

Ineffectiveness of the Binax NOW Malaria Test for Diagnosis of *Plasmodium ovale* Malaria

Malaria remains the most dangerous tropical disease for the French troops deployed overseas. In 2003, 768 cases in the French army were declared along with an increase of incidence of 400% in comparison with 2002, mainly due to the Licorne peacekeeping operation in Ivory Coast. In order to ensure fast diagnosis in the field, a rapid immunochromatographic test (NOW malaria test; Binax, Portland, Oreg.) has been provided to military doctors. This test detects the *Plasmodium falciparum*-specific HRP2 antigen and a panmalarial aldolase common to all species. The assay is sensitive for the detection of *P. falciparum* (1) and *Plasmodium vivax* (2) but, in our experience, is poorly sensitive for the detection of *Plasmodium ovale*, a species involved in cases of malaria among the French troops in western Africa.

Between November 2002 and August 2004, 114 samples from patients presenting a malaria attack were analyzed in the laboratory of the French military hospital of Bégin (Saint-Mande, France). The *Plasmodium* species identified were *P. falciparum* ($n = 93$), *P. ovale* ($n = 12$), *P. vivax* ($n = 9$), and *P. malariae* ($n = 1$). For each specimen, thin and thick blood films, the Binax NOW malaria test, and a specific SYBR green real-time PCR using the Lightcycler instrument (Roche Diagnostics, Meylan, France) were used for diagnosis. This technique is considered the "gold standard" in our laboratory. Sequences used for the design of primers were the 18S RNA genes of the four species (Table 1), which have been previously validated (data not shown). Thin and thick blood films were stained with a rapid coloration set (Diff-Quick; Dade Behring, Newark, Del.) and examined by two experienced microscopists during 20 min. The rapid immunochromatographic test was used according to the manufacturer's recommendations.

Among the 22 patients infected with non-*P. falciparum* species, 9 were positive for *P. vivax*, 12 were positive for *P. ovale*,

and 1 was positive for *P. malariae* by microscopic examination (Table 1). PCR was used to detect all the infections and confirm all microscopic identifications. The Binax NOW malaria test detected all cases of *P. vivax* infection (9 of 9) but only 3 of the 12 cases of *P. ovale* infection (25%). The test was positive in the only case of *P. malariae* infection. With *P. falciparum*, 89 cases were positive, and two false positives and one false negative were found.

Considering these data, it seems that the Binax NOW malaria test is not reliable for the detection of *P. ovale* infection. This has been previously described in a study including nine cases of *P. ovale* infection (2). The inability of the rapid immunochromatographic test to detect *P. ovale* has been also observed with the ICT Malaria P.f/P.v test (3, 4). The main explanation for the failure of the assay was low parasite density, but in this study all infections due to *P. ovale* were detected by microscopic examination. The inaccuracy could be due to low production of the aldolase by *P. ovale* or, as supposed by Mason et al., to regional variations in the genetic determinants of ICT panmalarial antigen (5). In case of suspicion of malaria challenge, the diagnosis of infection with *P. ovale* must not be elicited, and blood smear examination remains necessary for the elimination of the diagnosis.

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TABLE 1. Comparison of Binax NOW malaria test versus microscopic examination and PCR

Organism (n)	No. of positive results		
	Microscopy	Antigenemia assay	PCR ^a
<i>P. vivax</i> (9)	9	9	9
<i>P. ovale</i> (12)	12	3	12
<i>P. malariae</i> (1)	1	1	1
<i>P. falciparum</i> (93)	89	92	93

^aThe forward and reverse primer sequences were, respectively, as follows: for *P. vivax*, GAT ACT TCG TAT CGA CTT TGT GC (PVF) and CAA TCT AAG AAT AAA CTC CGA AGA GA (PVR); for *P. ovale*, CCC TAT TCT TCT TAA TTC GCA (POF) and AGT GGA GGA AAA CTA TA (POR); for *P. malariae*, TGT TTT TTT TAA TAA AAA CGT TCT TTT CC (PMF) and ACT TTT CAG TGG AGG AAA ACT ATA TAT TCT (PMR); and for *P. falciparum*, CTT TTG GCT TTA ATA CGC TTC (PFF) and TGA AGG AAG CAA TCT AAA AGT CAC (PFR).

Christine Bigaillon*
 Eléonore Fontan
 Jean-Didier Cavallo
 Eric Hernandez
 Laboratory of Medical Microbiology

André Spiegel
 North Department of Epidemiology and Public Health
 HIA Bégin
 94163 Saint-Mande Cedex, France

*Phone: 33 1 43 98 47 33
 Fax: 33 1 43 98 53 36
 E-mail: hia-begin-biologie@worldonline.fr