

Evaluation of the performance of "Advantage P.f. Malaria Card® " and " Advantage Malaria Pan+Pf Card® " two rapid diagnostic tests for biological confirmation of malaria cases in field situation in Togo

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Abstract

Background

In Togo, malaria remains a major public health problem and the management of suspected cases requires confirmation with appropriate biological methods. Indeed, malaria diagnosis has been improved through the introduction of rapid diagnostic tests (RDTs), recommended by the World Health Organization (WHO) for areas where microscopy is not available. To be used, these RDTs must meet performance criteria defined by the WHO. This study was conducted to evaluate the diagnostic performance of two RDTs: Advantage P.f. Malaria Card® detecting HRP2 antigen and Advantage Malaria Pan + Pf Card® detecting both HRP2 and pLDH antigens.

Methods

This was a cross sectional analytical study conducted from December 2019 to February 2020 on malaria suspected cases seen in three sentinel sites in Togo and from whom capillary blood was collected to perform the two RDTs according to the procedures described by the manufacturer. The performance was estimated comparing to thick/thin blood smear and molecular biology (PCR), considered as gold standard.

Results

The sensitivity of both Advantage P.f. Malaria Card® and Advantage Malaria Pan + Pf Card® compared to thick /thin blood smear was 91.8% and 91.3% respectively and for both the specificity was 94.7%. Compared to PCR, the sensitivity decreased to 89.4% and 88.9% respectively and their specificity to 72.2%.

Conclusion

The performance of the Advantage P.f. Malaria Card® and Advantage Malaria PAN + Pf Card® compared to microscopy, considered as the gold standard, according to WHO criteria were acceptable. They can therefore be used for the biological diagnosis of malaria.

Background

Malaria, one of the major endemic tropical diseases, is a febrile erythrocytopathy caused by a parasite of the genus *Plasmodium* and transmitted to humans by the infested female Anopheles. In 2020, World Health organization (WHO) estimated the number worldwide of malaria cases to 241 million and 627,000 deaths attributable to malaria, 95% of which occurred in the African region [1].

In Togo, malaria remains a public health problem, with children and pregnant women being the most affected. Indeed, its prevalence over the period from 2017 to 2019, in children aged 6 to 59 months, was 28% and its lethality increased from 3% in 2016 to 3.6% in 2019. The annual incidence increased from 153 cases per 1000 inhabitants in 2016 to 300 in 2019 [2].

Among the control strategies adopted to reduce morbidity and mortality linked to this parasitic disease, the malaria treatment policy has emphasized the early (within 24 hours) and effective management of confirmed cases. In line with WHO recommendations, uncomplicated case management should be based on confirmatory biological diagnosis and prompt treatment using artemisinin-based combination therapy (ACT) [3].

The gold standard method for biological confirmation of malaria remains thick/thin blood smear (TTBS), which demonstrates the different stages of the plasmodial species. However, the reliability of its results is highly dependent on the technical platform, the skills of the microscopist, the reading time and requires the implementation of a quality management system [4].

For more than a decade of the development of rapid diagnostic tests (RDTs) with the support of partners, malaria diagnosis has improved considerably, as biological confirmation can be done using these RDTs. The main advantages are the ease of use and the fact that they do not require electricity or equipment. In addition, results can be obtained within 30 minutes [3]. Based on the immunochromatography technique, they detect Plasmodium-specific antigens by immunocapture using antibodies, which can be specific to a single species, or "pan-specific" capable of recognizing multiple plasmodial species. Histidine-rich protein 2 (HRP2), plasmodial lactate dehydrogenase (pLDH) and less frequently aldolase are the proteins mainly used for commercially available RDTs. To ensure the quality of these RDTs for use in countries, in 2006, WHO and the Foundation for New and Innovative Diagnostic Tools (FIND) initiated a process of systematic evaluation and comparison of the effectiveness of commercially available malaria RDTs [5].

Furthermore, numerous evaluations of these RDTs have demonstrated a better performance compared to the TTBS which is the reference method. For preparing the introduction of two new RDTs: Advantage Pf. Malaria Card® and Advantage Malaria Pan + Pf Card®, this evaluation was conducted to determine their performance to diagnose *P. falciparum* infection in Togo.

Methods

Study design

This was a cross-sectional analytical study that took place from December 2019 to February 2020 in Togo. Malaria transmission is stable throughout the country, with two predominant facies: the sub-equatorial with two rainy seasons in the southern part of the country and the tropical facies with a single rainy season in the northern part. Thus, three sentinel sites for monitoring the effectiveness of ACTs used for the treatment of uncomplicated malaria, were used for this evaluation: First one, the Social Medical Center (SMC) of Cacaveli, a public health facility in Lomé, the capital of Togo, duplicated with SMC "UTB

Circulaire"; SMC Ahépé, a public health facility in the Maritime region, the second site, located 66 km from Lomé, also duplicated with the hospital "la Providence de Kouvé" and the third site represented by the polyclinic of Sokodé located in the central region approximately 340 km north of Lomé. The first two sites were sub-equatorial, located respectively in urban and rural areas, and the third site was tropical, located in semi-urban area.

Study population and sampling

The study population was patients with suspected clinical signs of malaria who were seen in consultation at the different sentinel sites and for whom TTBS were prescribed. They were divided into three groups: the first one, which representing the control group, has enrolled the patients whose had TTBS negative; the second, constituted by patients with parasitaemia considered low and the third with patients with a high parasitaemia.

For an estimated sensitivity and specificity (P) of 90%, a tolerated margin of error (E) of 5%; an accepted risk of error (α) of 5%; and $Z_{\alpha/2}$ at 1.96, the sample size with the Schwartz formula $N = \frac{Z_{\alpha/2}^2 P^*(1-P)}{E^2}$ was estimated 139 subjects per group.

The low parasitaemia is defined by a TTBS positive with the number of asexual parasitaemia per microliter ranged between 50 and 1000 and high parasitaemia by TTBS positive with the number of asexual parasitaemia per microliter parasitemia between 2000 and 10000. Signed written consent was obtained from each adult patient and from the parent/guardian of children before their enrolment in the study.

Questionnaire

A structured questionnaire was used to collect information on sociodemographic characteristics, clinical signs presented, history of the disease and existence of other diseases if applicable.

Laboratory tests

Each patient had a capillary blood sampling for a thick/thin blood smear, to test the RDTs evaluated for *Plasmodium spp* infection and for dried blood spots (DBS).

Thick and thin blood smear

The thick smear and the thin smear were made on the same slide, stained by Giemsa 3% after fixing thin blood smear by methanol for a few seconds. After drying, the slides were then read under an immersion microscope with an X100 objective to determine the positivity and identify the plasmodial species and estimate the parasitaemia. For the quality control, duplicated reading was done by two experienced microscopists of each TTBS. In case of the coefficient of variation between the results was greater than 5%, a third reading was performed by another independent microscopist. If there was a difference between the sites' parasitaemia and results found by the quality control, parasitaemia of the quality control was considered. The estimated parasitaemia was used to constitute the three groups.

Rapid diagnostic tests

Two types of Advantage brand RDTs were evaluated: Advantage P.f. Malaria Card® which is specific for *P. falciparum* and Advantage Malaria Pan + Pf Card® which can detect *P. falciparum*, *P. malariae*, *P. vivax* and *P. ovale* (J. Mitra & Co. Pvt. Ltd.). Both RDTs are based on the immunochromatographic technique: Advantage P.f. Malaria Card® using a monoclonal anti-Pf HRP2 antibody, detect HRP2 antigen specific of *P. falciparum* and Advantage Malaria Pan + Pf Card® in addition to the *P. falciparum* specific anti-Pf HRP2 monoclonal antibody, detect pLDH (Plasmodium Lactate Dehydrogenase) antigen which is common to all plasmodial species, using a specific anti-Pan pLDH monoclonal antibody. Both RDTs were evaluated for each enrolled subject according to the manufacturer's instructions.

Detection of the plasmodial genome by real time PCR

For each participant, a DBS were performed from the capillary blood on Wattman type III paper. Plasmodium DNA was extracted by the heating method described by Miura et al. (2013) [6]. The Qiagen® kit used according to the manufacturer's instructions was used to validate the heating extraction method and real-time PCR was performed using primers, probes, and reaction conditions described by Shokoples et al. (2009) [7], and Divis et al. (2010) [8] with the following modification, however, the fluorophores for the *P. falciparum* probes were replaced with Cy5-BHQ-1 [9].

RDTs, TTBS, and DBS were labelled identically with "TG followed by the patient's registration order number, the numbers assigned to each site, and the first three letters of each site's name."

Endpoints

TTBS and PCR were considered in this evaluation as gold standard to which both RDTs were compared. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are the estimated performance indicators of these two RDTs. They were determined by comparing to the TTBS, for low and high parasitaemia. For the calculation of the PPV and NPV of the RDTs, considering PCR as the reference method, due to the large difference between the population size of the positives ($N_p = 226$) and that of the PCR negatives ($N_n = 18$), the population size of the positives was adjusted to have the same size as that of the negatives ($N = 18$) while preserving its distribution (Table 1).

Table 1
Basis of adjustment for performance calculation against PCR as reference method

Test	Number of PCR performed		Number after adjustment for calculation	
	Negative	Positive	Negative	Positive
Negative	A	C	A	$N_n * C / N_p$
Positive	B	D	B	$N_n * D / N_p$
Grand total	N_n	N_p	N_n	$N_n * N_p / N_p$

Data management and analysis

All collected data were entered into a database in Excel 2019 and performance was calculated by EpiInfo™ version 3.5.1 software.

Ethical considerations

The study protocol obtained ethical clearance from the Bioethics Committee for Health Research (CBRS) of Togo (N° 046/2019/CBRS of November 21, 2019) before its implementation. In addition, signed consent was obtained from adults and children's parents/guardians. Any patient detected positive by at least one of the methods was referred to the clinicians at the sites for free management with an antimalarial drug available through the National Malaria Control Program.

Results

Characteristics of study participants

Of the 1758 people screened at the 3 sites, 448 were enrolled in the study, for an inclusion rate of 25.5%. Fifty-eight of them were excluded for quality control (impaired slide quality and parasitaemia outside the accepted range). The final number submitted for analysis was 390. The mean age of those included was 18 years (± 0.84) with extremes of 10 months and 85 years and a sex ratio (M/F) of 0.8. The main reason for consultation was fever (61.0%). The socio-demographic characteristics of the participants are summarized in Table 2.

Table 2
Sociodemographic characteristics and clinical signs
presented by the patients

	Frequency (n)	Percentage (%)
Age (years)		
0–5	111	28.5
6–9	36	9.2
10–14	55	14.1
15–19	55	14.1
≥ 20	133	34.1
Gender		
Female	214	54.9
Male	176	45.1
Clinical symptoms		
Fever	238	61.0
Headache	147	37.7
Asthenia	85	21.8
Anorexia	68	17.4
Vomiting	52	17.4
Nausea	32	8.2
Chills	29	7.4
Coughing	20	5.1
Abdominal pain	19	4.9
Diarrhea	6	1.5

Group characteristics

The evaluation of the Advantage P.f. Malaria Card® and Advantage Malaria Pan + Pf Card® in comparison with microscopy, considered 390 patients, of which 164 were in the control group, 142 in the low-parasitaemia group and 84 in the high-parasitaemia group (Table 3).

For the PCR analysis, the confetti of all TTBS positive cases regardless of the result of the 2 RDTs evaluated, the discordant TTBS-RDT cases and 10% of the concordant TTBS negative-RDT negative cases were submitted to the PCR analysis, i.e. a total of 250.

Detection of plasmodial DNA of *P. falciparum* was inconclusive in 6 of them; therefore, the estimation of the performance of the 2 TDRs evaluated in comparison to PCR was done on a total of 244 patients divided into 18 for the PCR-negative group and 226 for the *P. falciparum* PCR-positive group (Table 3)

Table 3
Total number of samples included by defined sites

Sites	Lomé	Kouvé	Sokodé
	n (%)	n (%)	n (%)
Microscopy (TTBS)			
Negative: N = 164	54 (13.8)	51 (13.1)	59 (15.1)
Positive			
<i>Low parasitaemia: N = 142</i>	27 (06.9)	97 (24.9)	18 (04.6)
<i>High-parasitaemia: N = 84</i>	27 (06.9)	38 (09.7)	19 (04.9)
Total (n = 390)	108 (27.7)	186 (47.7)	96 (24.6)
PCR			
Negative (n = 18)	8 (03.3)	3 (01.2)	7 (02.9)
fPositive (n = 226)	54 (22.1)	132 (54.1)	40 (16.4)
Total (n = 244)	62 (25.4)	135 (55.3)	47 (19.3)

Performance of RDTs evaluated comparing to microscopy

The sensitivity and specificity of the Advantage P.f. Malaria Card® compared to TTBS for the detection of *P. falciparum* were 91.8% [CI = 87.1–94.9] and 94.7% [CI = 90.0–97.4] respectively. The sensitivity and specificity of the Advantage Malaria Pan + Pf Card® RDT were 91.3% [CI = 86.6–94.6] and 94.7% [CI = 90.0–97.4] respectively. The PPV and NPV values are shown in Table 4.

Performance of RDTs evaluated comparing to PCR

The sensitivity of the Advantage P.F Malaria Card® RDT compared to PCR for *P. falciparum* infection detection was 89.4% and the specificity was 72.2%. This sensitivity increased to 88.9% for the Advantage Malaria PAN + P.F Card® RDT while specificity was the same (72.2%). The PPV and NPV values are shown in Table 4.

Table 4
Performance levels of RDTs evaluated against GE/FS and PCR for the detection of *P. falciparum* in 2020, Togo

	Advantage Malaria Pf. card®	Advantage Malaria Pan + Pf Card®
Microscopy as reference method		
Low parasitaemia		
SE* [IC à 95%]	88.7 [81.9–93.2]	87.9 [81.1–92.7]
PPV*	94.0	93.9
NPV*	90.8	90,2
High-parasitaemia		
SE [IC à 95%]	97.4 [90.2–99.6]	97.4 [90.2–99.6]
PPV	89.4	89.4
NPV	98.8	98.8
Total		
SE [IC à 95%]	91.8 [87.1–94.9]	91.3 [86.6–94.6]
SP* [IC à 95%]	94.7 [90.0–97.4]	94.7 [90.0–97.4]
PPV	95.7	95.7
NPV	90.0	89.5
PCR as reference method		
SE [IC à 95%]	89.4 [84.7–92.8]	88.9 [84.2–92.4]
SP (IC à 95%)	72.2 [46.4–89.3]	72.2 [46.4–89.3]
PPV	76.3	76.2
NPV	87.2	86.7

*: SE = sensitivity SP = specificity; PPV = positive predictive value; NPV = negative predictive value

Discussion

The World Health Organization recommends that malaria endemic countries and their partners procure pre-qualified RDTs for the biological confirmation of suspected malaria cases and as such the FIND group conducts regular evaluations and makes the results available to countries. The company “J.Mitra and Co. Pvt. Ltd”, in the context of the marketing of two new RDTs, wanted these RDTs to be tested in a

situation of use in current practice before being submitted to the WHO for this qualification. This study was therefore conducted at sentinel sites in Togo, where evaluation of the effectiveness of ACTs is often carried out, thus ensuring that evaluation procedures are respected.

The performance levels of the Advantage Pf. Malaria Card® and Advantage Malaria Pan + Pf Card® for the detection of *P. falciparum* compared to the microscopy, were 91.8% and 91.3% for sensitivity respectively and 94.7% for specificity. Our results are similar to other evaluations done that also considered microscopy as a gold standard method: these are the study conducted in northwest Ethiopia, evaluating CareStart™ with a sensitivity of 92.9 and specificity of 95.4 [10], that of the Central African Republic which found a sensitivity of 92.3 for the SD Bioline AgPf®(HRP2) RDT [11] and Margiano (2018) [12] which noted a specificity of 93.56% for the Alere HS RDT®.

In our study, the rate of sensitivity of the RDTs in relation to microscopy, increased with parasitaemia: indeed, it was higher for high parasitaemia for both evaluated TDRs (97.4% vs. 88.7% and 87.9% respectively). This trend corroborates with the finding by Wanja et al. [13] who in an evaluation of the diagnostic performance of four types of RDTs targeting HRP2 (2016) in Kenya, noted that the overall sensitivity which was above 90% for the four RDTs evaluated, fell below 90% for parasitaemia < 200 P/μL (parasitaemia between 79% and 89%). These data confirm the results of the study conducted in Togo by Dorkenoo et al, (2020) [14] as part of the evaluation of the performance of actors in malaria diagnosis by RDTs, where dried tube samples of *P. falciparum* of 100 to 200 parasites/μL were not reliably detected by the RDTs used.

This limitation of RDT detection for low parasitaemia [15] would explain the false negatives cases observed in our study, although other factors have been listed by other authors including the lack of specificity of the monoclonal antibodies used to design RDTs, the genetic diversity of the HRP2 parasite antigen, or the deletion of the gene coding HRP2, [16]. Indeed Koita et al. (2012) [17] showed that HRP2 deletions occur under field conditions and that some strains of *P. falciparum* without the histidine-rich repeat region of HRP2 or its gene product, would produce true malaria cases in sub-Saharan Africa.

In our study, the RDTs evaluated showed a relatively high specificity (94.7%) compared to microscopy. Indeed, WHO recommends that RDTs to be used for biological confirmation of malaria should have at least a specificity higher than 90% [18]. The low false positive rate (5.3%) found in our study could be related to the possible presence of rheumatoid factors in these patients [19] and/or to the persistence of HRP2 in the blood several days after parasite clearance. Indeed, Iqbal et al (2004) [20] showed in their study, the persistence of HRP2 antigenemia after antimalarial treatment, up to 14 days of follow-up. Other studies supporting this finding, have shown that this persistence can be up to 28 days [21, 22].

Significant positive and negative predictive values were found for these RDTs compared to TTBS in this study. Thus, the high PPV for both RDTs (95.7%) reinforces the sensitivity and specificity data found for these RDTs to be used for biological confirmation of malaria cases in malaria endemic areas. The NPVs of the Advantage Pf. Malaria Card® and Advantage Malaria Pan + Pf Card®, respectively 90.0% and 89.5%, are probably related to the time period of our study which started at the end of the rainy season

while the evaluations of the other authors were done during the period of high transmission. Indeed the predictive values (PPV and NPV) are a function of the prevalence of the disease in the study area [23].

Although microscopy is the gold standard detection technique for malaria, the performance of these RDTs was also estimated in relation to PCR, a technique with better sensitivity than TTBS. The performance of the RDTs were slightly lower than those obtained when considering TTBS. The sensitivity of the Advantage P.f. Malaria Card® and Advantage Malaria Pan + Pf Card®, which were 91.8% and 91.3%, respectively, when compared to microscopy, fell below 90% when PCR was used as a reference method (89.4% and 88.9, respectively). Indeed, PCR can detect parasites as low as 0.002 P/μL, implying that the lower sensitivity range observed, could be due to sub-microscopic infections in the population. Also, Matangila et al (2014) [24] in their study showed that 65% of the microscopy negative samples analyzed by PCR were actually submicroscopic infections.

Similarly, a lower specificity of the two RDTs assessed in our study, compared to PCR, was observed (72.2%) compared to microscopy (94.7%). This difference in specificity could be explained by the size of the negative group submitted to PCR. In addition, false positive results could be explained by the persistence of HRP2 circulation in the blood for more than two weeks even after antimalarial treatment [24]. The different factors such as false positives, false negatives, inability to detect submicroscopic infections, persistent HRP2 antigenemia and HRP2 polymorphism considered as biases in the interpretation of the real level of RDT performance, would require PCR to be adjoined for an improvement of the quality of its diagnosis [18, 25].

Our study has some limitations. Indeed, although the Advantage Malaria Pan + Pf Card® RDT detects species other than *P. falciparum*, the performance of this RDT to detect other plasmodial species has not been assessed due to the low number of positive cases for these other species. In addition, the low number of PCR-negative cases may have introduced a bias in the estimation of the performance of these RDTs compared to PCR. Thus, further studies should be conducted on a larger sample size.

Conclusion

The diagnostic performance of the Advantage P.F Malaria Card® and Advantage Malaria PAN + PF Card® RDTs for the biological confirmation of malaria cases compared to the microscopy as reference method, although varying according to the parasite density, have reached the WHO acceptability stage, even if considering the PCR as reference method, this performance has slightly regressed. As sub-microscopic infections are expected to be more frequent, an impact of the control strategies undertaken by Togo, the determination of the performance of the new RDTs should perhaps consider PCR in the evaluation procedure as part of the continuous improvement of these diagnostic tools with a view to achieving the elimination of malaria in the countries where it still remains endemic.

Declarations

Competing interests

All authors declare that they have no competing interests.

Availability of data and materials

The data supporting the conclusions of this paper are included within the paper. Raw data may be obtained from the corresponding author upon request.

Consent for publication

Not applicable.

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Authors' contributions

Diwaba C. TEOU, Ameyo M. DORKENOO, Kossi YAKPA and Issaka MAMAN conceived and designed the study. Ameyo M. DORKENOO led the training and oversaw implementation in Togo. Ameyo M. DORKENOO, Amegnona AGBONON, Essoham ATABA, Kossi YAKPA and Agueragna ABDOU-KERIM supervised the data collection. Diwaba C. TEOU, Ameyo M. DORKENOO and Essoham ATABA drafted the analysis plan and wrote the first draft of the manuscript. All authors contributed to data analysis through review and interpretation of the results. All authors read, revised and approved the final manuscript.

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