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Limited Level of Accuracy Provided by Available Rapid Diagnosis Tests for Malaria Enhances the Need for PCR-Based Reference Laboratories

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The rise of imported malaria cases and the high fatality rate in Europe make the search for new and easy diagnostic methods necessary. Rapid diagnosis tests (RDTs) are, in part, developed to cover the lack of diagnosis experience. Unfortunately, our data suggest that the accuracy of RDTs is insufficient and could increase the number of incorrect malaria diagnoses.

In recent years, countries in which malaria is not endemic have reported high and increasing numbers of imported malaria cases, with fatalities up from 3.8 to 20% (2). Preventing fatal outcomes in malaria cases requires early recognition of infection, accurate laboratory diagnosis, and prompt therapy (2). Unfortunately, health-care personnel in countries where malaria is not endemic frequently lack experience in the microscopic diagnosis of malaria. In Italy, 80% of cases had less than a 1-week elapse between the onset of malaria symptoms and the microscopic diagnosis, but the average diagnosis took 8.5 days and the range was 1 to 28 days (3). This fact makes the search for new and easy diagnostic methods necessary. Rapid diagnosis tests (RDTs) for malaria might offer a valid alternative to microscopy (5).

We studied 206 pre- and posttreatment samples from 169 patients in 1998 and 1999 by microscopic diagnosis and seminested multiplex PCR (4). These samples were also tested using three commercial RDT kits; 189 samples from 149 patients were tested with the ParaSight-F Kit (Becton Dickinson), 197 samples from 126 patients were tested with the OptiMal Kit (Flow Incorporated), and 54 samples from 41 patients were tested with the ICT *Pf*/*Pv* kit (Amrad). All patients (with ages of 16 months to 72 years) presented a history of fever and travel in the previous year to an area of malaria endemicity.

RDTs were performed according to the manufacturers' instructions. All microscopy-positive samples were confirmed by PCR (4). Furthermore, PCR detected 6 samples with mixed infections (4 *Plasmodium falciparum* plus *P. malariae* and 2 *P. falciparum* plus *P. ovale*) from samples that were characterized as *P. falciparum*-only by microscopy and 24 more positive samples (12 *P. falciparum*, 4 *P. ovale*, 6 *P. malariae*, and 2 *P. vivax*). The three RDT methods showed a high rate of false positives and false negatives (Table 1). Moreover, 29.2% of positive non-*P. falciparum* samples rendered a positive *P. falciparum* result when the ParaSight-F test was used, which suggests a high number of cross-reactions, as this test according to the manufacturer detects only *P. falciparum* infections. In the same way, according to the manufacturers, the OptiMal and ICT *Pf*/*Pv* kits are able to detect *P. falciparum* specifically and the other *Plasmodium* spp. unspecifically. Our data, however, show that

TABLE 1. False positives and false negatives of three RDTs compared to microscopy analysis confirmed by PCR for the detection of *Plasmodium* spp.

Method	No. of isolates with indicated result/total (%)					Avg time (days) of RDT-positive samples[e]
	False positive[a]	Positive after treatment	Positive, no *P. falciparum*[b]	False negative[c]	Negative, no *P. falciparum*[d]	
ParaSight-F	21/74 (28.4)	7/14 (50)	7/24 (29.2)	27/101 (26.7)		>10
OptiMal	15/62 (24.2)	4/9 (44.4)		28/126 (22.2)	15/24 (62.5)	>17
ICT *Pf*/*Pv*	1/14 (7.1)			6/54 (11.11)	5/6 (83.3)	

[a] Positive by RDT when microscopy and PCR were negative (only for samples before any treatment).
[b] RDT was positive for *Plasmodium* spp. other than *P. falciparum*. For the ParaSight-F test, these values could be considered false positives.
[c] Negative by RDT when microscopy and PCR were positive (only for samples before any treatment).
[d] RDT was negative for *Plasmodium* spp. other than *P. falciparum* infection characterized by microscopy and PCR. For the OptiMal and ICT *Pf*/*Pv* tests, these values could be considered false negatives.
[e] Average time in which a sample is still positive by RDT (negative by microscopy and PCR) after treatment.

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TABLE 2. Study of the sensitivity and specificity of RDTS compared to those of thin-thick blood smears and PCR

RDT	Sensitivity (%)		Specificity (%)		PPV[a]		PPN[a]		Kappa coefficient		No. positive[b]/total	
	Microscopy	PCR	Microscopy	PCR	Microscopy	PCR	Microscopy	PCR	Microscopy	PCR	Microscopy	PCR
ParaSight-F[c]	76.81	67.86	76.66	77.14	65.43	70.37	85.18	75.00	51.57	45.16	53/189	57/189
	92.00	84.48	80.20	81.72	69.70	74.24	95.29	89.41	66.80	64.53	46/151	49/151
OptiMal	66.12	62.67	81.48	84.43	62.12	71.21	83.97	78.63	47.79	48.21	41/197	47/197
	87.80	77.55	87.70	86.84	70.58	71.70	95.53	90.00	69.85	62.93	36/163	38/163
ICT *Pf*/*Pv*	81.25	68.42	97.36	97.14	92.85	92.85	92.50	85.00	81.56	69.76	13/54	13/54
	100	91.66	96.66	96.55	91.66	91.66	100	96.55	93.96	88.21	11/41	11/41

[a] PPV, positive predictive value; PPN, negative predictive value.
[b] Number of positive samples by both methods under evaluation.
[c] For each test, the first row of data shows a comparison including all pre- and posttreatment samples and positives for any *Plasmodium* sp. The second row shows a comparison without posttreatment samples and samples positive for *Plasmodium* spp. other than *P. falciparum*.

these two diagnostic methods missed, respectively, 62.5 and 83.3% of *Plasmodium* infections other than *P. falciparum* (Table 1). Also, a lack of sensitivity was detected at low levels of parasitemia (under 100 parasites/μl of blood). The comparison of the sensitivity and specificity of the RDTs versus microscopy or PCR (Table 2) indicated that the detection rate was low except for *P. falciparum*. After antimalaria treatment, approximately 55% of treated patients remained positive (>10 days on average for ParaSight-F and >17 days for OptiMal), while microscopy and PCR tests were negative.

The data presented here indicate that the accuracy of the three examined RDTs was insufficient and could lead to the incorrect diagnosis of malaria. The increasing use of RDTs in Spanish hospitals and the comparative results of sensitivity and specificity of RDTs versus PCR diagnosis indicate an essential need to enhance the role of reference laboratories with PCR-based diagnostic capabilities. Our data suggest that RDTs could help the initial assessment of malaria in returned travellers (1) and migrants, but this and other reported studies indicate the need to develop more specialized laboratories with available confirmatory diagnostic techniques (PCR). The main difficulty still encountered by the use of RDTs is the correct identification of *Plasmodium* species. False-positive results derived from patients with immunological disorders and/or rheumatoid factor have been partially corrected by the latest versions of kits targeting the HR-II protein of *P. falciparum*. However, the occurrence of false positives due to antigen persistence is still a serious constraint to the assessment of treatment failure (Table 1). Low sensitivity is apparent for patients with low parasite numbers and is commonly encountered for patients with low immunity or nonimmune patients treated with antimalarial chemoprophylaxis. The RDTs were, in part, developed to cover the lack of experience in microscopic malaria diagnosis, but unfortunately our data suggest that in the current stage these methods could increase the number of incorrect diagnoses. RDTs could play and will play in the future an important role in malaria diagnosis. However, for the present they should be used with great caution and should not replace conventional microscopy or PCR.

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