Short Report: Utility of a Point-of-Care Malaria Rapid Diagnostic Test for Excluding Malaria as the Cause of Fever among HIV-Positive Adults in Rural Rakai, Uganda

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Abstract. We compared results of a malaria rapid diagnostic test (Binax Now® Malaria, Binax-M, Inverness Medical Innovations, Inc., Waltham, MA) performed at rural mobile clinics in Uganda by clinicians evaluating febrile adult HIV patients to thick smear evaluated at a central laboratory by trained microscopists. Two hundred forty-six samples were analyzed, including 14 (5.7%) which were thick-smear positive for falciparum malaria. Sensitivity of Binax-M compared with thick smear was 85.7% (95% CI: 57.2–98.2), specificity 97.8% (95% CI: 94.9–99.3), positive and negative predictive values were 70.6% (95% CI: 44.0–89.7) and 99.1% (95% CI: 96.8–99.9), respectively. The rapid diagnostic test accurately ruled malaria “in or out” at the point-of-care, facilitating appropriate clinical management and averting unnecessary anti-malarial therapy.

INTRODUCTION

By the end of 2007, more than 2 million of HIV-infected sub-Saharan Africans had begun receiving HIV care and antiretroviral therapy when indicated.¹ In malaria-endemic areas, fever is often assumed to result from malaria, and treatment is frequently given on a presumptive basis without laboratory investigation.² This is especially true in settings where timely microscopy is not available.³ However, among HIV-positive adults in malaria-endemic areas who receive cotrimoxazole, antiretroviral therapy (ART) when indicated, and insecticide-treated nets (ITNs), malaria is uncommon.⁴

There is an urgent need for accurate, inexpensive, simple malaria rapid diagnostic tests (RDTs) for use in resource-poor settings, especially where conventional microscopy is logistically impractical. A point-of-care RDT with high specificity would allow clinicians to “rule out” malaria and to focus on the correct detection and management of other febrile illnesses.⁵ Here, we report the performance of an FDA-approved point-of-care malaria RDT, the Binax Now® Malaria Test (Inverness Medical Innovations Inc., Waltham, MA) for the detection of *Plasmodium falciparum* in blood samples from febrile HIV-positive Ugandan adults attending rural mobile clinics.

Study setting. This study was performed in the Rakai District of south-western Uganda, which is meso-hyperendemic for falciparum malaria.⁶ Malaria is suspected in 41.5% of outpatient visits to clinics and hospitals in the district.⁷ ITN usage in this region is historically low; only 13% of local households had an ITN in 2004–2005, although distribution programs have expanded since.⁸ Most participants in this study, however, had received ITNs as part of the basic care package provided through a President’s Emergency Plan for AIDS Relief (PEPFAR) funded rural mobile HIV clinic program. In this program, participants received cotrimoxazole prophylaxis regardless of CD4 count as per Uganda Ministry of Health guidelines, and antiretroviral therapy for CD4 count < 250 cells/mm³ or for World Health Organization Stage IV disease.

Study population. Participants were enrolled between November 1, 2006 and November 30, 2007 into a cross-sectional study of febrile HIV patients. At clinic sessions at which fever study activities were performed, all consenting clinic attendees ≥ 18 years of age, including first-time attendees, were screened for axillary temperature ≥ 37.5°C, and if febrile, were offered enrollment via written consent into the fever study, which had received Institutional Review Board approval in Uganda (Uganda Virus Research Institute Science and Ethics Committee) and the United States (Western Institutional Review Board).

MATERIALS AND METHODS

Point-of-care activities. At the rural mobile clinics, each participant underwent a standardized history and physical examination followed by aseptic venipuncture. Anti-coagulated whole blood samples were inverted > 10 times and placed in vertical tube racks in cool boxes. An aliquot of this blood was immediately tested for malaria using Binax Now® Malaria (Binax-M), which was performed at the point-of-care by the clinician who interpreted the test result 15 minutes later. If a venous sample could not be obtained, finger-stick blood was used for Binax-M testing and to make a thick blood smear. Malaria microscopy was not available at the rural clinic sites.

Binax Now® Malaria is an FDA-approved rapid chromatographic immunoassay for qualitative detection of two antigens: Histidine-Rich Protein 2, a *Plasmodium falciparum*-specific antigen, plus a second antigen, aldolase, common to all *Plasmodia*.⁹ The assay was performed according to manufacturer’s specifications. Appearance of a control band was used to judge validity and a second test was allowed for any sample without a control band on first test. If two sequential tests did not demonstrate control bands, testing was considered invalid. For this analysis, test results indicating *Plasmodium falciparum* or mixed infection were considered positive, whereas test results indicating non-falciparum species or no plasmodia were considered negative. Any test that the clinician was unable to interpret was classified as indeterminate.

Cases of suspected malaria (based on clinician’s overall assessment of signs and symptoms) received artemether-

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TABLE 1
Participant demographics by thick smear result

	Positive thick smear <i>N</i> = 14 (5.70%)	Negative thick smear <i>N</i> = 232 (94.3%)	Total <i>N</i> = 246	Univariate analysis
Median age (range)	31.5 (range 20–47)	32 (range 18–65)	32 (range 18–65)	–
Female sex	9/14 (64.3%)	159/232 (68.5%)	168/246 (68.2%)	<i>P</i> = 0.740
CD4+ T-cell count*				
≤ 250 cells/mm ³	4/12 (33.3%)	98/228 (43.0%)	102/240 (42.5%)	<i>P</i> = 0.512#
> 250 cells/mm ³	8/12 (66.7%)	130/228 (57.0%)	138/240 (57.5%)	
Antiretroviral therapy†				
Yes	4/13 (30.8%)	89/228 (39.0%)	93/241 (38.6%)	<i>P</i> = 0.553#
No	9/13 (69.2%)	139/228 (61.0%)	148/241 (61.4%)	
Cotrimoxazole‡				
Yes	7/14 (50.0%)	196/232 (84.5%)	203/246 (82.5%)	<i>P</i> = 0.003
No	7/14 (50.0%)	36/232 (15.5%)	43/246 (17.5%)	
Insecticide-treated net§				
Yes	7/14 (50.0%)	154/232 (33.6%)	161/246 (65.5%)	<i>P</i> = 0.218
No	7/14 (50.0%)	78/232 (66.4%)	85/246 (34.5%)	
Recent anti-malarial medication¶				
Any	1/13 (7.70%)	47/207 (22.7%)	48/220 (21.8%)	<i>P</i> = 0.232#
None	12/13 (92.3%)	160/207 (77.3%)	172/220 (78.2%)	

* Most recent CD4 count before enrollment if within 6 months, or if unavailable, most recent CD4 count after enrollment within 6 months; 6 participants are missing data.

† Reported taking ART, five participants are missing data.

‡ Reported taking daily cotrimoxazole.

§ Reported sleeping under mosquito net every night for 7 nights prior to enrollment.

¶ Reported use within 30 days before enrollment, 26 participants took “unknown” medicines and were excluded.

^{||} Chi-square test.

Fisher's exact test (2-sided) was used for any values < 5.

lumefantrine (Coartem®, Novartis, Switzerland) according to Uganda Ministry of Health guidelines. Follow-up assessment occurred at 2 weeks or sooner if needed.

Laboratory activities. Blood samples were transported within 4–6 hours to a central laboratory where malaria microscopy was performed within 24 hours of sample collection. Thick smears were prepared by trained laboratory technicians who performed Giemsa staining; microscopic evaluation and organism quantification were performed according to World Health Organization guidelines.¹⁰ Positive thick smear results were defined by the presence of one or more visualized malaria parasites. Thick smear-positive samples underwent thin smear microscopy for species identification. Laboratory technicians were blinded to Binax-M result and all participant characteristics. Clinicians were contacted regarding any thick smear-positive sample, and provided artemether-lumefantrine by home visit to any thick smear-positive patient who had not received it at the point of care. Data were entered using FoxPro (Microsoft Corporation, Seattle, WA, 2004), and analysis performed with STATA software (Stata Statistical Software: Release 10.0, Stata Corporation, College Station, TX, 2008).

RESULTS

Table 1 shows participant characteristics stratified by malaria status. Sixty-eight percent of 246 total participants were female, and median age was 32 years (range: 18–65). One hundred and two or 42.5% of participants had CD4 counts ≤ 250 cells/mm³. Ninety-three or 38.6% of participants were on antiretroviral therapy, 82.5% were on cotrimoxazole prophylaxis, and 65.5% of participants reported they had slept under an insecticide-treated net every night for the 7 nights prior to enrollment. Participants that were new to the program (7.4%) had not yet been offered cotrimoxazole prophylaxis, antiretroviral therapy (if indicated), or insecticide-treated nets. More than 20% (21.8%) of participants reported having taken at least one anti-malarial medication within 30 days prior to enrollment.

Fourteen of 246 (5.7%) samples were thick smear-positive (Table 2). No non-falciparum malaria infections were detected by microscopy. Median parasitemia among TS-positive samples was 600 organisms per microliter of blood (IQR 240–3860). Cotrimoxazole prophylaxis was significantly associated with absence of malaria in univariate analysis (*P* = 0.003) whereas age, sex, CD4 count, ART status, ITN usage, and recent use of anti-malarial drugs were not (Table 1).

Overall, 14/246 (5.7%) samples were Binax-M-positive, and 6/246 (2.4%) were indeterminate, including one sample which was invalid. Twelve of 246 (4.9%) samples were concordantly positive for Binax-M and thick smear (TS), and 221/246 (89.8%) samples were concordantly negative (Table 2). Five of 246 (2.0%) samples were discordantly TS-negative but Binax-M-positive. Two of 246 (0.8%) samples were discordantly TS-positive but Binax-M-negative, and these two samples were from patients with low-grade parasitemia (128 and 304 parasites/microliter, respectively).

Sensitivity of Binax Now® Malaria (Table 3) was 85.7% (exact 95% CI: 57.2–98.2%) for detection of *Plasmodium falciparum* when compared with thick smear. Specificity was 97.8% (95% CI: 94.9–99.3%), Positive Predictive Value was 70.6% (95% CI: 44.0–89.7%), and Negative Predictive Value was 99.1% (95% CI: 96.8–99.9%).

TABLE 2
Comparison of point-of-care Binax Now® Malaria Rapid Diagnostic Test vs. thick smear microscopy for detection of *Plasmodium falciparum* among adult febrile African HIV Patients

Binax Now® Malaria falciparum	Positive thick smear	Negative thick smear	Total
Positive	12 (4.9%)	5 (2.0%)	17 (6.9%)
Negative	2 (0.8%)	221 (89.8%)	223 (90.7%)
Indeterminate	0	6 (2.4%)	6 (2.4%)
Total	14 (5.7%)	232 (94.3%)	246

TABLE 3

Performance of point-of-care Binax Now® Malaria Rapid Malaria Diagnostic Test vs. thick-smear microscopy*

	Value	Exact binomial
		95% confidence interval
Sensitivity	85.7%	57.2–98.2%
Specificity	97.8%	94.9–99.3%
Positive predictive value	70.6%	44.0–89.7%
Negative predictive value	99.1%	96.8–99.9%

* Binax-indeterminate tests were excluded from calculations.

DISCUSSION

We found very few cases of malaria overall, which concurs with other studies that have shown that malaria incidence is greatly decreased among HIV-infected patients who receive cotrimoxazole, ITNs, and ART when indicated.⁴ Because of the low malaria case number, the confidence interval for the sensitivity of Binax-M in our setting was wide and was compatible with assay sensitivities previously reported in other settings. Apparent false-positive Binax-M tests, which occurred in 2% of tests, may be explained in part by the limited sensitivity of malaria microscopy, or may be an indicator of residual antigenemia after clearance of parasitemia, which is known to occur with Histidine Rich Protein-2 for approximately 2 weeks after resolution of parasitemia.¹¹ Apparent false-negative Binax-M results and indeterminate results which represented 0.8% and 2.4% of tests respectively, were likely due to low sensitivity of Binax-M for samples with low parasite density, or due to operator error although clinicians found the test easy to perform and interpret.

The Binax Now® Malaria RDT served as an effective point-of-care tool for fever management in HIV-positive adults in a malaria-endemic area. Its high negative predictive value of 99.2% indicates that in similar populations of febrile outpatient HIV-positive adults, a negative Binax-M test can be useful for “ruling out” malaria at the point of care. These findings should not be generalized to more acute healthcare settings, to HIV-negative individuals or HIV-positive individuals *not* receiving cotrimoxazole, antiretroviral therapy, and insecticide-treated nets, or to populations of pregnant women and children, for which the malaria burden would likely be higher.

Implementation of point-of-care rapid malaria testing for febrile HIV-positive adults in malaria-endemic areas has the potential to avert unnecessary anti-malarial treatment. In addition, malaria RDTs should be viewed as a tool not just for malaria management, but also for febrile illness management overall, because they can enable clinicians and health systems to focus diagnostic and therapeutic resources on more common and potentially more urgent causes of fever.

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