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Short Report: Evaluation of Two New Immunochromatographic Assays for Diagnosis of Malaria

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Abstract. We assessed the performance of two new commercially available rapid diagnostic tests (RDTs) for malaria (SD Bioline Malaria Ag Pf® test and Ag Pf/Pan® test) in 200 patients with uncomplicated malaria between August and October 2007 in Madagascar. Results of the two RDTs were compared with those obtained by microscopy and real-time polymerase chain reaction. The sensitivity and specificity for detection of *Plasmodium falciparum* were 93% and 98.9%, respectively, for the SD Bioline Malaria Ag Pf® test and 92.9% and 98.9% for the SD Bioline Malaria Ag Pf/Pan® test. The sensitivity of the SD Bioline Malaria Ag Pf/Pan® test was much lower for detection of other species (63.6%). The sensitivity of the two new assays decreased to 77.3% at parasitemia levels < 100 parasites/μL for detection of *P. falciparum*.

In most malaria-endemic countries, since the introduction of more effective but more expensive antimalarial drug combinations, such as artemisinin combination therapy as first-line treatment, parasitologic confirmation has become essential in routine malaria case management. This medical practice ensures that antimalarial drugs are administered to patients who need them. This is considered as a public health priority by the World Health Organization, in particular in limiting the unnecessary use of inappropriate treatments and thereby avoiding selection and spread of drug-resistant *Plasmodium falciparum* parasites.

Over the past two decades, malaria rapid diagnostic tests (RDTs) have been developed for use in any situation where the only realistic alternative was the clinical diagnosis of malaria. These diagnostic tests are fast and easy to perform, and do not require electricity or specific equipment.[1–3] Currently, 86 malaria RDT products from 28 different manufacturers are available.[4] They are all based on the same principle and use antibodies that detect only three groups of antigen. Most products are based on the detection of a *P. falciparum*-specific protein, either *P. falciparum* histidine-rich protein 2 (PfHRP2) or *P. falciparum* lactate dehydrogenase (pfLDH). Some tests detect *P. falciparum*-specific and pan-specific antigens (aldolase or pan-malaria pLDH) and distinguish a non-*falciparum* infection from *P. falciparum* or *P. falciparum*/mixed infections.

The purpose of this study was to assess the performance of two new commercially available immunochromatographic assays: the SD Bioline Malaria Ag Pf® (ref. 05FK50) test and the SD Bioline Malaria Ag Pf/Pan® (ref. 05FK60) test (Standard Diagnostics Inc., Suwon City, South Korea). These tests both contain a membrane strip encased in a flat plastic housing. The strip is precoated with two antibodies: one that is specific for *P. falciparum* HRP2 (both kits) and one that is pan-specific for pLDH from *Plasmodium* species (SD Bioline Malaria Ag Pf/Pan®).

Our study was carried out between August and October during the season of low malaria transmission at the primary health center in Ampasimpotsy, a rural area in the western foothill of the central highlands in Madagascar. Malaria transmission in this area is low and predominantly seasonal. The main vector is *Anopheles funestus* and the number of infective bites associated with *P. falciparum* is estimated to be 1–2 per person each year.[5,6] Patients with a fever, or who have had a fever within the past 24 hours, and with typical malaria symptoms were invited to participate in the study. Pregnant women and patients with signs of severe and complicated *P. falciparum* malaria, as defined by the World Health Organization (2001), were excluded.[7] The study protocol was reviewed and approved by the expert committee of the National Malaria Control Program of the Ministry of Health of Madagascar. All subjects included provided informed consent.

Venous blood samples (3 mL) were collected in EDTA anti-coagulation tubes, and thick and thin blood films were prepared by a trained technician. Immunochromatographic tests were immediately carried out according to the manufacturers' instructions. All tests were kept at room temperature and opened just before use to avoid humidity damage. An RDT result was considered positive when both the internal control and the test band were stained (irrespective of the intensity of the staining). A test result was considered negative if only the internal control was stained. The result of an RDT was considered invalid if the internal control was not stained. Patients with a positive result for the RDT were promptly treated, as specified by the National Malaria Policy, with a combination of artesunate and amodiaquine (Falcimon®; Cipla Ltd., Mumbai, India).[8] Blood and blood films were sent to the malaria unit laboratory within 24 hours in a controlled cool box at 4°C. Thick and thin blood smears were stained with 10% Giemsa for 10 minutes. A microscopist who was blind to the results of clinical diagnosis and RDTs examined the blood films for parasites and identified the parasite species. A minimum of 200 consecutive fields were counted in the thick blood film before a slide was classified as negative. Depending on the parasite density, parasites in thick blood films were counted against 200 or 500 leukoytes. Parasite density was estimated assuming 8,000 leukocytes/μL of blood. As previously described,[9] parasite DNA was extracted and *Plasmodium* species were detected using a real-time polymerase chain reaction (PCR) with a RotorGene 3000 thermocycler (Corbett Life Science, Sydney, New South Wales, Australia)

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TABLE 1

Patients positive for *Plasmodium* spp. by the reference method (microscopy/PCR), the SD Bioline Malaria Ag Pf (ref. 05FK50) test, and the SD Bioline Malaria Ag Pf/Pan (ref. 05FK60) test, Madagascar, August–October 2007*

Microscopy/PCR	No. (%)	SD Bioline Malaria Ag Pf			SD Bioline Malaria Ag Pf/Pan			
		Negative	*Pf*	Invalid	Negative	*Pf*	Non-*Pf*	Invalid
Negative	91 (45.5)	89	1	1	89	1	1	0
P. falciparum	85 (42.5)	6	78	1	6	77	0	1
P. vivax	199 (9.5)	–	–	–	0	7	12	0
P. malariae	3 (1.5)	–	–	–	1	0	2	0
P. falciparum plus *P. vivax*	1 (0.5)	0	1	0	0	1	0	0
P. falciparum plus *P. malariae*	1 (0.5)	0	1	0	0	1	0	0

* PCR = polymerase chain reaction; *Pf* = *Plasmodium falciparum*.

carried out by technicians blind to the results of microscopy and RDT testing.

Data were entered, processed, and analyzed using Epi-Info version 3.3.2 software (Centers for Disease Control and Prevention, Atlanta, GA). The RDT results were compared with the reference method (combination of real-time PCR and Giemsa stain microscopy results) for sensitivity and specificity. Sensitivity was calculated as the proportion of samples with malaria parasites detected using the reference method that gave positive RDT results. Specificity was measured by the proportion of samples negative by the reference method that showed negative RDT results. Positive and negative predictive values were the proportion of all positive samples that were true positive samples and the proportion of all samples negative that were true negative samples.

A total of two hundred patients 0.5–63 years of age (mean ± SD age = 13.8 ± 14.9 years) were recruited; 40% were < 5 years of age, 26.5% were 5–15 years of age, and 33.5% were > 15 years of age. The male:female ratio was 1.2:1. The mean ± SD axillary temperature was 38.1 ± 1°C (range = 36.1–40.7°C) and the mean ± SD parasitemia was 16,757 ± 42,631 parasites/μL (range = 16–285,000 parasites/μL). Thirteen percent of these patients reported that they had received anti-malarial therapy before consultation (sulfamethoxazol-trimethoprim in 56%, chloroquine in 28%, sulfadoxine-pyrimethamine in 8%, and quinine in 8%).

Three results were discordant between microscopy and real-time PCR: among two isolates classified as *P. falciparum* malaria by real-time PCR, one was negative and one was classified as *P. vivax* malaria by microscopy; the third isolate was classified as mixed *P. falciparum/P. vivax* malaria by real-time PCR was classified as *P. falciparum* malaria by microscopy. The three isolates showed the same results as the real-time PCR results. The reference method was positive for 109 (54.5%) of the 200 malaria cases: *P. falciparum* was present in 78%, *P. vivax* in 17.4%, *P. malariae* in 2.8%, and mixed infections with *P. falciparum/P. vivax* or *P. falciparum/P. malariae* in 0.9% of the positive specimens (Table 1). Three results were invalid: two (1%) with the SD Bioline Malaria Ag Pf® (ref. 05FK50) test and one (0.5%) with the SD Bioline Malaria Ag Pf/Pan® (ref. 05FK60) test. The performance of the two RDTs is shown in Table 2. The sensitivity of the RDTs for *Plasmodium* spp. at different levels of parasitemia is summarized in Table 3. Consistent with the World Health Organization recommendation for RDT performance, the two RDTs had sensitivities greater than 95% for samples with parasitemia levels ≥ 100 parasites/μL (98.4%, 95% confidence interval = 92.5–99.9% for both tests).

This study provides the first evaluation of performance of two new commercially available immunochromatographic assays. The levels of performance were similar to those found in previous studies.[9–13] We used a combination of real-time PCR and microscopy as a reference method for classification of isolates. This method thereby had the advantage of combining the high sensitivity (especially at low parasite density) and specificity (to correctly identify the parasite species) of real-time PCR, with the ability to estimate the parasite density by microscopy. We observed only three discordant results (1.5%) and an agreement rate of 0.97, which is comparable to our published data.[9] Moreover, as previously reported, we also found that the sensitivities of the two tests decreased with the level of the parasitemia.[14–17] For *P. falciparum*, the sensitivity of the both tests started to decrease at levels of parasitemia < 500 parasites/μL. For *P.* non-*falciparum*, the sensitivity of the SD Bioline Malaria Ag Pf/Pan test started to decrease at levels of parasitemia 10-fold higher (< 5,000 parasites/μL). We found six false-negative results with both tests for *P. falciparum* malaria. For all isolates, parasite density was < 100/μL, with the exception of one isolate with a parasite density of 420/μL. The only false-positive case for *P. falciparum* malaria was in a patient previously treated with sulfadoxine-pyrimethamine. A substantial proportion of results

TABLE 2

Diagnostic performance of the SD Bioline Malaria Ag Pf (ref. 05FK50) test and the SD Bioline Malaria Ag Pf/Pan (ref. 05FK60) test for detection of *Plasmodium* spp. in field study patients, Madagascar, August–October 2007*

Characteristic	SD Bioline Malaria Ag Pf	SD Bioline Malaria Ag Pf/Pan	
	Pf (n = 176)	*Pf* (n = 175)	Non-*Pf* (n = 112)
Sensitivity (95% CI)	93.0% (86.0–97.1%)	92.9% (88.9–97.1%)	63.6% (42.4–81.5%)
Specificity (95% CI)	98.9% (94.6–99.9%)	98.9% (94.6–99.9%)	98.9% (94.6–99.9%)
Positive predictive value (95% CI)	98.8% (94.1–99.9%)	98.7% (94.0–99.9%)	93.3% (71.3–99.7%)
Negative predictive value (95% CI)	93.7% (87.3–97.4%)	93.7% (87.3–97.4%)	91.7% (84.8–96.1%)
Accuracy (95% CI)	96.0% (92.3–98.2%)	96.0% (92.2–98.2%)	91.9% (85.8–96.0%)

* *Pf* = *Plasmodium falciparum*; CI = confidence interval.

TABLE 3

Sensitivity of SD Bioline Malaria Ag Pf (ref. 05FK50) test and the SD Bioline Malaria Ag Pf/Pan (ref. 05FK60) tests for detection of *Plasmodium* spp. at different levels of parasitemia in field study patients, Madagascar, August–October 2007*

Parasitemia/microliter of blood	No. of isolates	Sensitivity (95% CI)		
		SD Bioline Malaria Ag Pf	SD Bioline Malaria Ag Pf/Pan	
		Pf (n = 176)	*Pf* (n = 175)	Non-*Pf* (n = 112)
> 50,000	8	100% (68.8–100%)	100% (68.8–100%)	ND
5,001–50,000	43	100% (92.0–100%)	100% (92.0–100%)	100% (60.7–700%)
501–5,000	32	100% (82.9–100%)	100% (82.9–100%)	93.3% (71.3–99.7%)
100–500	5	75.0% (24.2–98.7%)	75.0% (24.2–98.7%)	100% (5.0–100%)
< 100	21	77.3% (56.6–91.2%)	77.3% (56.6–91.2%)	ND

* *Pf* = *P. falciparum*; CI = confidence interval; ND = no data.

obtained by the SD Bioline Malaria Ag Pf/Pan test resulted in misclassification or were false-negative: 37% (7 of 19) of *P. vivax* malaria isolates classified as *P. falciparum* malaria (two isolates with parasitemias of 5,000–50,000/μL and five isolates with parasitemias of 500–5,000/μL) or 33% (1 of 3) for *P. malariae* malaria isolates, which were negative. Both tests were fairly easy to use and interpret, had consistent results, and were simple to store with no cold chain requirement.

In conclusion, these two new RDTs performed to a similar level as other commercially available devices. These results are consistent with World Health Organization recommendations for RDT performance and offer a good alternative tool for the diagnosis of malaria in disease-endemic areas.

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