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Performance of Malaria Rapid Diagnostic Tests as Part of Routine Malaria Case Management in Kenya

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Abstract. Data on malaria rapid diagnostic test (RDT) performance under routine program conditions are limited. We assessed the attributes of RDTs performed by study and health facility (HF) staffs as part of routine malaria case management of patients ≥ 5 years of age in Kenya. Expert microscopy was used as our gold standard. A total of 1,827 patients were enrolled; 191 (11.6%) were parasitemic by expert microscopy. Sensitivity and specificity of RDTs performed by study staff were 86.6% (95% confidence interval [CI]: 79.8–93.5%) and 95.4% (95% CI: 93.9–96.9%), respectively. Among tests performed by HF staff, RDTs were 91.7% (95% CI: 80.8–100.0%) sensitive and 96.7% (95% CI: 92.8–100.0%) specific, whereas microscopy was 52.5% (95% CI: 33.2–71.9%) sensitive and 77.0% (95% CI: 67.9–86.2%) specific. Our findings suggest that RDTs perform better than microscopy under routine conditions. Further efforts are needed to maintain this high RDT performance over time.

INTRODUCTION

Microscopy is considered the most accurate method for the diagnosis of malaria, but almost exclusively when performed by experienced laboratory technicians.[1–3] In addition, its implementation and large-scale use are hampered by the need for relatively expensive equipment and a reliable supply of reagents.[4] In the past, the poor performance of microscopy as part of routine care and the availability of relatively inexpensive antimalarials (e.g., chloroquine) have led to the presumptive malaria treatment of febrile patients without laboratory confirmation.[3,5]

Because the newer artemisinin-based combination therapies (ACTs) are more expensive, malaria diagnostic testing has recently been integrated into malaria case management policy for patients ≥ 5 years of age in many African countries. In addition to helping avoid malaria overtreatment and unnecessary patient exposure to ACTs, the use of a diagnostic laboratory test offers the opportunity for other diseases to be investigated and treated once malaria is ruled out. Malaria rapid diagnostic tests (RDTs) detect circulating *Plasmodium* antigens, are easy to use and interpret, and could offer a diagnostic alternative in areas with limited microscopy availability.[6,7]

Current malaria RDTs rely on the detection of one or more of three antigens: *Plasmodium falciparum*–specific histidine-rich protein 2 (HRP2), pan-specific *Plasmodium* lactate dehydrogenase (pLDH), and pan-specific *Plasmodium* aldolase. RDTs based on these three antigens have been studied in different settings with satisfactory performance.[8,9] Moreover, the sensitivity of HRP2-based assays has been reported to be > 90%.[7,10] Quality control and quality assurance programs involving pre-certification by the World Health Organization (WHO), lot testing against standardized positive controls, and evaluations post-deployment in the field are recommended to evaluate the accuracy of these tests on a routine basis.[6]

Few studies have provided estimates of RDT performance in the hands of health workers as part of routine programs. Therefore, we set out to evaluate the sensitivity, specificity, and positive and negative predictive values of an HRP2-based RDT used by study and health facility (HF) staffs as part of malaria case management in western Kenya.

METHODS

Setting. This study was conducted from July through September 2006 in government HFs in Kericho, Bondo, and Siaya districts in Kenya as part of a cluster randomized trial of the effect of an ACT- and RDT-based case management of uncomplicated malaria in patients ≥ 5 years of age (Skarbinski and others, unpublished data). New malaria case management guidelines recommending the use of artemether-lumefantrine (AL) and RDTs were being implemented at the time of the study in those districts. Sixty HFs where the Kenya Division of Malaria Control (DOMC) had implemented the new ACT guidelines were selected using stratified sampling. A total of 7 hospitals, 23 health centers, and 30 dispensaries were selected in seasonal (N = 30) and perennial (N = 30) transmission areas.

Study design and interventions. We randomized HFs into intervention (N = 30) and comparison (N = 30) groups, and conducted a baseline survey assessing outpatient malaria case management of patients ≥ 5 years of age who presented for initial consultations in all HFs. Study staff observed consultations, re-examined patients, performed RDTs, and collected blood smears on all consenting patients. If a health worker decided to request or perform a laboratory test for malaria (RDT or microscopy), study staff would specifically collect that test result information. The decision to request any malaria test, however, was not influenced by the study staff.

Following the baseline survey, we provided refresher training sessions at HFs on the newly revised national malaria treatment guidelines. Refresher training, offered to all HF staff present on the day of the visit, consisted of a half-day, on-site session using interactive discussions in small groups (1–5 participants) on RDT use, the revised national malaria treatment guidelines for patients ≥ 5 years of age, dosing and administration of AL, and management of severe malaria. We trained HF staff to perform RDTs and allowed them to practice until

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they could successfully perform an RDT independently. HFs in the intervention arm received RDTs and supplies for safe use and disposal of RDTs for the duration of the study. We recommended that RDTs be stored in a cool and dry place and provided thermometers to monitor daily storage temperatures. The provision of RDTs was the only difference between comparison and intervention HFs.

Approximately 2 weeks after training, study staff performed a supervisory visit during which they observed at least 5 health worker–patient consultations and provided feedback using a structured supervision form. A second survey assessing malaria case management of patients ≥ 5 years of age was conducted approximately 5 weeks after the baseline data collection.

Laboratory testing. We used the HRP2-based Paracheck Pf cassette device RDT, lot number 31422 with expiration date in April 2008 (Orchid Biomedical Systems, Goa, India). Study laboratory staff were all laboratory technicians and were trained to perform the test according to manufacturer's guidelines.

Thick and thin blood smears were stained with Giemsa and read by two independent expert microscopists who were blinded to the RDT result at the malaria laboratories of the Kenya Medical Research Institute (KEMRI) and U.S. Centers for Disease Control and Prevention (CDC) in Kisumu. Microscopists at KEMRI/CDC receive regular training at the Malaria Diagnostics and Control Center of Excellence of the U.S. Army Medical Research Unit in Kenya and consistently attain acceptable competency scores during such training sessions. A thick smear was considered negative if no parasites were identified in 100 high-power fields. The numbers of asexual and sexual parasites were counted in the same fields until 500 white blood cells (WBC) were observed. Parasite density was estimated by assuming a count of 8,000 WBC/μL. In case of discordance ≥ 20%, a third microscopist, blinded to all previous test results, reviewed the smear. The geometric mean of the two closest readings was used as the final parasitemia measurement.

RDT lot testing. Before the start of the study, 100 RDT devices were selected from the purchased lot. These were evaluated at the Malaria Branch, CDC, in Atlanta. The RDTs were tested using two *in-vitro* cultured *P. falciparum* strains from Southeast Asia (W-2) and Nigeria (RAN), and one wild-type patient isolate prepared at the Research Institute for Tropical Medicine (RITM) for WHO/West Pacific Regional Office (WPRO) in the Philippines. The three *P. falciparum* specimens were adjusted to parasite levels of 200, 500, 1,000, and 5,000 parasites per μL by standardized quantitative thick blood film methods. The objective of this testing was to check for RDT performance before deployment to the field. A second sample of 196 RDTs was collected at the end of the study from participating HFs and also evaluated at CDC using the WHO/WPRO wild-type *P. falciparum* standard. As a comparison, RDTs collected before the beginning of the study and stored at 4–8°C were tested in parallel with the RDTs collected at the end of the study. The objective of this parallel testing was to compare the performance of RDTs stored under optimal conditions with those that had been kept at HFs during the study.

Statistical analysis. We used SAS version 9.1 (SAS Institute, Cary, NC). The results of the microscopy tests at the KEMRI/CDC malaria laboratory were considered to be the gold standard and used to calculate all test attributes presented. The RDT sensitivity, specificity, and positive and negative predictive values when performed by study staff were calculated using the survey analysis tool, which accounts for stratification, cluster sampling, and unequal selection probabilities. Of note, 120 clusters were considered in the analysis, representing 2 days of data collection per each of the 60 HFs. We present frequency results reflecting this weighted analysis unless otherwise noted. We also calculated RDT and microscopy sensitivity and specificity when performed by HF staff as part of routine case management using the survey analysis tool.

Ethical considerations. Informed consent was obtained from all adult participants and from parents or caregivers of minors. This study was reviewed and approved by the ethical review boards of KEMRI (SSC 1057) and CDC (Identifier: 4701), and was registered under ClinicalTrials.gov (Identifier: NCT00336388). The funding source for this study had no role in study design; data collection, analysis, and interpretation; or writing of this report.

RESULTS

A total of 2,027 patients ≥ 5 years of age presented for initial consultation and were approached and invited to participate in this study (Figure 1). Of those, 2,004 (98.9%, unweighted result) provided informed consent and agreed to participate in the study. Among those who consented, 177 (8.8%, unweighted result) patients were excluded because they refused to provide laboratory samples and/or full clinical information. A total of 1,827 (91.2%, unweighted result) of 2,004 consented patients were included in this analysis.

All participating patients had an RDT and a microscopy test performed by the study staff. Of the 1,827 samples, 153 (8.4%, unweighted result) had discordant results by the first two microscopists and required a third reading. Gold-standard microscopy was positive in 191 (11.6%) of the 1,827 samples. Most samples were positive for *P. falciparum* (188 samples, 10 of which were also positive for *P. malariae*), followed by *P. malariae* only (2), and *P. ovale* only (1). Among the positive samples, the geometric mean of asexual parasitemia was 940 parasites/μL (range: 16–193,684 parasites/μL).

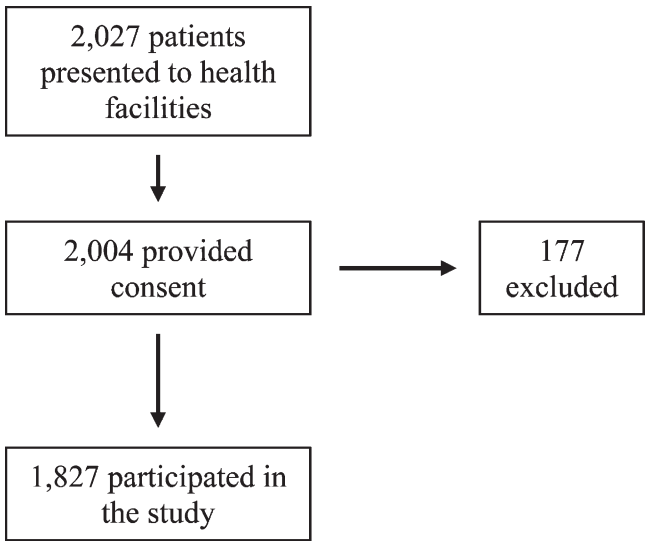

FIGURE 1. Patients ≥ 5 years of age invited to participate in the study (N = 2,027).

TABLE 1
Attributes of malaria rapid diagnostic tests (RDTs) performed by study staff, stratified by parasitemia levels,* Kenya, 2006 (N = 1,827)

Parasitemia	Sensitivity (95% confidence interval)	Specificity (95% confidence interval)	Positive predictive value (95% confidence interval)	Negative predictive value (95% confidence interval)
Any parasitemia	86.6% (79.8–93.5%)	95.4% (93.9–96.9%)	71.2% (64.2–78.1%)	98.2% (97.2–99.2%)
≥ 200 parasites/μL	93.3% (88.0–98.6%)	92.4% (90.2–94.6%)	50.2% (41.4–59.0%)	99.4% (98.9–99.9%)
≥ 5,000 parasites/μL	96.5% (89.7–100.0%)	88.7% (85.8–91.6%)	22.7% (17.0–28.4%)	99.9% (99.6–100.0%)

* Parasitemia levels determined by expert microscopy.

Attributes of RDTs performed by the study staff were stratified by different levels of parasitemia and are shown in Table 1. The RDT sensitivity and specificity were 86.6% and 95.4%, respectively, when all parasitemia levels were considered. The concordance between gold-standard microscopy and study staff RDTs was 94.9% (unweighted result). There were 28 false-negative RDTs; among those, the geometric mean of asexual parasitemia was 123 parasites/μL (range: 16–9,721 parasites/μL), lower than that observed among true positive patients; and 18 (64%, unweighted result) had < 200 parasites/μL. Three of these 28 infections were non–*P. falciparum* infections (2 due exclusively to *P. malariae* and 1 exclusively to *P. ovale*) and 10 (35.7%, unweighted result) of these infected patients did not have fever or history of fever. Overall, there were 65 false-positive RDTs; among those, three (4.6%, unweighted result) were positive for *Plasmodium* gametocytes only. Gametocytemia in these patients ranged from 16 gametocytes/μL (two cases) to 22.6 gametocytes/μL (one case).

The sensitivity of RDTs performed by the study staff increased to 93.3% and 96.5% for parasitemia levels ≥ 200 and 5,000 parasites/μL, respectively. Specificity decreased to 92.4% and 88.7% for these same parasitemia ranges, respectively.

Because HF staff used clinical judgment to decide when to use a laboratory test and when to rely on clinical signs and symptoms to diagnose malaria, only a total of 141 RDTs were performed by them (Table 2). When analyzing the performance of RDTs in the hands of HF staff, we found sensitivity and specificity of 91.7% and 96.7%, respectively. Microscopy was performed by HF staff in 429 cases and found to have a sensitivity of 52.5% and a specificity of 77.0% when compared with gold-standard microscopy performed by study staff.

Among the RDTs sent to CDC before initiation of the study for lot testing, 100% (15 out of 15 RDTs) detected 200 parasites/μL and greater using the WHO/WPRO wild-type standard and 95% (19/20) detected the RAN *in-vitro* strain at 200 parasites/μL and greater. They also detected the W-2 culture strain at 200 parasites/μL and greater in 65% (13/20) of RDTs evaluated. When all tests were considered, 97% (33/34) of RDTs detected concentrations of 500 parasites/μL or greater. The RDTs collected from participating HFs at the end of the study were tested with only the WHO/WPRO wild-type standard produced at RITM in the Philippines, because of the limited amount of RDTs collected at each individual HF and the need to run the same reference samples on all RDTs collected. Overall, 86% (169/196) of RDTs collected at the end of the study detected 200 parasites/μL or greater; and 100% (112/112) detected 500 parasites/μL or greater. In comparison, 90% (44/49) of the RDTs collected at the beginning of the study and stored at CDC at 4–8°C detected 200 parasites/μL or greater; and 100% (28/28) detected 500 parasites/μL or greater.

When considering the lot testing using the WHO standard at a density of 200 parasites/μL or greater, the performance of RDTs tested before the study compared with those stored at CDC and tested at the end of the study was not significantly different (P = 0.33, by Fisher's exact test). Similarly, the performance of RDTs collected at the beginning of the study and stored at CDC compared with those collected from the field at the end of the study was not significantly different either (P = 0.64, by Fisher's exact test).

TABLE 2
Attributes of rapid diagnostic tests (RDTs) and microscopy in the hands of health facility (HF) staff as part of routine malaria case management, Kenya, 2006

Test	Sensitivity (95% confidence interval)	Specificity (95% confidence interval)	Number of samples
HF RDTs	91.7% (80.8–100.0%)	96.7% (92.8–100.0%)	141
HF microscopy	52.5% (33.2–71.9%)	77.0% (67.9–86.2%)	429

DISCUSSION

Clinical diagnosis of malaria, leading to presumptive treatment, has been a common practice in sub-Saharan Africa for patients ≥ 5 years of age. However, with the advent of ACTs, which are more expensive and have a less well-defined safety profile than the previous treatments (e.g., chloroquine and sulfadoxine-pyrimethamine) presumptive treatment of malaria patients without laboratory confirmation becomes less acceptable, both clinically and economically.[11] In this context, RDTs may be an option to improve malaria diagnosis in areas with limited resources. As part of this study, we observed high sensitivity and specificity of these tests when used by both study and HF staffs during a cross-sectional study. These results are similar to that observed in controlled studies and demonstrate the potential added benefit of including RDTs as part of malaria treatment guidelines.[7,10]

Microscopy is commonly accepted as the gold-standard test for malaria in clinical settings, with a sensitivity threshold of 50, or even 20, parasites/μL.[12] However, this high performance requires good quality reagents and equipment, and highly trained microscopists, which may not be readily available in developing countries. Additionally, microscopy is labor-intensive and time-consuming, which limits its performance as part of routine care. Under field conditions, as opposed to those found in reference laboratories, microscopy sensitivity may be less than that achieved with RDTs.[11,13] Consistent with these previous observations, we found HF microscopy to have a sensitivity of 52.2% and specificity of 77.0%, both lower than that of RDTs performed by both study and HF staffs. Reasons for the poor accuracy of microscopy might include HF microscopists' lack of skill and heavy workload, poor slide preparation

techniques, and poor quality of laboratory equipment and/or reagents. Our study did not evaluate these potential risk factors associated with poor performance of microscopy.

Because both HF and study staffs were either trained or retrained on RDTs as part of this study, our findings may not be generalizable to other HFs where this focused training has not occurred. However, RDT training sessions done as part of the study were both simple and short, taking roughly half a day. Similar approaches should be considered by malaria control programs as they implement RDTs as part of malaria case management. Additionally, the lower number of RDTs tests performed by HF staff compared with microscopy tests, when both were part of the new policy, highlights the need for malaria control programs to not only train health workers in performing RDTs, but also assess for and address potential factors that may impair acceptance of these tests, such as lack of confidence in test accuracy and/or in case management guidelines, by health workers.

We chose to use an HRP2-based RDT in this study because of its higher sensitivity and lower cost compared with pLDH-based ones.[10,14] The HRP2 is a water-soluble antigen produced by both asexual stages and young gametocytes of *P. falciparum*.[8] Because malaria treatment often targets asexual stages only, HRP2-based RDTs may yield false-positive results for active infection up to a couple of weeks after adequate malaria treatment as a result of the persistence of antigens produced by residual circulating gametocytes.[7] However, the majority of false-positive results in our study were likely not a result of a recently resolved infection, because only 4.6% of the false-positive results occurred in patients with gametocytemia only.

The HRP2-based tests are *P. falciparum*–specific and are not expected to detect other human malaria species. However, most malaria infections in sub-Saharan Africa are due to *P. falciparum,* which would be adequately diagnosed with these tests. In this study, we found 28 (15%) of 191 infections to have false-negative results by study staff RDTs. Most of the false-negative results occurred in patients with < 200 parasites/μL, the detection limit of HRP2-based RDTs; and three of these cases were non–*P. falciparum* malaria cases, which are not expected to be detected by HRP2-based RDTs. In areas of moderate or high malaria transmission, the clinical consequences associated with missing parasitemic patients because of false-negative results are probably minor in older children and adults, who likely have acquired immunity and therefore are less vulnerable to developing severe disease.[3,7] RDT-negative febrile cases should be assessed appropriately for other causes of fever and be advised to return to HFs for revaluation if symptoms persist.

Performance of RDTs collected before and after the study appeared to be adequate as judged against laboratory standards, except for the results obtained with the W-2 culture samples.[4] The discrepant results obtained with W-2 culture can be associated with variable HRP2 reactivity among strains of *P. falciparum* because of lower antigen expression and/or low repeat copy number in the HRP2 gene.[15] Although not statistically significant, the decrease in sensitivity between RDTs collected at the beginning and end of the study underscores the need for continued monitoring of RDT performance at the HF level. Alternatives for this post-deployment monitoring include microcopy at reference laboratories and potentially the use of recombinant protein–positive controls.[16]

Although RDTs in the hands of HF staff performed well and had higher sensitivity than that of HF microscopy, the value of microscopy cannot be dismissed. Laboratory technicians should still receive adequate training and ongoing supervision in microscopy as this is still considered the gold-standard diagnostic method for malaria. Microscopy is also needed to provide information on species identification, parasite quantification, and treatment response.[17,18] In addition, high quality microscopy is often used as a tool for RDT quality control.

Malaria control programs could consider a combination of RDTs and microscopy for malaria diagnosis, prioritizing RDT in HFs where it is not feasible to implement good quality microscopy, such as primary HFs.[6] The challenges of scaling up good quality microscopy as part of national malaria control programs should not be underestimated, because it requires complex and potentially costly continued training and supervision of laboratory technicians. Of note, expertise and proficiency in malaria microscopy is also scarce in developed countries.[4]

In the era of ACTs, it is important to first consider in which situations or among which patient groups laboratory confirmation, with either RDTs or microscopy, is warranted. WHO recommends that, in areas of high malaria transmission, patients < 5 years of age with fever or history of fever and no other apparent cause should be treated empirically for malaria without laboratory confirmation.[19] For older patients in these areas and for patients of all ages in areas of low malaria transmission, treatment should be based on laboratory diagnosis. To effectively implement this practice, it is necessary to address the issue of health worker adherence to laboratory test results, whether microscopy or RDTs, and to provide appropriate differential diagnosis and treatment of sick patients once malaria is excluded.[20] There is also evidence that, for RDT-negative patients, malaria treatments other than ACTs are commonly prescribed, a practice that should be discouraged.[21]

Our results suggest that RDTs performed by both study and HF staffs have higher sensitivity and specificity compared with routine microscopy and may have an important role in malaria case management. The RDTs if performed correctly and used by health workers to guide treatment would help to prevent unnecessary use of ACTs, which could in turn decrease the possibility of adverse events and prompt health workers to assess RDT-negative febrile patients for other diseases. Both RDT and microscopy implementation, however, require the development and implementation of training and quality assurance programs to ensure continued high performance of these tests as part of routine case management of malaria.

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