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Full Length Research Paper

Field evaluation of SD Bioline Malaria Antigen *P.f*® for *Plasmodium falciparum* malaria diagnosis in Nanoro, Burkina Faso

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A study was conducted to assess the performance of the SD Bioline Malaria Antigen P.f® compared to the ''gold standard'' microscopy as for the diagnosis of *Plasmodium falciparum* malaria in Nanoro, Burkina Faso. Microscopy and RDT diagnosis were performed on 254 blood samples. 78.09% (189/242) were positive by microscopy compared to 93.8% (227/242) diagnosed by RDT. The RDT test reported 39 false-positive results and one false-negative result. The RDT showed high sensitivity of 99.47% [IC95%: 98.56-100], with sensitivity increasing with increasing parasite density. RDT specificity was 26.42% [IC95%: 20.86-31.97]. Low specificity limits accuracy for malaria diagnosis. However, in areas of hyperendemic malaria, high sensitivity is preferable to over diagnosis.

Keywords: Malaria, *Plasmodium falciparum*, Rapid Diagnosis Tests, SD-Bioline Malaria Antigen P.f test, Accuracy.

INTRODUCTION

Worldwide use of Artemisinin-based combination therapies (ACT) combined with vector control measures (i.e. bed nets and indoor residual insecticide spraying) has led to significant decreases in malaria transmission and thus subsequent malaria-related morbidity and mortality in many endemic countries (WHO, 2011b). However, recent reports on the decreasing susceptibility of *P. falciparum* to artemisinin derivatives along the Thailand and Myanmar border are a concern as it may repeat the same patterns observed for the spread of chloroquine resistance (Dondorp et al., 2009; Gharbi et al., 2013; Lim et al., 2009; Maude et al., 2009; Noedl et al., 2010; Rogers et al., 2009). It is thus essential that a more targeted treatment approach is required in order to reduce the drug pressure which may lead to increasing resistant parasites strains (Bisoffi et al., 2009). This need has prompted the WHO to propose new guidelines to recommend that malaria case management be based on parasite-based diagnosis in all cases prior to treatment (WHO, 2011a).

Microscopy is currently considered as the „"gold standard"" for malaria diagnosis. However, despite its apparent simplicity, it requires some conditions which are not always accessible for many peripheral health centers (Haditsch, 2004). Rapid diagnostic tests (RDTs) have been suggested as a viable alternative for malaria diagnosis, and in particular in areas where microscopy is not available (WHO, 2011a).

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In Burkina Faso, a new malaria treatment policy was adopted in 2005: For uncomplicated falciparum malaria, artesunate-amodiaquine (ASAQ) or alternatively artemether-lumefantrine (AL) are recommended as first line treatments, whereas quinine is recommended for severe malaria (Gansané et al., 2009). Availability and wide-scale use of RDTs has dramatically increased over the past few years as a result of a policy change in 2009, which recommended the use of RDT named SD Bioline Malaria Antigen P.f® for malaria diagnosis in patients who attend health facilities where microscopy is not available (Tiono et al., 2013). Although several clinical trials have reported good test performances, factors such as shelf-life, and heat stability vary which could impact on quality and reliability. Currently information on the performance of RDT in real life conditions in Burkina Faso are limited. Here we report a field evaluation of the performance of SD Bioline Malaria Antigen P.f® test in Nanoro, Burkina Faso.

MATERIAL AND METHODS

Study area

This study was carried out between October and December 2012 by the Clinical Research Unit of Nanoro (CRUN) in four primary health centers (Nanoro, Godo, Seguedin, Nazoanga) of the Nanoro health district (NHD) in Burkina Faso. Nanoro is situated at approximately 85 km from Ouagadougou, the capital of Burkina Faso. Malaria is hyperendemic with a seasonal transmission from July to December with *Plasmodium falciparum* as the predominant malaria parasite. The commonest vectors are Anopheles gambiae ss, An. funestus and An. Arabiensis and the entomological inoculation rate is estimated at 50–60 infective bites/man/year (Tinto et al., 2002). The majority of the population practice subsistence farming.

Study participants

This field evaluation is part of a larger study that investigated the therapeutic efficacy of AL and ASAQ in patients > 6 months of age in Nanoro, Burkina Faso (ClinicalTrials.gov Identifier: NCT01697787). Details of the study methodology have been described in detail elsewhere (ClinicalTrials.gov Identifier: NCT01697787). However, briefly, patients with fever (axillary temperature of 37.5°C) or history of fever in the preceding 24 hours with a suspicion of malaria were screened after informed consent. All patients screened were systematically considered for the RDT performance evaluation whereas only patients with positive microscopy (parasitaemia ≥ 2,000 to 200,000 parasites/µL) were enrolled in the therapeutic efficacy assessment. This study was reviewed and approved by the Center Muraz local Ethical Review Committee (N /Réf. R09-2012/CE-CM).

Laboratory procedures

Blood samples were collected by finger prick to perform both microscopy slides and RDT testing. Blood smears were stained with 3% Giemsa for 30 minutes and then read by light microscopy at each health facility. Parasite density was determined by counting the number of asexual parasites per 200 white blood cells, and calculating per micro liter of blood by assuming the white blood cells at 8,000 per µL. A blood smear was considered negative when the examination of 100 thick-film fields did not reveal the presence of any asexual parasites. Blood smears were examined by two readers and, in the case of discordant results, by a third reader. Discordant results were defined as a difference between the two readers in (i) Plasmodium species, (ii) positive and negative, (iii) with parasite >400/µL; if the higher count divided by the lower count was >2, (iv) with parasite≤400/µL; if the higher reading is >Log10 higher than the lower reading. The RDT SD Bioline Malaria Antigen P.f® (Standard Diagnostics, Hagal-Dong, Korea), is the RDT recommended by the national malaria control programme of Burkina Faso and detects the protein *PfHRP2*. Following the manufacturer‟s instructions, the test uses approximately 5 L of blood and is readable after 15 minutes. In this study, the tests were performed immediately after blood collection (following the manufacturer instructions) by trained laboratory staff before slides were read. The results were recorded in comparison with the control line as positive if a unique *PfHRP2* line appears indicating *P. falciparum* infection. In case of absence of the control line the test was considered invalid. Test line intensities were scored as negative, faint, weak, medium, or strong compared to the control line by a reader who was blinded to the result of microscopy (Maltha et al., 2014).

Data analysis

Data were double entered by two independent data clerks and a verification program was used to correct errors by referring to the original case record forms. Statistical analysis was performed using STATA (IC), version 10.0 software. Descriptive analysis was performed using the proportions to estimate categorical variables and means or median to estimate quantitative variables. Parasites densities were expressed as geometric means with 95% confidence intervals. The performance of RDT was evaluated against microscopy by calculating the sensitivity, specificity, negative and positive predictive values of the test. Cohen‟s Kappa coefficient was computed to assess the agreement between the two tests. The performance of the RDTs according to parasite density was also assessed. For that,

the microscopy positive samples were stratified as ≤ 100, 101–1,000 and ≥1,000 parasites/µL. A p-value ≤0.05 was considered statistically significant.

RESULTS

A total of 246 suspected malaria patients attending the four primary health centers were screened. Microscopy examination showed four patients were infected by species other than falciparum (three with *P. malaria* and one with *P. ovalea*) and thus were excluded from the analysis. The baseline characteristics of the remaining 242 patients included are summarized in table 1.

The majority of the study population were less than five years of age (70.7%) and males and females were equally represented (50.4% vs 49.69%**)**. 78.09% (189/242) were malaria positive as determined by microscopy compared to 93.8% (227/242) as determined by RDT (Table 2). Using microscopy as the reference, the RDT reported 39 false-positive results and 1 false-negative result.

The validity of RDT compared to the "gold standard" microscopy test is summarized in table 3.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the RDT were 99.47% [IC95%: 98.56-100], 26.42% [IC95%: 20.86-31.97], 82.82% [IC95%: 78.07-87.57] and 93.33% [IC95%: 90.19-96.48] respectively. The agreement between the microscopy and the RDT was moderate with a Cohen"s Kappa coefficient of 0.35 ($p < 0.00001$). The sensitivity of the RDT increased with increasing parasite density. The proportion of positive results by RDT was 83.3% for parasite density ≤ 100 parasites/µL. For parasite densities of 101-1000 parasites/µL and ≥1000 parasites/µL, the proportion increased to 100% (Table 4).

DISCUSSION

The decision of Burkina Faso National Malaria Control Program (NMCP) to select SD Bioline Malaria Antigen P.f® for malaria diagnosis was motivated by the high sensitivity and specificity reported in the WHO panel studies for this test (WHO, 2011a). WHO recommends as a condition to be chosen in a policy, the RDT must have both sensitivity and specificity of at least ≥95% (WHO, 2011a).The high sensitivity found in this field evaluation (>99%) with a high Negative Predictive Value (NPV) confirms the relevance of such choice. This high sensitivity is similar to the results reported in other studies where this RDT was tested (Chinkhumba et al., 2010; Djallé et al., 2014; Kyabayinze et al., 2008; Liu et al., 2013; Makler et al., 1998). With such high sensitivity this test is then able to detect almost all positive infections in our study area. This is very important as this is a guarantee that all true cases of malaria infections will be promptly detected and correctly treated. Moreover the high NPV make health practitioners more confident to diagnose negative-test as non-malaria patients so that further causes of fever can be investigated (Kyabayinze et al., 2008).

Previous studies have reported that Malaria antigen concentration depends on the parasite biomass and thus, lower levels of HRP2 due to low parasite density might not be completely detected by RDTs (Desakorn et al., 2005; Dondorp et al., 2005). However, the level of the RDT detection for the low parasite density found in our study was very good. Indeed for all parasite densities >100 parasites/µL the sensitivity was 100% when the WHO recommend a score of at least 75% for a parasite density of 200 parasites/µl (WHO, 2011a). Moreover, we had only one discrepancy for the very low parasite densities (1-100 parasites/µL).

In contrast, we observed a very low specificity of 26% with a high Positive Predictive Value (PPV), resulting in 39 false-positive results. This low specificity is a concern, as it could lead to high false positive results that would consequently result in unnecessary anti-malarial treatment but also a missed diagnosis of the true cause of non-malaria fever (Chinkhumba et al., 2010). Few studies have reported low specificity rates with this RDT (Chinkhumba et al., 2010; McMorrow et al., 2010; Swarthout et al., 2007). However, most studies report a specificity of at least 40% (Chinkhumba et al., 2010; Djallé et al., 2014; Kyabayinze et al., 2008). Only one study, also in Burkina Faso but using a different RDT , FirstSignTM Malaria Pf (Unimed International Inc, South San Francisco, USA) but also detecting the *P. falciparum*-specific HRP-2 (Tiono et al., 2013) reported a very low specificity of 25.4% during the high malaria transmission season compared to a specificity of 63.7% during the low transmission season. Our study was performed during the high transmission season in Burkina Faso (October to December) and are thus consistent with these findings. The moderate Cohen"s Kappa coefficient agreement obtained in this study can be explained by this low specificity. Studies where the specificity was better, this agreement coefficient was higher (Kyabayinze et al., 2008). Low specificity could be due to the quality of the slides readers. However, all microscopists in the study area are submitted to regular certification programs in which only certified microscopists are allowed to read the slides (Tinto et al., 2014). It is more likely that low specificity is related to biological factors of the HRP2. This protein is known to persist at detectable levels for several weeks without live parasites detectable by the microscopy (Dougnon et al., 2015). In a study conducted in Uganda, persistence of HRP2 antigenicity was detectable in the majority of the study participants at three weeks following successful treatment (Kyabayinze et al., 2008). Therefore, the test can detect positives results regardless of the presence of parasites in the blood stream or not (Dougnon et al., 2015; Swarthout et al., 2007). This is particularly import-

Table 1. Baseline characteristics of 242 suspected malaria patients enrolled at four health centers in Nanoro, Bukrina Faso between October and December 2012.

Characteristics		**n (%)**
Age in years :	0 – 4	171 (70.66)
	5 – 17	62 (25.62)
	≥ 18	9 (3.72)
Sex :	Male	122 (50.41)
	Female	120 (49.59)
Weight in kg , median (P25 –P75)		11 (9 – 16)
Parasite density , geometrics means (IC)		16283.96 (12332.56 – 21501.41)
Hemoglobin at day 0, means (SD)		9.46 (2.00)

Table 2. Malaria diagnosis among 242 malaria suspected patients by microscopy and rapid diagnostic test (RDT).

	GE Positive n (%)	GE Negative n (%)	Total n (%)
RDT Positive	188 (77,7)	39 (16,1)	227 (93,8)
RDT Negative	1 (0,4)	14 (5,6)	15 (6,2)
Total	189 (78,1)	53 (21,9)	242 (100)

Table 3. Validity of RDT compared with microscopy (as gold standard) for detecting malaria infection among 242 malaria suspected patients enrolled in the study.

Accuracy parameters	Values	Confidence intervals (95% C.I)
Sensitivity	99.47 %	98.56 – 100
Specificity	26.42 %	20.86 – 31.97
Positive predictive value	82.82 %	78.07 – 87.57
Negative predictive value	93.33 %	90.19 – 96.48

ant for malaria hyperendemic settings such as our study area, where high levels of recurrent parasiteamia in clinical trials have been reported because people are subject to frequent infections during the high transmission season (The Four Artemisinine-Based Combinations (4ABC) Study Group, 2011).When we compare the SD Bioline detecting the HRP2 with other RDTs tested in Burkina Faso such as the OptiMAL-IT® detecting the plasmodium lactate dehydrogenase (pLDH), the specificity of the latter is much better (Diarra et al., 2012; Valéa et al., 2009). This confirms the biological factor raised above as in opposite of HRP2, the pLDH detected by the OptiMAL-IT® is a protein present in the blood of currently or recently infected people (Makler et al., 1998). Another key factor that might affect RDT field performance is the stability of the tests under heat conditions (Pattanasin et al., 2003; Tiono et al., 2013). However as this study was conducted in the rainy season when the average temperature is moderate (25°C-35°C), it is unlikely that the quality of the RDTs was affected as, following the manufacturer instructions, the test could be stored in temperatures up to 40 °C (WHO, 2013). This low specificity should be further investigated and monitored over time. Further studies which include the use of Polymerase Chain Reaction (PCR), which can detect very low parasiteamia that cannot be detected by the microscopy would provide better diagnosis.

CONCLUSION

The SD Bioline Malaria Antigen P.f® test showed a very high sensitivity for malaria diagnosis in this study. However,

Table 4. Sensitivity of the rapid diagnostic test (RDT) according to the microscopy parasiteamia (parasite/µL).

Microscopy (parasites/µL)	Number of samples	RDT positive	RDT negative	Sensibility (%)
1 – 100	6	5	1	83.33
101 – 1000	12	12	0	100
>1000	171	171	0	100

the poor specificity could limit the validity of this test for malaria diagnosis in our study area. This is of concern as it would result in unnecessary anti-malarial treatment and hence increase the risk of drug resistant parasites selection. Nevertheless, having low specificity is of less concern than low sensitivity which could have a more dramatic impact in a setting where malaria is hyper-endemic (Kyabayinze et al., 2008). Our findings need to be confirmed by further investigations on this test to understand the low specificity reported.

COMPETING INTERESTS

The authors declare that they have no competing interest.

Authors' contributions

HT, SP, IV, RR designed the study, HT, OS, FL, IV, MDK, RR contributed for data collection and supervision in the field, AK carried out the data management and statistical analysis, HT, RR drafted the manuscript and all authors read and approved the manuscript.

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