Malaria Chemotherapy, Control & Elimination

Akotet et al., Malar Chemoth Cont Elimination 2014, 3:2
DOI: 10.4172/2090-2778.1000125

Research Article Open Access

Performances of SD Bioline Malaria Ag-P.F/Pan RDT for the Diagnosis of Malaria in Febrile Patients Living In Gabon, Central Africa

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Abstract

The SD Bioline malaria Ag- Pf/Pan performances were evaluated for malaria species detection in 215 febrile patients living in Gabon, using microscopy as gold standard. Malaria parasites were identified in 94 (43.7%) individuals by microscopy, and 104 (48.4%) patients tested positive by the RDT. The SD Bioline Ag-*Pf*/Pan global sensitivity was 96.8%. All the non-falciparum malaria species infections were correctly diagnosed by the rapid test. The specificity was of 89.3% and the false positive (FP) rate of 12.5%. The test sensitivity significantly increased with parasitaemia, being of 88.9% for parasite density below 100/ µL and 98.5% at density higher than 500 parasites/ µL ($p<0.01$). Among the patients with a negative blood smear, the proportion of FP results was 21.0% in those previously-treated with an antimalarial drug before the consultation, and 8.8% in individuals without self-medication. SD Bioline Ag-Pf/Pan RDT represents a good alternative to microscopy for the diagnosis of *Plasmodium spp* infection.

Keywords: Malaria; Diagnosis-HRP2- pLDH-; Self-medication; Gabon

Abbreviations: Se- sensitivity; Sp- specificity; Spp- species; FP- false positive; FN- false negative; NPV- negative predictive value; PPV- positive predictive value; PBS-positive blood smear(s); NBS- negative blood smear(s); MNCP- malaria national control program; RHM- regional hospital of Melen

Introduction

The Test-Treat-Track (T3) initiative, launched in 2012 by the WHO, is a clear indication that malaria management should be based on evidences. Performing an accurate diagnosis of malaria before treatment, through parasitological confirmation with either microscopy or rapid diagnostic tests (RDTs) is recommended by the World Health Organization (WHO) [1]. Although several evaluations of existing RDTs are already available, field and operational data which take into account the local epidemiology of malaria, the heterogeneity of these tests performance variability and comparative results of the different tests are required to select the suitable ones for routine use within a country. The Gabonese Ministry of Health recently included RDTs in national guidelines as essential tools for malaria diagnosis; and their performance evaluation was recommended. To date, none is recommended in public health centers. Although several tests are marketed in the country, the majority are not included in the list of the Global Fund Quality Assurance Policy [2,3]. Single RDTs detecting the Histidine rich protein 2 (HRP2) or the lactate deshydrogenase (pLDH), and a HRP2/aldolase combo RDT performances were analyzed in field conditions [4,5]. They provided good results. However, the pan HRP2/pLDH RDTs, the most frequently recommended tests in African countries, have not been studied in the country [6-9]. The SD Bioline Malaria Ag P.f/Pan test is a rapid, qualitative and differential test for the detection of HRP-II antigen of *Plasmodium(P.) falciparum* and common Plasmodium lactate dehydrogenase (pLDH) of Plasmodium species in human whole blood. It was one of the best performing assays in the WHO/TDR (Special Program for Research and Training in Tropical Diseases)/ FIND (Foundation for Innovative New Diagnostics)/CDC (Centers for Disease Control) evaluation. Field studies in several countries showed its stability and its high sensitivity and specificity when it was compared to other HRP2 and pan RDT for the detection of falciparum and non-falciparum malaria infections [9,10-12]. Moreover, Pan RDTs which combined HRP2 with other antigens are a good alternative to single HRP2 tests as hrp2 gene deletions that are responsible for HRP2 RDT false negative results, are now reported in African settings [13,14]. In order to provide additional reliable data to help the policy makers for the decision on the selection of RDTs for routine use in all health facilities, the present study was performed to determine the performances of the SD Bioline Malaria Ag-P.f/Pan for the diagnosis of malaria in a sentinel site of Gabon. Microscopy was used as gold standard.

Patients and Methods

Study area and population

The study was carried out between July and September 2013, at a suburban area located few kilometers from Libreville, the capital city of Gabon. Volunteer patients attending the outpatient unit of the Regional Hospital of Melen (RHM) were included based on the following criteria: axillary temperature ≥ 37.5°C or report of fever in the previous 24 hours. After oral informed consent obtained, body temperature, history of fever, age, sex and a history of self-medication with an antimalarial drug prior to the consultation were collected.

Laboratory procedures

Sampling methods

A volume of one mL of venous blood was taken from each patient into an EDTA tube. RDTs and microscopy were immediately carried

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Received August 06, 2013; **Accepted** September 27, 2013; **Published** October 04, 2014

Citation: Akotet BMK, Nkare CA, Mbouoronde OC, Mawili-Mboumba DP (2014) Performances of SD Bioline Malaria Ag-P.F/Pan RDT for the Diagnosis of Malaria in Febrile Patients Living In Gabon, Central Africa. Malar Chemoth Cont Elimination 3: 125. doi: 10.4172/2090-2778.1000125

out by well-trained staff. RDT results were communicated immediately to the clinicians. Each lab technician involved in the RDT realization was blinded to the results of thick and thin smears.

Malaria parasite microscopic detection

Matched thick and thin blood smears were prepared and stained with 20% Giemsa. Thick smears were screened for the presence of malaria parasites according to the Lambaréné method [15,16]. Carefully, 10 µL of blood were placed on a 10 by 18 mm area of a microscope slide, then dried and stained. The parasitemia was expressed as number of parasites per microliter of blood (p/ µL), and parasite species were identified in the matched thin blood smears. Smears were read by two experienced technicians using a light microscope (× 100 oil immersion lenses). Smears were considered negative if no parasite was seen after the examination of at least 100 oil immersion fields in a thick blood smear. The definition of a malaria case was a febrile patient with a positive blood smear (PBS).

The SD Bioline malaria P.f/Pan RDT

The SD Bioline Malaria Ag-Pf/Pan is a rapid three-band lateral-flow chromatographic immunoassay for the qualitative detection of P. falciparum specific HRP2 antigens and/or Panmalarial LDH found in *P. falciparum, P. vivax, P. ovale*, and *P. malariae*. The test allows the differentiation between a *P. falciparum* infection and a plasmodial infection due to another malaria parasites species or a mixed infection in a cassette format. The RDT was carried out with five microliters of whole blood according to the manufacturer's instructions. A negative result was indicated by the presence of a single line, the 'C' control line, in the result window. A *P. falciparum*-positive result was indicated by presence of colour bands in the *P. falciparum* test line and the "C" control line. The presence of the "Pan" test line and the "C" control line (again, two colour bands) indicate a *P. ovale* or *P. malariae*-positive result or a mixed non-falciparum infection. The presence of the "*P. falciparum*" the "Pan" test and the "C" control lines indicates a *P. falciparum*-positive result or a mixed infection. In absence of the control line, the results were interpreted as invalid and the test repeated with a new device. Timers were used to ensure that tests were read at exactly 15 minutes.

Operational quality control

RDTs were stored between 19°C–25°C within the manufacturer's recommended temperature ranges of 4°C–30°C. They were used with in the indicated shelf life. Proper storage and use of the devices was ensured by supervisory staff, each package was examined for the integrity of the desiccant before use. The blood smears were read by two experienced microscopists and in case of discordant results (presence or lack of asexual/sexual blood stages, mismatch species or parasite density), the slides were reviewed by a third technician who resolved any discrepancy. For parasite density determination, the mean of the two closest parasitaemia was taken.

Ethical considerations

The study was approved by the Ministry of Health. As reference laboratory for the MNCP, DPM has health authorities' approval to monitor the evolution of malaria morbidity, to evaluate RDTs and to analyze the evolution of drug resistance parasite evolution in the whole country through sentinel sites. The RHM is one of the five sentinel sites for malaria survey. After information and appropriate explanations, adult participants, parents or legal guardians of all children willing to participate in the study gave their oral consent before sampling. All tests were free of charge and patients were treated by physicians according to the results of microscopy and/or RDTs.

Data analysis

All data were recorded on a CRF and entered in Epi-info version 3.3.2 (2005 CDC Atlanta). Analysis was performed with Statview 5.0 (SAS Institute,Cary, NC, USA). Microscopy was considered as the gold standard method. RDTs results were distributed into four categories: negative, *P. falciparum* mono-infection, non-falciparum malaria species single and mixed infection (including *P. falciparum* and non- falciparum malaria parasites). The sensitivity (Se), specificity (Sp), negative predictive value (NPV), positive predictive value (PPV), likelihood ratio (LR) of a positive or a negative test result were calculated with 95% confidence intervals (CI). The proportions of false positive (FP) and false negative (FN) results were also determined. The Kappa coefficient (k), representing the proportion of agreements beyond chance, was used to quantify the level of agreement between the RDT and the 'gold standard', microscopy; a $k \geq 0.8$ was considered to indicate high reliability [16]. As the aim of this study was to provide additional and comparable data for the comparison of previously evaluated RDTs performances in the country with the SD Bioline Malaria Ag-Pf/Pan, the parasite density was stratified as follows: <100p/ µL, <500p/ µL, >100p/ µL and >500p/ µL for the Se determination according to the parasitaemia [4,5]. A p-value less than 0.05 were considered significant.

Results

Patient characteristics

From June to October 2013, 287 febrile patients were screened for malaria. Data from 215 were available and analyzed: 47.4% (n= 102) were female (Table 1). The median [IQR] age was 2 (1-8) years old: 147 (68.4%) were aged below five years old; 44 (20.5%) between five to ten years old and 24 (11.2%) were older than 10 years old (Table 1). The majority (91.2%; n=196/215) had fever the day of the consultation and 15.3% had taken an antimalarial drug prior to the visit.

Plasmodium spp microscopic detection

Malaria parasites were detected in 94 (43.7%) blood smears by microcopy, 92 (97.8%) were identified as P. falciparum mono-infection. Single *P. malariae* infection was found in one (1.1%) slide and *P. falciparum-P. malariae* mixed infection in an (1.1) sample. Among the infected patients, the median parasite density in *P. falciparum* samples was 7000 [490-46900] p/ µL. The *P. malariae* parasitaemia was 2800 p/ µL. According to the parasite density, 22 (23.4%) patients had less than 100 p/ µL and 67 (71.3%) had more than 500 p/ µL.

SD Bioline Malaria Antigen P.f/Pan

SD Bioline RDT revealed 104 (48.4%) positive samples of which 101 (97.1%) were single *P. falciparum* infections, 1 (1.0) was *P. falciparum* or mixed infections and 1 (1.0%) a non *P. falciparum* malaria infection. Among positive samples, 13 (12.5%) were microscopy negative (false

	N	%
	215	
Female	102	47.4
Age below 5 years old	147	68.4
Self-medication with an antimalarial drug	33	15.4

Table 1: Patient characteristics

positive, FP) and identified as *P. falciparum* single infection (Table 2). There were three false negative (FN) results with one from a sample having 11600p/ µL. One hundred eleven (51.6%) were found negative. Table 3 shows the performances of the SD Bioline Malaria Ag-P.f/Pan. The sensitivity (Se) was 96.8% and the negative predictive value (NPV) was 97.3%, whereas the specificity (Sp) and the PPV were 89.3% and 87.5% respectively. The *P. malariae* infected sample was correctly identified as a non-falciparum infection by the RDT, as well as the mixed *P. falciparum/P. malariae* infection (Table 2). The Se calculated for parasite density above 100 p/ µL was of 97.4 [92.6-99.2]%. There was a trend towards an improved RDT Se with increasing parasitaemia; Se varied from 88.9[69.9-98.0]% (at parasite density below 100 p/ µL of blood) to 98.5[91.0-99.1] % at more than 500 p/ µL of blood. The positive LR (9.1) and the negative LR (0.04) were good as well as the agreement degree between tests (Kappa coefficient) which was high (>80%) (Table 3). Self-medication with an antimalarial drug prior to the consultation was reported by 33 patients; 14 were found malaria-infected by both techniques. Among the 19 ones with a microscopy negative blood smear, 21.0% (n=4/19) displayed a positive RDT result.

In contrary, the proportion of positive RDT results was lower in the group of patients without a previous antimalarial treatment who had no detectable parasite in their blood smears (8.8%; n=9/102 versus 42.4% among self-treated patients, n=14/33) (p>0.05). The SD Bioline Se was thus of 100.0[76.6-100.0]% in case of previous medication and of 96.3[89.4-99.2]% in absence of medication (p=0.15).

Discussion

HRP2/pLDH comboRDTs have not been evaluated in Gabon. The present data showed overall good performances for the SD Bioline malaria Ag/Pf/Pan in the identification of *P. falciparum* and non-falciparum malaria infection in febrile patients living in Gabon. Results are consistent with those from other previously obtained with HRP2 or pLDH alone or HRP2/aldolase RDTs in the country and those displayed by the SD Bioline RDT when its performances were assessed elsewhere [4,5,9,11,12,17]. Se increased with the level of parasitaemia, as frequently observed with the majority of RDTs. Three samples with microscopic *P. falciparum* infection gave negative results. One had more than 10000p/ µL. These observations corroborate with those from other studies which report the well-known limitation of tests performances with low parasitaemia and false negative results with parasite densities above 500 parasites/ µL [5,18-20]. Although the inability of RDTs to detect some high parasitaemia is a rare event, it is not parasite species or antigen specific and it was previously reported by others [4,5,18-21]. The prozone effect and the deletion of the histidine rich repeat region might explain such negative result with high parasitaemia [14,14,22]. RDT false negative results in some malaria positive cases were noticed for all the RDT evaluated in the country, highlighting the necessity of investigating the frequency the hrp 2 gene deletion in Gabonese populations [4,5]. The proportion of FP results was comparable to the rates of other comboRDTs [3,7]. It was also higher in self-treated patients with an antimalarial drug prior to presentation at the health facility. These individuals may have residual circulating antigens as usually described [4,6,23,24]. Patient self-medication that is frequent in the country should be noticed before the test realization. The misclassification of some samples as malaria-infected by the Sd Bioline test (FP) could also be due to cross reactivity with human auto-antibodies, low level of gametocytaemia, although it was not the case in the present study [4,14,25-28].

There was no species mismatch. Indeed, SD Bioline Malaria Ag-Pf/Pan correctly classified the two samples with single or mixed *P. malariae* infection as reported in RCA [6]. Although the prevalence rate of non-falciparum malaria is low in the study population, this result should be taken into account. Non negligible frequencies of *P. falciparum*-non falciparum mixed infections or single non-falciparum malaria infections misclassifications were recorded with single or comboRDTs evaluated within the country and in other settings [18,29]. The main limitation of this study is that molecular DNA amplification that displays higher Se for low parasitaemia detection and thereby reduces the frequency of FP results was not used as gold standard. However, the procedure of blood smears examination and the rigorous quality control of the slide reading may have reduced the risk of misdiagnosis due to human error. Additionally, immediate blood testing, as recommended for a rapid patient care management, was performed during the whole study. It ensures the best probability of detecting lived parasites and represents the best case scenario. The present data show that the SD Bioline malaria Ag-P*f*/Pan is a useful test for the diagnosis of malaria in febrile patients and its position as a good alternative to microscopy is also confirmed. Self-medication should be recorded to improve HRP2-based RDT result interpretation.

	PositiveBloodSmears			NBS	Total
	P.falciparum	***P.malariae***	***P.falciparum/P. malariae***		
P.falciparum	89	0	0	13	102
Non *falciparum*	0	1	0	0	0
Mixed infection	0	0	1	0	1
Negative RDT	3	0	0	108	111
Total	92	1	1	121	215

Table 2: SD Bioline malaria Ag-Pf/pan results according to parasite species

	%	95%CI
Over all		
Sensitivity	96.8	90.9-99.3
Specificity	89.3	82.3-94.1
False positive	12.5	6.8-20.4
False negative	2.7	0.6-7.7
PPV	87.5	79.6-93.2
NPV	97.3	92.3-99.4
Kappa	85.1	71.8-98.4
Positive LR	9.1	5.1-16.8
Negative LR	0.04	0.01-0.11
Only *P.falciparum*		
Sensitivity	96.7	90.1-99.1
Specificity	89.3	79.7-94.1
Non falciparum species		
Sensitivity	100.0	99.5-100.0
Specificity	100.0	97.4-100.0

Table 3: Performance characteristics of SD Bioline malaria Ag-*Pf/pan* compared to microscopy

Authors' contribution

MKBA designed the study, drafted the manuscript and analyzed the data. MKBA and DPMM directed and supervised the field study. CAN, COM and PM participated in the samples testing.

All authors revised and approved the final manuscript.

Acknowledgement

We thank all the adults, children parents/guardians, as well as staff of the outpatient unit of the RHM.

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