

Short Report: Performance of Two Malaria Rapid Diagnostic Tests in Febrile Adult Patients with and without Human Immunodeficiency Virus-1 Infection in Blantyre, Malawi

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Abstract. The performance of two histidine-rich protein type-2–based malaria rapid diagnostic tests (mRDTs) was examined in a rural area with a high prevalence of malaria and human immunodeficiency virus type-1 (HIV-1) infection in 113 and 445 febrile patients ≥ 15 years of age with and without HIV-1 infection, respectively. Patients were tested for HIV-1 infection by using a standard assay and for *Plasmodium falciparum* by using two mRDTs and microscopy. When microscopy was used as the gold standard, both mRDTs performed similarly in patients with and without HIV-1 infection: Bioline SD Malaria Antigen P.f, sensitivity 94.4% (95% confidence interval [CI]: 81.3–99.3%) versus 97.1% (95% CI: 92.8–99.2%) and specificity 50.6% (95% CI: 39.0–62.2%) versus 47.2% (95% CI: 41.4–53.1%); and ICT diagnostics Malaria Pf, sensitivity 94.4% (95% CI: 81.3–99.3%) versus 97.1% (95% CI: 92.8–99.2%) and specificity 50.6% (95% CI: 39.0–62.2%) versus 50.3% (95% CI: 44.4–56.1%). Infection with HIV-1 does not appear to affect the performance of these histidine-rich protein type-2 (HRP-2)-based mRDTs.

Malaria and human immunodeficiency virus (HIV) are among the most important health problems in sub-Saharan Africa. Malaria accounted for 781,000 deaths in 2009, of which more than 80% occurred in sub-Saharan Africa.¹ In addition, approximately 22.5 million adults and children in sub-Saharan Africa have HIV infection and an estimated 1.3 million deaths in the region were caused by acquired immunodeficiency syndrome (AIDS) in 2009.² There is increasing evidence of an interaction between malaria and HIV-1, and the impact of co-infection is most apparent in areas with generalized HIV-1 epidemics and stable malaria transmission.^{3–8} In Malawi, more than 90% of the population is exposed to year-long, intense malaria transmission and HIV-1 prevalence in adults is estimated to be 12%.⁹ In adults, HIV-1 infection is associated with increased episodes of uncomplicated malaria and patients with low CD4 cell counts are particularly vulnerable to malaria.^{5,8,10,11}

Resistance to first-line malaria treatments in sub-Saharan Africa has made national malaria control programs switch to more expensive but effective artemisinin combination therapy.¹² This change in treatment policy, renewed calls for malaria elimination, the need for reporting accurate surveillance data, and innovations in malaria antigen detection formats have contributed to revised recommendations for obtaining an accurate diagnosis on the basis of parasitologic confirmation before administering treatment. Currently, the World Health Organization recommends that malaria case management should be based on parasitologically confirmed diagnosis in all cases. Given their relative ease of use, mRDTs form a vital part of this strategy.¹³

Infection with HIV-1 in adults is associated with high-density parasitemia,¹⁴ and performance of mRDTs is affected by parasitemia density and the amount of circulating antigenemia.^{15,16} HIV-positive adults are more likely to have fever than HIV-negative adults, and acceptable mRDT performance is critical for diagnosis of malaria and to rule out malaria as a cause of fever.¹⁷ The World Health Organization has set the

assessment of how malaria/HIV co-morbidity affects mRDT results as one of the priority areas for operational research.¹⁸ In this study, we assessed the sensitivity and specificity of two commercially available histidine-rich protein type-2 (HRP-2)–based mRDTs in patients ≥ 15 years of age with and without HIV-1 infection who came to a rural health center with an acute febrile episode.

In a cross-section study design, patients were enrolled in March–April 2009 at the outpatient department of Lirangwe Health Center, a rural facility in the Blantyre District of Malawi where there is year-round malaria transmission that peaks during November–April. Patients with fever or a history of fever within 48 hours and ≥ 15 years of age were eligible for enrollment. Pregnant women and ill patients were excluded from the study.

Eligible patients who provided informed consent or assent (if < 18 years of age) were tested for HIV-1 by using Unigold (catalog no. 1206502; Trinity Biotech Plc., Bray, Ireland) and for malaria by using SD Bioline Malaria Antigen Pf (catalog no. 05FK50, Standard Diagnostics, Seoul, South Korea), ICT Malaria Pf Cassette Test (ICT Diagnostics, Cape Town, South Africa) and a thick blood smear examination by light microscopy. Blood collected by a finger prick by trained community health workers was used to prepare thick blood smears. The blood smears were then sent to a College of Medicine laboratory to be stained with Field's Stains A and B (azure dye and eosin) and read independently by two expert microscopists. Discordant readings were resolved by a third microscopist.

A thick smear was considered positive if one or more malaria parasite was visualized and considered negative if no trophozoites were seen after examining 100 high-power fields (100 \times objective). The microscopists were blinded to each other, mRDT and HIV test results. Both mRDTs were conducted by the same trained health workers according to the manufacturer's instructions. The HIV-1 tests were performed in the facility Voluntary, Counseling, and Testing room by a ministry of health–certified counselor who was blinded to mRDT results. Treatment of malaria was according to mRDT results. Sensitivity, specificity, positive and negative predictive values of mRDTs in HIV-positive and HIV-negative patients was assessed by using microscopy as the gold standard.

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TABLE 1

Demographic, clinical and laboratory characteristics of HIV-positive and HIV-negative patients ≥ 15 years of age with an acute febrile episode, Lirangwe Health Center, Blantyre, Malawi, 2009

Characteristic	HIV-positive (n = 113)	HIV-negative (n = 445)	P*
Age in years (mean)	32.2	26.8	0.01†
Weight in kilograms (mean)	48.5	45.5	0.01†
Female (%)	69.7	60.3	0.07
<i>P. falciparum</i> asexual parasitemia count (mean)‡	2,542.2	3,848.0	0.24†
<i>P. falciparum</i> asexual parasitemia prevalence (%)	31.8	32.3	0.92
Febrile patients, temperature $\geq 37.5^\circ\text{C}$ (%)	47.1	45.2	0.71
Febrile patients with parasitemia (%)	40.0	46.7	0.39
Used any mosquito net previous night (%)	89.2	83.9	0.29
Self-treated with antimalarial drugs before visit (%)	04.7	04.3	0.87

* By Pearson χ^2 test unless otherwise noted.

† By Student's *t*-test.

‡ n = 36 for HIV-positive patients and n = 141 for HIV negative patients. Mean parasitemia count is per microliter.

HIV = human immunodeficiency virus.

Data were entered into SPSS version 12.0 (SPSS Inc., Chicago, IL) and analysis was performed by using STATA version 10 (StataCorp, College Station, TX). Statistical testing was done by using Pearson's chi-square test or *t*-test, as appropriate. Agreement between the two mRDTs was measured by using the kappa statistic. Significance was defined as $P < 0.05$. The study protocol was approved by the Institutional Review Board at the University of Malawi, College of Medicine.

A total of 650 eligible adult patients were invited to participate in the study and 598 (92.0%) provided informed consent and were enrolled. We included 558 (93.3%) patients with available HIV test results in the analysis. Of these patients, 113 (20.2%) were HIV positive (Table 1). The prevalence of *Plasmodium falciparum* parasitemia was 31.8% and 31.6% in HIV-positive and HIV-negative patients, respectively. The proportion of febrile patients (temperature $\geq 37.5^\circ\text{C}$) who were parasitemic in HIV-positive and HIV-negative groups was 40.0% and 46.7% respectively, but this difference was not statistically significant ($P = 0.39$). HIV-positive patients were more likely to be older than HIV-negative patients ($P < 0.01$).

Only 36 (6.4%) of the 558 patients were co-infected with HIV-1 and *P. falciparum*. Among these patients, 34 had true-positive results (microscopy positive, RDT positive) for both RDTs. Two patients had false negative results (microscopy positive but RDT negative) for both RDTs. The parasite counts for the two patients were 780 parasites/ μL and 2,210 parasites/ μL , respectively.

Conversely, in the HIV-negative group, 137 patients had true positive results for both RDTs and 4 patients had false negative results and parasite counts of 10, 60, 200, and 590 parasites/ μL . For HIV-negative patients with false positive results (microscopy negative but RDT positive), results of the Bioline SD Malaria Antigen P.f and ICT Diagnostics

Malaria Pf were positive for 155 and 146 patients, respectively. Both RDTs showed positive results for 145 patients. The level of agreement between the two mRDTs was 98% (kappa = 0.96, $P < 0.001$).

There was no significant difference in the sensitivity, specificity, positive and negative predictive value of either Bioline SD Malaria Antigen P.f or ICT Diagnostics Malaria Pf Cassette Test for detecting *P. falciparum* asexual parasitemia in HIV-positive versus HIV-negative patients (Table 2). Although sensitivity was adequate ($> 90\%$) for both mRDTs regardless of HIV status, specificity was low for both mRDTs (47.2–50.6%) regardless of HIV status.

We evaluated the accuracy of two HRP-2-based mRDTs in HIV-positive and HIV-negative patients with acute febrile illness seeking care at a rural health facility. The sensitivity of both mRDTs was adequate ($> 90\%$) in HIV-positive and HIV-negative patients. Given that HIV-associated immunosuppression increases malaria incidence in HIV-positive patients^{5,8,10,11} and that HIV positive status is associated with risk of getting an incorrect presumptive malaria diagnosis,¹⁹ our findings are reassuring because most HIV-positive patients with *P. falciparum* malaria would be appropriately diagnosed by using these HRP-2-based mRDTs. Only one study has assessed performance of mRDTs in febrile HIV-positive adults in sub-Saharan Africa. Using the Binax Now[®] Malaria Test (Inverness Medical Innovations Inc., Waltham, MA), Mills and others reported comparable sensitivity (85.7%; 95% confidence interval: 57.2–98.2%) but high specificity (97.8%; 95% confidence interval: 94.9–99.3%) for detection of *P. falciparum* compared with a thick blood smear.²⁰

Both mRDTs evaluated in this study had low specificity in HIV-positive and HIV-negative patients. Previous studies in Malawi²¹ and elsewhere^{22,23} have also reported low specificity

TABLE 2

Performance of malaria rapid diagnostic tests compared with microscopy in HIV-positive and HIV-negative patients ≥ 15 years with an acute febrile episode, Lirangwe Health Center, Blantyre, Malawi, 2009

Characteristic	SD Bioline malaria antigen Pf			ICT Diagnostics Malaria Pf Cassette Test		
	HIV positive, n/N (% , 95% CI)*	HIV negative, n/N (% , 95% CI)*	P†	HIV positive, n/N (% , 95% CI)*	HIV negative, n/N (% , 95% CI)*	P†
Sensitivity	34/36 (94.4, 81.3–99.3)	137/141 (97.1, 92.8–99.2)	0.4	34/36 (94.4, 81.3–99.3)	137/141 (97.1, 92.8–99.2)	0.4
Specificity	39/77 (50.6, 39.0–62.2)	139/294 (47.2, 41.4–53.1)	0.6	39/77 (50.6, 39.0–62.2)	148/294 (50.3, 44.4–56.1)	0.9
Positive predictive value	34/72 (47.2, 35.3–59.3)	137/292 (46.9, 41.0–52.8)	0.9	34/72 (47.2, 35.3–59.3)	137/283 (48.4, 42.4–54.3)	0.8
Negative predictive value	39/41 (95.1, 83.4–99.4)	139/143 (97.2, 92.9–99.2)	0.5	39/41 (95.1, 83.4–99.4)	148/152 (97.3, 93.3–99.2)	0.4

* Binomial exact 95% confidence interval.

† By Pearson's chi-square test.

HIV = human immunodeficiency virus; PF = *Plasmodium falciparum*.

of mRDTs. Possible explanations for low specificity include persistent circulating antigenemia after treatment with an effective antimalarial drug, presence of sub-microscopic parasitemia, operator error in reading the mRDT test result and other factors. Self-treatment with antimalarial drugs was 4.7% and 4.3% in the HIV-positive and HIV-negative groups, respectively. Among these groups, 1 (20%) of 5 HIV-positive patients had false positive results compared with 9 (48%) of 19 HIV-negative patients.

Although providing the correct diagnosis and treatment of the true underlying cause of fever is critical in all febrile patients, it is particularly important in HIV-positive patients because they have multiple alternative causes for fever, including opportunistic or other infections. A high rate of false-positive results due to poorly specific mRDTs might lead to over-treatment of malaria and under-treatment of other causes of fever in this vulnerable population. However, it is worth noting that consistent with findings of a large study of mRDTs in areas with medium-to-high malaria transmission rates in Uganda,²⁴ the negative predictive values of HRP-2-based RDTs in this study and those in the study of Mills and others²⁰ are high (> 95%). This finding implies that a negative HRP-2-based RDT result can be an important point-of-care tool for guiding clinicians to look for other causes of febrile illness in HIV-positive patients.

In this study, malaria parasitemia prevalence among all patients and parasite density among those infected with malaria did not vary significantly between HIV-positive and HIV-negative patients. These findings are similar to those of a hospital-based study conducted in the region,¹⁹ but inconsistent with results of another study, which reported substantially higher malaria prevalence in HIV-positive patients than in HIV-negative patients.²⁵ Similar levels of malaria in HIV-positive and HIV-negative groups in our setting might be the result of the fact that all patients in the study lived in an area with similar malaria transmission intensity and had equal access to malaria control interventions such as mosquito net use (89.2% versus 83.9%; $P = 0.29$) and malaria case management. Because 98% of HIV-positive patients were diagnosed for the first time in the study, it is also possible that HIV-positive patients did not have substantial immunosuppression that might have made them more susceptible to malaria. Lastly, the prevalence of HIV in patients attending the health facility for an acute febrile illness episode (20.2%) was almost twice the prevalence of HIV in the general population,⁹ which underscores the need for provider-initiated counseling and testing for all adult patients with an acute febrile illness.

This study had several limitations. First, to estimate mRDT sensitivity of $90 \pm 5\%$, with 80% power, we needed to enroll 138 HIV-positive parasitemic patients. However, we were able to enroll only 36 HIV-positive parasitemic patients and were thus underpowered.²⁶ Second, we were unable to measure CD4 cell counts in the HIV-positive patients and were thus unable to assess the relationship between degree of immunosuppression and mRDT test performance. Studies on larger samples of adult patients with higher malaria/HIV co-morbidity and that take into account CD4 cell counts are needed to confirm these findings.

In summary, we found no difference in HRP-2-based mRDT performance in HIV-positive and HIV-negative patients with acute febrile illness in an area of high HIV and malaria prevalence. Clinicians in areas with high HIV and malaria preva-

lence should be vigilant for possible co-infection and should use laboratory-based diagnostic tools for both diseases to guide clinical management.

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