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CONCISE ARTICLE

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Rapid Immunochromatographic Malarial Antigen Detection Unreliable for Detecting *Plasmodium malariae* and *Plasmodium ovale*

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Abstract In order to determine the reliability of two commercial tests for the rapid detection of plasmodial antigen in cases of infection with *Plasmodium ovale* and *Plasmodium malariae*, the products were evaluated in four centers and a search of the relevant literature was performed. The results of the present and previous studies were compared. With overall sensitivities ranging between 18.8% and 47.6% for *Plasmodium malariae* and between 20% and 31.3% for *Plasmodium ovale*, it is evident that neither test is reliable for the detection of *Plasmodium ovale* and *Plasmodium malariae* infections.

Introduction

During the last 5 years, it has been established that rapid immunochromatographic malarial antigen detection assays (RDTs) have the potential for enhancing speed and accuracy in the diagnosis of both falciparum and vivax malaria, especially in nonspecialized laboratories [1]. Newer RDTs employ a second pan-malarial antibody, which is supposed to detect all four malarial species that infect humans.

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Currently, two RDTs are available that employ this second pan-malarial antibody. The ICT Malaria P.f./P.v. test (ICT Diagnostics, Australia), which was recently marketed under various labels [2], targets *Plasmodium falciparum*-specific histidine-rich protein 2 and a pan-specific aldolase produced by all four *Plasmodium* spp. The OptiMal test (Flow, USA), which is currently marketed by DiaMed, Switzerland [3], detects a *Plasmodium falciparum*-specific lactate dehydrogenase (LDH) and a second pLDH, common to all four malarial species that infect humans. A detailed description of the test principles can be found elsewhere [1].

In the current product inserts provided by the manufacturers of both tests, it is stated explicitly that each product can detect all four malarial species. Yet, the performance of both tests in the detection of *Plasmodium ovale* and *Plasmodium malariae* has been poor so far [4, 5, 6, 7, 8]. In order to provide more data on the accuracy and usefulness of these tests, the present study was conducted.

Materials and Methods

A total of four institutions in Germany and Portugal participated in the study. Each institution determined the sensitivity and specificity of both the ICT Malaria P.f./P.v. and the OptiMal tests for the detection of *Plasmodium malariae* and *Plasmodium ovale* in cases of imported malaria in a retrospective analysis. Over a period of 4 years, RDTs had been performed on patients presenting with febrile disease suggestive of malaria. Results obtained using ICT Malaria P.f./P.v. and/or OptiMal were compared with the results of standard expert microscopy as described elsewhere [9].

For our review and analysis of earlier reports on the performance of these RDTs for detecting *Plasmodium ovale* and *malariae*, we screened the English-language peer-reviewed journals in mid-April 2002 for relevant reports using Medline.

Results and Discussion

A total of 38 samples were tested in our study. Thirty-three samples (25 *Plasmodium ovale* and 8 *Plasmodium malariae*) were tested with the ICT Malaria P.f./P.v. test.

Table 1 Detection of *Plasmodium ovale* and *Plasmodium malariae* infection by microscopy, ICT Malaria P.f./P.v. and OptiMal in a total of 78 samples (own study group results, *n*=38; results retrieved from the literature, *n*=40; see text)

Test kit result	Thick-film microscopy result	
	P. ovale	*P. malariae*
ICT Malaria P.f./P.v.		
Positive[a]	5	3
Negative	20	13
Wrong species[b]	0	0
Sensitivity (%)	20	18.8
OptiMal		
Positive[a]	5	10
Negative	11	11
Wrong species[b]	0	0
Sensitivity (%)	31.3	47.6

[a] Indicating non-falciparum plasmodial infection
[b] Indicating infection with *Plasmodium falciparum*

All eight of the *Plasmodium malariae* samples tested false negative. Of the 25 *Plasmodium ovale* specimens, only 5 tested correctly positive, whereas the remaining 20 tested false negative. Five *Plasmodium ovale* samples were tested with the OptiMal test, and all of the results were false negative.

Only monoinfections were encountered in our study, and not all of the participating centers used both RDTs concomitantly. Thus, no data was available for the performance of the OptiMal test for detecting *Plasmodium malariae*. No false-positive RDT results indicating non-falciparum malaria were found. False species identification did not occur.

Our review of the literature revealed five reports related to the performance of RDTs [4, 5, 6, 7, 8]. Hunt-Cooke et al. [4], using OptiMal in Gambia, found that only one of three *Plasmodium malariae* infections and only one of four *Plasmodium ovale* infections were correctly identified by this test. Parasite densities were not reported. In a large cohort of patients with imported malaria in the UK, Moody et al. [5] found that 8 of 17 *Plasmodium malariae* infections and four of seven *Plasmodium ovale* infections tested correctly positive with OptiMal.

In two larger study cohorts in Indonesia, Dyer et al. [6] found three cases of *Plasmodium malariae* infection. All three tested negative with the ICT Malaria P.f./P.v. test. Parasite loads ranged between <50 and 4,080/µl, and all stages (schizonts, trophozoites, gametocytes) were present in blood. Mason et al. [7] noted that three of four cases of *Plasmodium malariae* from Thailand and Myanmar tested correctly positive with the ICT Malaria P.f./P.v. test. However, a single *Plasmodium malariae* isolate found by Iqbal et al. [8] in imported malaria infections in Kuwait tested negative with both RDTs. An analysis of all study results is presented in Table 1.

Another recent series from Mason et al. [10] reported on 34 cases of non-falciparum malaria from Myanmar. However, since the organisms were not identified to the species level, they were not included in the analysis. Nevertheless, these authors found a sensitivity of just 2.9% with the ICT Malaria P.f./P.v. test. For OptiMal, the sensitivity was 47.1% for non-falciparum plasmodia.

It has been suggested that test sensitivity correlates with the level of parasitaemia [7]. Yet, in one small series of non-falciparum cases[6], a negative test result also occurred in a sample with a parasite load of 4,080/µl, a level that is well above that at which the pan-malarial antibody was found to reliably detect both *Plasmodium vivax* and *Plasmodium falciparum* infections [11].

In contrast, Moody et al. [5] hypothesized that a lower affinity of the monoclonal antibodies for *Plasmodium ovale* or *Plasmodium malariae* might account for the reduced test sensitivity in both of the combination RDTs. However, Mason et al. [10] reasoned that the near-zero sensitivity reported for the HRP-2/aldolase kit as well as the previously reported low sensitivity of a pLDH kit for *Plasmodium falciparum* detection were most likely attributable to huge variations in batch quality. This raises concerns about the ability of these RDTs to detect the more dangerous *Plasmodium falciparum* and *Plasmodium vivax* infections.

Another important issue to consider is the possible decline in test quality due to reduced shelf life under tropical conditions. For the ICT test, the manufacturer's recommendation is to use the test kit within 3 months of storage under ambient conditions. To the best of our knowledge, one published study has already addressed this topic with regard to malarial RDTs. In their study of the shelf life of the ParaSight-F dipstick test in Ethiopia, Mengesha et al. [12] showed that after 1 year of storage at room temperature test results were similar to those found originally; thus, stability and long durability of the test strips is indicated. However, additional studies on other test formats, such as those used in this study, are lacking so far.

Although the number of tests performed in this study is small, we can conclude that with sensitivities well below 50% for the detection of *Plasmodium ovale* and *Plasmodium malariae*, the performance of both of the currently available combination RDTs is unacceptably poor. Consequently, expert microscopy remains the essential diagnostic tool for detecting cases of infection with these species.

References

1. Moody A (2002) Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev 15:66–78
2. Garcia M, Kirimoama S, Marlborough D, Leafasia J, Rieckmann KH (1996) Immunochromatographic test for malaria diagnosis. Lancet 347:1549
3. Piper R, Lebras J, Wentworth L, Hunt-Cooke A, Houze S, Baum MK, Chiodini P, Makler M (1999) Immunocapture diagnostic assays for malaria using *Plasmodium* lactate dehydrogenase (pLDH). Am J Trop Med Hyg 60:109–118

4. Hunt-Cooke A, Chiodini PL, Doherty T, Moody AH, Ries J, Pinder M (1999) Comparison of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (Optimal®) with microscopy for the detection of malaria parasites in human blood samples. Am J Trop Med Hyg 60:173–176
5. Moody A, Hunt-Cooke A, Gabbett E, Chiodini P (2000) Performance of the OptiMal malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital for Tropical Diseases, London. Br J Haematol 109:891–894
6. Dyer M, Tjitra E, Currie BJ, Anstey NM (2000) Failure of the 'pan-malarial' antibody of the ICT Malaria P.f./P.v. immunochromatographic test to detect symptomatic *Plasmodium malariae* infection. Trans R Soc Trop Med Hyg 94:518
7. Mason DP, Wongsrichanalai C, Lin K, Miller RS, Kawamoto F (2001) The panmalarial antigen detected by the ICT Malaria P.f./P.v. immunochromatographic test is expressed by *Plasmodium malariae*. J Clin Microbiol 39:2035
8. Iqbal J, Hira PR, Sher A, Al-Enezi AA (2001) Diagnosis of imported malaria by *Plasmodium* lactate dehydrogenase (pLDH) and histidine-rich protein 2 (PfHRP-2)-based immunocapture assays. Am J Trop Med Hyg 64:20–23
9. Jelinek T, Grobusch MP, Harms G (2001) Evaluation of a dipstick test for the rapid diagnosis of imported malaria among patients presenting within the network TropNetEurop. Scand J Infect Dis 33:752–754
10. Mason DP, Kawamoto F, Lin K, Laoboonchai A, Wongsrichanalai C (2002) A comparison of two rapid field immunochromatographic tests to expert microscopy in the diagnosis of malaria. Acta Trop 82:51–59
11. Tjitra E, Suprianto S, Dyer M, Currie BJ, Anstey NM (1999) 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. J Clin Microbiol 37:2412–2417
12. Mengesha T, Gebreselassie H, Mohammed H, Assefa T, Woldemichael T (1999) Parasight-F dipstick antigen test in the diagnosis of falciparum malaria in Ethiopia. East Afr Med J 76:626–629