severe infection [1]. In contrast, the requirement in endemic countries is for tests that are easy to perform and interpret, particularly in remote areas [10]. For these reasons, it is interesting to evaluate new non-microscopic rapid diagnostic tests. According to WHO recommendations, new assays should have a sensitivity of >95% compared with the standard of microscopy [5].

As reported previously, the Now Malaria assay is able to detect *P. falciparum* and non-*P. falciparum* antigens with different sensitivities and specificities [14–20]. These results should be interpreted with regard to the reference used and should be compared carefully because of the heterogeneity of the study designs (prospective or retrospective) and the pre-analytical phase (e.g., whole blood sample conservation, test-kit transportation and storage). Detection of *P. falciparum* has shown a range of sensitivities, from 68.4% to 100%, whereas the sensitivity for detection of non-*P. falciparum* parasites ranges from 39.3% to 84.7% [14–20]. Preliminary results from a comparison of the Now Malaria and OptiMAL IT

assays with QBC tests and thin blood films indicated that the sensitivity and specificity of the Now Malaria assay for *Plasmodium* spp. were 96.3% and 98.8%, respectively [21]. In the present study, the sensitivity and specificity of the Now Malaria assay for detection of *P. falciparum* infection compared with microscopic methods were >95%, suggesting that this test could improve accuracy in malaria diagnosis [22], although perhaps performing less well than some other traditional methods, such as thick blood films (capable of detecting 0.001% of RBCs infected, or 50 parasites/μL, when used by experienced microscopists) [6]. This could explain why two false-negative results were obtained with the Now Malaria assay for two *P. falciparum* infections with a very low level of parasitaemia.

All discrepant positive results for *P. falciparum* in the present study with the Now Malaria assay could be explained by a recent malaria attack, treated or untreated. Circulating HRP-2 antigens can persist for weeks following parasitaemia [10]. Indeed, HRP-2 is a protein of *P. falciparum* that can be detected as a result of circulating non-viable parasites, whereas *Plasmodium* lactate dehydrogenase, the antibody for which is also used in dipstick tests for rapid diagnosis of malaria, is produced only by viable parasites and is detected by rapid diagnostic tests earlier than HRP-2 [10]. Since the discrepant positive results obtained in the present study were correlated with the occurrence of a recent malaria attack, other possible causes of false-positive results (e.g., rheumatoid factor) need not be considered. The possibility that the IgM subtypes used in the Now Malaria assay are less reactive with rheumatoid factor than the IgG subtypes used in some other dipstick tests has been discussed previously [22,23]. With respect to non-*P. falciparum* infection, the sensitivity and specificity results were in agreement with those reported in previous studies [14–20]. The lack of sensitivity could be explained by the weaker affinity for the malaria pan-specific antigen (aldolase) than for HRP-2 antigen [10].

In conclusion, the Now Malaria rapid diagnostic test enables a prompt positive diagnosis of malaria with high specificity. However, occasional false-negative results mean that there is a risk of misdiagnosing malaria caused by *P. falciparum*. For this reason, the test cannot be recommended without also recommending confirmation of a negative result by a traditional microscopic method. Nevertheless, if the clinical history of the patient is taken into account, the good negative predictive value permits this test to be included as part of a comprehensive diagnostic strategy.

REFERENCES

1. Legros F, Danis M. Surveillance of malaria in European Union countries. *Euro Surveill* 1998; **3**: 45–47.
2. Ryan ET, Kain KC. Health advice and immunizations for travelers. *N Engl J Med* 2000; **342**: 1716–1725.
3. Danis M, Legros F, Thellier M, Caumes E. Current data on malaria in metropolitan France. *Med Trop* 2002; **62**: 214–218.
4. Hanscheid T. Current strategies to avoid misdiagnosis of malaria. *Clin Microbiol Infect* 2003; **9**: 497–504.
5. World Health Organization. *New perspectives: malaria diagnosis.* Report of a joint WHO/USAID informal consultation, 25–27 October 1999. WHO/CDS/RBM/2000.14 and WHO/MAL/2000. 1091. Geneva: WHO, 2000.
6. Moody A. Rapid diagnostic tests for malaria parasites. *Clin Microbiol Rev* 2002; **15**: 66–78.
7. Morassin B, Fabre R, Berry A, Magnaval JF. One year's experience with the polymerase chain reaction as a routine method for the diagnosis of imported malaria. *Am J Trop Med Hyg* 2002; **66**: 503–508.
8. Brenier-Pinchart MP, Pinel C, Grillot R, Ambroise-Thomas P. Diagnosis of malaria in non-endemic countries: value, limitations and complementarity of existing methods. *Ann Biol Clin Paris* 2000; **58**: 310–316.
9. Hanscheid T, Grobusch MP. How useful is PCR in the diagnosis of malaria? *Trends Parasitol* 2002; **18**: 395–398.
10. Murray CK, Bell D, Gasser RA, Wongsrichanalai C. Rapid diagnostic testing for malaria. *Trop Med Int Health* 2003; **8**: 876–883.
11. Brenier-Pinchart MP, Pinel C, Croisonnier A *et al.* Diagnosis of malaria in non-endemic countries by the Para-Sight-F test. *Am J Trop Med Hyg* 2000; **63**: 150–152.
12. Uguen C, Rabodonirina M, De Pina JJ *et al.* ParaSight-F rapid manual diagnostic test of *Plasmodium falciparum* infection. *Bull WHO* 1995; **73**: 643–649.
13. 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 a presumptive clinical diagnosis of malaria in Eastern Indonesia. *J Clin Microbiol* 1999; **37**: 2412–2417.
14. Richter J, Harms G, Muller-Stover I, Gobels K, Haussinger D. Performance of an immunochromatographic test for the rapid diagnosis of malaria. *Parasitol Res* 2004; **92**: 518–519.
15. Farcas GA, Zhong KJ, Lovegrove FE, Graham CM, Kain KC. Evaluation of the Binax NOW ICT test *versus* polymerase chain reaction and microscopy for the detection of malaria in returned travelers. *Am J Trop Med Hyg* 2003; **69**: 589–592.
16. Iqbal J, Khalid N, Hira PR. Comparison of two commercial assays with expert microscopy for confirmation of symptomatically diagnosed malaria. *J Clin Microbiol* 2002; **40**: 4675–4678.
17. Playford EG, Walker J. Evaluation of the ICT malaria P.f/P.v and the OptiMal rapid diagnostic tests for malaria

in febrile returned travellers. *J Clin Microbiol* 2002; **40**: 4166–4171.

18. Gatti S, Bernuzzi AM, Bisoffi Z, Raglio A, Gulletta M, Scaglia M. Multicentre study, in patients with imported malaria, on the sensitivity and specificity of a dipstick test (ICT Malaria P.f./P.v.) compared with expert microscopy. *Ann Trop Med Parasitol* 2002; **96**: 15–18.
19. Jelinek T, Grobusch MP, Harms G. Evaluation of a dipstick test for the rapid diagnosis of imported malaria among patients presenting within the network TropNetEurop. *Scand J Infect Dis* 2001; **33**: 752–754.
20. Rubio JM, Buhigas I, Subirats M, Baquero M, Puente S, Benito A. Limited level of accuracy provided by available rapid diagnosis tests for malaria enhances the need for PCR-based reference laboratories. *J Clin Microbiol* 2001; **39**: 2736–2737.
21. Peyron F, De Monbrison F, Gérôme P, Chaulet JF, Wallon M, Picot S. Comparison of the Now® ICT malaria P.f./P.v. and the OptiMAL® IT rapid diagnostic tests for malaria in a nonendemic area. *Clin Microbiol Infect* 2004; **10**(suppl 3): 103.
22. Milne LM, Kyi MS, Chiodini PL, Warhurst DC. Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. *J Clin Pathol* 1994; **47**: 740–742.
23. Mishra B, Samantaray JC, Kumar A, Mirdha BR. Study of false positivity of two rapid antigen detection tests for diagnosis of *Plasmodium falciparum* malaria. *J Clin Microbiol* 1999; **37**: 1233.