Field evaluation of rapid diagnostic tests for malaria in Yaoundé, Cameroon

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**ABSTRACT**

Rapid diagnostic tests (RDTs) are affordable, alternative diagnostic tools. The present study aimed to evaluate RDTs available in Cameroon and compare their characteristics to follow the parasitological response of patients for 28 days. Malaria diagnosis was assessed in 179 febrile patients using conventional microscopy as the reference method. Parascreen detects both *Plasmodium falciparum*-specific histidine-rich protein 2 (PF HRP-2) and Pan-specific plasmodial lactate dehydrogenase (pLDH) in all four human *Plasmodium* spp. Diaspot is based on the detection of PF HRP-2, OptiMAL-IT (pLDH specific for *P. falciparum* and pLDH for all four human *Plasmodium* spp.) was assessed for comparison. The reliability of RDTs was evaluated by calculating the sensitivity, specificity, positive predictive value, negative predictive value, false-positive rate, false-negative rate, and likelihood ratio. The clinical outcome of 18 children treated with atovaquone-proguanil and followed for 28 days was evaluated using microscopy and RDTs. Of 179 samples, 133 (74.3%) were pure *P. falciparum*-positive smears, 4 (2.2%) pure *P. malariae*-positive smears, and 42 (23.5%) negative smears. Parascreen and Diaspot had high sensitivity (>92%) and positive predictive values (>94%). The specificities for Parascreen and Diaspot were 81.0% and 90.5%, respectively. The false-positive rates and the false-negative rates were 19.0% and 4.5% for Parascreen and 9.5% and 8.3% for Diaspot, respectively. Most false-negatives occurred in samples with low parasitaemia (<500 asexual parasites/\mu L). The performance of RDTs was better at higher parasitaemia (>500 asexual parasites/\mu L). Four pure *P. malariae* were only detected by the pan-*Plasmodium* bands of Parascreen and OptiMAL-IT. In blood samples from patients treated and followed-up for 28 days, HRP2-based RDTs remained positive in most samples until Day 28. Despite negative smears, OptiMAL-IT remained positive in several patients until Day 7 but was negative in all patients from Day 14 onwards. RDTs can improve the management of febrile patients. The validity, ease of use, and cost of HRP2-based tests were comparable. However, one of the current weaknesses of the RDT-based strategy using the tests available in Cameroon is inadequate sensitivity for low parasitaemia. In some cases, RDT results may require correct interpretation based on clinical history, clinical examination, and microscopic diagnosis.

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1. Introduction

The attainment of the global goal to reduce morbidity and mortality due to malaria depends on prompt diagnosis and early treatment of patients (World Health Organization [WHO], 2000a). At present, a large majority of patients in Africa are diagnosed to have malaria on the basis of clinical signs and symptoms. Presumptive clinical diagnosis is highly unreliable since the differential diagnosis of malaria includes many febrile illnesses of viral and bacterial origin. Moreover, as the majority of sub-Saharan African countries, including Cameroon, resort to artemisinin-based combination therapy (ACT), which are much more expensive than synthetic compounds like chloroquine and sulfadoxine–pyrimethamine and limited in quantity due to the necessity to extract artemisinin from *Artemisia annua*, a reliable

**Abbreviations:** ACT, artemisinin-based combination therapy; HRP2, histidine-rich protein 2; pLDH, plasmodial lactate dehydrogenase; RDT, rapid diagnostic test; WBC, white blood cell.

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and rapid laboratory diagnosis of malaria will improve the cost and effectiveness of the management of malaria-infected patients (Sayang et al., 2009).

Microscopic examination of Giemsa-stained blood smears is the gold standard for malaria diagnosis. The procedure is labour-intensive and requires at least 1 h (usually more in a health centre with numerous suspected cases of malaria) from blood collection to results. Moreover, many health facilities in Africa do not possess a microscope, and even if there is a microscope in good working condition, well-trained and experienced microscopists are scarce. High-quality Giemsa stain and immersion oil may also be lacking in peripheral health centres. Within this context, rapid diagnostic tests (RDTs) are affordable alternative diagnostic tools in the absence of a microscope (WHO, 2000b; Long, 2009). RDTs require a drop of fingerpricked capillary blood (5–20 μL) to detect malaria-specific proteins, some of which are specific to the genus Plasmodium (pan plasmodial lactate dehydrogenase, pLDH), while others are specifically expressed by a given species (e.g. histidine-rich protein 2 [HRP2] or pf pLDH by Plasmodium falciparum and pv pLDH by P. vivax) by immunochromatographic methods. Some RDTs detect P. falciparum, with or without the other Plasmodium species, and non-P. falciparum (i.e. P. vivax, P. ovale, and/or P. malariae).

Our previous study showed the effectiveness of the RDT-based treatment with ACT over the presumptive treatment approach in Yaoundé (Sayang et al., 2009). This survey also demonstrated the importance of the characteristics of RDTs. The present study was conducted with the aim to evaluate two RDTs available through local commercial suppliers and one RDT imported from Europe. We compared the diagnostic characteristics of these RDTs in a dispensary and compared them to follow-up the parasitological response of patients for 28 days.

2. Patients, materials and methods

2.1. Patients

2.1.1. Group 1, patients enrolled for RDT only
All febrile adults (no age limits) and children (or history of fever within the past 48 h) attending the Nlongkak Catholic missionary dispensary, Yaoundé, for presumptive uncomplicated malaria, were enrolled in the study in 2008–2009 after a written informed consent. Giemsa-stained blood smear was prepared from fingerpricked capillary blood and examined under light microscope. The parasite density was expressed as the number of asexual parasites per μL of blood, assuming white blood cell (WBC) density of 8000/μL. In case of low parasitaemia (i.e. <10 asexual parasites per 200 WBC), parasites were counted against 500 WBCs. A blood smear was considered to be negative in the absence of asexual parasites against 500 WBCs. All smears were read by two experienced microscopists, and the final parasite densities were the mean calculated from two counts. A third microscopist examined and determined the parasite density in case of >15% discordant results. Patients with a positive blood smear were treated with a 3-day regimen of artemunate–amodiaquine (artemunate, 4 mg/kg/day + amodiaquine, 10 mg/kg/day) as the first-line and artemether–lumefantrine as second-line treatment, according to the current Cameroonian anti-malarial treatment guidelines.

2.1.2. Group 2, patients enrolled for RDT evaluation for clinical follow-up
Eighteen children aged less than 5 years were enrolled after an informed written consent of the parents or legal guardians. The inclusion criteria as recommended by the 2003 WHO protocol were as follows: rectal temperature ≥ 38.0°C, P. falciparum parasitaemia between 2000 and 200,000 asexual parasites/μL, easy access to the dispensary, absence of malnutrition and danger signs of severe and complicated falciparum malaria, and absence of history of allergic reactions to antimalarial drugs (WHO, 2003). These 18 patients were treated with oral atovaquone–proguanil (atovaquone, 20 mg/kg/day +proguanil, 8 mg/kg/day, once daily for 3 days) and followed-up for 28 days. Each dose was administered under medical supervision. Fingerpricked capillary blood was obtained on Days 0, 2, 3, 7, 14, 21, and 28 for blood examination and RDTs. The clinical outcomes were classified as early treatment failure, late clinical failure, late parasitological failure, or adequate clinical and parasitological response, according to the 2003 WHO definitions (WHO, 2003). Patients who failed to respond to atovaquone–proguanil treatment were treated with artemether–lumefantrine. This study was reviewed and approved by the Cameroonian national ethics committee and the Cameroonian Ministry of Public Health.

2.2. RDT

Two RDTs that are commercially available in Yaoundé, Cameroon during the study period were assessed. DiaSpot P. falciparum rapid test device (batch numbers MAL6090104, MAL6110020, MAL7050030; Acumen Diagnostics Inc., Livermore, CA) is a chromatographic immunoassay for the qualitative detection of the P. falciparum HRP2 in whole blood within 15 min. This RDT does not detect P. vivax, P. malariae, or P. ovale. Parascreen (batch no. 101098; Zephyr Biomedicals, Goa, India; distributed by International Dispensary Association Foundation, Amsterdam, The Netherlands) is an immunoassay that detects P. falciparum-specific HRP2 and Plasmodium genus-specific (P. falciparum, P. vivax, P. ovale, and P. malariae) pLDH within 15 min. In the presence of P. falciparum, either the ‘P’ HRP2 line alone (usually in the presence of low parasitaemia) or two bands on the ‘P’ and ‘Pan’ lines may appear. The latter case indicates P. falciparum or mixed infection. If P. falciparum is absent but one of the three other Plasmodium species (or mixed infections without P. falciparum) is present, the ‘Pan’ band is visible.

In addition to these two RDT devices that are available in Cameroon, Optimal-IT (batch no. 46110.23.01; DiaMed AG, Cressier sur Morat, Switzerland) was ordered in Europe for comparison, mostly in patients who were treated with atovaquone–proguanil and followed for 28 days. This RDT was also assessed in blood samples with low parasitaemia ≤500 asexual parasites/μL of blood. Optimal-IT is an immunochromatographic test that detects P. falciparum-specific LDH (‘P’ band) and Plasmodium sp. LDH (‘P’ band for all 4 human Plasmodium spp. LDH species) using monoclonal antibodies within 20 min.

All three RDTs were performed according to the manufacturers’ instructions. Two- to three-page users’ guides provided by the manufacturers in French were distributed to the health personnel, and test procedures were explained and demonstrated for each RDT during a 1-h training session. The ease of application of each RDT was evaluated during a feedback session. The interpretation of results was controlled by one or two experienced researchers.

2.3. Data interpretation and analysis

The gold standard was microscopic examination of Giemsa-stained thick blood smears. All RDTs assessed in the present study have an internal control that ensures that the test is valid. Parascreen RDT was interpreted as positive if ‘P’ (HRP2) or ‘Pan’ band (LDH) (or both) was visible. Likewise, the result of Optimal-IT was positive if ‘P’ and/or ‘P’ band(s) were visible. Diaspot was interpreted as positive when a single band was visible. The performance of RDTs, including 95% confidence intervals, was assessed by calculating the
sensitivity, specificity, positive predictive value, negative predictive value, false-positive rate (i.e. the number of false-positive samples [positive test in a person without malaria] as a percentage of all patients without malaria according to the reference method), false-negative rate (i.e. the number of false-negatives [negative test in a person who does have malaria] as a percentage of all patients with malaria), and likelihood ratio, i.e. sensitivity/(1 – specificity), using InStat (GraphPad Software, Inc., San Diego, CA). Logarithmic values of parasitaemia were compared between groups by the Student’s unpaired t-test. The coefficient of determination ($r^2$) was calculated from the parametric Pearson correlation coefficient. Statistical significance was fixed at 0.05.

3. Results

3.1. Comparison of three RDTs in patient group 1

A total of 179 blood samples were collected to assess the performance of the RDTs. There were 133 (74.3%) pure *P. falciparum*-positive smears, 4 (2.2%) pure *P. malariae*-positive smears, and 42 (23.5%) negative smears (Table 1). Among *P. falciparum*-positive patients, the geometric mean parasitaemia was 9420 asexual parasites/µl of blood (range, 33–230,000 asexual parasites/µl). The parasitaemia of four pure *P. malariae* samples ranged from 1130 to 7650 asexual parasites/µl. *P. ovale* and *P. vivax* were not detected by microscopy in the present study. *P. vivax* has never been reported from Cameroon.

Parascreen and Diaspot were highly sensitive and had similar positive predictive values (94.1–96.8%) (Table 2, excluding 4 *P. malariae* samples). Eleven false-negatives with Diaspot occurred in samples with 33, 45, 48, 61, 126 (+gametocytes), 155, 212, 250, 430, 3890, and 5870 asexual parasites/µl; five of these samples yielded a positive ‘P’ band and a negative ‘Pan’ band with Parascreen (33, 126 [presence of gametocytes], 250, 3890, and 5870 asexual parasites/µl), while six showed negative ‘P’ and ‘Pan’ bands with Parascreen (45, 48, 61, 155, 212, 430 asexual parasites/µl). Three false-positives were observed with both Diaspot and Parascreen in the same samples. Five additional false-positives were obtained with Parascreen (positive ‘P’ band and negative ‘Pan’ band), but not with Diaspot. One of these five patients with a false-positive result started self-medication with artesunate–amodiaquine 24 h before consultation. Another patient had a negative smear for asexual parasites but carried *P. falciparum* gametocytes (Diaspot and OptiMAL-IT negative). For the remaining patients, the false-positive result was unexplained. Three patients carried *P. falciparum* gametocytes and asexual parasites: (i) 668 asexual parasites/µl Diaspot positive, Parascreen ‘Pan’ and ‘P’ bands positive, OptiMAL-IT not done; (ii) 126 asexual parasites/µl, Diaspot negative, Parascreen ‘Pan’ band negative, Parascreen ‘P’ band positive, OptiMAL-IT negative; and (iii) 92 asexual parasites/µl, Diaspot positive, Parascreen ‘Pan’ band negative, Parascreen ‘P’ band positive, and OptiMAL-IT negative.

The ‘Pan’ band and the ‘P’ band of Parascreen RDT were discordant, i.e. negative ‘Pan’ band and positive ‘P’ band, in 8 of 42 smear-negative samples (8 false-positives) and 30 of 133 *P. falciparum*-positive samples. Blood samples with discordant results had a lower parasitaemia (geometric mean, 783 asexual parasites/µl; range, 33–54,000 asexual parasites/µl, $P < 0.05$), compared to *P. falciparum*-positive samples with concordant positive ‘Pan’ and ‘P’ band results (geometric mean, 28,100 asexual parasites/µl; range, 668–230,000 asexual parasites/µl). Four pure *P. malariae* were only detected by the ‘Pan’ band of Parascreen. Therefore, Parascreen (also OptiMAL-IT in one sample tested) correctly detected the samples infected with *P. malariae* with a positive ‘Pan’ band and a negative ‘P’ band, while Diaspot did not, and cannot detect *P. malariae*.

OptiMAL-IT was assessed in 26 random samples (11 *P. falciparum*-positive samples [8000–118,000 asexual parasites/µl], 1 pure *P. malariae*-positive sample, and 14 smear-negative samples) and 13 samples with low parasitaemia (range, 38–430 asexual parasites/µl). OptiMAL-IT yielded 9 false negative results and 1 false positive with *P. falciparum* samples. False negatives occurred in samples with low parasitaemias (<500 asexual parasites/µl): 38, 45, 48, 61, 92 (with *P. falciparum* gametocytes), 126 (with *P. falciparum* gametocytes), 155, 212, and 430 asexual parasites/µl. The threshold parasite density for OptiMAL-IT-positive results could not be determined, as the RDT was positive in few samples with low parasitaemias (75, 92, 205, 260 asexual parasites/µl). For all 38 *P. falciparum*-positive samples, ‘P’ and ‘P’ bands yielded the same results, i.e. either both positive or both negative.

The sensitivity of each of the assessed RDTs in relation to *P. falciparum* parasite density is summarised in Table 3. The sensitivity was low (<67%) for blood samples with low parasitaemia (<200 asexual parasites/µl and <500 asexual parasites/µl) with

<table>
<thead>
<tr>
<th>RDT</th>
<th>N</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
<th>False-positive rate (95% CI)</th>
<th>False-negative rate (95% CI)</th>
<th>Likelihood rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaspot</td>
<td>175</td>
<td>91.7 (85.7–95.8)</td>
<td>90.5 (77.4–97.4)</td>
<td>96.8 (92.1–99.1)</td>
<td>77.6 (63.4–88.2)</td>
<td>9.5 (8.3–10.8)</td>
<td>8.3 (7.5–9.1)</td>
<td>9.6</td>
</tr>
<tr>
<td>Parascreen</td>
<td>175</td>
<td>95.5 (90.4–98.3)</td>
<td>81.0 (65.9–91.4)</td>
<td>94.1 (88.6–97.4)</td>
<td>85.0 (70.2–94.3)</td>
<td>19.0 (16.5–21.5)</td>
<td>4.5 (3.8–5.2)</td>
<td>5.0</td>
</tr>
<tr>
<td>OptiMAL-IT</td>
<td>38</td>
<td>62.5 (40.6–81.2)</td>
<td>92.9 (66.1–99.8)</td>
<td>93.8 (69.8–99.8)</td>
<td>59.1 (36.3–79.3)</td>
<td>7.1 (5.6–8.6)</td>
<td>37.5 (32.7–42.3)</td>
<td>8.8</td>
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</table>

* For Parascreen and OptiMAL-IT, only the results of the *P. falciparum*-specific bands are presented here. Four additional samples were pure *P. malariae*. OptiMAL-IT was assessed in samples with low parasitaemia, which explains its low sensitivity and high false-negative rate in the present study. N, number of samples; PPV, positive predictive value; NPV, negative predictive value; 95% CI, 95% confidence interval.
Table 3

<table>
<thead>
<tr>
<th>Parasite density (asexual parasites/μL)</th>
<th>N*</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diapost</td>
<td>Parascreen</td>
<td>OptiMAL-IT</td>
</tr>
<tr>
<td>&lt;200</td>
<td>12 (9)</td>
<td>50.0</td>
</tr>
<tr>
<td>&lt;500</td>
<td>18 (13)</td>
<td>50.0</td>
</tr>
<tr>
<td>500–5000</td>
<td>27 (0)</td>
<td>96.3</td>
</tr>
<tr>
<td>&gt;5000</td>
<td>88 (11)</td>
<td>98.9</td>
</tr>
</tbody>
</table>

* N, number of blood samples tested with Diapost and Parascreen (the numbers of samples tested with OptiMAL-IT in parentheses). Samples with >500 asexual parasites/μL include those with <200 asexual parasites/μL.

all 3 RDTs. Parascreen and Diapost were highly sensitive, i.e. 100% and 96.3%, respectively, for blood samples with >500 asexual parasites/μL. The performance of OptiMAL-IT, as well as that of Parascreen and Diapost, was better at higher parasitaemia.

3.2. RDTs as a complementary tool to evaluate therapeutic efficacy

Diapost, Parascreen, and OptiMAL-IT were used as a complementary tool to monitor the therapeutic efficacy of atovaquone–proguanil during the 28-day follow-up period in 18 patients (Fig. 1). On Day 0 (before treatment), *P. falciparum* parasitaemia ranged from 3840 to 192,000 asexual parasites/μL of blood (geometric mean parasitaemia, 40,200 asexual parasites/μL). All three RDTs were positive before treatment. For unknown reasons, the ‘Pan’ band, but not the ‘PF’ band, of Parascreen in 2 patients (parasitaemia, 3840 and 120,000 asexual parasites/μL) was negative before treatment, and in all subsequent samples obtained from these 2 patients, the ‘Pan’ band remained negative until Day 28.

On Day 3, 15 of 18 (83%) patients had a negative smear, and 3 had low parasitaemia (65–866 asexual parasites/μL). OptiMAL-IT remained positive in 10 patients (8 smear negative and 2 smear positive), but was negative in 8 patients, including one patient with 866 asexual parasites/μL. On Day 7, all patients had a negative smear. Diapost remained positive in all but one patient, Parascreen ‘P’ band was positive in all patients, and Parascreen ‘Pan’ band was negative in all patients. On Day 7, OptiMAL-IT remained positive in 5 smear-negative patients. From Day 14, only Diapost and OptiMAL-IT were evaluated. All 18 patients had negative smears on Day 14 and Day 21, and 17 had negative smears on Day 28. Diapost was still positive in 17 of 18 patients on Day 14, 10 of 18 patients on Day 21, and 7 of 17 patients on Day 28. With OptiMAL-IT, all 5 patients who had a positive result on Day 7 became negative on Day 14 and Day 21. One patient had a late parasitological failure on Day 28, with a parasite density of 2420 asexual parasites/μL. Diapost, Parascreen, and OptiMAL-IT were positive for this patient.

4. Discussion

This survey assessed the comparative validity of RDTs available in Cameroon. Results lay down the needs for a procurement chain analysis of RDT and its implementation in the field. The present study was conducted in low malaria transmission settings where a majority of patients infected with malaria parasites are symptomatic due to a relatively low level of acquired immunity and both adults and children attend health facilities with malaria-associated symptoms throughout the year. Entomological infection rate varies between 0 and 33 infective mosquito bites per year and per person, depending on the district (Fondjo et al., 1992; Manga et al., 1992, 1993). In urban malaria, asymptomatic carriage is rare, requiring specific antimalarial treatment when blood smear is positive. Moreover, it is known that the majority (about 95%) of malaria infections are due to *P. falciparum*. Within this context, RDTs with a high sensitivity and low false-negative rate are required. The ability to distinguish between *P. falciparum* and non-*falciparum* species, with either a combination of HRP2 and pLDH, as in the case of Parascreen, or a combination of pan-*Plasmodium* and *P. falciparum*-specific pLDH, is less important, as all cases of malarial infections are treated with ACT in Cameroon.

The high positive predictive values of RDT (Diapost and Parascreen) reflect their sensitivity and partly depend on malaria prevalence. In Yaoundé, where malaria prevalence among febrile children and adults is relatively high, these RDTs proved to be useful screening tests. Based on the likelihood rate, which estimates the probability for a patient with a positive test is likely to be malaria-infected, as compared to a patient with a negative test, HRP2-based Diapost showed the best performance, followed by Parascreen. The results obtained with OptiMAL-IT cannot be compared with those of Diapost and Parascreen due to a much smaller sample tested with OptiMAL-IT. Moreover, in the present study, OptiMAL-IT was preferentially assessed in samples known to have low parasitaemias. Nonetheless, the present study confirms the higher sensitivity of HRP2-based RDTs for low parasitaemias, as compared with pLDH-based detection, as supported by the observation of more discordance between ‘PF’ and ‘Pan’ bands on Parascreen in samples with low parasite densities. These results are in agreement with previous studies (Hopkins et al., 2007, 2008; Rakotonirina et al., 2008; Ashley et al., 2009).

According to the manufacturer, Diapost yields a 100% sensitivity, 100% specificity, 100% positive predictive value, 0% false-positive rate, and 0% false-negative rate (*n* = 332 samples). As with Diapost, the manufacturer of Parascreen claims 100% sensitivity, 100% specificity, 100% positive predictive value, 0% false-positive rate, and 0% false-negative rate (*n* = 226, of which 16 *P. falciparum*-positive by microscopy). Neither of the manufacturers mentions the minimal density required for a positive test. In the present study, the performance of these RDTs was less than what has been claimed by the manufacturers.

In the present study, few samples were tested with OptiMAL-IT. This RDT was considered to be of less importance because of the following reasons: (i) Parascreen incorporates both *P. falciparum*-specific HRP2 and *Plasmodium* genus-specific pan-pLDH, (ii) OptiMAL-IT is more expensive than other RDTs and (iii) is not available locally, and (iv) many other studies have evaluated...
OptiMAL-IT elsewhere in Africa and Madagascar, both in earlier and recent years (Cooke et al., 1999; Mankhambo et al., 2002; Rakotonirina et al., 2008; Valéa et al., 2009; WHO, 2009; Ansah et al., 2010). Only viable parasites are thought to yield a positive OptiMAL-IT test. The manufacturer claims that a successful treatment yields a negative test within 2–4 days and that a positive test 5–7 days after treatment may indicate resistant parasite. Although the number of patients followed with both microscopy and OptiMAL-IT is limited, data presented in the present study do not support the use of either HRP2-based or pLDH-based RDTs as a complementary tool to evaluate therapeutic response within 7 days post-treatment. At present, RDTs are not recommended for follow-up of treatment. Microscopic examination of blood smears is still indispensable for evaluation of therapeutic efficacy and correct classification of clinical and parasitological outcomes.

One of the major confounding factors in the field is self-medication prior to medical consultation, which may lead to a negative smear and a positive HRP2-based RDT. The present study has confirmed the persistence of HRP2 antigen for at least 1 month and detection of pLDH up to 7 days after a successful treatment with highly effective drug combination. False-positive RDTs in such a context may result in overprescription of antimalarial drugs unless a complete and accurate history of previous antimalarial drug consumption is recorded. In our experience, approximately 50% of febrile patients in Yaoundé resort to self-medication with “traditional medicine,” quinine, sulfadoxine–pyrimethamine, or, more recently, artesunate–amodiaquine before consultation. Many of these self-administered drugs, in particular quinine and sulfadoxine–pyrimethamine, are purchased from informal outlets, which are known to supply low-quality or counterfeit drugs (Basco, 2004). Such drugs may influence microscopy and RDT results, depending on the dose and drug quality. False-positive RDT may also result from a misdiagnosis by microscopy if RDT is more sensitive than microscopy (Bell et al., 2005). Diagnosis by PCR may be able to correct such a discrepancy. However, the present study was designed to compare RDT results to microscopy as the gold standard. Moreover, PCR diagnosis is currently not an appropriate technology in the field in most sub-Saharan African countries.

False-negative RDTs have a more serious impact on individual patients as they may not receive adequate treatment unless microscopy is performed. The major known cause of false-negative RDTs is low parasitaemia. Higher sensitive RDTs will be required to detect malaria parasites in these cases. Another cause of false-negative HRP2-based RDTs can be non-expression of the HRP2 protein and therefore non-detection by HRP2-based RDTs, as described in the South American Amazon region (Gamboa et al., 2010). HRP2 sequence variation was previously thought to influence RDT sensitivity but was not fully supported by subsequent studies (Baker et al., 2005, 2010). The extent of hsp2 gene polymorphism in Cameroonian P. falciparum isolates is currently unknown. So far, hsp2 gene sequence of only 4 Cameroonian P. falciparum strains has been determined (Baker et al., 2005, 2010), but the existence of hsp2- and/or hsp3-deleted P. falciparum strains has never been reported in this country. Another possible cause of false-negative HRP2-based RDT results is the probeon effect (defined as false-negative immunological tests due to high antigen [or antibody] concentrations) that may be observed in blood samples with hyperparasitaemia (Gillet et al., 2009, 2011).

All three RDTs assessed in the present study are comparable in terms of ease of application. Diaspot has a single well into which both the blood sample and lysis buffer are placed. Parascreen has two wells, one for the blood sample and the other for lysis buffer, but once they are placed correctly, there is no further handling required. Errors with the use of these wells are unlikely to occur with Parascreen after a short training session. As for OptiMAL-IT, there are two wells; blood sample and lysis buffer are mixed in the first well and the stick is transferred to the second well containing lysis buffer after 10 min. Therefore, OptiMAL-IT requires an extra handling step. Diaspot was purchased for 1.14 euros per cassette at the time of the study. Parascreen cost was 1.17 euros (29.25 euros or 38.00 USD for 25 tests) per cassette without shipping and custom fees. OptiMAL-IT costs 3 times more (about 3.00 euros per cassette), without taking into consideration shipping and custom fees. Based on the results of our studies, the Cameroonian Ministry of Public Health plans to include a RDT as part of the management of febrile illnesses. However, more studies on RDTs are necessary to select the most appropriate RDT and assess its usefulness in different epidemiological situations in the country.

5. Conclusion

The present study showed that Diaspot and Parascreen are useful laboratory tools in a dispensary in Yaoundé, Cameroon. Their performance, ease of use, and cost were comparable. However, Parascreen has the advantage of being previously evaluated in Ethiopia, Peruvian Amazon, and India, and its performance has been published (Endeshaw et al., 2008, 2010; Ashton et al., 2010; Bendeuz et al., 2010; Singh et al., 2010). Diaspot has not been evaluated elsewhere due to unavailability in other countries. All three RDTs, in particular pLDH-based RDT, performed poorly for samples with low (<500 asexual parasites/μL) parasite density. The results of the present study suggest that HRP2-based RDTs are not useful to follow therapeutic response, while pLDH-based RDT may have a limited role beyond 7 days post-treatment. However, the general conclusions of the present study are only applicable to the RDTs that were available in Cameroon and assessed in the field. RDTs are important, cost-effective laboratory tools that improve patient management and avoid presumptive treatment based only on clinical symptoms, in particular in remote areas where microscopy is not available or is of poor quality. There are persistent technical problems (inadequate sensitivity for low parasitaemia, HRP2 antigen variation, and low [or absence of] HRP2 expression) that need to be overcome. Clinical acumen and microscopic diagnosis are still needed while waiting for highly sensitive RDTs.

Competing interest

The authors declare that they have no competing interest.

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References


