Rapid Diagnostic Tests for Malaria at Sites of Varying Transmission Intensity in Uganda

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Background. In Africa, fever is often treated presumptively as malaria, resulting in misdiagnosis and the overuse of antimalarial drugs. Rapid diagnostic tests (RDTs) for malaria may allow improved fever management.

Methods. We compared RDTs based on histidine-rich protein 2 (HRP2) and RDTs based on *Plasmodium* lactate dehydrogenase (pLDH) with expert microscopy and PCR-corrected microscopy for 7000 patients at sites of varying malaria transmission intensity across Uganda.

Results. When all sites were considered, the sensitivity of the HRP2-based test was 97% when compared with microscopy and 98% when corrected by PCR; the sensitivity of the pLDH-based test was 88% when compared with microscopy and 77% when corrected by PCR. The specificity of the HRP2-based test was 71% when compared with microscopy and 88% when corrected by PCR; the specificity of the pLDH-based test was 92% when compared with microscopy and >98% when corrected by PCR. Based on *Plasmodium falciparum* PCR-corrected microscopy, the positive predictive value (PPV) of the HRP2-based test was high (93%) at all but the site with the lowest transmission rate; the pLDH-based test and expert microscopy offered excellent PPVs (98%) for all sites. The negative predictive value (NPV) of the HRP2-based test was consistently high (>97%); in contrast, the NPV for the pLDH-based test dropped significantly (from 98% to 66%) as transmission intensity increased, and the NPV for expert microscopy decreased significantly (99% to 54%) because of increasing failure to detect subpatent parasitemia.

Conclusions. Based on the high PPV and NPV, HRP2-based RDTs are likely to be the best diagnostic choice for areas with medium-to-high malaria transmission rates in Africa.

In Africa, diagnostic tests are not routinely available and episodes of fever are typically treated as malaria without laboratory confirmation. In many settings, empirical treatment results in substantial overuse of antimalarial drugs and delays the diagnosis of other febrile illnesses [1–4]. Presumptive treatment of all fevers as malaria becomes increasingly problematic as older antimalarial drugs are replaced with new combination therapies that are more costly, in limited supply, and potentially more toxic [5, 6]. Despite these concerns, presumptive treatment is endorsed because light microscopy, which for decades has been the standard for malaria diagnosis, remains inaccessible to most patients because of the laboratory infrastructure and technical expertise it requires [7, 8].

Rapid diagnostic tests (RDTs) for malaria may offer a practical solution to the challenge of malaria diagnosis in Africa. RDTs are simple immunochromatographic tests that identify antigens of malaria parasites in whole blood. They are relatively simple to perform and interpret, and therefore might be made available on a wider scale than microscopy in regions where malaria is endemic. The RDTs that currently appear most suitable for clinical use identify histidine-rich protein 2 (HRP2) or *Plasmodium* lactate dehydrogenase (pLDH). HRP2-based tests have been available in various formats for several years, have shown good sensitivity in a variety of field settings, and they are increasingly recommended for use in settings where reliable microscopy is not available [5, 9]. A concern with respect to HRP2-based assays is the persistence of detectable circulating antigen for up to several weeks after parasites have been eradicated [10–13]. Persistent antigenemia may limit the usefulness of HRP2-based assays in areas of intense malaria trans-
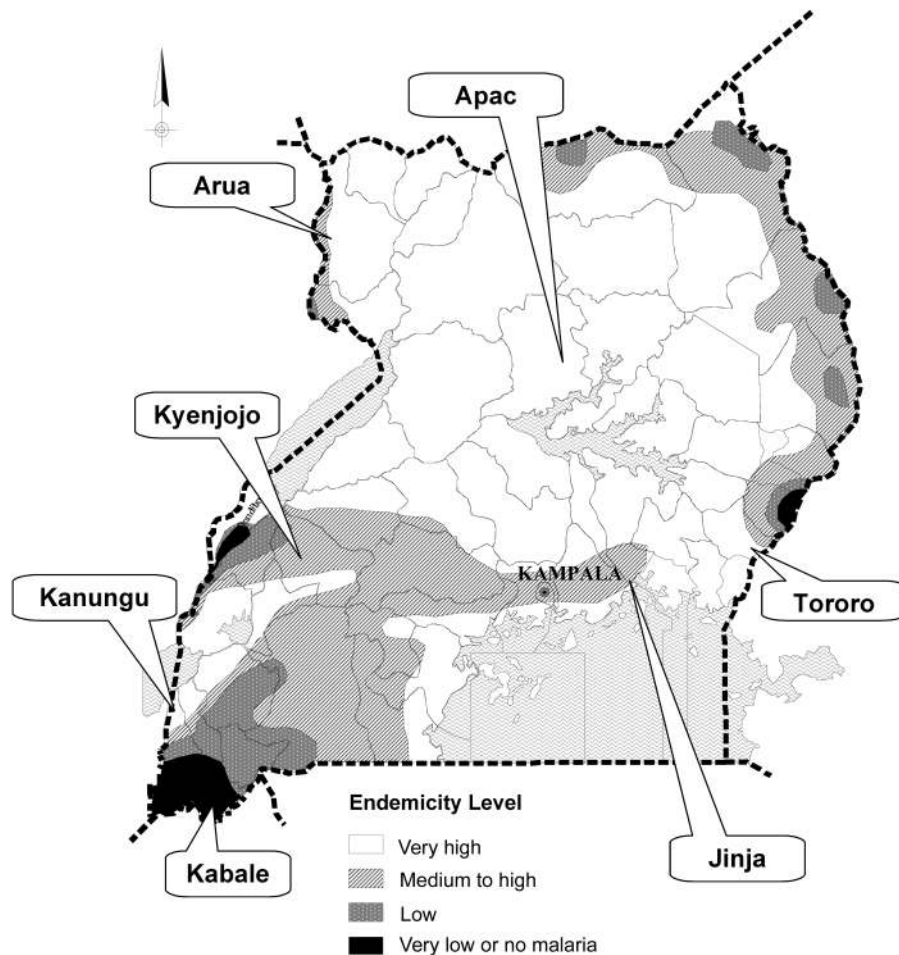
Figure 1. Map of sentinel site health centers

mission, where positive tests may commonly be the result of prior infections that are no longer clinically relevant. pLDH-based RDTs appear to be less sensitive than tests that detect HRP2, but they are more specific, as pLDH is rapidly cleared from the bloodstream and becomes undetectable at about the same time blood smears become negative after antimalarial therapy [14–16]. HRP2- and pLDH-based tests also differ in the parasite species they detect: the HRP2 test detects only *Plasmodium falciparum,* whereas the pLDH test detects all 4 species that cause human malaria.

Although RDTs clearly show promise as new diagnostic tools for Africa, it is not clear where RDTs should replace presumptive therapy or light microscopy, nor is it clear which RDT is most appropriate for different epidemiological settings. Few studies have compared RDT performance among areas with different levels of endemicity and populations, and these studies have not identified consistent associations between malaria transmission rate and RDT performance [17, 18]. In addition, many prior studies have used light microscopy results as the gold standard when measuring the diagnostic accuracy of RDTs [17, 19–21]. This approach may underestimate the performance of RDTs, because RDTs may detect parasites below the limits of light microscopy. Recent studies have

highlighted the significance of subpatent parasitemia, both clinically and as a reservoir for transmission [22–26]. We therefore compared the diagnostic accuracy of expert microscopy, an HRP2-based RDT, and a pLDH-based RDT, considering PCR-corrected microscopy as the gold standard, at sites of varying transmission intensity across Uganda.

METHODS

Study sites

The study was conducted from May 2006 through February 2007 at 7 sentinel sites that were established in 1998 by the Uganda Ministry of Health. The sites were selected to represent the geographic diversity of malaria transmission intensity in Uganda (figure 1). Health clinics at these sites are operated by the Ugandan government and provide care at no charge.

Study population

At each site, we used a cross-sectional study design to enroll 1000 consecutive outpatients who had been referred to the laboratory for malaria blood smears in accordance with the usual standard

of care at the health centers. Study personnel were not involved in the decision to refer patients to the laboratory. At the time of enrollment, the patient's age and sex were recorded, and a finger-prick blood sample was obtained for thick smear, both RDTs, and storage on filter paper for molecular testing. The study was approved by the Uganda National Council of Science and Technology and by the institutional review boards of Makerere University and the University of California, San Francisco.

Laboratory procedures

RDTs. RDTs were selected for evaluation on the basis of ease of use, safety, appropriate packaging for transport and storage in tropical environments, availability of published and unpublished data from field trials, low market price, and reliability of supply. The RDTs studied were Paracheck (for the detection of HRP2; Orchid Biomedical Systems) and Parabank (for the detection of pLDH; Zephyr Biomedicals). RDTs were obtained directly from the manufacturers and stored in their original packaging at room temperature before transport to the sentinel sites, and they were stored at room temperature in health center storerooms at the sentinel sites. The temperature and humidity of the storage areas and the areas in which the tests were kept during transport were monitored, but not controlled. Storage temperatures ranged from 19°C–31°C, generally within the manufacturer's recommended range of 4°C–30°C. The relative humidity during storage and transport was 39%–87%. Prior to the beginning of the study, positive and negative control blood samples were obtained and stored at −80°C, for quality control testing of RDTs throughout the study. Each batch of RDTs underwent quality control testing when opened and at 8–12 week intervals thereafter.

RDTs were performed and interpreted at each site by members of the study team (including W.K., H.H., and L.B.). Study staff performed and interpreted RDTs according to manufacturers' instructions, with results interpreted and recorded after 15 to 30 min. Study staff were advised that if the background of the RDT test window remained pink (bloody) after 15 min, they should allow the background to clear before interpreting the RDT (up to an additional 15 min). A single reader recorded each RDT result as either positive or negative; faint test lines were considered positive. RDT results did not influence patient care.

Microscopy. Thick smears were stained with 10% Giemsa for 10 min and interpreted by health center laboratory staff who were unaware of the RDT results. Blood smears were fixed and transported to a central laboratory in Kampala, Uganda. Expert microscopists who were blinded to results from the initial field readings reviewed all smears. Asexual parasitemia of any level was reported as a positive smear; smears were considered negative if the examination of 100 high power fields did not reveal asexual parasites. When results of the field reading and the first expert reading were discrepant, smears were reviewed by a second expert microscopist. A third expert reader resolved any discrepancies between the first and second expert readers.

PCR correction. Samples that were positive for parasites by expert microscopy and both RDTs were considered to be positive. Samples that were negative by microscopy and both RDTs were considered negative. If a sample was positive by microscopy but negative by either of the 2 RDTs, PCR was performed to detect any of the 4 malaria species. If a sample was negative by microscopy but positive only by the HRP2-based test, PCR was performed to detect subpatent *P. falciparum* infection. If a sample was negative by microscopy but positive by the pLDH-based test, PCR was performed to detect subpatent infections caused by any of the 4 malaria species. DNA was extracted from filter paper samples by use of chelex resin [27]. Genus-specific PCR followed by nested species–specific PCR of 18S small subunit ribosomal RNA [28] for *P. falciparum, P. malariae, P. ovale,* and *P. vivax* was performed, using oligonucleotide primers from the Malaria Research and Reference Reagent Resource Center as described elsewhere [29]. PCR products were analyzed by electrophoresis using 2% agarose gels.

Statistical methods

Data were double entered using Epi Info (version 6.04; Centers for Disease Control and Prevention) and analyzed with Stata (version 8.0; Stata). Sensitivity, specificity, and positive and negative predictive values were calculated using different gold standards, as described in Results. Categorical variables were compared using the χ^2 or Fisher exact test. A P value of $<.05$ was considered statistically significant.

RESULTS

Study sites. We evaluated 1000 patients who were referred for microscopy at each of 7 sites ($N = 7000$) across Uganda (table 1). These sites vary widely in malaria endemicity, ranging from Kabale, a highland area with very low transmission rates where indoor residual spraying of the entire district had been completed 3 months earlier, to 3 sites where transmission intensity is holoendemic and the entomological inoculation rate (i.e., the number of infective *Anopheles* mosquito bites per person per year [EIR]) has been estimated at more than 1 infectious bite per person per day [30, 31]. In general, at sites with high transmission rates, children under 5 years made up a large proportion of those referred for microscopy, whereas at sites with low transmission rates, more older children and adults were referred. The percentage of referred patients who had positive smears was only 4% in Kabale, and at the other sites, the percentage ranged from 33%–85% for children under 5 years of age and from 13%–49% for older children and adults (table 1).

Sensitivity and specificity of microscopy and RDTs. Of the 7000 samples tested, 2355 (33.6%) were positive by expert microscopy, the HRP2-based test, and the pLDH-based test, whereas 3029 (43.3%) were negative by all 3 diagnostic tests (figure 2). The most common categories of discordant results in-

Table 1. Baseline characteristics of study sites and site data.

	Site						
Characteristic	Kabale (n =1000)	Kanungu (n =1000)	Jinja (n =1000)	Kyenjojo (n =1000)	Arua (n =1000)	Tororo (n =1000)	Apac (n =1000)
EIR	<1[a]	6[b]	6[b]	7[b]	397[b]	562[b]	1586[b]
Dates of study	Sept–Oct 2006	Nov 2006	Jan–Feb 2007	Dec 2006–Jan 2007	Nov–Dec 2006	Feb 2007	May 2006
Median patient age (IQR), years	20 (3.5–30)	4.5 (1.4–16)	10 (2.5–24)	13 (3.0–28)	17 (2.0–30)	3.3 (1.2–22)	2.0 (0.9–4.0)
Patients <5 years, no. (%)	279 (28)	510 (51)	354 (35)	335 (34)	350 (35)	540 (54)	793 (79)
Smears for patients <5 years positive by expert microscopy	11 (4)	240 (47)	117 (33)	209 (62)	222 (63)	321 (59)	672 (85)
Smears for patients ≥5 years positive by expert microscopy	28 (4)	123 (25)	154 (24)	237 (36)	206 (32)	61 (13)	102 (49)
Smears for patients <5 years positive by PCR-corrected[c] microscopy	14 (5)	274 (54)	187 (53)	250 (75)	263 (75)	439 (81)	738 (93)
Smears for patients ≥5 years positive by PCR- corrected[c] microscopy	38 (5)	154 (31)	259 (40)	333 (50)	284 (44)	152 (33)	140 (68)

NOTE. Data are no. (%) of smears, unless otherwise indicated. EIR, entomological inoculation rate; number of infective *Anopheles* mosquito bites per person per year; IQR, interquartile range; PCR, polymerase chain reaction.

[a] Reference [30].
[b] Reference [31].
[c] PCR correction for all 4 malaria species.

cluded 910 samples (13%) that were positive only by the HRP2-based test and 335 samples (5%) that were positive by both RDTs but negative by microscopy. Among the 1245 samples that were positive by the HRP2-based test but negative by microscopy, 830 (67%) were positive by PCR. The proportion of samples that were negative by microscopy but had subpatent parasitemia detected by the HRP2-based test ranged from 1.5% at the site with the lowest transmission rate to 45% at the site with the highest transmission rate. Among the 358 samples that were positive by the pLDH-based test but negative by microscopy, 328 (92%) were positive by PCR. The proportion of microscopy-negative samples that had subpatent parasitemia detected by the pLDH-

based test ranged from 0.1% at the site with the lowest transmission rate to 27% at the site with the highest transmission rate. A total of 271 samples were positive by microscopy and the HRP2-based test, but negative by the pLDH-based test, and 264 (97%) of these were confirmed positive by PCR. Thirty samples were positive by microscopy and the pLDH-based test, but negative by the HRP2-based test, and the majority of these (19 of 30 [63%]) were positive by PCR only for non-falciparum species. Forty-seven samples were positive by microscopy, but negative by both RDTs. Of these, 25 (53%) were positive by PCR for *P. falciparum,* 11 (23%) were positive for non-falciparum species, and 11 (23%) were negative by PCR.
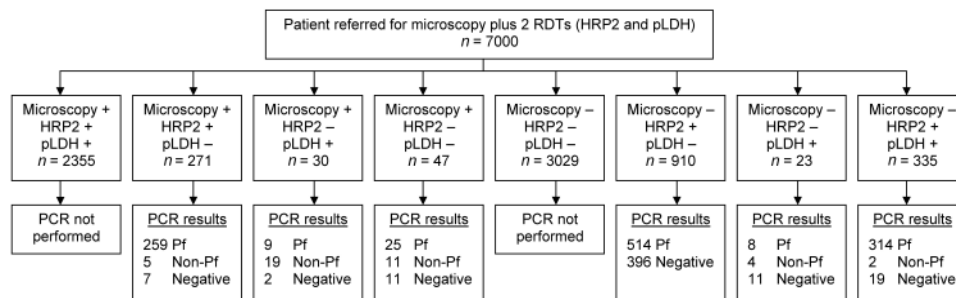

Figure 2. Polymerase chain reaction correction for discordant results

Table 2. Accuracy of diagnostic tests for malaria, compared with different gold standards.

	Gold standard					
	Sensitivity, % (95% CI)			Specificity, % (95% CI)		
Diagnostic test	Microscopy	Microscopy corrected by PCR for *Plasmodium falciparum*[a]	Microscopy corrected by PCR for all species[b]	Microscopy	Microscopy corrected by PCR for *Plasmodium falciparum*[a]	Microscopy corrected by PCR for all species[b]
HRP2	97.2 (96.5–97.7)	98.8 (98.4–99.1)	97.8 (97.3–98.3)	71.0 (69.6–72.4)	87.8 (86.7–88.9)	87.9 (86.7–88.9)
pLDH	88.2 (87.0–89.4)	77.1 (75.7–78.5)	76.9 (75.5–78.3)	91.7 (90.8–92.5)	98.4 (97.9–98.8)	99.1 (98.7–99.4)
Microscopy	–	76.0 (74.6–77.4)	76.1 (74.7–77.5)	–	98.4 (98.0–98.8)	99.4 (99.1–99.6)

NOTE. HRP2, histidine-rich protein 2–based test; PCR, polymerase chain reaction; pLDH, *Plasmodium* lactate dehydrogenase–based test.

[a] PCR positive for *P. falciparum* considered true-positive; PCR negative for *P. falciparum* considered true-negative.

[b] PCR positive for any species considered true-positive; PCR negative for all 4 species considered true-negative.

The accuracy of the diagnostic tests was assessed by comparison with 3 gold standards (table 2). First, results from the 2 RDTs were compared, and expert microscopy was used as the gold standard. Second, results from both RDTs and expert microscopy were compared, and expert microscopy corrected by PCR to confirm the presence or absence of *P. falciparum* was used as the gold standard. Third, results from both RDTs and expert microscopy were compared, and expert microscopy corrected by PCR to confirm the presence or absence of any of the 4 malaria species was used as the gold standard. Overall, the HRP2-based RDT showed excellent sensitivity, detecting over 97% of the infections identified by expert microscopy or PCR. The pLDH-based test had lower sensitivity, detecting 88% of infections identified by expert microscopy and only 77% of infections when corrected by PCR. Expert microscopy had a sensitivity of only 76% when corrected by PCR. If expert microscopy was used as the gold standard, the pLDH-based RDT showed superior specificity, giving a negative result for 92% of microscopy-negative samples, compared with only 71% for the HRP2-based RDT. After correction by PCR, both expert microscopy and the pLDH-based RDT had excellent specificities of over 98% when corrected only for *P. falciparum* infections and over 99% when corrected for infection with any of the 4 species. The specificity of the HRP2-based RDT improved to 88% when corrected by PCR. Compared to PCR correction only for *P. falciparum*, PCR correction for all 4 species had minimal effects on the sensitivities and specificities of the RDTs.

Predictive values of microscopy and RDTs. Predictive values were calculated for all 3 RDTs with expert microscopy corrected by PCR for *P. falciparum* used as the gold standard, because this species contributes by far the largest share of malaria morbidity in Uganda and is the most likely to lead to poor clinical outcomes if not appropriately identified and treated. For all 3 RDTs, the positive predictive values (PPVs) were significantly higher for children under 5 years, compared with older patients (HRP2-based RDT, 92% vs. 85% [$P < .001$]; pLDH-based RDT, 99% vs. 96% [$P < .001$]; expert microscopy, 99% vs. 97% [$P < .001$]). At

the site with the lowest transmission rate, the PPV for the HRP2-based RDT (20%) was significantly lower than the PPV for the pLDH-based RDT (82%) and the PPV for expert microscopy (85%) ($P < .001$, for both pair-wise comparisons). For the remaining 6 sites, the PPVs were consistently high, although they were significantly higher for the pLDH-based test (98%) and expert microscopy (98%) than for the HRP2-based test (93%) ($P < .001$, for both pair-wise comparisons). At the 6 sites with higher transmission rates, there were no consistent trends in the PPV of each RDT on the basis of age (figure 3).

The negative predictive value (NPV) for the HRP2-based RDT was >97% at all 7 sites, and the NPV was similar for children under 5 years and older patients (99% vs. 99%; $P = .71$) (figure 4). In contrast, the NPVs were lower for children under 5 years, compared with older patients, for both the pLDH-based RDT (78% vs. 83%; $P = .001$) and for expert microscopy (72% vs. 84%; $P < .001$). In addition, there was a trend for decreasing NPV as the intensity of transmission increased across the 7 sites for both the pLDH-based test (range, 98% to 66%) and expert microscopy (range, 99% to 54%) (figure 4).

Gametocytes may be responsible for positive RDT results in the absence of asexual parasites. Of 836 samples that were positive by either RDT, negative by expert microscopy, and positive for *P. falciparum* by PCR, 782 (94%) were available for reexamination, and 98 (13%) were positive by microscopy for *P. falciparum* gametocytes. The percentage of positive samples ranged from 2%–10% at the 4 sites with lower transmission intensity (EIR, <10) to 16%–25% at the 3 sites with higher transmission intensity (EIR, >300).

DISCUSSION

In Uganda and other African countries, RDTs are increasingly available in the private healthcare sector and are widely advocated for use in the public sector, although clear guidelines for their use are not yet available. We compared the accuracies of an HRP2-based RDT, a pLDH-based RDT, and expert microscopy on samples from 7000 patients referred for malaria microscopy
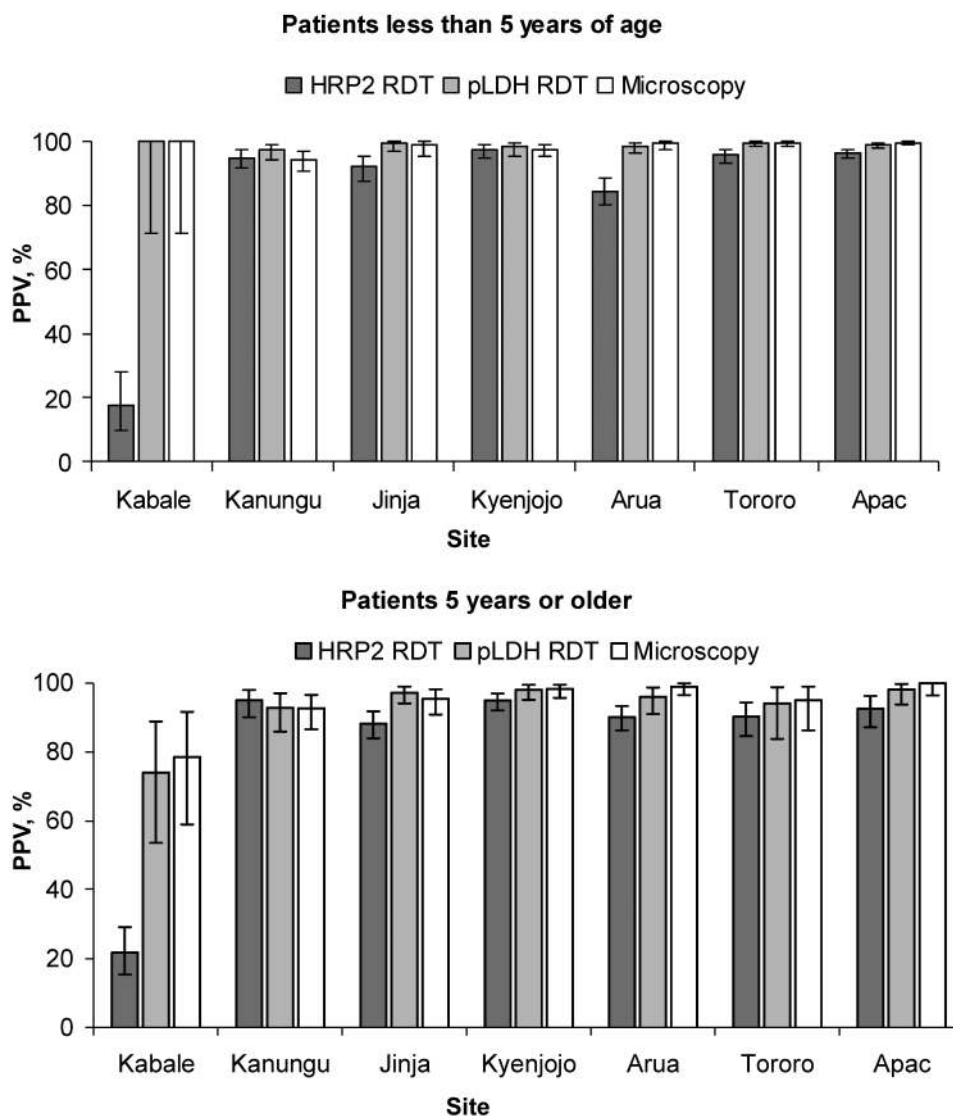
Patients less than 5 years of age

■ HRP2 RDT □ pLDH RDT □ Microscopy

Patients 5 years or older

■ HRP2 RDT ■ pLDH RDT □ Microscopy

Figure 3. Positive predictive values (PPVs) of rapid diagnostic tests (RDTs) for malaria, stratified by age. The comparison gold standard is expert microscopy, corrected by polymerase chain reaction for the presence or absence of *Plasmodium falciparum.* Error bars represent 95% confidence intervals. HRP2, histidine-rich protein 2–based test; pLDH, *Plasmodium* lactate dehydrogenase–based test.

at 7 sites of varying malaria transmission intensity across Uganda. The accuracies of the tests were determined by use of different gold standards, including expert microscopy alone and expert microscopy corrected by PCR to confirm presence or absence of parasites. If expert microscopy was used as the gold standard, the HRP2-based test showed superior sensitivity, whereas the pLDH-based test showed superior specificity. The sensitivity of the HRP2-based test was approximately the same when the gold standard was either microscopy alone or PCR-corrected microscopy; in contrast, the sensitivity of the pLDH-based test decreased significantly when compared with PCR-corrected microscopy results. Specificity for both RDTs rose when PCR-corrected microscopy results were used as the gold standard, but the specificity of the pLDH-based test remained significantly higher than that of the HRP2-based test. When

compared with PCR-corrected microscopy, the accuracy of the pLDH-based test and expert microscopy were very similar. If *P. falciparum* PCR-corrected microscopy was used as the gold standard, both expert microscopy and the pLDH-based test offered excellent PPV across all sites and age ranges, whereas the PPV of the HRP2-based test was slightly lower at all but 1 site. The NPV for the HRP2-based test was consistently high across sites and patient age groups. In contrast, the NPV for both the pLDH-based test and expert microscopy declined significantly as transmission intensity increased.

A number of studies of RDTs have been conducted, although measures of accuracy have varied widely, likely as a result, in part, of differences in methodology and study site epidemiology. If expert microscopy alone was used as the gold standard, the accuracy of the RDTs evaluated in our study was very similar to
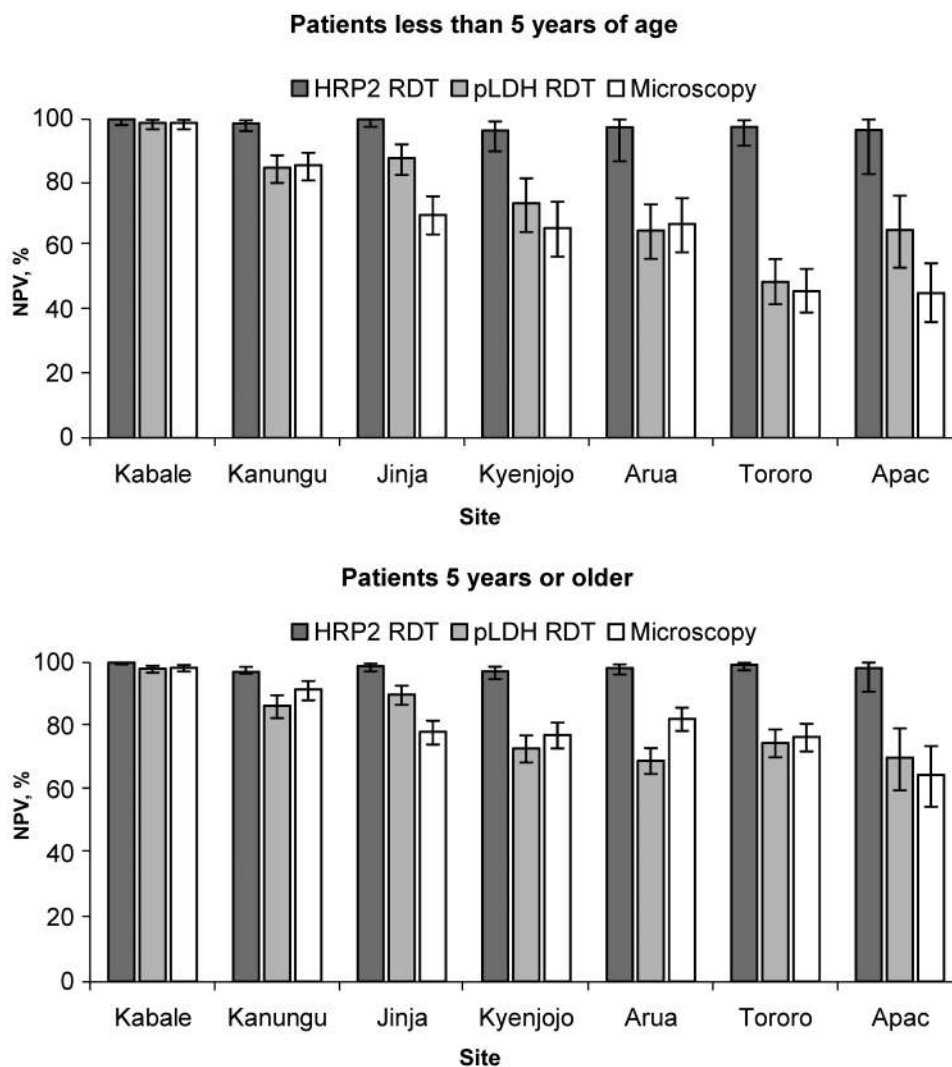
Patients less than 5 years of age

Patients 5 years or older

Figure 4. Negative predictive values (NPVs) of rapid diagnostic tests (RDTs) for malaria stratified by age. The comparison gold standard is expert microscopy, corrected by polymerase chain reaction for the presence or absence of *Plasmodium falciparum.* Error bars represent 95% confidence intervals. HRP2, histidine-rich protein 2–based test; pLDH, *Plasmodium* lactate dehydrogenase–based test.

the accuracy levels described in other reports from East Africa. Two earlier studies in western Uganda compared HRP2-based tests with microscopy. One study, which used an older HRP2-based assay, found a sensitivity of 99.6% for parasitemia >500 parasites/μL and a specificity of 92.7% for patients with fever [17]. The other study, which used the same HRP2-based test as we used in our evaluation, found a sensitivity of 97% and a specificity of 88% for *P. falciparum* infection [20]. A Tanzanian study compared HRP2-based and pLDH-based tests with microscopy and found sensitivities of 100% for the HRP2-based test and 94% for the pLDH-based test; specificities were 74% and 100%, respectively [32]. Our results confirm the higher sensitivity of the HRP2-based test and the higher specificity of the pLDH-based test, although all estimates were somewhat lower in our study.

Important differences in the accuracy of the RDTs, particularly their NPV, were identified when we used microscopy corrected by PCR for *P. falciparum* as the gold standard for malaria diagnosis. The improved sensitivity of PCR over microscopy for low-density malaria infections is well-established [22, 33, 34]. However, the value of PCR-based diagnosis is uncertain; microscopy has served as a reasonable gold standard for decades, and an overly sensitive diagnostic test might lead to inappropriate antimalarial treatments in Africa. On the other hand, it might be argued that symptomatic individuals with any degree of parasitemia should receive antimalarial treatment. Recent studies have noted the significance of subpatent parasitemia both for symptomatic patients and as a reservoir for transmission [23, 24, 26]. However, the clinical relevance of subpatent parasitemia has not yet been established at sites with higher transmission rates in

Africa, where a proportion of the population carries low levels of parasitemia at any given time [29, 35, 36]. In addition, gametocytemia may cause positive RDT results in the absence of asexual parasitemia, although in our study, gametocytes were identified in only 13% of samples that were RDT-positive and PCR-positive but smear-negative. Antimalarial treatment of gametocytemic patients, especially with artemisinin-based combination therapies that have some antigametocyte activity, may be beneficial in reducing transmission [37].

Few studies have directly compared RDT results and microscopy results with PCR for detection of *P. falciparum* in areas where malaria is endemic. In one study, the proportion of positive test results for symptomatic children increased from 33% by use of microscopy to 44% by use of PCR at a site with a medium transmission rate in Kenya ($n = 184$) and from 2% by microscopy to 8% by PCR at a site with a low transmission rate in Tanzania ($n = 154$) [25]. However, RDTs detected only a minority of episodes of subpatent parasitemia in this study. Another study performed in an area with a low transmission rate in the Philippines found that a large number of the HRP2-based test results that had been categorized as false-positive when compared with microscopy were reclassified as true-positive when compared with PCR [22]. Our previous evaluation of RDTs in an urban setting in Uganda found that most samples that were positive according to the HRP2-based test but negative by microscopy were also negative by PCR [38]. The results of the current study add the important finding that, particularly at sites with high transmission rates, a significant proportion of positive HRP2-based test results that are categorized as false-positive when compared with microscopy may be positive due to subpatent parasitemia.

Very few previous studies have considered RDT performance across sites of varying malaria endemicity. An earlier trial in southwestern Uganda, which compared an older HRP2-based test with expert microscopy, found no differences in sensitivity, specificity, or predictive value among regions of with varying malaria endemicity [17]. Another evaluation of the same test in Zimbabwe found that, although RDT sensitivity and NPV remained relatively constant, PPV was higher in the regions with higher transmission rates, and lowest in an area with a low transmission rate [18]. In the present study, when RDT results were compared with microscopy, there was no clear relationship between diagnostic accuracy and transmission intensity. However, when PCR-corrected microscopy was used as the gold standard, a clear pattern emerged. As transmission intensity increased, the HRP2-based test detected an increasing number of episodes of subpatent parasitemia, compared with the pLDH-based test and microscopy.

There are some potential limitations in generalizing our results to predict the success of implementing RDT in fever case management across Africa. We stored RDTs for a relatively short time, and our study staff was well trained in test preparation and interpretation. In addition, microscopy was performed by expert technicians with good-quality equipment. Our study was conducted in Uganda, where *P. falciparum* is the dominant malaria species; RDT accuracy may be different in areas where non-falciparum species are more prevalent.

To summarize, when RDT results were compared with expert microscopy, the HRP2-based assay had higher sensitivity and the pLDH-based assay had higher specificity. However, if results were compared with PCR-corrected microscopy, the pLDH-based RDT and expert microscopy failed to detect an increasing number of malaria cases due to subpatent parasitemia as transmission intensity increased. How should these results help to guide the use of RDTs in Africa? Considering the potential severity of each *P. falciparum* infection, it is probably better to err on the side of treatment if the clinical significance of a positive test result is uncertain. The excellent accuracy of the HRP2-based test across multiple sites suggests that this test should be the first choice for areas in Africa with medium-to-high transmission rates, which includes much of the continent. At sites with low transmission rates, the pLDH-based test or good-quality microscopy appear to offer superior PPV, and they may be better options to reduce unnecessary antimalarial treatments. In areas with very high transmission rates, fevers are commonly the result of malaria, and empirical treatment of febrile children under the age of 5, as recommended by many current guidelines, may remain the safest and most cost-effective approach. Further evaluations of the utility and cost-effectiveness of RDTs in fever case management that consider varied epidemiological settings are greatly needed. Ideally, RDTs will help to target antimalarial treatment to African patients who have malaria, but for whom reliable laboratory diagnosis is currently not available.

Acknowledgments

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