Performance of rapid DiaMed OptiMal-IT[®] malaria test in an endemic Ghanaian setting

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Abstract At peripheral health facility levels, the diagnosis of malaria is difficult due to lack of infrastructure. In the study reported here, the diagnostic performance of a batch of ‘DiaMed OptiMAL-IT’ (Cressier, Switzerland) rapid antigen tests were examined in parallel with routine microscopy for a select population of high risk individuals: 202 pregnant women and 200 children less than 5 years old in an endemic setting in Ghana. Results of both diagnostic methods were compared to that of cross-checking microscopy, taken as gold standard, at the Navrongo Health Research Centre. Of the 402 patients, 218 (54.2%) were confirmed with *Plasmodium falciparum* infections by cross-checking. All 218 patients (100%) were accurately diagnosed with malaria by routine microscopy. Of these, 151 (69.3%) were positive by DiaMed OptiMAL-IT test (26 false positives, 67 false negatives). DiaMed OptiMAL-IT had the following performance indicators for detection

of *P. falciparum* among pregnant women and children less than 5 years respectively: Sensitivity—50.5% [95% CI (40.6–60.3)], 87.7% [95% CI (78.7–92.1)]; Specificity—82.5% [95% CI (73.1–89.1)], 89.6% [95% CI (80.8–94.8)]; Predictive values for positive tests—75.7% [95% CI (63.7–84.8)], 91.6% [95% CI (85.2–95.8)]; Predictive values for negative tests—60.6% [95% CI (51.7–68.9)], 83.8% [95% CI (74.5–90.3)]; Likelihood ratio for positive tests—2.88, 10.8; Likelihood ratio for negative tests—0.59, 0.19; Cohens Kappa values—0.33 [95% CI (0.20–0.43)], 0.76 [95% CI (0.65–0.83)]. Our results suggest that DiaMed OptiMAL-IT tests should not replace microscopy in our endemic setting.

Keywords Malaria · Ghana · Rapid · Diagnosis · Performance

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Introduction

Malaria is hyper-endemic in Ghana and the highest cause of mortality, accounting for 18% of deaths reported at health facilities (Ghana Health Service Annual Report 2007). Each year, malaria accounts for 38.6% of outpatient attendance and 20% of mortality among children under 5 years. Among pregnant women, malaria accounts for about 8% of hospital admissions (Ghana Statistical Service 2009). Early diagnosis and prompt treatment of malaria is a major key strategy for control. However, in many countries where malaria is endemic, diagnosis at the peripheral health facility levels is limited by lack of reliable microscopy, and often restricted to clinical signs and symptoms. Microscopy, which has been the traditional standard for laboratory diagnosis of malaria, is often not available in most peripheral health facilities. As a possible solution,

one-step rapid diagnostic tests (RDTs) that are simple to perform, and able to provide accurate and immediate results for treatment have been advocated.

The DiaMed OptiMAL-IT (Cressier, Switzerland) is a rapid immunochromatographic test that both identifies and differentiates *Plasmodium falciparum* from non-*P. falciparum* (*P. vivax*, *P. ovale*, and *P. malariae*) species on the basis of *Plasmodium*-specific lactate dehydrogenase (pLDH) in patients' whole blood (Iqbal et al. 2003). Several investigations have reported on the performance of OptiMAL-IT assays in various European and American settings (Moody 2000). Although the efficiency of DiaMed OptiMAL-IT may vary from one geographical location to the other, no systematic survey on the performance of DiaMed OptiMAL-IT has been conducted in Ghana and the extent of its potential application remains unclear. In the study reported here, the diagnostic performance of a batch of 'DiaMed OptiMAL-IT' rapid antigen tests were examined in parallel with routine microscopy for a select population of high risk individuals: pregnant women and children less than 5 years old symptomatic for malaria in the Navrongo area of Kassena-Nankana District of Ghana.

Methods

Study setting

As part of routine health-service delivery, patients with clinical symptoms suspicious of malaria, and with no history of antimalarial treatment during the last 2 weeks, were enrolled through passive malaria case detection at the Navrongo War-Memorial Hospital (NMH) in the Kassena-Nankana District. NMH is a district hospital that serves as a referral point, and the only facility that renders laboratory services for four health centres positioned across the whole district. The District has the Navrongo Health Research Centre (NHRC). NHRC has a multidisciplinary team of scientists engaged in a wide range of activities encompassing biomedical research. The Kassena-Nankana District covers an area of about 1,674 sq km of the Sahelian savannah, in the Upper East Region of Ghana, on the Northern border with Burkina Faso (Kassena Nankana district 2011, <http://www.kassenanankana.ghanadistricts.gov.gh>, 2011). The district has a population of about 150,000 [48% male, 52% female, 25% children (12% for children under 5 years of age)].

Sample collection

In general, physician-identified patients (prospective children and pregnant women) who presented with symptoms consistent with malaria [history of fever with or without

chills (axillary temperature $>37.5^{\circ}\text{C}$), sweating, headache] and referred to the laboratory for a malaria test, between October and December 2006, were included in the study after their informed consents were obtained. Venous blood was drawn into EDTA-coated syringes, distributed into sterile test tubes, and placed immediately on ice. Additional samples comprised blood specimens referred secondarily from other laboratories in the Kassena-Nankana District for confirmation. Routinely, DiaMed OptiMAL-IT detection tests were performed according to manufacturer's instructions in parallel with thin and thick film microscopy on all specimens by separate operators blinded to the results of the other assays.

Microscopy

Thin films (*for speciation*) and thick films (*for determination of parasitaemia*) were prepared from primarily referred whole-blood specimens and stained with 2% Giemsa stain (Warhurst and Williams 1996) by standard procedures within an hour of receipt. A trained operator examined each film for at least 20 min under $100\times$ oil immersion lens such that at least 300 fields were examined before a slide was counted negative. Parasite densities were determined by the number of parasites per 200 white cell count against a standard 8,000 white cell count/ μl value (Shute 1988). If a parasite count was more than 100 parasites per field, the number of parasitized red blood cells (RBCs) per 1,000 RBCs against the baseline erythrocyte counts of patients was used to calculate the parasitaemia (parasites/ μl).

Rapid diagnostic tests

Each whole-blood specimen was tested immediately on receipt by using the DiaMed OptiMAL-IT assays and results interpreted according to manufacturer's instructions. If the results were borderline (faint or ambiguous lines), a third party was consulted for a consensual decision.

Validation

Whole blood samples were re-examined and cross-checked at NHRC for *Plasmodium* infections by an expert microscopist without reference to results of field microscopy and DiaMed OptiMAL-IT assays.

Statistical analysis

All data were recorded in Excel 2000 software (Microsoft Corp., Redmond, WA, USA). Specimens were classified as true positive, true negative, false positive, or false negative for each test under evaluation compared with validation

microscopy. The following performance indices were calculated: Sensitivity, specificity, positive predictive values for positive and negative tests, Likelihood ratios for positive and negative tests and Cohens' Kappa values. Comparison of independent means was performed using a two-sample *t* test, and comparison of independent proportions was performed using a χ^2 test. All significance tests were two tailed at *P* value <0.05.

Results

A total of 402 symptomatic malaria patients comprising 202 pregnant women and 200 children under 5 years old were included in this study. The mean age of pregnant women was 32 ± 14 years (range: 21–38 years), and that of children was $2 \text{ years} \pm 5 \text{ months}$ (range, 4 days–5 years). Table 1 displays the differential diagnosis obtained by DiaMed OptiMAL-IT and the results of primary field microscopy at NMH. No discrepancies were observed between the primary routine microscopy and the validation microscopy results from the research laboratory. Overall, 54.2% (218/402) of patients enrolled to the study were diagnosed with malaria by field microscopy, whereas 44.0% (177/402) were positive for malaria by the rapid DiaMed OptiMAL-IT test (26 false positives, 67 false negatives). Using DiaMed OptiMAL-IT, *P. falciparum* diagnosis among pregnant women showed a higher proportion of false positives, 24.3% [95% CI (15.2–36.3)], compared to that of children under 5 years, 8.4% [95% CI (4.2–15.7)]. Likewise, false negatives amongst pregnant women diagnosed of malaria by DiaMed OptiMAL-IT was significantly higher, 39.4% [95% CI (31.1–48.3)], compared to that of children less than 5 years, 16.1% [95% CI (9.6–25.5)]. Table 2 shows the comparative performance of DiaMed OptiMAL-IT detection assay calculated on the basis of our confirmed microscopy results. DiaMed OptiMAL-IT demonstrated a significantly lower sensitivity

(*P* value = 0.001) for detection of *P. falciparum* in pregnant women, 50.5% [95% CI (40.6–60.3)], compared to children less than 5 years old, 86.7% [95% CI (78.7–92.1)]. Overall, the sensitivity of DiaMed OptiMAL-IT was 69.3% [95% CI (62.6–75.2)], with a specificity and positive predictive value of 85.9% [95% CI (79.8–90.4)], and 89.3% [95% CI (80.6–89.2)], respectively. The DiaMed OptiMAL-IT had the following performance indicators for detection of *P. falciparum* among pregnant women and children less than 5 years respectively: Specificity—82.5, 89.6%; Predictive values for positive tests—75.9, 91.6%; Predictive values for negative tests—60.6, 83.8%; Likelihood ratio for positive tests—2.88, 10.81; Likelihood ratio for negative tests—0.59, 0.19; Cohens Kappa values—0.33, 0.76.

Discussion

In this study, we have evaluated the performance of the rapid diagnostic tests, DiaMed OptiMAL-IT, against conventional microscopy on a population of high risk individuals symptomatic for malaria.

Provided cross-checking microscopy was accurately read for all smears, routine microscopy was 100 percent sensitive and specific for *P. falciparum* and gametocytes identified in the pregnant women and children less than 5 years old. DiaMed OptiMAL-IT showed unacceptable performance for malaria diagnosis under routine conditions for pregnant women. The sensitivity of the DiaMed OptiMAL-IT assay for detection of *P. falciparum* in pregnant women (50.5%) was lower than that observed for children participants (86.7%). Subsequent parasite counts showed that the low sensitivity of DiaMed OptiMAL-IT in pregnant women was due to low parasitaemias (below 100 parasites/ μ l) observed within this group. There was significant difference (*P* value = 0.001) in mean parasite counts between pregnant women, 146.9 [95% CI

Table 1 Differential diagnosis of malaria cases by DiaMed OptiMAL-IT rapid antigen testing versus Microscopy in pregnant women and Children less than 5 years

DiaMed OptiMAL-IT	Result of field microscopy ^a (number of samples)			
	Pf +ve only	Non-Pf	Gametocytes	Negative tests
Pf ^b only				
Pregnant women (<i>n</i> = 202)	45	0	8	17
Children (<i>n</i> = 200)	98	0	0	9
Non-Pf				
Pregnant women (<i>n</i> = 202)	0	0	0	0
Children (<i>n</i> = 200)	0	0	0	0
Negative				
Pregnant women (<i>n</i> = 202)	52	0	0	80
Children (<i>n</i> = 200)	15	0	0	78

^a There were no discrepancies between field and cross-checking microscopy readings

^b All confirmed positive malaria cases were with *Plasmodium falciparum* (Pf) only

Table 2 Performance of DiaMed OptiMAL-IT rapid detection assay calculated on the basis of confirmed microscopy results

Microscopy results		Results by DiaMed OptiMAL-IT							
No. of cases	Pf/μl	No. of positive tests	Percent sensitivity (95% CI)	Percent specificity (95% CI)	Percent predictive values		Likelihood ^a ratios		Kappa values (95% CI)
					Positive tests (95% CI)	Negative tests (95% CI)	Pos tests	Neg tests	
Pregnant women (<i>n</i> = 105)	1–100	38/89	50.5 (40.6–60.3)	82.5 (73.1–89.1)	75.7 (63.7–84.8)	60.6 (51.7–68.9)	2.88	0.60	0.33 (0.20–0.43)
	<100	15/16							<i>P</i> value = 0.001
Children less than 5 years (<i>n</i> = 113)	1–100	29/39	86.7 (78.7–92.1)	89.6 (80.8–94.8)	91.6 (85.2–95.8)	83.8 (74.5–90.3)	10.80	0.19	0.76 (0.65–0.83)
	<100	69/74							<i>P</i> value = 0.001

^a Likelihood Ratios for positive (*Pos*) and negative (*Neg*) test results

(33.6–60.1)] parasites/μl and children less than 5 years, 1088 [95% CI (711–1464)] parasite/μl. Likelihood ratios determine the accuracy of a positive or negative result and are independent of the prevalence of a disease conditions in populations (Marx et al. 2005). The calculated ratios for positive and negative tests for the two study groups suggest that the diagnostic efficiency of DiaMed OptiMAL-IT is highly dependent on parasitaemia: the higher the parasite densities, the higher the probability that patients with positive test results would be truly infected and the vice versa. Indeed, the performance of OptiMAL-IT tests have considerably improved in other studies with parasite densities >100 parasites/μl (World Health organization (WHO)/Foundation for Innovative New Diagnostics (FIND) 2009a, b; WHO/FIND 2010; Mawili-Mboumba et al. 2010). It must be emphasized that Kappa values for DiaMed OptiMAL-IT using microscopy as reference standard, were 0.33 for pregnant women and 0.76 for children participants; suggesting a very weak concordance and low reliability of DiaMed OptiMAL-IT test for the former who significantly had low parasitaemias. These observation are consistent with reports of other investigations that have showed variations in DiaMed OptiMAL-IT to be strain specific- and parasite density-dependent (Moody et al. 2000; Mason et al. 2002). Moreover, compared with the field microscopy, the DiaMed OptiMAL-IT test did not perform as well in differentiating *P. falciparum* infections from gametocytaemia (8 cases from pregnant women). Mueller et al. (2007) monitored treatment outcomes with Diamed OptiMAL-IT in an endemic malaria region in Papua New Guinea using in vivo samples from treatment failure malaria cases. The study showed that the sensitivity of Diamed OptiMAL-IT is compromised by the presence of *P. falciparum* gametocytes. The presence of gametocytes, which also produce the pLDH, greatly reduce the utility of DiaMed OptiMAL-IT assays in monitoring patients' recovery from asexual parasitemia (Tjitra and Anstey 2001).

It is well known that, preferably, RDTs should achieve a sensitivity of >90% at densities of above 100 parasites/μl of blood (Moody 2000). Overall, the sensitivity (69.3%) and specificity (85.9%) of DiaMed OptiMAL-IT for the diagnosis of malaria cases in our study were lower than the 98 and 100% obtained in a validity assessment reported by Palmer et al. 2003. Palmer et al. (2003) did not stratify their study population and used the more relevant Polymerase Chain Reaction (PCR) to validate the DiaMed OptiMAL-IT results. Similar results and observations for sensitivity (87.0%) and specificity (97.9%) of DiaMed OptiMAL-IT have been recorded in Venezuela by Rodulfo et al. (2007). In the present study, although PCR was not performed, we find the DiaMed OptiMAL-IT antigen assay to have relatively low performance indices, especially for malaria infections with low parasite densities. Our results suggest that DiaMed OptiMAL-IT tests for malaria should not replace microscopy in our endemic setting.

Even though the DiaMed OptiMAL-IT use required simple training for peripheral health staff, it showed low performance indicators when compared with microscopy for diagnosis of malaria amongst pregnant women and children less than 5 years. Across geographical settings, performance of DiaMed OptiMAL-IT appears to be variable. In Mozambique and Tanzania, Hendriksen et al. (2011) reported an overall sensitivity and specificity of 88.0 and 88.3% respectively for DiaMed OptiMAL-IT assays. Buhalata and Massaga (2011) recorded a low sensitivity of 17% in north-western Tanzania. In Burkina Faso, Valéa et al. (2009) reported a high sensitivity and specificity of 98.7 and 96.2% respectively for DiaMed OptiMAL-IT tests among children aged 6 to 59 months old. DiaMed OptiMAL-IT results may vary as a result of parasite density-dependent sensitivity, parasite-strain-specific differences and variations in performance between batches. In our study, the project coordinator ensured that, routinely, stability testing for batches of DiaMed OptiMAL-IT Kits were performed. The DiaMed OptiMAL-IT kits were kept

in temperature-controlled store-rooms and dispatched to project sites by cold-chain transport. All batches of DiaMed OptiMAL-IT kits used for this study had expiration dates far beyond the project duration.

In conclusion, priority should be given to microscopy and expanding its utility in the conventional diagnosis of malaria. The DiaMed OptiMAL-IT assays should not be recommended for treatment screening in our malaria endemic environment from the results of this study. In non endemic settings, DiaMed OptiMAL-IT may be considered under field conditions for quick screening of study participants for inclusion of people with *P. falciparum* in research studies where additional methods are to be used for confirmation.

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