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# Accuracy of Two Malaria Rapid Diagnostic Tests (RDTS) for Initial Diagnosis and Treatment Monitoring in a High Transmission Setting in Uganda

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*Abstract.* Malaria rapid diagnostic tests (RDTs) may improve fever management in areas without microscopy. We compared the accuracy of histidine-rich protein 2 (HRP2) and *Plasmodium* lactate dehydrogenase (pLDH)-based RDTs, using expert microscopy as a gold standard, for initial diagnosis, treatment monitoring, and diagnosis of recurrent malaria in a cohort of children followed longitudinally in a high-transmission area in Uganda. For 305 initial fever episodes, sensitivity was 98% for HRP2 and 87% for pLDH, whereas specificity was 55% and 96%, respectively. The HRP2 gave 51% false-positive results on Day 28, whereas pLDH gave no false positives after Day 7. For 59 recurrent fever episodes during follow-up, sensitivity was 100% for HRP2 and 91% for pLDH, whereas specificity was 33% and 100%, respectively. The HRP2-based RDTs are useful for initial diagnosis of malaria caused by superior sensitivity; however, as a result of superior specificity, pLDH-based RDTs are more appropriate to monitor treatment and diagnose recurrent malaria.

## INTRODUCTION

Uganda has some of the highest malaria transmission intensities reported in the world.[1] High malaria transmission areas are characterized by a high prevalence of parasitemia in the population and frequent malaria episodes, especially in children < 5 years of age. Parasitological diagnosis using either microcopy or malaria rapid diagnostic tests is recommended before antimalarial treatment.[2] Despite this recommendation, clinical diagnosis of malaria is still practiced in many areas leading to significant over-diagnosis caused by symptom overlap with other causes of fever, indiscriminate use of antimalarials, and failure to diagnose and treat alternative causes of fever.[3–5] Over-diagnosis of malaria and indiscriminate treatment with artemisinin-based combination therapy (ACT) leaves patients without correct diagnosis and treatment, may result in increased pressure toward resistant parasites, leads to significant wastage, and is costly and unsustainable. Malaria rapid diagnostic tests (RDTs), which detect parasite antigen in blood, are increasingly favored for diagnostic confirmation because of their simplicity, low infrastructure requirements, and rapid results.[6] The most popular RDTs currently in use identify histidine-rich protein 2 (HRP2) and/or *Plasmodium* lactate dehydrogenase (pLDH). The HRP2-based RDTs have been recommended for Uganda by the Ministry of Health on the basis of accuracy and ease of use.[7,8] Currently, HRP2 assays appear to be somewhat more sensitive than pLDH-based tests.[8,9] However, HRP2 antigen remains in the bloodstream for several weeks after parasite clearance, thus contributing to false-positive results and limiting specificity.[10–14] The pLDH-based assays may be more useful for monitoring patients' recovery after treatment and avoiding unnecessary retreatment of malaria because they turn negative soon after parasite clearance from the blood.[15] Additional information on RDT performance in high transmission areas will be helpful in guiding policy and practice. We compared the performance of one HRP2- and one pLDH-based RDT with expert microscopy, in a cohort of children in a high transmission area to 1) determine RDT accuracy for initial diagnosis of uncomplicated malaria, 2) compare duration of persistent antigenemia of HRP2 and pLDH after efficacious treatment of uncomplicated malaria, and 3) determine RDT accuracy for malaria diagnosis in patients returning with fever after efficacious treatment of uncomplicated malaria.

## MATERIALS AND METHODS

**Study site.** The study was conducted in Tororo District Hospital in eastern Uganda, a malaria hyperendemic area between November 4, 2011 and January 14, 2012. The entomological inoculation rate in this area was estimated at 562 infective bites per person per year in 2002,[1] and more recent reports indicate persistent very high incidence of malaria in childhood despite use of long-lasting insecticide-treated nets and ACT.[16] The study was carried out alongside the Tororo Child Cohort (TCC), a longitudinal antimalarial drug efficacy trial that began in 2007. The primary objectives of the TCC were to compare the incidence of malaria in a cohort of children stratified by human immunodeficiency virus (HIV) status, mother's HIV status, and use of trimethoprim-sulfamethoxazole (TMP/SMX) prophylaxis in an area where malaria is highly endemic; to compare the efficacy, safety, and tolerability of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) for the treatment of uncomplicated falciparum malaria among HIV-infected and uninfected children; and to assess the effect of TMP-SMX prophylaxis on the incidence of malaria after cessation of prophylaxis. Details of the screening and recruitment of this cohort have been published previously.[17] Briefly, children were recruited from the antenatal and pediatric clinics of Tororo Hospital and Tororo branch of The AIDS Support Organization (TASO) if they fulfilled all of the following eligibility criteria: 1) age 6 weeks to 12 months, 2) documented HIV status of mother and child, 3) agreement to come to the study clinic for any febrile episode or other illness, 4) agreement to avoid medications administered outside the study protocol, 5) willingness of parents or guardians to provide informed consent, and 6) residence within 30 km radius of the study clinic. Children in the study cohort were seen at least monthly at the study clinic and encouraged to attend the clinic in case of any illness and to avoid any medication not given at the study

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clinic. At the beginning of the trial, participants were randomized to always receive either DP or AL in case of microscopy confirmed malaria. Children who presented to the study clinic with fever (tympanic temperature ≥ 38°C and/or history of fever in the previous 24 hours) and had a positive blood smear were treated with antimalarials and followed up for 28 days. Children with negative blood smears did not receive antimalarials and were treated according to standard treatment algorithms and the study physician's judgment.

**Sample size estimation.** Sample size was based on episodes of fever, and was estimated using a formula by Buderer for diagnostic tests.[18] Assuming a prevalence of malaria in the study population of 50.5%,[3] 274 episodes of fever were required to obtain sensitivity of 90% (precision of 5% and alpha error of 0.05). To obtain a specificity of 90%, 280 episodes of fever were required. The higher sample size of 280 was used plus an additional 10% to allow for invalid or inaccurate results to give a final sample size of 308 episodes of fever. Febrile patients who fulfilled the inclusion criteria were consecutively enrolled until the sample size was attained.

**Recruitment of study participants.** Children were screened for enrollment into this RDT sub-study, according to the following eligibility criteria:1) age between 6 months and 5 years, 2) presence of fever (temperature ≥ 38°C) or history of fever in the previous 24 hours, 3) absence of World Health Organization (WHO) symptoms and signs of severe malaria or danger signs,[2] 4) willingness of parents/guardians to provide written informed consent, 5) residence within 30 km of the clinic, 6) absence of another obvious cause of fever (as determined by the study physicians), and 7) absence of non-falciparum malaria species on blood smear. Eligible children who presented to the study clinic during the study period were consecutively enrolled until the target sample size of 308 fever episodes was attained. Of this total, a convenience sample of the first 140 fever episodes with microscopy confirmed malaria, all of whom received antimalarial treatment according to the TCC study protocol, were asked to participate in the follow-up component of the study until Day 28 or until development of a recurrent episode of microscopy confirmed malaria. Follow-up study measurements were used to compare duration of persistent antigenemia and to determine RDT accuracy for malaria diagnosis in patients returning with fever after efficacious malaria treatment.

**Clinical and laboratory procedures.** Demographic and clinical information was obtained for patients who fulfilled the inclusion criteria. Children who presented to the clinic with a documented fever (tympanic temperature ≥ 38.0°C) or history of fever in the previous 24 hours had blood obtained by finger prick for a thick and thin blood smear and for RDTs. The RDTs used were selected on the basis of performance in the WHO Malaria RDT Product Testing Program (rounds 1–3 results as available in 2011).[9] They included CareStart Malaria HRP2 Lot D21MO (September 2013) and CareStart Malaria pLDH (P.f) Lot H11MY (July 2013) (Access Bio, Inc., Somerset, NJ). Test kits were purchased directly from the manufacturer and kept in their original packaging in the study clinic at room temperature. The RDTs were prepared and read according to manufacturer's instructions by trained laboratory technicians who were blinded to blood smear results. Blood smears were stained with 2% Giemsa for 30 minutes and read by experienced laboratory technologists. Thick smears were used to determine presence or absence of *Plasmodium* parasites and gametocytes, and to calculate parasite density. Parasite densities were calculated by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes, if < 10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/μL. A blood smear was considered negative when the examination of 100 high-power fields did not reveal asexual parasites. Thin smears were used for confirmation of the parasite species. All blood slides were read by microscopists with extensive experience in research malaria microscopy. Quality control of microscopy was performed as follows: all slides were reread by a second expert microscopist and any discordance regarding presence or absence of parasites was resolved by a third expert reading. Microscopists were not aware of previous blood smear readings or RDT results.

**Diagnosis and treatment of malaria.** If a child's thick blood smear was positive, s/he was diagnosed with malaria regardless of the parasite density and given directly observed therapy with DP or AL according to the TCC trial protocol. If the thick blood smear was negative, the patient was not given antimalarial therapy and was managed at the discretion of the study physicians. The RDT results were not provided to treating clinicians, and were not used for treatment decisions.

**Follow-up of study participants.** A convenience sample of the first consecutive 140 smear-positive participants treated for malaria were asked to return on Days 2, 7, 14, 21, and 28, and any other day they felt ill during the 28-day follow-up period. At each follow-up visit, history of fever and tympanic temperature were recorded and blood was obtained by finger prick for thick and thin blood smears and RDTs. Patients with recurrent microscopy confirmed malaria during the 28 days were discontinued from further follow-up.

**Statistical analysis.** Data were entered using EPI DATA 3.1 and analyzed using STATA version 12 (Stata, College Station, TX). Comparison of characteristics of patients that were and were not followed up were made using a *t* test for continuous variables and $\chi^2$ or Fisher's exact test for categorical variables. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the RDTs were estimated using expert microscopy results as the comparison standard. The percentage of false-positive RDT results was defined as the percentage of RDT results that remained positive during follow-up once the blood sample became negative by microscopy. Comparisons of measures of diagnostic accuracy were made using generalized estimating equations with adjustment for repeated measures in the same study participant. A $P$ value of $< 0.05$ was considered statistically significant.

**Ethical approval.** The study protocol was approved by Makerere University School of Medicine Research and Ethics Committee and the Uganda National Council for Science and Technology. Written informed consent was obtained from participants' parents or legal guardians at enrollment.

## RESULTS

**Study participants and baseline characteristics.** The TCC study participants presenting with 311 consecutive episodes of fever were screened for this sub-study. Of these six were excluded because of severe malaria (1), concomitant febrile illnesses (4), and non-falciparum malaria (1); 305 fever

TABLE 1
Demographic and clinical characteristics of enrolled patients

| Characteristic | Enrolled patients, $N = 305$ | BS positive patients that were followed up, $N = 131$ | BS positive patients that were not followed up, $N = 62$ |
|---|---|---|---|
| Median age in months (IQR) | 52 (50–55) | 53.3 (50.9–56.5) | 54.4 (52.4–56.5) |
| Female, n (%) | 144 (47) | 75 (57) | 30 (48) |
| Median weight in kg (IQR) | 15.2 (13.9–16.5) | 15.5 (14.0–16.9) | 15.5 (14.1–17.2) |
| Median height in cm (IQR) | 101 (98–104) | 102 (98–104) | 102.5 (98.8–105.0) |
| Median temperature °C (IQR) | 37.3 (36.8–38.3) | 37.5 (36.8–38.5) | 37.9 (37.1–38.7) |
| Mean parasite density/μL (SD) | 29204.6 (42498.3) | 47130.4 (48984.9) | 37530 (36674.1) |
| Patients with gametocytes, Day 0, n (%) | 9 (3) | 5 (4) | 4 (6) |
| HIV-positive, n (%) | 22 (7) | 7 (5) | 2 (3) |
| Drug use in the past one month | | | |
| Antimalarial, n (%) | 84 (28) | 24 (18) | 14 (23) |
| Cotrimoxazole prophylaxis, n (%) | 22 (7) | 7 (5) | 2 (3) |

BS = blood smear, IQR = interquartile range.

episodes, recorded in 176 children, were evaluated. Study participants' median age was 52 months (interquartile range [IQR] 50–55) and 53% were male (Table 1). Blood smears were positive by expert microscopy for *Plasmodium falciparum* in 202 (66%) fever episodes. A convenience sample of children presenting with 140 consecutive smear-positive fever episodes were followed up for 28 days (Figure 1). There were no differences in the demographic and clinical characteristics between the 140 included and the 62 not included in the follow-up component (Table 1).

**Accuracy of HRP2 and pLDH RDTs for initial diagnosis of malaria.** For initial diagnosis of malaria, HRP2 had higher sensitivity of 98% and NPV of 92% compared with pLDH with sensitivity of 87% and NPV of 78% ($P$ value < 0.001). In contrast, pLDH had higher specificity of 96% and PPV of 98% compared with HRP2 with specificity of 54% and PPV of 81% (Table 2).

**Association of sensitivity of HRP2 and pLDH RDTs with parasite density.** The HRP2 result was negative in 60 of 305 initial episodes of fever and of these, 4 (7%) were false negative when compared with expert microscopy. All the false-negative HRP2 results were associated with a low parasite density (32–112/μL).There were 124 negative pLDH results of which 26 (21%) were false negative. Of the false-negative pLDH results, 17 (65%) occurred at a parasite density < 500/μL, 4 (15%) at parasite density between 500–5,000/μL, 3 (12%) between 10,000 and 12,000/mL, and 2 (8%) > 50,000/mL. We compared sensitivity of HRP2 and pLDH at different parasite density categories. At parasite density < 200/μL, pLDH sensitivity was 0% compared with 67% for

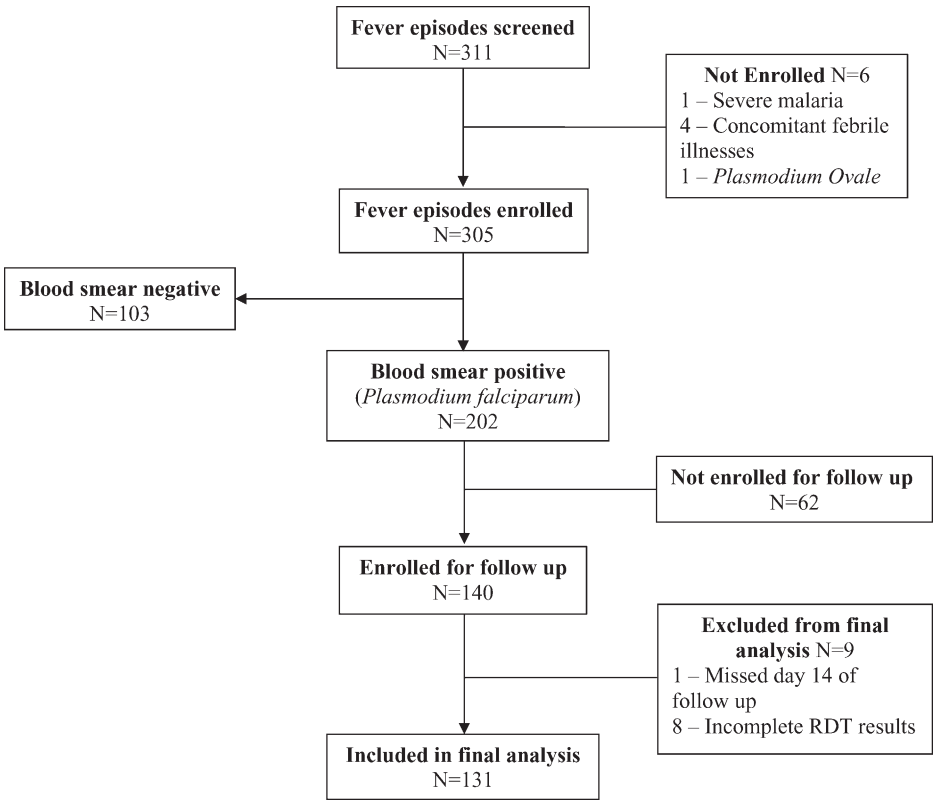


FIGURE 1. Study profile. Trial profile showing screened and enrolled patients, blood smear results, and those enrolled for follow-up. Of the 140 cases that were enrolled for follow-up, 131 were included in the final analysis.

TABLE 2

Accuracy of HRP2- and pLDH-based malaria rapid diagnostic tests (RDTs) for initial diagnosis of malaria in 305 children using expert microscopy as a gold standard

| Accuracy | HRP2 RDT | pLDH RDT | *P* value |
|---|---|---|---|
| Sensitivity (%) (95% CI) | 98 (94–99) | 87 (81–91) | < 0.001 |
| Specificity (%) (95% CI) | 54 (45–64) | 96 (90–98) | < 0.001 |
| Positive predictive value (%) (95% CI) | 81 (76–86) | 98 (94–99) | < 0.001 |
| Negative predictive value (%) (95% CI) | 92 (82–96) | 78 (70–85) | < 0.001 |

HRP2 = histidine-rich protein 2; pLDH = *Plasmodium* lactate dehydrogenase; CI = confidence interval.

HRP2. At parasite density 200 to < 1,000 HRP2 sensitivity was 100%, whereas that of pLDH was sensitivity 60% (Figure 2).

**Association of specificity of HRP2 and pLDH RDTs with duration since last episode of malaria.** The HRP2 result was positive in 245 of 305 (80%) initial episodes of fever; of these, 46 (19%) were false positive when compared with expert microscopy. Of the 46 false-positive HRP2 results, 29 (63%) occurred in children who had a documented malaria episode in the previous 4 weeks and 43 (93%) occurred in children who had a documented malaria episode in the previous 8 weeks. For the same 305 fever episodes, there were 181 (59%) positive pLDH tests of which 4 (2%) were false positive when compared with expert microscopy. All the false-positive pLDH tests occurred in children who had a documented malaria episode in the previous 5 weeks. We stratified duration since the last documented episode of malaria into four groups. For patients who had had a malaria episode in the previous 2 weeks, HRP2 had a very low specificity of 11% compared with 94% for pLDH. Specificity of HRP2 was 89% for patients who had had a malaria episode within the previous 10 weeks compared with 100% for pLDH (Figure 3).

**Persistence of HRP2 and pLDH antigenemia during follow-up.** Among the 140 malaria episodes that were followed up, 131 were included in the final analysis (Figure 1). Of the 131, 52 (40%) had recurrent peripheral parasitemia during follow-up and were not included in the analysis of duration of persistent antigenemia. There were 94% false-positive HRP2 test results on Day 2 and 51% false-positive test results on Day 28. On Day 2, pLDH had 5% false-positive results and no false-positive result on Day 14, 21, and 28 (Figure 4).

**Association of pretreatment parasite density with duration of antigenemia.** Pretreatment parasite density was found to predict the duration of persistent antigenemia of the HRP2-based RDT. We stratified parasite density into four groups and compared the percentage of false-positive HRP2 results on each day of follow-up with pretreatment parasite density. Among patients with the highest parasite density > 100,000, 89% had false-positive HRP2 results on Day 28 compared with 4% of those with parasite density < 10,000 (Figure 5).

**Accuracy of HRP2 and pLDH RDTs for diagnosis of malaria in children with recurrent fever after treatment of uncomplicated malaria.** There were 59 episodes of recurrent fever after completion of antimalarial treatment. Of these, 46 (78%) occurred after Day 14 of follow-up. Of the 59 episodes of recurrent fever, 32 (54%) were blood smear positive for *Plasmodium falciparum* and these all occurred after Day 17 of follow-up. For diagnosis of malaria in children with recurrent fever after treatment, HRP2 had higher sensitivity of 100% and NPV of 100% compared with pLDH with sensitivity of 91% and NPV of 90%. In contrast, pLDH had higher specificity of 100% and PPV of 100% compared with HRP2 with specificity of 33% and PPV of 64% (Table 3). There were 50 positive HRP2 results of which 18 (36%) were false positive. All of the 18 false-positive results occurred in children who had had a documented malaria episode and used an antimalarial in the previous 1 month. Of the 18 children, 17 (94%) had a malaria episode in the previous 21 days. There was no false-positive pLDH result among the 29 positive pLDH results obtained. There was no false-negative HRP2 result among the 9 negative HRP2 results. There were 30 negative pLDH results of which 3 (10%) were false negative. All the false-negative pLDH results were associated with relatively low parasite densities of 80/μL, 400/μL, and 520/μL.

## DISCUSSION

For initial diagnosis of malaria in this high transmission area, as expected the HRP2-based malaria RDT showed a

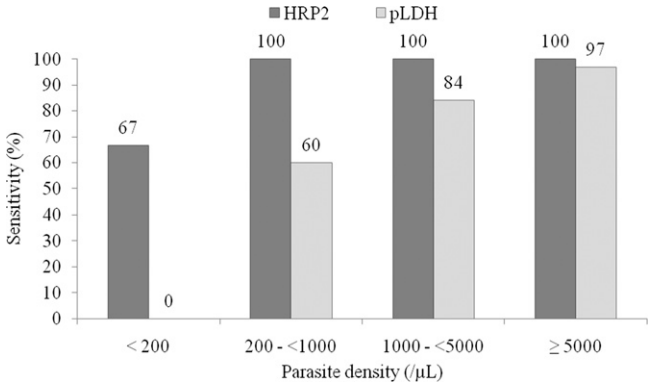


FIGURE 2. Relationship between parasite density and sensitivity of histidine-rich protein 2 (HRP2) and *Plasmodium* lactate dehydrogenase (pLDH) rapid diagnostic tests (RDTs). At parasite densities < 1,000/μL, the sensitivity of pLDH is very low compared with HRP2.

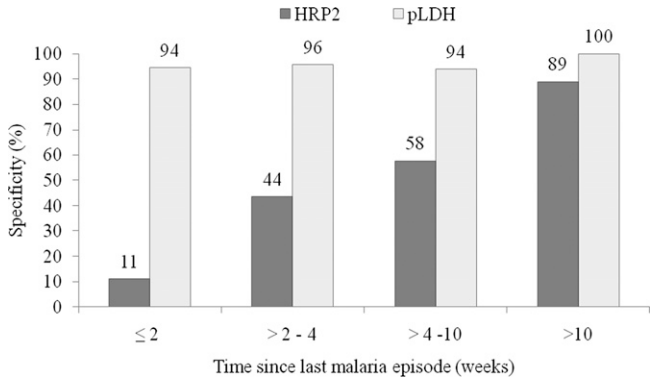


FIGURE 3. Relationship between specificity of histidine-rich protein 2 (HRP2) and *Plasmodium* lactate dehydrogenase (pLDH),and time since last malaria episode. The pLDH had acceptable specificity when used in patients with a recent malaria episode, unlike HRP2, which showed poorer specificity.

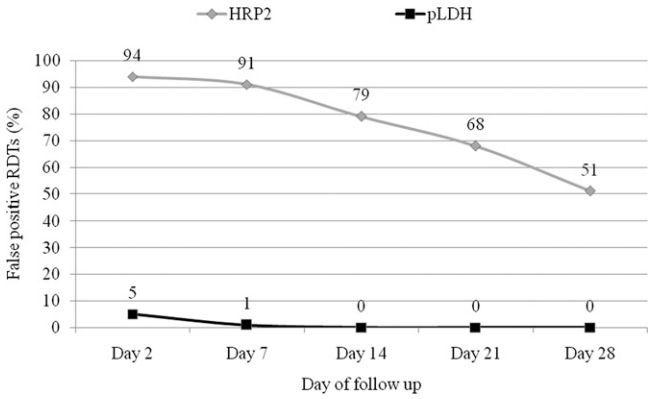


FIGURE 4. Duration of antigenemia detected by histidine-rich protein 2 (HRP2) and *Plasmodium* lactate dehydrogenase (pLDH)-based rapid diagnostic tests (RDTs) during follow-up. The figure shows duration of antigenemia of HRP2 and pLDH during follow-up represented by percentage of false-positive HRP2 and pLDH RDTs, as compared with expert microscopy, obtained on each day of follow-up in 79 children without recurrent parasitemia. There was no false-positive pLDH result after Day 7, although 51% of HRP2 results were false positive on Day 28.

higher sensitivity and NPV compared with pLDH, whereas the pLDH assay showed a higher specificity and PPV. The lower sensitivity and NPV of pLDH could be attributed mainly to its declining ability to detect antigen at lower parasite densities. Although highly sensitive for initial diagnosis of malaria, the HRP2-based RDT had low specificity because of a large number of false-positive results, most of which were obtained in patients who had received treatment of microscopy confirmed malaria in the previous 1 month. This implies that persistent antigenemia of HRP2 in these patients contributed to the low specificity of the HRP2- based RDT, as suggested by previous studies.[10,11] Another factor that contributes to false-positive HRP2 results is sub-patent parasitemia, i.e., presence of parasite density levels below the detection threshold for expert microscopy of 10–50 parasites/μL.[8,19] A study done at sites of varying malaria transmission intensity in Uganda found up to 45% of samples that were

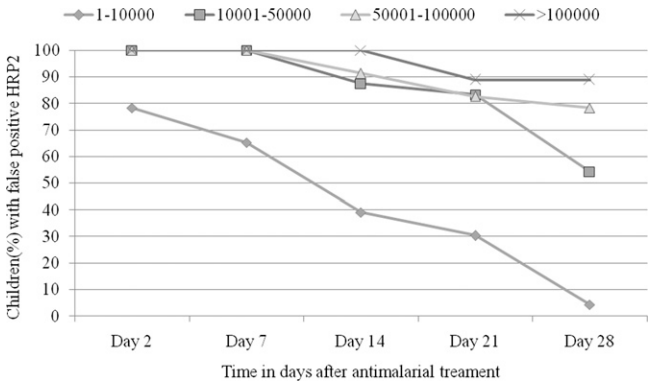


FIGURE 5. Proportion of children with continued false-positive histidine-rich protein 2 (HRP2) results during follow-up, stratified by Day 0 parasite density. The figure shows the proportion of children with false-positive HRP2 results on each day of follow-up, stratified into four groups of pretreatment parasite density, in 79 children without recurrent parasitemia during follow-up. Children with initial parasite density ≤ 10,000 had a smaller proportion of false-positive results on each day of follow-up when compared with those with parasite density > 100,000.

negative for malaria by microscopy to be positive by PCR at the site with the highest transmission rate.[8] The high sensitivity and NPV of the HRP2-based RDT implies that true malaria cases would rarely be missed making it an appropriate test for initial diagnosis of malaria. However, the high number of false-positive HRP2 results and low specificity could lead to over-diagnosis of malaria in patients presenting with alternative causes of fever. These results imply that when a positive HRP2 result is obtained in a patient with history of recent malaria treatment, clinicians should look for alternative causes of fever to improve patient management and prevent misuse of ACTs.

The pLDH-based malaria RDT showed rapid clearance of antigenemia after treatment of uncomplicated malaria compared with the HRP2-based RDT. Persistent antigenemia of HRP2 has been shown for up to 28–42 days after successful treatment and clearance of malaria parasites in peripheral blood.[10,11,13] Although the mechanism of HRP2 clearance is not well understood, some features that appear to be associated with its persistence after efficacious treatment include high pretreatment parasite density, presence of subpatent parasitemia, and presence of gametocytes, which continue to secrete antigen. In this study, children with a higher pretreatment parasite density had longer duration of persistent HRP2 antigenemia. Some studies have identified presence of antigen-secreting gametocytes as another possible cause of persistent HRP2 or pLDH antigenemia.[20] However, gametocytes were not found to significantly affect duration of antigenemia in other assessments.[10,11] In this study the gametocyte rate of 4% in patients who were followed up was too low to identify a significant correlation with the duration of antigenemia. Other studies using molecular methods to identify gametocytes have suggested that microscopy dramatically underestimates gametocytemia.[21] Thus, some of the HRP2 positives might be explained by subpatent gametocytemia, in addition to persistence of antigen from recently cleared blood-stage parasites. In addition, in a large surveillance project that included patients of all ages, patient age was also found to affect false-positive HRP2 results. Abeku and others[22] found false-positive rates of HRP2-based RDT to decline in older age groups; the authors suggest this may be a result of acquired immunity in clearing parasite antigens. In a clinical setting, persistent antigenemia detectable by HRP2-based RDTs limits their use in monitoring treatment of malaria. This study conducted in a high transmission setting confirms that a positive HRP2 result obtained within 28 days of antimalarial treatment has low specificity and may lead to over-diagnosis of malaria. The pLDH-based RDT however showed higher specificity soon after treatment and may be more useful in monitoring response to antimalarial treatment.

The pLDH-based RDT showed acceptable sensitivity and specificity for diagnosis of malaria in children with recurrent episodes of fever after administration of ACT for uncomplicated malaria. Positive predictive value of HRP2 for initial diagnosis of malaria was relatively low at 81% and even lower at 64% for diagnosis of malaria in children with recurrent episodes of fever within 28 days of efficacious treatment. This is of concern particularly in high transmission areas that still exist across much of East and Central Africa, where in the setting of frequent prolonged antigenemia, even with RDT use fever may be over-diagnosed as malaria as a result of the detection of antigen from previous infections, leading to

TABLE 3

Accuracy of HRP2- and pLDH-based malaria rapid diagnostic tests (RDTs) for diagnosis of malaria in children with recurrent fever within 28 days after treatment of uncomplicated malaria, using expert microscopy as a gold standard

| Accuracy | HRP2 RDT | pLDH RDT | *P* value |
|---|---|---|---|
| Sensitivity (%) (95% CI) | 100 (89–100) | 91 (76–97) | < 0.001 |
| Specificity (%) (95% CI) | 33 (19–52) | 100 (88–100) | < 0.001 |
| Positive predictive value (%) (95% CI) | 64 (50–76) | 100 (88–100) | < 0.001 |
| Negative predictive value (%) (95% CI) | 100 (70–100) | 90 (74–97) | < 0.001 |

HRP2 = histidine-rich protein 2; pLDH = *Plasmodium* lactate dehydrogenase; CI = confidence interval.

misuse of antimalarials and failure to identify and treat alternative fever etiologies in these patients.

Significantly, a majority of the false-positive HRP2 tests occurred in children who had been diagnosed and treated for malaria in the previous 21 days, implying that an HRP2 test result obtained within 3 weeks of antimalarial treatment has a high likelihood of being false positive. These results suggest that for fever management in high transmission areas, HRP2-based tests are of limited use within a minimum of 3 weeks following a malaria episode. On the other hand, the pLDH-based RDT showed excellent specificity and positive predictive value and could be recommended for evaluation of patients who return with fever after completion of treatment of an initial malaria episode. The potential for false-negative pLDH results, although mostly occurring in cases of low parasite density, remains a point of caution, and repeat evaluation for persistent symptoms is warranted.

To our knowledge, there is only one previous, small assessment of HRP2 and pLDH RDT accuracy for malaria diagnosis in a higher transmission zone after completion of efficacious antimalarial treatment. A study of 53 children by Aydin-Schmidt and others,[23] in a "moderately high" transmission area in Tanzania, found that the HRP2-based RDT detected only two of 10 recurrent infections because of persistent positivity up to the day of recurrent infection, whereas the pLDH-based RDT was able to detect eight of the 10.

This study used expert microscopy, and not a more sensitive nucleic acid-based assay, as a gold standard. The use of a gold standard with relatively low sensitivity likely contributed to the low specificity of the HRP2-based RDT, as some results classified as false positives would have been true positives with a more sensitive gold standard. Nonetheless, the majority of false-positive HRP2-based results were in children recently treated for malaria, suggesting that the main limitation to specificity was the ability of the HRP2-based assay to identify antigen well after clearance of parasites. Furthermore, the Uganda National Malaria Control Program policy currently recommends confirmation of malaria infection with microscopy or RDT, and it is not clear that more sensitive assays for diagnosis would be helpful clinically, therefore considering results based on the microscopy gold standard is most relevant for current malaria control policy.

In addition to accuracy, other factors such as economic considerations play a role in decisions about RDT implementation. The cost effectiveness of using RDTs, compared with microscopy or presumptive treatment of malaria, varies across different patient populations and malaria prevalences, and is also affected by prescribers' response to RDT results. For example, in high transmission areas, some studies have found RDTs to be cost-effective compared with microscopy and presumptive treatment of malaria.[24] However, among children < 5 years of age in high transmission settings, some investigators suggest that presumptive treatment of malaria may be more cost effective than RDTs given the high prevalence of malaria in this age group.[25] Economic factors may be weighed alongside other potential benefits of RDT use in decision-making for given regions.

In this highly endemic setting, HRP2-based RDTs should be used for initial diagnosis of malaria; however, in patients who have had a malaria episode in the previous 3 weeks, pLDH-based RDTs appear to be more useful for monitoring response to treatment and diagnosis of new episodes of malaria after treatment. However, a recommendation to use two different RDTs for two different indications may be very challenging to implement in routine clinical practice. At least, where HRP2-based RDTs are used, clinicians should be trained to search for an alternative diagnosis in patients presenting with fever following a recent malaria episode.

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