Use of a Dual-Antigen Rapid Diagnostic Test to Screen Children for Severe *Plasmodium falciparum* Malaria in a High-Transmission, Resource-Limited Setting

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Background. In rural areas, many patients with malaria seek care at peripheral health facilities or community case management programs. While this strategy is effective for the management of uncomplicated malaria, severe malaria necessitates prompt detection and referral to facilities with adequate resources.

Methods. In this prospective, observational cohort study, we assessed the accuracy of a dual-band (histidine-rich protein-2/*pan*-lactate dehydrogenase [HRP2/pLDH]) rapid diagnostic test (RDT) to differentiate uncomplicated from severe malaria. We included children aged <12 years who presented to a rural clinic in western Uganda with a positive HRP2 or HRP2/pLDH RDT. We estimated the test characteristics of a dual-antigen (HRP2+/pLDH+) band positive RDT compared to World Health Organization–defined clinical and laboratory criteria to detect severe malaria.

Results. A total of 2678 children underwent testing for malaria with an RDT, and 83 (9.0%) satisfied criteria for severe malaria. The sensitivity and specificity of a HRP2+/pLDH+ result for severe malaria was 97.6% (95% confidence interval [CI], 90.8%–99.6%) and 75.6% (95% CI, 73.8%–77.4%), respectively. An HRP2+/pLDH+ result was significantly more sensitive (97.6% vs 68.7%, $P < .001$) for the detection of severe malaria compared to algorithms that incorporate screening for danger signs.

Conclusions. A positive dual-antigen (HRP2/pLDH) RDT has higher sensitivity than the use of clinical manifestations to detect severe malaria, making it a promising tool in the triage of children with malaria in low-resource settings. Additional work is needed to operationalize diagnostic and treatment algorithms that include dual-antigen RDTs to avoid over referral.

Keywords. malaria; severe malaria; rapid diagnostic tests; community case management.

Each year millions of children are infected with *Plasmodium falciparum* malaria, and approximately 300 000 die from manifestations of severe disease [1]. In Uganda, like much of sub-Saharan Africa, malaria is a leading cause of child mortality and has historically accounted for nearly half of inpatient pediatric deaths [2, 3]. Many who suffer from severe malaria, however, do not reach a health facility, and thus a large proportion of deaths take place outside the formal healthcare sector [4–6].

Patients may initially seek care at peripheral health facilities that are staffed by health workers with only basic training in malaria case management. In Uganda, these level II and level III facilities may lack the requisite infrastructure to perform microscopy or other tests that may yield evidence of severe malaria (Table 1) [7, 8]. Malaria-endemic countries have also increasingly adopted community case management strategies, which provide for malaria diagnosis and treatment by lay members of the community outside of health facilities [9, 10].

While these programs improve access to care [11], community health workers and peripheral health centers are not equipped with the laboratory infrastructure (eg, lactate and bicarbonate testing) required to diagnose severe malaria. Instead, providers are instructed to refer suspected cases to a higher level of care [12, 13]. The decision to refer, however, depends on the performance of a detailed physical exam and the identification of abnormal clinical signs, which may not be recognized by ancillary staff and lay health workers [14, 15].

One advance in rapid diagnostic test (RDT) technology is the development of assays that combine different antigens on a single membrane. Some reports suggest that the pattern of antigen band positivity can provide a semiquantitative estimate of *P. falciparum* parasite density [16–18], with dual-antigen band-positive tests signifying higher parasite densities. Given the association between higher levels of parasitemia and severe malaria [19], we hypothesized that dual-band positive tests may help discriminate between uncomplicated and severe malaria among children in rural Uganda.

Our objective in this prospective, observational study was to evaluate the validity of a dual-antigen RDT as a screening

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Table 1. Study Criteria for Severe Malaria Required *Plasmodium falciparum* Parasitemia on Microscopy and Any One of the Following

Impaired consciousness	Blantyre coma score <3 for children aged <5 years or Glasgow coma score <11 for children aged >5 years
Metabolic acidosis	Plasma bicarbonate of <15 mmol/L or venous plasma lactate >5 mmol/L
Hypoglycemia	Blood or plasma glucose <40 mg/dL (<2.2 mmol/L)
Severe anemia	A hemoglobin concentration <5 g/dL in children aged <12 years and <7 g/dL in adults together with a parasite count >10 000/uL
Kidney injury	Plasma or serum creatinine >3 mg/dL and blood urea nitrogen ≥20 mg/dL
Pulmonary edema	Oxygen saturation <92% on room air with a respiratory rate >30/min
Abnormal bleeding	Hemoglobinuria of ≥4+ on dipstick urinalysis (250 red blood cells)
Shock	Systolic blood pressure <70 mm Hg
Convulsions	History of seizures during present illness
Hyperparasitemia	*Plasmodium falciparum* parasitaemia >250 000/μL

tool for severe malaria and compare its performance to standard, clinical-based diagnostic criteria. Our overarching goal was to identify a low-cost, easy-to-interpret method for detecting severe malaria and guide referral decisions in community-based fever management programs and at peripheral health centers in resource-limited settings where microscopy and advanced laboratory capacity are not routinely available.

METHODS

Study Setting

The Bugoye Level III Health Center in the Kasese District of western Uganda serves a rural population of approximately 50 000 residents. Clinical officers, nurses, midwives, and laboratory technicians employed by the Ugandan Ministry of Health staff the health center. The climate in Bugoye permits year-round malaria transmission marked by semiannual transmission peaks typically following the end of the rainy seasons [20].

Study Design

The RDTs for Severe Malaria study was a prospective, observational cohort study of patients with a parasitological diagnosis of malaria conducted from May 2015 to November 2015. Study staff recorded demographic information, clinical history, and vital signs on all individuals who presented to the outpatient department. Health facility staff, typically nurses and clinical officers, performed clinical evaluations of all patients in accordance with local protocols. Individuals with fever (axillary temperature ≥38°C) or other symptoms suspicious for malaria as determined by the attendant provider were eligible for inclusion. Individuals aged >12 years and those who presented without a caregiver were excluded.

The health center laboratory staff performed initial testing for malaria using the Standard Diagnostics 05FK60 Malaria Ag *P.f*/Pan RDT assay (Hagal-Dong, Korea). The RDT, which has been prequalified by the World Health Organization (WHO), is an antigen detection test with 3 bands signifying the control, the histidine-rich protein-2 (HRP2), and the *pan*-lactate dehydrogenase (pLDH) antigens [21, 22]. The presence of a single HRP2 line (herein referred to as a HRP2+/LDH– result) denotes infection with *P. falciparum,* whereas a unique pLDH line (herein referred to as HRP2–/pLDH+) indicates infection with 1 or more of the other *Plasmodium* species. The presence of a positive HRP2 line together with a pLDH line (HRP2+/pLDH+) indicates an infection with *P. falciparum* or a mixed-species infection. RDTs for the study were obtained directly from the manufacturer, stored in the original packaging at room temperature, and used prior to the expiration date. We included participants with at least 1 single-band positive RDT in this analysis.

Study staff with training in laboratory medicine prepared thin and thick blood smears for all children with a positive RDT result. Children with a negative RDT were defined as not having severe malaria and did not undergo further testing, except for the 15% of individuals who underwent microscopy for the purpose of quality control. Smears were fixed with methanol and stained in 10% Giemsa. Microscopists who had undergone validation testing (Shoklo Malaria Research Unit) and were blinded to the RDT results reviewed all slides in accordance with WHO/Tropical Disease Research guidelines [23]. Two independent microscopists read all slides. A third, senior microscopist reviewed slides when there were discrepancies between the first 2 reads in the presence of parasitemia, species identification, or differences (>10%) in parasite density. Dried blood spots were obtained from a subset of participants over a 1-month period, and real-time polymerase chain reaction (PCR) was performed to assess the accuracy of microscopy [24].

Hemoglobin levels were measured using the Hemocue Hb 201+ analyzer (Brea, California), while serum chemistry and venous blood gas values were obtained using the Abbott iStat analyzer (Princeton, New Jersey) with the CHEM8+ and CG4+ cartridges. Estimates of hemoglobinuria were made using a UroColor 11 dipstick urinalysis assay (Standard Diagnostics, Hagal-Dong, Korea). All point-of-care tests were calibrated daily by study staff and performed in accordance with the manufacturers' instructions.

Patients with any positive RDT result were treated by health center staff in accordance with local standards of care. We recorded treatment and disposition plans, including admission to the inpatient ward and referral to higher-level facilities.

Statistical Analyses

Data were double-entered into Microsoft Excel (Redmond, Washington) and analyzed with Stata 12.1 (College Station,

Texas). We summarized patient characteristics and compared them between those with HRP2+/pLDH– and HRP2+/pLDH+ results using Student *t* test for continuous variables and Pearson χ^2 test for categorical variables. Parasite densities were log-transformed and reported as geometric means.

For our primary analysis, we assessed the validity of the dual-antigen RDT to detect severe malaria in children aged <12 years. To do so, we defined severe malaria in accordance with the guidelines for research and epidemiological studies [4]. We used the threshold of 250 000 parasites/μL (approximately 5%) to define hyperparasitemia. We defined abnormal bleeding as the presence of gross hemoglobinuria (4+ on urinalysis). These criteria are outlined in Table 1.

We estimated the sensitivity, specificity, and negative and positive likelihood ratios for a HRP2+/pLDH+ result (vs a HRP2+/pLDH– result) to identify severe malaria. Using our estimated sensitivity and specificity results and a range of severe malaria prevalence from 1% to 30%, we then assessed the negative (NPV) and positive predictive values (PPV) of the tests. We repeated these analyses restricted to children aged <5 years. We performed sensitivity analyses of these estimates by liberalizing thresholds for a number of criteria that define severe malaria.

Finally, we evaluated the test characteristics of the RDT for the detection of severe malaria in comparison to clinician impressions, which comprise key physical exam findings often termed "danger signs" (eg, impaired consciousness, shock, acidotic breathing) in the setting of *P. falciparum* parasitemia, as used by the health center clinical staff under routine conditions [4]. We recorded disposition plans (eg, discharge, admit, refer) as a surrogate marker for the presence of such danger signs. We also evaluated the test characteristics of the RDT compared to a "bedside exam," which we defined as the presence of outward clinical manifestations of severe malaria according to the criteria listed in Table 1 (eg, impaired consciousness, pulmonary edema, seizures) prior to a confirmed parasitological diagnosis or other laboratory-based evidence of severe malaria.

Ethics Statement

The institutional review boards of Partners Healthcare, the University of North Carolina at Chapel Hill, the Mbarara University of Science and Technology, and the Uganda National Council for Science and Technology provided ethical approval of the study. Written informed consent was obtained from all study participants.

RESULTS

Diagnostic Testing and Clinical Manifestations

A total of 2701 children aged <12 years presented with a clinical history and/or symptoms concerning for malaria. Baseline characteristics of the study cohort are shown in Table 2.

RDT results were available on 99.2% (2678/2701) of patients, nearly half (1248/2678, 46.6%) of whom displayed at least 1 positive antigen band. The HRP2+/pLDH+ result was the most common, accounting for 67.8% of all positive results, while an isolated HRP2 band (HRP2+/pLDH–) was seen in 30.7%, and a single pLDH band (HRP2–/pLDH+) in less than 2% of positive results. Of those participants with any positive RDT result, 75% (936/1248) chose to pursue additional diagnostic testing in the study protocol and were subsequently included in the analysis.

Table 2. Baseline Demographic and Clinical Characteristics Stratified by Rapid Diagnostic Test Result

Baseline Characteristic	All	HRP2+/pLDH–	HRP2+/pLDH+	*P* Value
Patients (n, %)	2678	383 (14.3)	846 (31.6)	—
Age, y (median, IQR)	5 (2–8)	6 (3 - 9)	6 (3–9)	.79
5–12	1438 (53.2)	246 (64.2)	545 (64.4)	.95
<5	1263 (46.8)	137 (35.8)	301 (35.6)	
Male sex (n, %)	1331 (50.2)	**190 (50.0)**	438 (52.4)	.44
Days since symptom onset (median, IQR)	2 (2–3)	3 (2 - 3)	2 (2 - 3)	.001
Reported symptoms (n,%)				
Fever	2192 (81.2)	351 (91.6	794 (93.9)	.16
Cough	1443 (53.4)	214 (55.9)	381 (45.0)	<.001
Rhinorrhea	719 (26.6)	105 (27.4)	185 (21.9)	.03
Arthralgias/Myalgias	316 (11.7)	52 (13.6)	135 (16.0)	.28
Diarrhea	408 (15.1)	43 (11.2)	114 (13.5)	.27
Convulsion	43 (1.6)	**5 (1.3)**	**20 (2.4)**	**.22**
Vital signs (n, %)				
Febrile	474 (18.2)	57 (15.5)	262 (31.9)	<.001
Tachycardic	464 (18.3)	64 (17.4)	543 (31.0)	<.001
Hypotensive/Shock	**79 (3.3)**	**8 (2.2)**	**24 (3.0)**	**.45**
Pulmonary edema	**60 (2.6)**	**7 (1.9)**	**21 (2.7)**	**.45**

Text in bold represents measures that define criteria for severe malaria.

Abbreviations: HRP2/pLDH, histidine-rich protein-2/pan-lactate dehydrogenase; IQR, interquartile range.

Nonparticipants were younger (median age 4 vs 7 years, $P < .001$) and more likely to have a HRP2+/pLDH– RDT result (31.1% vs 12.3%, $P < .001$) than participants included in the study. There were similar rates of fever (31.3% vs 29.4%, $P = .56$), tachycardia (26.3% vs 30.9%, $P = .15$), hypoxia (12.6% vs 8.7%, $P = .06$), hypotension (2.9% vs 2.6%, $P = .8$), and reported seizures (3.2% vs 1.7%, $P = .13$) between those included and those not included. Rates of admission (29.9% vs 25.3%, $P = .21$) and referral (1.1% vs 0.3%, $P = .19$) were also similar.

Using microscopy as the reference standard, the overall sensitivity of the RDT for the diagnosis of *P. falciparum* malaria was 98.2% (95% confidence interval [CI], 96.8%–99.0%). The specificity was 89.1% (95% CI, 85.9%–91.7%) for HRP2+/pLDH+ results and 52.1% (95% CI, 47.3%–57.0%) for HRP2+/pLDH– results ($P < .001$). Among the subset of samples that underwent parallel real-time PCR (n = 112), slide reading demonstrated a high degree of agreement ($\kappa = 0.94$). *Plasmodium falciparum* accounted for 95.4% of infections, while *Plasmodium malariae, Plasmodium ovale*, and mixed *P. falciparum* accounted for 2.3%, 1.2%, and 1.2% of infections, respectively, as determined by microscopy.

Results of blood and urine testing to assess for severe malaria are shown in Table 3. Those with HRP2+/pLDH+ results had significantly higher levels of parasitemia compared to individuals with HRP2+/pLDH– results (geometric mean, 13 051 parasites/μL vs 1126 parasites/μL, $P < .001$). All cases above the clinically relevant threshold of 100 000 parasites/μL and all 17 cases of hyperparasitemia were observed in the HRP2+/pLDH+ group.

Severe Malaria

A total of 83 (9.0%) children with a positive HRP2 or HRP2/pLDH RDT satisfied criteria for severe malaria. The sensitivity and specificity of a HRP2+/pLDH+ result for severe malaria was 97.6% (95% CI, 90.8%–99.6%) and 75.6% (95% CI, 73.8%–77.4%), respectively (Table 4). Two children with single-antigen HRP2+/pLDH– results met criteria for severe malaria. One had pulmonary edema, tachycardia, elevated lactate (2.5 mmol/L), and a parasite density of 10 087/μL, while another had shock (systolic blood pressure 61 mm Hg), elevated lactate (2.3 mmol/L), and a parasite density of 21 208/μL.

Using a severe malaria prevalence of 3.5% (83/2349) among all children who presented for testing, a HRP2+/pLDH+ result demonstrated a near-perfect NPV for the diagnosis of severe malaria (99.9%, 95% CI, 99.5%–99.9%) and a PPV of 12.8% (95% CI, 10.4%–15.7%). These estimates were robust to ranges of severe malaria prevalence from as low as 1% to as high as 30% (Supplementary Table 1).

When we restricted the analysis to children aged <5 years (Table 4), the proportion of children with severe malaria increased to 14.0% among those with a positive RDT. All 37 cases of severe malaria were identified by a HRP2+/pLDH+ RDT, with a resulting sensitivity of 100% (95% CI, 86.6%–100.0%), PPV of 20.9% (95% CI, 15.3%–27.8%), and NPV of 100% (95% CI, 99.5%–100.0%).

In multiple sensitivity analyses, we found that changes in the diagnostic criteria for severe malaria resulted in the identification of more cases but did not significantly impact the high

Table 3. Laboratory Results for Patients With Confirmed *Plasmodium falciparum* Parasitemia Stratified by Rapid Diagnostic Test Result

Characteristic	HRP2+/pLDH–	HRP2+/pLDH+	*P* Value
Patients (n, %)	77	579	—
Microscopy results			
Parasitemia (geometric mean, 95% CI)	1126/μL (686–1849)	13 051/μL (11 201–15 208)	<.001
Density >10 000 (n,%)	13 (16.3)	340 (59.4)	<.001
Density >100 000 (n,%)	0 (0.0)	81 (14.1)	<.001
Hyperparasitemia	**0 (0.0)**	**17 (3.0)**	**.12**
Gametocytes (n, %)	5 (5.8)	33 (5.7)	.99
Anemia			
Hemoglobin (mean, 95% CI)	11.5 g/dL (11.3–11.7)	11.0 g/dL (10.8–11.1)	<.001
Hb <5 g/dL (n,%)	**0 (0.0)**	**2 (0.4)**	**1.00**[a]
Hb <7 g/dL (n,%)	0 (0.0)	21 (3.8)	.09
Hypoglycemia (n,%)	**0 (0.0)**	**2 (0.4)**	**1.00**[a]
Kidney injury (n, %)	**0 (0.0)**	**0 (0.0)**	**-**
Metabolic acidosis (n,%)			
Lactate (mean, 95% CI)	1.73 mmol/L (1.52–1.94)	2.18 mmol/L (2.08–2.27)	.001
Lactate >5 mmol/L	**0 (0.0)**	**13 (2.6)**	**.19**
HCO_3 (mean, 95% CI)	23.2 mmol/L (22.6–23.9)	22.6 mmol/L (22.2–22.9)	.20
HCO3 < 15 (n,%)	**0 (0)**	**8 (1.6)**	**.61**[a]
Hematuria (n,%)	**0 (0)**	**11 (2.1)**	**.22**

Text in bold represents measures that define criteria for severe malaria.

Abbreviations: CI, confidence interval; HCO_3, bicarbonate; HRP2/pLDH, histidine-rich protein-2/*pan*-lactate dehydrogenase.

[a]Two sided *P* value obtained using Fisher exact test.

Table 4. Test Performance of 2-Antigen Band Rapid Diagnostic Test for the Diagnosis of Severe Malaria Among Children

	Malaria diagnosis		
Children Aged <12 Years[a]			
RDT	Nonsevere	Severe	**Total**
Negative and HRP2+/pLDH-	11 714 (99.9)	2 (0.1)	**1716 (100.0)**
HRP2+/pLDH+	552 (87.2)	81 (12.8)	**633 (100.0)**
	2266 (96.2)	**83 (3.5)**	**2349 (100.0)**
Children Aged <5 Years[b]			
Negative and HRP2+/pLDH-	901 (100.0)	0 (0.0)	**901 (100.0)**
HRP2+/pLDH+	140 (79.1)	37 (20.9)	**177 (100.0)**
	1041 (96.6)	**37 (3.4)**	**1078 (100.0)**

Abbreviations: CI, confidence interval; HRP2/pLDH, histidine-rich protein-2/pan-lactate dehydrogenase; NPV, negative predictive value; PPV, positive predictive value; RDT, rapid diagnostic test; SENS, sensitivity; SPEC, specificity.

[a]**SENS**: 97.6% (95% CI, 90.8%–99.6%), **SPEC**: 75.6% (95% CI, 73.8%–77.4%), **NPV**: 99.9% (95% CI, 99.5%–100.0%), and **PPV**: 12.8% (95% CI, 10.4%–15.7%).

[b]**SENS**: 100.0% (95% CI 86.6–100.0%), **SPEC**: 86.6% (95% CI 84.3–88.5%), NPV: 100.0% (95% CI 99.5–100.0%), and **PPV**: 20.9% (95% CI 15.3–27.8%).

sensitivity and NPV of the RDT, as all additional cases were identified in the HRP2+/pLDH+ positive group (Supplementary Table 2).

The use of clinical findings to identify severe malaria among those with a positive RDT result had relatively poor diagnostic validity compared to a HRP2+/pLDH+ test. The combination of a positive HRP2 RDT and the clinical criteria for severe malaria had significantly lower sensitivity (57/83, 68.7% vs 81/83, 97.6%, $P < .001$) but higher specificity (90.5% vs 75.6%, $P < .001$) compared to the multiple-antigen RDT. The most common manifestations of severe malaria in cases missed by the clinical criteria were lactic acidosis (10, 38.5%), hyperparasitemia (7, 26.9%), and metabolic acidosis (6, 23.1%). The geometric mean level of parasitemia in the missed cases was not statistically different from those identified using the bedside criteria ($P = .32$).

Similarly, clinician impression and disposition decisions had poor diagnostic performance. Clinicians identified and made appropriate plans for admission or referral in only 40 of 76 cases of severe malaria. The sensitivity of clinical decision making was 52.6%, ($P < .001$ compared to HRP2+/pLDH+ RDT) and the specificity was 81.6% ($P < .001$).

Using HRP2+/pLDH+ results to trigger referrals for microscopy and parenteral antimalarials, however, would result in more than twice as many referrals as current protocols in widespread use, which incorporate HRP2 RDTs and the bedside criteria, but would identify an additional 23 (27.7%) cases of severe malaria. Notably, when this analysis was restricted to children aged <5 years (Table 5), we found that the HRP2+/pLDH+ RDT alone performed with significantly higher sensitivity (100.0% vs 83.8%, $P < .001$) and similar specificity (86.6% vs 89.1%, $P = .08$) compared to the combination of screening with a HRP2 RDT and clinical exam. Additionally, the increase in the number of referrals was small (n = 33, 3.1%, $P = .05$).

DISCUSSION

A positive dual-antigen (HRP2+/pLDH+) RDT result has high sensitivity (97.6%) and NPV (99.9%) for the detection of severe malaria among Ugandan children aged <12 years. The assay was more sensitive than current protocols that use clinical measures to distinguish uncomplicated malaria from severe malaria and does not require a detailed physical exam. These test characteristics along with the low cost, wide distribution, and ease of use in the absence of laboratory infrastructure make it a promising screening tool to guide triage algorithms at peripheral health centers and to guide referral decisions in community-based fever management programs in resource-limited settings.

The relatively poor sensitivity of clinical symptoms and the clinical impression of health center staff to detect severe malaria was unexpected. More than half (16/26, 61.5%) of the cases missed by clinical scoring had severe lactic or metabolic acidosis, which has been associated with neurological involvement and increased mortality [25–29]. In contrast, the dual-band RDT result correctly identified all but 1 case of acidosis as well as all patients with hyperparasitemia.

When used to examine the study population of children aged <12 years, the assay was relatively nonspecific, and our results suggest that adoption of an algorithm based on dual-antigen RDTs would trigger significantly more referrals to health facilities than existing protocols (Table 5). The relatively low specificity of the assay might require alternate algorithms appropriate to local context in order to avoid overburdening fragile healthcare systems. Yet among children aged <5 years, in whom the

Table 5. Test Characteristics of Various Algorithms for the Diagnosis of Severe Malaria Among Children in Resource-Limited Settings

Diagnostic approach	Referred (n, %)	Sens (%)	Spec (%)	NPV (%)	PPV (%)
Children Aged <12 Years					
Clinical impression	211 (23.3)	52.6	81.6	94.8	21.1
Bedside exam	997 (42.1)	68.7	58.9	98.1	5.7
HRP2 RDT+ and bedside exam	273 (11.6)	68.7	90.5	98.7	20.9
HRP2+/pLDH+ RDT	**633 (27.0)**	**97.6**	**75.6**	**99.9**	**12.8**
Children Aged <5 Years					
Clinical impression	90 (36.4)	60.0	66.8	92.4	20.0
Bedside exam	722 (66.9)	83.8	33.8	98.3	4.3
HRP2 RDT+ and bedside exam	144 (13.4)	83.8	89.1	99.4	21.5
HRP2+/pLDH+ RDT	**177 (16.4)**	**100.0**	**86.6**	**100.0**	**20.9**

Clinical impression represents the disposition plan of the treating provider where a decision to admit or refer was considered evidence of "danger signs" and thus severe malaria. The bedside exam represents children who exhibited the outward clinical manifestations of severe malaria according to the criteria listed in Table 1 (eg, impaired consciousness, pulmonary edema, seizures) but did not have a confirmed parasitological diagnosis or other laboratory-based evidence of severe malaria. The HRP2 RDT+ and bedside exam category represents children with a positive HRP2-only RDT who displayed danger signs and thus is consistent with current World Health Organization guidelines for the diagnosis of severe malaria.

Abbreviations: HRP2/pLDH, histidine-rich protein-2/pan-lactate dehydrogenase; NPV, negative predictive value; PPV, positive predictive value; RDT, rapid diagnostic test; Sens, sensitivity; Spec, specificity.

prevalence of severe malaria was higher, this strategy demonstrated high sensitivity (100.0%) and fair specificity (86.2%) for the detection of severe malaria. The PPV of 20.9% suggests that approximately 1 in 5 referred children would have severe malaria, which is similar to the number of referrals produced by current protocols. Further studies are needed to validate and operationalize diagnostic and treatment algorithms that incorporate the dual-antigen RDT.

The strengths of our study include the large sample size, comprehensive laboratory analysis, and comparison to "real-world" clinically based diagnoses. Our study also has a number of limitations. First, we used expert microscopy rather than PCR as the reference standard for RDT accuracy, which may have resulted in a small misclassification bias. However, quality control testing on a subset of our results comparing microscopy to PCR demonstrated excellent agreement ($\kappa = 0.94$) between the 2 methods. Additionally, false-negative microscopy results were likely due to low levels of parasitemia, which is less commonly associated with severe malaria.

Second, our overarching goal was to identify a low-cost tool for detecting severe malaria and for guiding referral decisions in community-based fever management programs and at peripheral health centers where microscopy and advanced laboratory capacity are not routinely available. However, our study was conducted at a level III health center with the ability to diagnose and manage severe malaria. Thus, the study population may not be directly generalizable to more peripheral sites. However, to account for this, we assessed the NPV and PPV of the assay across a range of severe malaria prevalence estimates.

Additionally, we conducted the study in a setting of *P. falciparum* predominance, where non–*P. falciparum* infections account for an extremely low proportion (3.5%) of infections. Therefore, our findings, which rely on a pan-LDH antigen to provide a semiquantitative estimate of parasite density, are not generalizable to areas where other *Plasmodium* species are common.

Finally, we had a higher rate of study nonparticipation among younger children whose parents may have declined additional testing once the RDT results were known. However, we found little evidence in differences in clinical status between those who did and did not participate, partially mitigating this concern.

CONCLUSIONS

A dual-antigen RDT that detects HRP2 and pan-pLDH has high sensitivity for the detection of severe malaria in an area of high transmission and *P. falciparum* predominance, making it a promising tool in the triage of children with malaria in similar low-resource settings. The test was particularly sensitive among children aged <5 years. Notably, the RDT demonstrated signifi-cantly higher sensitivity for the detection of severe malaria than commonly used clinical criteria. Given the imperfect specificity of a dual-band positive assay, however, further work is needed to validate and operationalize diagnostic and treatment algorithms so as not to overwhelm referral networks.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. R. B. and M. S. conceived the study, collected the data, participated in the design of the study, performed the statistical analysis, and drafted the manuscript. R. R., M. M., M. N., and E. M. helped collect the data and draft the manuscript. All authors, external and internal, had full access to all of the data (including statistical reports and tables) from the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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