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EVALUATION OF THREE RAPID TESTS FOR DIAGNOSIS OF *P. FALCIPARUM* AND *P. VIVAX* MALARIA IN COLOMBIA

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Abstract. The diagnostic capacity of three malaria rapid diagnostic tests (RDTs), NOW-Malaria-ICT, OptiMAL-IT, and Paracheck-Pf, was evaluated against expert microscopy in Colombia. We tested 896 patients, of whom microscopy confirmed 139 *P. falciparum*, 279 *P. vivax*, and 13 mixed *P.f/P.v* infections and 465 negatives. Paracheck-Pf and NOW-malaria-ICT were more accurate in detecting *P. falciparum* (sensitivities 90.8% and 90.1%, respectively) in comparison with Optimal-IT (83.6%). NOW showed an acceptable *Pf* detection rate at low densities (< 500/μL), but resulted in a higher proportion of false positives. For *P. vivax* diagnosis, Optimal-IT had a higher sensitivity than NOW (91.0% and 81.4%, respectively). The choice between the two *Pf/Pv* detecting RDTs balances *P. falciparum* and *P. vivax* detection rates. Considering some degree of *P. falciparum* overtreatment and failure to detect all *P. vivax* cases as more acceptable than missing some cases of *P. falciparum*, we recommend careful implementation of NOW-malaria-ICT in areas where microscopy is lacking. The price is however still a constraint.

INTRODUCTION

In Colombia, malaria represents an important health problem, affecting mainly populations living in rural areas. Remote areas of the country have now become inaccessible because of a lack of control and constant threat of violence. The indigenous communities that live in these areas often travel several hours or days to reach the nearest health services. In the *Zona Atlantica*, the northern coastal area of the country, *Médecins sans Frontières* (MSF) provides healthcare to these groups through rural health-posts and mobile clinics. Diagnosis and treatment of malaria is an essential service.

In this area of low transmission, treatment of malaria cases should ideally be based on biologic diagnosis because of the nonspecific nature of malaria symptoms,[1] and the fact that infections with *P. falciparum* and *P. vivax* cannot be distinguished clinically, although different treatment is required. Detection of parasites in the blood by microscopy remains the most common method for the diagnosis of malaria in Colombia, but materials, supply lines, and trained staff are not sufficient in the isolated rural areas where MSF works nor easily applied in mobile clinics. Accurate malaria rapid diagnostic tests (RDTs) would greatly improve the quality of diagnosis and treatment of malaria in these remote settings.

Several rapid diagnostic test kits for malaria exist, which are fast, easy to perform, and can be carried out by relatively unskilled staff. The most commonly used tests for *P. falciparum* are based on the immuno-chromatographic detection of the histidine-rich protein-2 (HRP-2), a protein produced by asexual stages and young gametocytes of *P. falciparum*[2] or of *Plasmodium* lactate dehydrogenase, pLDH.[3] pLDH can be either species-specific antigens detecting *P. falciparum* or *P. vivax* or 'pan-malarial' pLDH, detecting all four species of *Plasmodium*.[4] In addition, there is another antigen, aldolase, which can detect all species of *Plasmodium*.[5,6] The rapid tests we were interested in were (1) the Paracheck-Pf, a *P. falciparum* specific test, based on detection of parasite HRP-2, which has proven its accuracy and usefulness in many MSF-projects worldwide, (2) the Optimal-IT, a test that can detect *P. falciparum* as well as other *Plasmodium* species by Pf-specific PLDH and pan-malarial PLDH, and (3) the NOW malaria ICT, a test that combines Pf-specific HRP-2 with pan-malarial aldolase.

Most rapid tests have shown high accuracy in laboratory and field-based studies, though their sensitivity declines at low parasitemias (< 300–500/μL).[7,8] Test performance may vary for different geographical populations, levels of disease prevalence, and presence of different parasite species.[9] It has been suggested that natural immunity in endemic areas may reduce the sensitivity, but this has not been proven.[10] To determine the usefulness of RDTs in the specific situation of low-endemic, mixed *P. falciparum* and *P. vivax* malaria in southern American Colombia, we compared the diagnostic capacity of Optimal-IT and NOW Malaria ICT with the capacity of the MSF-standard, Paracheck test and that of expert microscopy, the latter considered as our 'gold standard'. Additionally, the ease of use of the various tests was evaluated.

MATERIALS AND METHODS

Study area. The survey was performed in a Malaria Center in Tierralta, *Zona Atlantica*, Colombia. Colombia is an area of hypo-endemic malaria transmission with 2–5% annual parasite rate in the one third of the population that lives at risk of the disease, which is due to both *P. vivax* (54%) and *P. falciparum* (46%).[11] Rural/jungle areas below 800 meters are most affected. It is one of the Latin American countries where malaria morbidity is rising again, due to climate factors and drug resistance among other factors.[9] Chloroquine-resistant *P. falciparum* exists widely (level 44–97%) and resistance to sulfadoxine/pyrimethamine (0–27%) and amodiaquine (0–50%) is also reported.[12,13]

Patients. Patients of all ages with suspected malaria were recruited according to routine criteria of the health workers in the Malaria Center (i.e., fever or a history of fever and/or other complaints indicating a possible malaria infection). Persons who came for follow-up visits of an earlier episode of malaria or within 4 weeks after a (confirmed and treated) malaria infection were excluded. Patients were asked for their informed consent and when accepted, they had their blood sampled for blood slides and 3 RDTs. Patients whose results

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were positive for malaria (for any test) were treated according to the National Protocol with Amodiaquine + Sulfadoxine-Pyrimethamine + single dose Primaquine for *P. falciparum* and Chloroquine + 14 days Primaquine for *P. vivax*.

Sample size. The sample size was calculated assuming RDT sensitivities in the range of 70–90%. A number of 140 positive patients had to be tested to reach a precision of 5% for a sensitivity of 90%, or 7% for a sensitivity of 80%, with alpha error = 0.05. For proper assessment of sensitivity of the *Pf/Pv* tests, this number was required for both *P. falciparum* and *P. vivax*. Applying similar calculations to the specificity, also 140 negative patients had to be tested. Recruitment was continued until the required number of *P. falciparum* patients (more rare than *P. vivax* or negative) was reached.

Data and sample collection. A patient form was filled with basic clinical and demographic information. The rapid test kits were opened only after the patient had been selected and interviewed by the medical staff. Capillary blood was collected by finger-prick, sampling a standard volume of blood for each test according to the manufacturer's instructions, with the sampling device provided. Finger-pricking was repeated when needed to collect enough blood. Each selected patient had his/her blood examined by four methods: Optimal-IT, Paracheck-Pf, NOW malaria, and microscopy. The RDTs were compared by the bacteriologists scoring a list of issues on ease-of-use and other characteristics.

RAPID DIAGNOSTIC TESTS

1. Paracheck-Pf (Orchid Biomedical Systems, Goa, India), individually packed test cassettes diagnosing *P. falciparum* infections by HRP-2 detection, requiring one drop of blood (5 μL) to be collected with a loop-shaped plastic sampling tool included with the device; there is one test line that demonstrates *P. falciparum* infection when it turns pink and results are read at 15 minutes.
2. Optimal-IT (Diamed AG, Switzerland), individually packed dipstick kits, detecting parasite pLDH specific for *P. falciparum* in one capture site and pan-pLDH detecting all four *Plasmodium* species in a separate capture line. Blood sampling 8–12 μL is done with a plastic capillary pipette provided. The test device consists of two tubes, in which the dipstick stands for 10 minutes each, so results are read after 20 minutes.
3. NOW Malaria ICT (Binax, Portland, USA), a card-type test with one capture line specific for *P. falciparum* through *Pf* HRP-2 detection and the second line detecting all *Plasmodium* species based on aldolase. The blood sample (15 μl), collected with a small glass capillary, is applied to one side of the card, where it runs up first; the card is then closed. Wash reagent clears the strip in about 10 minutes until control and/or test lines appear as pink-colored bands in a reading window.

Rapid diagnostic tests were read by the same bacteriologist and confirmed by a second independent reader when needed, all according to the manufacturer's instructions. The first person performed, read and recorded the results of the three tests and after that a second opinion was obtained from a second person reading again the same tests and recording the results. Each person read the RDT without knowing the result of the other reader or of the blood film. Results were compared and discussed to come to a consensus in case of different readings. At the end of this procedure, results were recorded on the patient's individual record form.

Microscopy diagnosis. Two thick smears were taken on one slide and one thin smear on a separate slide. Thick smears were submerged in methylene blue for 1 second, washed with buffer solution and left to dry, thereafter stained horizontally with Field solution (one drop of solution A and one drop of solution B per 10 mL) in phosphate buffer B for 10 minutes, in accordance with nationally standard methods. Thin smears were fixed with methanol but not stained until necessary for species determination or better examination of the infection. Thick smears were evaluated by a well-trained, experienced microscopist, unaware of RDT results. A thick smear was considered negative if no parasites were seen in at least 200 fields. For positive smears, the number of parasites was counted in the number of fields needed to reach 200 white blood cells (WBCs) or 500 WBCs for low densities. Parasite density per μl was calculated assuming a standard of 8000 WBCs per μl of blood as per WHO guidelines.[14] Presence of gametocytes or schizonts was also recorded. Thin smears were used for species verification.

Quality control. For internal quality control, a second independent reading was done by a different microscopist on about one third of the slides, especially low-density parasitemias and mixed infections. Slides with discordances between the two microscopists or between rapid tests and slide-reading (in terms of positivity and species determination), and a random sample of 20% of other slides, were sent to the University of Antioquia for external cross-checking. Disagreement results between the two were sent on to a third laboratory, of the National Health Institute in Bogota. In cases in which both reference laboratories agreed on one diagnosis different from ours, results were corrected accordingly.

The RDTs had a guaranteed history of proper storage (temperature 4–30°C, low humidity) and transport conditions, and were used within shelf life. Only tests from one batch were used.

Analysis. The performance of Paracheck-Pf, NOW ICT Malaria, and Optimal-IT tests was expressed by calculating the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV), for *P. vivax* and *P. falciparum* separately, taking microscopy results as the "gold standard". To assess the performance of the Optimal-IT test and NOW Malaria for diagnosis of *P. vivax,* cases with mixed infections with *P. falciparum* were excluded, because the pan-malaria antigen turns up positive due to *P. falciparum* infection. For performance on *P. falciparum* detection, mixed infections with *P. falciparum* were included. Slides with gametocytes only were regarded as negative for further analyses. Data were analyzed in SPSS 12.0 (Chicago, IL) and Epi-info 6.04 (CDC, Atlanta, GA). Proportions were compared using the χ^2 test. Agreement (kappa statistic, κ) between RDT and microscopy provided an estimation of the reliability of the RDT ($\kappa > 80\%$ was considered as a measure of very good reliability).

Ethical considerations. The protocol was reviewed and approved by the Ethical Board of MSF (a committee of external experts) and the Ethical Board of the University of Antioquia and received approval from the National Institutes of Health, Bogotá. The provincial and local health authorities in Tier-

TABLE 1
Baseline characteristics of study participants, MSF Tierralta, Colombia, 2005

	Under 5 years N (%)	5 to < 15 years N (%)	15 years and older N (%)	Total N (%)
Number of patients	73 (8.1)	120 (13.4)	703 (78.5)	896
Sex: M/F (% F)	29/44 (39.7)	42/78 (35.0)	180/523 (25.6)	251/645 (28.0)
Age*	2.4 ± 1.1 (1–4.5)	9.5 ± 2.9 (5–14)	28.9 ± 11.7 (15–73)	24.2 ± 13.9 (1–73)
Temperature* (°C)	37.0 ± 1.1 (34.2–40.2)	37.0 ± 1.1 (34.9–40.4)	36.6 ± 0.8 (34.2–40.0)	36.7 ± 0.9 (34.2–40.4)
Fever (T > 37.5°C)	20 (27.4)	33 (27.5)	95 (13.5)	148 (16.6)
Fever history in 2 days	68 (93.2)	113 (94.2)	634 (90.2)	815 (91.0)
P. falciparum	5 (6.8)	20 (16.7)	114 (16.2)	139 (15.5)
P. vivax	23 (31.5)	31 (25.8)	225 (32.0)	279 (31.1)
Mixed *Pf/Pv*	0	2 (1.7)	11 (1.6)	13 (1.5)
Negative	45 (61.6)	67 (55.8)	353 (50.2)	465 (51.9)
Parasite density *P.f.*† (per μl)	1862 (40–25,600)	4654 (39–45,898)	2200 (39–87,560)	2438 39–87,560)
Parasite density *P.v.*† (per μl)	6520 (40–38,990)	4653 (40–35,400)	1778 (39–42,912)	2196 (39–42,912)

* Values given as mean ± SD (standard deviation) and range (min-max value).
† Parasite density given as geometric mean and range.

ralta were informed of the plans of study and supported the study by spreading information to their staff in rural health centers and the local population. Selected patients or the caregivers of children under 15 years were asked for their informed, written consent. The patients in study were taken care of in exactly the same way as non-study patients, except for the few for whom an extra finger-prick was needed to collect enough blood.

RESULTS

From May 10, 2005 to July 11, 2005, a total of 2937 patients visited the Malaria Center in Tierralta, of which 896 patients were included in our study. According to the microscopy results, 139 had *P. falciparum* infections, 279 *P. vivax*, 13 mixed infections of *Pf/Pv*, and 465 patients were negative for malaria, including two with *P. falciparum* gametocytes only. The majority of patients were adults (79%) (Table 1). Most of the patients were male (646 of 896, 72%), often workers from the forest-based agricultural locations around Tierralta. The proportion positive for *P. falciparum* was 17% and for *P. vivax* 33%. The parasite densities of patients were for the most part below 5000 parasites per μl of blood. The geometric mean parasite density was similar, about 2300 p/μl for both the *falciparum* and the *vivax* infections.

Quality control of 226 slides in the first reference laboratory resulted in 16 different slide results; these were re-read in the second reference laboratory finally leading to 11 results for which diagnosis differed from the MSF bacteriologists, hence a 'disagreement rate' of 4.9%. Discordances were six infections classified as mixed *P. falciparum*/*P. vivax*, which were diagnosed as *P. vivax* by the other laboratories, four low-density *P. falciparum* infections (39–240 trophozoites per μl of blood) that were regarded as negative in the two other laboratories and one mixed infection that the others classified as *P. falciparum* only. For further calculations these 11 cases were adapted to the diagnosis of the reference laboratories.

Rapid diagnostic tests versus microscopy. The results of microscopy were in agreement in 93% of cases for Optimal-IT, 87% with NOW malaria ICT, and 98% of cases for Paracheck (*P. falciparum* only). Diagnosis by Optimal-IT gave 10 false positives (3 *P. falciparum*, 6 Pan-*Plasmodium*, and 1 Pf + Pan-Pl) and 46 false negatives (19 *P. falciparum*, 25 *P. vivax*, and 2 mixed). The NOW resulted in 47 false positives (44 *P. falciparum*, 1 pan-Pl, and 2 Pf + Pan-Pl) and 54 false negatives (4 *P. falciparum* and 48 *P. vivax* and 2 mixed). Paracheck gave 1 false *P. falciparum* and 15 false negatives (9 *P. falciparum* and 6 mixed). NOW and Paracheck gave a positive result for pure *P. falciparum* when microscopy indicated a pure *P. vivax* infection in four and three cases, respectively (Table 2).

Validity of the rapid diagnostic tests. The sensitivity of the NOW test for *P. falciparum* was similar to that of Paracheck (91% and 90% with 95% CI: 85–95 and 84–94), whereas Optimal-IT had a somewhat lower sensitivity (84%, 95% CI: 77–89), but this difference was not significant (Table 3). The specificity for *P. falciparum* of NOW malaria ICT was signifi-

TABLE 2
Results of malaria blood tests of study patients by microscopy versus RDT: Optimal-IT, NOW malaria ICT, and Paracheck Pf. MSF Tierralta, Colombia, 2005

		Optimal-IT				NOW malaria ICT				Paracheck	
Microscopy	N	Neg	Pf	Pf + Pan-*Pl**	Pan-*Pl** only	Neg	Pf	Pf + Pan-*Pl**	Pan-*Pl** only	Neg	Pf
Negative	465	455	3	1	6	418	44	2	1	464	1
P. falciparum	139	19	1	119	0	4	29	106	0	9	130
P. vivax	279	25	0	9	245	48	4	20	207	276	3
Mix Pf/Pv	13	2	0	7	4	2	1	8	2	6	7
Total	896	501	4	136	255	472	78	136	210	755	141

* Pan-*Pl* = Pan-*Plasmodium* line positive.

TABLE 3
Diagnostic performance of rapid tests to detect malaria parasites: A) for *P. falciparum* and B) for *P. vivax*. MSF Tierralta, Colombia, 2005

	Optimal-IT	NOW ICT	Paracheck	χ^2
P. falciparum	(n = 896)	(n = 896)	(n = 895)	*P* values
Sensitivity (95% CI)	83.6 (76.5–88.9)	90.8 (84.7–94.7)	90.1 (84.0–94.2)	0.097
Specificity (95% CI)	98.3 (96.9–99.0)	90.6 (88.2–92.5)	99.5 (98.5–99.8)	< 0.001
PPV (95% CI)	90.7 (84.3–94.8)	66.3 (59.4–72.7)	97.2 (95.0–99.1)	< 0.001
NPV (95% CI)	96.7 (95.1–97.8)	98.0 (96.5–98.8)	98.0 (96.7–98.8)	0.18
κ (95% CI)	0.84 (0.78–0.90)	0.71 (0.65–0.77)	0.92 (0.86–0.98)	
Non-*P. falciparum**	(n = 883)	(n = 883)		
Sensitivity (95% CI)	91.0 (86.9–94.0)	81.4 (76.2–85.7)		0.001
Specificity (95% CI)	98.6 (97.0–99.4)	99.4 (98.2–99.9)		0.189
PPV (95% CI)	97.3 (94.4–98.9)	98.7 (96.2–99.7)		0.28
NPV (95% CI)	95.0 (92.8–96.8)	90.5 (87.6–92.8)		0.003
κ (95% CI)	0.91 (0.83–0.99)	0.84 (0.76–0.92)		

* Mixed infections were excluded from calculations for non-*P. falciparum*.

cantly lower (91% [88–93]) than both Paracheck (100% [99–100]) and Optimal-IT (98% [97–99]). The *Pf* Positive Predictive Value of NOW was low (66% [(59–73]). For *non-P. falciparum* NOW scored lower in sensitivity than Optimal-IT (81% [76–86]) versus 91% (CI 87–94). The specificity for *non-P. falciparum* in both Optimal-IT and NOW test was high (99% each). Kappa κ values showed that for *P. falciparum* Paracheck was most reliable, with a κ value of 0.92; the NOW test scored lower (0.71) and Optimal-IT intermediate (0.84). For *non-P. falciparum*, the κ values of Optimal-IT and NOW were 0.91 and 0.84, respectively.

Sensitivity at different parasitemia levels. Table 4 shows the sensitivity of the tests for *P. falciparum* and *non-P. falciparum* infections in different classes of parasite density. For *P. falciparum* infections, the NOW test performed better at lower densities as compared with the Optimal-IT, while the sensitivity of Paracheck was between the two. For *non-P. falciparum* both Optimal-IT and NOW showed a higher detection limit than for *P. falciparum*; Optimal-IT was better than NOW test.

Evaluation of ease-of-use of the rapid tests. Overall, both Paracheck and NOW malaria ICT tests were evaluated as very easy to perform, though the sampling methods for blood collection needed some practice. Optimal-IT was evaluated as less practical due to difficulties with the sampling pipette, added to the fact that this test device has a wobbly design (standing up) and the dipstick needs changing from the first to the second well, timed halfway through the procedure, which takes 20 minutes. The NOW test yielded a high number of lines recorded as doubtful: 72 of 214 *P. falciparum* lines were scored 'weak' or 'very weak' by the reader. Of these, eight were true *Pf*-positive of which four had low parasitemias (< 500/μL) and 64 were false *Pf*-positive, which included 20 infections diagnosed as *P. vivax* by microscopy and one case of *Pf*-gametocytes. Also 83 pan-malaria lines were annotated (very) weak of which 72 were true *Plasmodium*-positive of which 4 with low *Pf* and 11 with low *Pv* parasitemias. Likewise, some of the test lines for Optimal-IT were (very) weak: (i) 28 *P. falciparum* lines with 21 true *Pf*-positive cases of which eight had low parasitemias and seven false *Pf*-positives, which included six *P. vivax* infections and (ii) 28 pan-malaria lines with 22 true *Plasmodium*-positives of which 4 with low *Pf* and 12 with low *Pv* parasitemia. The weak lines were not seen with Paracheck tests, but these had occasionally a problem with dry white patches (partly) preventing the control or test line to become visible.

DISCUSSION

Rapid diagnostic test capacities. Here we have presented the results of a study on the diagnostic capacity of three rapid diagnostic tests for malaria in an area of low malaria transmission of *P. falciparum* and *P. vivax,* in South America. Our data show that the rapid diagnostic tests are potentially useful tools in the diagnosis of malaria in this setting. The levels of

TABLE 4
Sensitivity to detect (A) *P. falciparum* and (B) *P. vivax* at different parasite densities. MSF Tierralta, Colombia, 2005

P. falciparum		Optimal-IT	NOW ICT	Paracheck	Microscopy	χ^2
Parasite density	N	% detected				*P* values
< 100 par/μl	16	25.5%	68.8%	50.0%	15 Pf, 1 Pf+Pv	0.002
100–500 par/μl	14	42.9%	92.8%	78.6%	13 Pf, 1 Pf+Pv	0.01
500–5000 par/μl	53	90.6%	96.3%	92.5%	48 Pf, 5 Pf+Pv	0.5
> 5000 par/μl	69	100.0%	100.0%	100.0%	64 Pf, 5 Pf+Pv	–
P. vivax						
< 100 par/μl	19	15.8%	0.0%		19 Pv	0.2
100–500 par/μl	46	80.4%	32.6%		41 Pv	< 0.001
500–5000 par/μl	91	100.0%	97.8%		91 Pv	0.2
> 5000 par/μl	123	100.0%	100.0%		123 Pv	–

sensitivity ranged from 84–91% for *P. falciparum* and from 81–91% for *P. vivax*.

For *P. falciparum* detection the HRP2 test, the NOW malaria ICT, as well as Paracheck-Pf, appeared to be more sensitive than the pLDH test, Optimal-IT. The NOW test had a better capacity to detect lower density *P. falciparum* infections, but it gave a relatively high number of false-positive results (11% of all positives), so that its specificity and PPV were lower than that of the other two tests. For non-*P. falciparum* infections, here *P. vivax,* Optimal-IT (pLDH) was more sensitive than NOW malaria ICT (aldolase). Both Optimal-IT and NOW ICT revealed a relatively large number of *P. vivax* false negatives, missing respectively 9% and 17% of the infections. At lower *P. vivax* densities the tests performed less accurately than for *P. falciparum*. Doubtful, weakly colored test lines, found in positive as well as negative cases, were a problem encountered with NOW and Optimal-IT. Some of these, but not all, had low parasitemias.

The limitation of the study in this setting is the potential overestimation of the accuracy of microscopy. Hypothetically, the RDTs might be more sensitive than microscopy. If so, at least part of the 41 cases in which the NOW test indicated a *P. falciparum* infection as opposed to microscopy and the results of the other two RDTs, and the six cases in which Optimal-IT diagnosed *P. vivax* but microscopy, Paracheck, and NOW were all negative, might have been false-negative microscopy results rather than false-positive RDT results. *Vice versa*, some false-negative RDT results may have been false-positive microscopy results [e.g., slides read as very low density *P. falciparum* ($N = 4$), *P. vivax* ($N = 23$), or mixed infections ($N = 2$)] for which all three RDTs gave a negative result. Also, the few cases where microscopy detected *P. vivax* only and the RDTs indicated *P. falicparum* also ($N = 5$) may have been microscopy errors. We have applied maximum efforts to achieve expert reading in field conditions, with rigorous quality control procedures in place, such as double reading of difficult slides in our laboratory and blinded re-reading of a considerable number of slides in two reference laboratories. Expert microscopy is judged by Moody to detect parasite densities down to 50 par/μl[6] and remains the current universal 'gold standard,' which is widely available.[7,8] However, taking a blood sample on filter paper to confirm parasitemia by means of polymerase chain reaction can be considered for future studies.

Our results are in line with findings from other studies in areas of low to medium endemicity for malaria. Optimal was evaluated positively by most researchers but not all: in Latin America, sensitivities for *P. falciparum* averaged 82% (range 42–100%) and for *P. vivax* 88% (65–100), including studies from Colombia,[15–17] Honduras,[4,18] Mexico,[19] Peru,[20] and Brazil;[21] in Asia it showed about 87% (79–94) sensitivity for *P. falciparum* and 80% (65–95) for *P. vivax* (Afghanistan,[22] Thailand,[23] Pakistan,[24] Kuwait[8]). The NOW test and its predecessor ICT Pf/Pv were reported to be very sensitive for *P. falciparum,* about 96% (range 89–100) and a bit lower but acceptably sensitive for non-*P. falciparum* infections, about 87% (range 75–100, Colombia [Mendoza and others, unpublished data], Indonesia,[5] Thailand[25,26]). Paracheck *Pf* generally showed good diagnostic capacity, 96% (range 92–100) in Thailand,[27] Vietnam,[28] and India.[29]

High-endemic versus low-endemic areas. Reports on the same rapid tests from high-endemic malaria areas are scarce, but they generally show a higher sensitivity: the older version of the NOW-test, ICT Pf/Pv 100% and Optimal 94% (Tanzania[30]), and Paracheck 97% (Uganda[31]). The hypothesis of Fryauff and others[8]—that natural immunity against malaria might reduce the sensitivity of RDTs—is not confirmed by this rough comparison. The main factor explaining the difference in sensitivity between high-endemic and low-endemic areas seems to be the parasite density. In our study group of symptomatic malaria patients, geometric mean parasite density was about 2300/μL for both species, whereas worldwide it is said to be 20,000 for *P. vivax* and 20,000 to 500,000 for *P. falciparum.*[32] A total of 40% of *P. falciparum* infections and 38% of *P. vivax* infections had a parasite density below 2000 par/μl, and nearly 20% and 25% were below 500 par/μl. This proportion is higher than in high-endemic areas such as in Africa: in studies in DRC and Sudan (data from [33–35]) we saw that only 8–11% of *P. falciparum* infections of clinically ill children under 5 years were below 2000 par/μl. Hence, in areas of low and moderate malaria transmission, such as South America and Asia, rapid tests require a high sensitivity at lower densities of infection, to serve the non-immune populations that can suffer from clinical disease at much lower infection grades, as opposed to people in high-endemic areas in sub-Saharan Africa.

The PPV and NPV depend on the proportion of positive patients seen. The PPV reduces with lower prevalence, whereas the NPV increases.[36] In the group of patients selected for study, 17% had a *P. falciparum* infection; however, of all patients visiting the Malaria Center in the period of study, only 10% were *P. falciparum* positive. This is higher than the annual parasite rates reported for Colombia.[10] Health posts and mobile clinics where the RDT will be applied will probably see a lower positivity rate than in this specific Malaria Center where patients come for malaria diagnosis and treatment specifically. Thus, the PPV for *P. falciparum* of the NOW malaria ICT can be even lower than the 66% we reported here, related to a proportional increase in false positives among the few testing positive.

Implementation of rapid diagnostic tests. In Colombia, the tests that detect all *Plasmodium* species have an obvious added value above those detecting *P. falciparum* only. A test with HRP-2 for *P. falciparum* and pLDH for *P. vivax* detection would have given the best combined results, with both sensitivities over 90%; however, the tests available now combine HRP2–aldolase (NOW ICT) and *Pf* pLDH-pan pLDH (Optimal-IT). The NOW test appears to be more sensitive for *P. falciparum*. It will however lead to more false- positive results. But if we accept some degree of overtreatment and prioritize *P. falciparum* over *P. vivax*, then NOW is the test of choice. The NOW test was considered easier to perform than the Optimal-IT, and as a card test is also very easy to read. The scoring of weak positive lines should be addressed in training.

In areas in Colombia where microscopy is in use and quality requirements of trained staff and proper equipment can be met, this is still the more accurate way to diagnose malaria in this zone of mixed *Pf/Pv* prevalence. The RDTs are quicker, but still far from perfect in the diagnosis of different *Plasmodium* species or mixed infections.

The disadvantage of the Pf/Pv combination rapid tests is that their price (US $2.5) is about 5 times more than the price of the 'Pf-only' test (US $0.5), whereas microscopy is esti-

mated to cost 0.12 to 0.40 US$ in endemic countries. The Colombian health system is privatized and health centers and hospitals often operate on a cost-recovery scheme; therefore a large proportion of the costs must be paid for by the patients themselves. RDTs should not replace microscopy in Colombia in areas where there is a good network of skilled technicians and where microscopy remains the best option. Nevertheless, RDTs will be a useful tool in remote, deprived settings.

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