

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



LSHTM Research Online

Hendriksen, ICE; Mtove, G; Pedro, AJ; Gomes, E; Silamut, K; Lee, SJ; Mwambuli, A; Gesase, S; Reyburn, H; Day, NPJ; +3 more... White, NJ; von Seidlein, L; Dondorp, AM; (2011) Evaluation of a PfHRP(2) and a pLDH-based Rapid Diagnostic Test for the Diagnosis of Severe Malaria in 2 Populations of African Children. *Clinical infectious diseases*, 52 (9). pp. 1100-1107. ISSN 1058-4838
DOI: <https://doi.org/10.1093/cid/cir143>

Downloaded from: <http://researchonline.lshtm.ac.uk/827/>

DOI: <https://doi.org/10.1093/cid/cir143>

Usage Guidelines:

Please refer to usage guidelines at <http://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: <http://creativecommons.org/licenses/by-nc-nd/2.5/>

<https://researchonline.lshtm.ac.uk>

Evaluation of a PfHRP₂ and a pLDH-based Rapid Diagnostic Test for the Diagnosis of Severe Malaria in 2 Populations of African Children

Ilse C. E. Hendriksen,^{1,2} George Mtove,⁴ Alínia José Pedro,⁷ Ermelinda Gomes,⁷ Kamolrat Silamut,¹ Sue J. Lee,^{1,2} Abraham Mwambuli,⁵ Samwel Gesase,⁶ Hugh Reyburn,^{3,5} Nicholas P. J. Day,^{1,2} Nicholas J. White,^{1,2} Lorenz von Seidlein,⁸ and Arjen M. Dondorp^{1,2}

¹Mahidol-Oxford Research Unit, Bangkok, Thailand; ²Centre for Tropical Medicine, Churchill Hospital, Oxford University, Oxford, United Kingdom; ³London School of Tropical Medicine and Hygiene, London, UK; ⁴National Institute for Medical Research, Amani Centre, Tanga, Tanzania; ⁵Joint Malaria Programme, Tanzania; ⁶National Institute for Medical Research, Tanga, Tanzania; ⁷Hospital Central da Beira, Beira, Mozambique; and ⁸Menzies School of Health Research, Casuarina, Northern Territory, Australia

Background. Rapid diagnostic tests (RDTs) now play an important role in the diagnosis of falciparum malaria in many countries where the disease is endemic. Although these tests have been extensively evaluated in uncomplicated falciparum malaria, reliable data on their performance for diagnosing potentially lethal severe malaria is lacking.

Methods. We compared a *Plasmodium falciparum* histidine-rich-protein2 (PfHRP₂)-based RDT and a *Plasmodium* lactate dehydrogenase (pLDH)-based RDT with routine microscopy of a peripheral blood slide and expert microscopy as a reference standard for the diagnosis of severe malaria in 1898 children who presented with severe febrile illness at 2 centers in Mozambique and Tanzania.

Results. The overall sensitivity, specificity, positive predictive value, and negative predictive values of the PfHRP₂-based test were 94.0%, 70.9%, 85.4%, and 86.8%, respectively, and for the pLDH-based test, the values were 88.0%, 88.3%, 93.2%, and 80.3%, respectively. At parasite counts <1000 parasites/μL (*n* = 173), sensitivity of the pLDH-based test was low (45.7%), compared with that of the PfHRP₂-based test (69.9%). Both RDTs performed better than did the routine slide reading in a clinical laboratory as assessed in 1 of the centers.

Conclusion. The evaluated PfHRP₂-based RDT is an acceptable alternative to routine microscopy for diagnosing severe malaria in African children and performed better than did the evaluated pLDH-based RDT.

The clinical diagnosis of severe malaria is unreliable, because signs and symptoms overlap with other life-threatening febrile illnesses, including pneumonia, meningitis, and bacterial sepsis [1–3]. Because severe malaria

kills rapidly, prompt diagnosis and management are vital [4, 5]. On the other hand, overdiagnosis of severe malaria in African children is common and diverts attention from other infectious causes, which has been shown to contribute to mortality [6]. A rapid and reliable parasitological diagnosis of severe malaria is thus essential for proper management of patients with severe febrile illness. Microscopy remains the reference standard [7], but this requires the availability of a good microscope, significant technical skills, good-quality reagents, and clean slides. The diagnostic quality of microscopy is highly variable in routine hospitals in sub-Saharan Africa [8, 9].

Compared with microscopy, malaria rapid diagnostic tests (RDTs) do not require extensive training or well-maintained equipment. They are increasingly used for malaria diagnosis. Malaria RDTs are immunochromatographic tests that identify malaria antigens, most

Received 26 November 2010; accepted 08 February 2011.

The study is published with the permission of the Director General of the Tanzanian National Institute for Medical Research, Dar-Es-Salaam, Tanzania.

Correspondence: Arjen M. Dondorp, MD, PhD, Mahidol-Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, 3/F, 60th Anniversary Chalermprakiat Bldg, 420/6 Rajvithi Rd, Bangkok 10400, Thailand (arjen@tropmedres.ac).

Clinical Infectious Diseases 2011;52(9):1100–1107

© The Author 2011. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. 2011. All rights reserved. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/2.5/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

1058-4838/2011/529-0001\$37.00

DOI: 10.1093/cid/cir143

commonly *Plasmodium falciparum* histidine-rich-protein2 (PfHRP₂) or *Plasmodium* lactate dehydrogenase (pLDH). Disadvantages are that test results are qualitative and do not provide prognostic information, such as parasite staging and neutrophil pigment [10, 11].

RDTs have been evaluated extensively for the diagnosis of uncomplicated malaria but not for severe malaria [12], and diagnostic test requirements are different in severe disease; eg, a high sensitivity is of utmost importance, because missing a case may result in inappropriate treatment and death. We therefore compared the diagnostic performance of a commonly used PfHRP₂-based RDT (Paracheck; Orchid Biomedical) and a pLDH-based RDT (OptiMAL-IT; DiaMed) with that of expert microscopy, which was used as the reference standard, for the diagnosis of severe malaria in children with severe febrile illness who were admitted to 2 African hospitals in areas with different malaria transmission intensities.

METHODS

The study sites were in Teule Hospital in Muheza, Tanzania, and Hospital Central da Beira in Beira, Mozambique. The sites were chosen because of their different transmission dynamics, because this determines the a priori probability of the diagnosis, which influences test performance. The study was part of a large multicenter clinical trial that compared quinine and artesunate for the treatment of severe malaria [5]. Ethical approval was obtained from Comité Nacional de Bioética para a Saúde in Mozambique and the Tanzania Medical Research Coordinating Committee. Written informed consent for enrolled patients was obtained from attending relatives.

Teule Hospital is a rural 300-bed district hospital in Muheza in North-Eastern Tanzania. Malaria transmission is high, with an estimated Entomological Inoculation Rate (EIR) of 149 in 2000 [13]. In-patient pediatric human immunodeficiency virus (HIV) prevalence in children presenting with febrile illnesses was reported as 3.9% [14]. Beira Central Hospital is an 800-bed tertiary referral hospital in Beira in Central Mozambique. EIR has not been documented here, but the observed age distribution, including older children and occasionally including adults, suggests significantly lower transmission intensity than in Muheza [15]. HIV infection prevalence is high and was reported to be 16% in adults in 2005 [16].

Children (weight, ≥ 5 kg; age, < 15 years) who presented with severe febrile illness according to modified World Health Organization (WHO) clinical criteria for severe malaria [17] were screened using 2 malaria RDTs and a peripheral blood slide. Severity criteria included decreased consciousness (coma or severe prostration), convulsions, respiratory distress or acidotic breathing, shock, severe symptomatic anemia, hypoglycemia, hemoglobinuria, or severe jaundice. Health care workers

were trained to recognize the criteria for severe febrile illness, perform the RDTs, and prepare a peripheral blood slide, and they were supervised by the study site coordinator. In Tanzania, health workers were clinical officers and nurses who were employed for the research project. In Mozambique, hospital nurses performed the screening as part of routine care.

Definitions

Fever was defined as an axillary temperature $\geq 37.5^{\circ}\text{C}$ or by a history of recent fever. Coma was defined as a Blantyre Coma Score ≤ 2 for children < 2 years of age or a Glasgow Coma Score ≤ 10 for older children. Prostration was defined as the inability to sit unsupported (for children > 6 months of age) or the inability to drink or breast-feed in younger children. Convulsions were recorded in cases in which the duration was > 30 min or the frequency was ≥ 2 within the 24 h preceding hospital admission. Compensated shock was defined as a peripheral capillary refill time ≥ 3 sec or the presence of a temperature gradient with a normal systolic blood pressure (≥ 70 mmHg). Decompensated shock was defined as a systolic blood pressure < 70 mmHg. Severe respiratory distress was defined as nasal alar flaring, costal indrawing or recession or the use of accessory muscles, or severe tachypnoea, whereas severe acidosis was suspected if deep breathing was present. A blood glucose level < 3 mmol/L or clinical improvement in the level of consciousness immediately after administration of 10% dextrose was regarded as hypoglycemia. Anemia was defined as severe pallor combined with respiratory distress. Hemoglobinuria was assessed by caretaker history or observation of dark or black discoloration of the urine. Jaundice was assessed by clinical examination.

Sampling and Slide Reading

Paracheck test (Orchid Biomedical; Mumbai, India; 0.65 USD/test), OptiMAL-IT test (DiaMed AG; Cressier, Switzerland; 1.70 USD/test) and peripheral blood slides were prepared from blood samples taken by finger prick. Thin and thick blood films were stained with 5% Giemsa for 20 min. These slides were read by local laboratory technicians (routine practice slide reading), with later assessment by expert microscopy at the reference laboratory at the Mahidol Oxford Tropical Medicine Research Unit in Bangkok, Thailand.

In Beira, the slides were read by microscopists working in the routine hospital laboratory. These microscopists were blinded to the RDT results and reported thick film results using a semi-quantitative method with a 5-point scale [18]. In Muheza, microscopists were unblinded, and hence their findings were not included in the analysis.

Microscopists in the reference laboratory were blinded to the RDT results. The slide findings were reported as negative if no parasites were encountered per 500 white blood cells (WBCs) counted. Parasitemia was quantified in the thick film if < 1

parasite was encountered per 1000 red blood cells (RBCs) in the thin film, using the formula parasites/ μL = (parasites/200 WBCs) \times 40, assuming a WBC count of 8000 WBCs/ μL . Parasitemia was quantified in the thin film if >5 parasites were seen per field in the thick film, using the formula parasites/ μL = (parasites/1000 RBCs) \times 30 \times 125.6, assuming a hematocrit of 30%. Slides with gametocytes but no asexual parasites were scored as negative. Hemoglobin testing, HIV testing, and blood cultures were not performed routinely.

Data Management and Analysis

Data were double-entered using Access database software (Microsoft) and analyzed using Stata, version 10.0 (Stata). Sensitivity, specificity, and positive and negative predictive values were calculated using expert laboratory microscopy as the reference standard. Categorical variables were compared using the χ^2 or Fisher's exact test, and continuous variables were compared using Student's *t* test or the Mann-Whitney *U* test, depending on the distribution of the data. Sensitivities and specificities between methods were compared using McNemar's test [19]. To determine the prognostic significance of the clinical signs and symptoms, a logistic regression model was constructed with the positive malaria slide by the reference laboratory as the dependent variable and the age group and signs and symptoms of severe disease as independent variables. Age groups <3 and ≥ 3 years were chosen on the basis of the age distribution within sites. Using a backwards stepwise approach, only variables with $P < .05$ were retained in the final model.

RESULTS

From July 2005 through April 2009, 2190 patients were screened (Figure 1). Paired PfHRP₂ and pLDH test and slide results were available for 1898 patients, after excluding 40 patients (2%) who did not fulfill clinical severity criteria, 235 patients (10.7%) who lacked an evaluable slide for the reference laboratory, and 17

patients (0.8%) who were without a valid PfHRP₂- or pLDH-based test result. Excluded patients did not differ with respect to age, sex, or severity criteria.

Patients differed in age and clinical signs and symptoms between sites (Table 1). In Muheza, related to the higher malaria transmission rate, children were younger, and severe respiratory distress and prostration were the most frequent presenting symptoms. Severe anemia with respiratory distress was more prevalent at the Muheza site than it was at the Beira site. In Beira, the most common presenting symptoms were convulsions and coma. Hemoglobinuria and severe jaundice were rare at both sites. Peripheral blood parasitemia at hospital admission did not differ significantly between sites ($P = .711$).

One slide from the Muheza site showed mixed infection of *P. falciparum* and *P. ovale*. In 141 slides (7.4%), *P. falciparum* asexual parasites were detected but could not be quantified because of poor slide quality, which was most commonly caused by precipitations of the Giemsa stain.

A total of 345 patients had discordant results between the 3 diagnostic tests (Table 2). Most frequent were the combinations of a positive PfHRP₂ test result and negative slide findings (in 197 [10.4%] of 1898 patients) and a negative pLDH test result with positive slide findings (147 [7.7%] of 1898). Patients with a positive peripheral blood slide finding and a negative PfHRP₂-based test result all also had negative pLDH-based test results.

A small number of the patients with false-positive RDT results (5 [2.5%] of 197 PfHRP₂-based test results and 1 [1.3%] of 79 pLDH-based test results) showed *P. falciparum* gametocytes on the slide. The presence of gametocytes in the patients with negative blood slide findings was associated with a positive PfHRP₂ test result (5 of 197 with false-positive results vs 1 of 480 with true negative results; $P = .009$).

The PfHRP₂-based test was more sensitive than was the pLDH-based test (94.0% vs 88.0%; $P < .001$), but the pLDH test was more specific (88.3% vs 70.9%; $P = .001$). This difference in sensitivity and specificity was observed at both sites. Both RDTs performed better at the Muheza site than at the Beira site (Table 3).

RDT sensitivity correlated positively with peripheral blood parasitemia, as shown in Figure 2. The sensitivity of both tests was $<50\%$ with parasite counts <100 parasites/ μL . For the PfHRP₂-based test, sensitivity increased substantially with higher parasite densities (85% at parasite counts of 100–1000 parasites/ μL and $>90\%$ at parasite counts >1000 parasites/ μL). With the pLDH-based test, the sensitivity increased $>90\%$ only at parasite densities $>10,000$ parasites/ μL .

The peripheral blood slides assessed by hospital microscopists in Beira were compared with slide readings of the reference laboratory. Results were available for 861 of 874 patients. Using expert microscopy as the reference standard, the sensitivity of routine slide reading was significantly less than that of both

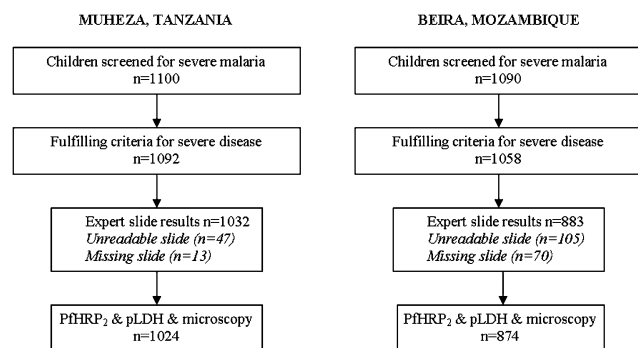


Figure 1. Study profile comparing 2 different rapid diagnostic tests with expert microscopy in African children presenting with severe febrile illness. PfHRP₂, *Plasmodium falciparum* histidine-rich-protein2; pLDH, *Plasmodium lactate* dehydrogenase.

Table 1. Baseline Characteristics of African Pediatric Patients with Severe Febrile Illness Screened for Severe Malaria, by Study Site

Variable	Muheza (n=1024)	Beira (n=874)	P
Data collection period	Dec 2006–Mar 2008	Jul 2005–Apr 2009	
Male sex	527 (51.5)	463 (53.0)	.512
Age, median months (IQR)	18 (9–35)	36 (24–65)	<.001
Presenting symptoms			
Severity criteria per patient, median no. of criteria (range)	2 (1–7)	2 (1–6)	.006
Coma	177 (17)	417 (48)	<.001
Prostration	412 (40)	338 (39)	.488
Convulsions	211 (21)	724 (83)	<.001
Decompensated shock	16 (2)	10 (1)	.434
Compensated shock	76 (7)	NA ^a	
Severe respiratory distress and/or acidotic breathing	636 (62)	27 (3)	<.001
Hypoglycemia	109 (11)	36 (4)	<.001
Severe anemia with respiratory distress	156 (15)	85 (10)	<.001
Hemoglobinuria	3 (<1)	16 (2)	.001
Severe jaundice	12 (1)	17 (2)	.171
Slide results/parasitemia			
Slide negative	409 (40)	268 (31)	<.001
Slide positive	530 (52)	550 (63)	
Slide positive but no Pf count	85 (8)	56 (6)	
Geometric mean parasites/ μ L ^b (95% CI)	35,196 (27,533–44,990)	32,974 (25,841–42,076)	.711

NOTE. Data are no. (%) of patients, unless otherwise indicated. CI, confidence interval; IQR, interquartile range; Pf, *Plasmodium falciparum*.

^a Compensated shock was not recorded at the moment of screening in Beira.

^b From slide-positive patients with Pf count.

RDTs: 78.0% (95% confidence interval [CI], 74.4%–81.2%) versus 92.2% for the PfHRP₂-based test and 84.8% for the pLDH-test ($P < .001$ for both). The specificity of routine slide reading was 84.0% (95% CI, 79.0%–88.2%), which is higher than that of the PfHRP₂ test (64.9%; $P < .001$) and the same as that of the pLDH test (82.5%, $P = .552$). The positive and negative predictive values of routine slide reading using expert microscopy as a reference test were 91.8% (95% CI, 89.0%–94.0%) and 62.5% (95% CI, 57.2%–67.6%), respectively.

The sensitivity of the RDTs improved with increasing numbers of presenting signs and symptoms of severe disease (data not shown). The sensitivity of both RDTs was highest in patients

who presented with reduced consciousness, severe anemia with respiratory distress, and hypoglycemia (Table 4).

A logistic regression model was used to identify independent predictors of slide positivity on the basis of clinical parameters and age group (<3 or ≥ 3 years of age). Reduced consciousness (adjusted odds ratio [AOR], 4.0; 95% CI, 3.2–5.1; $P < .001$), and convulsions (AOR, 1.7; 95% CI, 1.3–2.3; $P < .001$) were associated with slide positivity in the final model, adjusted for site. There was a significant interaction between age and severe anemia ($P = .024$), indicating a higher risk of severe anemia with younger age (for the <3-year-old age group: AOR, 5.8; 95% CI 3.6–9.4; $P = .001$; for the ≥ 3 -year-old age group: AOR, 2.2; 95% CI, 1.1–4.4; $P = .024$). Shock, severe respiratory distress,

Table 2. Rapid Diagnostic Test Results Compared with Expert Microscopy Findings

Variable	Slide positive (n=1221)		Slide negative (n=677)	
	pLDH positive	pLDH negative	pLDH positive	pLDH negative
pfHRP ₂ positive	1074	74	78	119
pfHRP ₂ negative	0	73	1	479

NOTE. PfHRP₂, *Plasmodium falciparum* histidine-rich-protein2; pLDH, *Plasmodium* lactate dehydrogenase.

Table 3. Comparison of the Performance of 2 Malaria Rapid Tests for the Diagnosis of Pediatric Severe *Falciparum* Malaria Compared with Expert Microscopy as the Reference Standard

Variable	Muheza (n=1024)	Beira (n=874)	Combined (n=1898)
Slide positive	615 (60)	606 (69)	1221 (64)
pfHRP₂-based test			
PfHRP ₂ -based test positive	692 (68)	653 (75)	1345 (71)
Sensitivity, % (95% CI)	95.8 (93.9–97.2)	92.2 (89.8–94.3)	94.0 (92.5–95.3)
Specificity, % (95% CI)	74.8 (70.3–79.0)	64.9 (58.9–70.6)	70.9 (67.3–74.3)
Positive predictive value, % (95% CI)	85.1 (82.2–87.7)	85.6 (82.7–88.2)	85.4 (83.4–87.2)
Negative predictive value, % (95% CI)	92.2 (88.7–94.8)	78.7 (72.7–83.9)	86.8 (83.7–89.5)
pLDH-based test			
pLDH-based test positive	592 (58)	561 (64)	1153 (61)
Sensitivity, % (95% CI)	91.1 (88.5–93.2)	84.8 (81.7–87.6)	88.0 (86.0–89.7)
Specificity, % (95% CI)	92.2 (89.1–94.6)	82.5 (77.4–86.8)	88.3 (85.7–90.7)
Positive predictive value, % (95% CI)	94.6 (92.5–96.3)	91.6 (89.0–93.8)	93.2 (91.5–94.5)
Negative predictive value, % (95% CI)	87.3 (83.8–90.3)	70.6 (65.2–75.6)	80.3 (77.2–83.1)

NOTE. CI, confidence interval; PfHRP₂, *Plasmodium falciparum* histidine-rich-protein2; pLDH, *Plasmodium* lactate dehydrogenase.

hypoglycemia, hemoglobinuria, and severe jaundice were not independent predictors of slide positivity.

Discussion

This is a large comparative study of RDTs for diagnosing severe malaria in severely ill children presenting to African hospitals. In our evaluation, the PfHRP₂-based test was a reliable alternative to routine microscopy for the diagnosis of pediatric severe malaria and was more sensitive than was the pLDH-based RDT, but this was at the expense of a lower specificity. The

PfHRP₂-based test sensitivity of 96.9% (95% CI, 95.7%–97.9%) for parasite densities >100 parasites/μL is above the WHO-recommended threshold of 95% [20]. The pLDH-based test had a sensitivity of 91.2% (95% CI, 89.3%–92.9%) for parasite densities >100 parasites/μL. Severe malaria requires a diagnostic test with high sensitivity, because missing the diagnosis and withholding treatment may well cause death. Conversely, sub-optimal specificity leads to underdiagnosis of other severe infections [6, 21].

It should be reminded that, even with slide-proven severe malaria, a substantial proportion of children have concomitant invasive bacterial infections that warrant antimicrobial treatment [14, 22].

The a priori probability of severe malaria depends on malaria transmission intensity and the prevalence of alternative diseases causing severe febrile illnesses, notably HIV/AIDS. Because HIV/AIDS prevalence is high in sub-Saharan Africa, including Mozambique [16], and with decreasing malaria transmission in several African countries [23], alternative diagnoses will become increasingly prevalent. In our study, all children met at least 1 of the WHO-defined criteria for severe malaria [17], but these clinical signs did not have a strong predictive value for peripheral blood parasitemia, confirming the findings from earlier studies [6]; only reduced consciousness had a predictive value for the diagnosis. Severe anemia was more common in the younger age group, as reported in other studies [24, 25].

We identified only 2 small studies that evaluated RDT performance for the diagnosis of severe or cerebral malaria [12, 26], whereas numerous studies have compared the performance of various PfHRP₂-based and pLDH-based RDTs in the laboratory or for diagnosis of uncomplicated malaria in the field. The

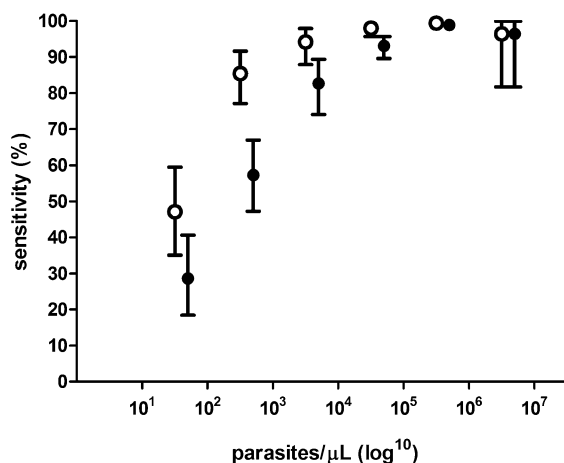


Figure 2. RDT sensitivity according to the level of peripheral blood parasitaemia (expressed as log₁₀ parasites/μL) from n=1080 patients with positive slide and parasite count. ●, *Plasmodium* lactate dehydrogenase (pLDH)-based test; ○, *Plasmodium falciparum* histidine-rich-protein2 (PfHRP₂)-based test. Bars represent 95% confidence intervals.

Table 4. Parasitaemia and Rapid Diagnostic Test Performance by Presenting Clinical Signs

Variable	Total, no. (%) of patients	Positive slide results, no. (%) of patients	Parasite count, geometric mean parasites/ μ L (95% CI)	PfHRP ₂		pLDH	
				Sensitivity, % (95% CI)	Specificity, % (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)
Clinical signs							
Reduced consciousness ^a	1344 (71)	999 (74)	42,730 (35,607–51,279)	96.1 (94.7–97.2)	58.6 (53.2–63.8)	91.1 (89.2–92.8)	79.7 (75.1–83.8)
Convulsions	935 (49)	669 (72)	34,972 (27,744–44,082)	94.3 (92.3–96.0)	61.3 (55.1–67.2)	87.7 (85.0–90.1)	80.5 (75.2–85.0)
Shock ^b	102 (5)	72 (71)	50,789 (24,707–104,403)	97.2 (90.3–99.7)	53.3 (34.3–71.7)	90.3 (81.0–96.0)	80.0 (61.4–92.3)
Severe respiratory distress and/or acidotic breathing	663 (35)	354 (53)	41,675 (30,271–57,377)	93.8 (90.7–96.1)	80.9 (76.1–85.1)	90.1 (86.5–93.0)	94.5 (91.3–96.8)
Hypoglycemia	145 (8)	111 (77)	132,645 (78,968–222,809)	98.2 (93.6–99.8)	50.0 (32.4–67.6)	98.2 (93.6–99.8)	73.5 (55.6–87.1)
Severe anemia with respiratory distress	241 (13)	207 (86)	76,895 (53,610–110,294)	98.1 (95.1–99.5)	35.3 (19.8–53.5)	96.6 (93.2–98.6)	61.8 (43.6–77.8)
Hemoglobinuria	19 (1)	17 (89)	29,713 (6,255–141,151)	100 (80.5–100)	50.0 (1.3–98.7)	100 (80.5–100)	50.0 (1.3–98.7)
Severe jaundice	29 (2)	20 (69)	92,446 (31,960–267,405)	100 (83.2–100)	88.9 (51.8–99.7)	100 (83.2–100)	88.9 (51.8–99.7)

NOTE. CI, confidence interval; PfHRP₂, *Plasmodium falciparum* histidine-rich-protein2; pLDH, *Plasmodium* lactate dehydrogenase.

^a Reduced consciousness included coma or prostration.

^b Shock included compensated or decompensated shock.

WHO and the Foundation for Innovative New Diagnostics (FIND) evaluated 68 RDT, including both RDTs used in our study [10, 11]. Although assessed under laboratory conditions, the FIND evaluation confirmed that, with parasite counts <200 parasites/ μ L, detection rates decreased substantially for most RDTs. The WHO/FIND recommendation for RDT procurement requires a minimum detection score of 50% at a *P. falciparum* parasite count of 200 parasites/ μ L [27]. The pfHRP2-based test evaluated in our study complied with this, whereas the pLDH-based test did not.

In field studies evaluating pfHRP2- and pLDH-based RDTs for the diagnosis of uncomplicated malaria, some studies report sensitivities >95% for both RDTs [28–31], but most studies that directly compare PfHRP2 with pLDH-based tests confirm a higher sensitivity (and lower specificity) for RDTs detecting PfHRP2 [32]. For example, a study by Hopkins et al [33] found a sensitivity of 92% for a PfHRP2-based test and 85% for a pLDH-based test, which was mainly attributable to a better performance of the PfHRP2-based test at low parasite densities.

Although, in our evaluation, both RDTs performed poorly at very low parasite densities, even at very low parasite densities, the sensitivity of the PfHRP2-based test was significantly better, and this better performance was apparent at parasite counts up to 100,000 parasites/ μ L (Figure 2).

The persistence of PfHRP2 in the bloodstream for an extended period of up to 1 month following successful malaria parasite clearance is well documented [34–36]. This is in contrast with the kinetics of pLDH, in which enzyme activity is no longer detectible after parasite clearance [37], which contributes to the higher specificity of the pLDH test. In addition, gametocytes are known to produce PfHRP2, which contributes to false-positive PfHRP2 test results [38, 39]. Indeed, in patients with negative slide findings, the presence of gametocytes was associated with false-positive PfHRP2-based test results.

False-negative PfHRP2-based test results occurred in 1% of patients with parasite counts >100,000 parasites/ μ L, including 1 patient with a parasite count of 1,073,880 parasites/ μ L. This could be related to the so-called prozone effect that has been reported for PfHRP2-based RDTs, which is the phenomenon that an excess of either antigen or antibodies can cause a false-negative test result. The prozone effect has not been observed in association with pLDH-based tests [40]. Alternative explanations for false-negative RDT results are PfHRP2 gene polymorphisms that potentially change the antigenicity of PfHRP2 [41–43]. This has also been postulated for the gene encoding pLDH but, to date, this has not been substantiated.

RDT performance was better at the site in Tanzania than at the site in Mozambique. Most likely, these differences are explained by operator-related issues, because in Tanzania, the tests were operated by research staff. A contributing factor could

have been that the pLDH-based test was perceived as more complicated to operate, because it requires multiple steps [10, 11, 31]. Additionally, weak positive results are easier to detect with the PfHRP₂-based test, in which a thin test band can be observed, than with the pLDH-based test, in which a faint test band indicates a weak positive result.

A limitation of our study was the presence of only a single observer to report the RDT result and the absence of batch or stability testing. However, the RDTs were shipped to the study sites by cold-chain transport, and the study site coordinator and the independent monitor assured that batches were used within the expiration date, as well as stored in air-conditioned, temperature-controlled rooms. The PfHRP₂-based tests are relatively heat-stable, but some of the pLDH-based tests, particularly the one evaluated in this study, are known to be heat-unstable [10, 11, 31]. At both study sites, the staff were regularly trained and supervised in slide preparation and RDT operation.

RDTs have mainly been promoted for outpatient management of uncomplicated malaria, but the challenges of microscopy in sub-Saharan Africa are likely to extend their use to inpatient settings [8, 44]. At the study site in Mozambique, which is a tertiary care center with experienced microscopists, the sensitivity of routine microscopy was significantly lower than that of both RDTs, despite the provision of good quality reagents and training. Where the current WHO guidelines leave uncertainty about the best method for a parasitological diagnosis of severe malaria in young children, the findings of our study suggest that a PfHRP₂-based RDT is considerably better than routine microscopy. To optimize the diagnosis of severe malaria and severe illness, a diagnostic algorithm could be employed in which only negative RDT results should be confirmed by reliable microscopy. A reduction in the workload of the hospital laboratory could improve microscopy quality. A negative RDT result should trigger contemplation of an alternative diagnosis. A positive RDT result does not exclude co-existing bacterial infections, and antimicrobial treatment is recommended.

In conclusion, this study shows that the PfHRP₂-based RDT is a reliable and easy-to-perform alternative to routine microscopy for the diagnosis of severe malaria in African children and performs better than does a pLDH-based test.

Acknowledgments

We thank the patients and their parents; all clinical staff and laboratory technicians from the “Banco de Socorros” from Hospital Central da Beira and the Joint Malaria Research staff from Teule Hospital in Muheza; and Forradee Nuchsongsin, Benjamas Intharabut, Ketsanee Srinamon, and Kesinee Chotivanich of the Malaria Laboratory of Mahidol-Oxford Research Unit in Bangkok, Thailand.

Financial support. The Wellcome Trust (grants 076908 and 082541), and was coordinated as part of the Wellcome Trust Mahidol University Oxford Tropical Medicine Research Programme funded by the Wellcome Trust of Great Britain.

Potential conflicts of interest. All authors: no conflicts.

References

1. English M, Punt J, Mwangi I, McHugh K, Marsh K. Clinical overlap between malaria and severe pneumonia in Africa children in hospital. *Trans R Soc Trop Med Hyg* **1996**; 90:658–62.
2. Molyneux E, Walsh A, Phiri A, Molyneux M. Acute bacterial meningitis in children admitted to the Queen Elizabeth Central hospital, Blantyre, Malawi in 1996–97. *Trop Med Int Health* **1998**; 3:610–8.
3. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med* **2005**; 352:39–47.
4. Marsh K, Forster D, Waruiru C, et al. Indicators of life-threatening malaria in African children. *N Engl J Med* **1995**; 332:1399–404.
5. Dondorp AM, Fanello CI, Hendriksen IC, et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet* **2010**; 376:1647–57.
6. Reyburn H, Mbatia R, Drakeley C, et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ* **2004**; 329:1212.
7. World Health Organization (WHO). Guidelines for the treatment of malaria. Geneva, Switzerland: WHO, 2010. ISBN 9241546948.
8. McMorro ML, Masanja MI, Abdulla SM, Kahigwa E, Kachur SP. Challenges in routine implementation and quality control of rapid diagnostic tests for malaria—Rufiji District, Tanzania. *Am J Trop Med Hyg* **2008**; 79:385–90.
9. Nankabirwa J, Zurovac D, Njogu JN, et al. Malaria misdiagnosis in Uganda—implications for policy change. *Malar J* **2009**; 8:66.
10. World Health Organization (WHO). Malaria rapid diagnostic test performance. Results of WHO product testing of malaria RDTs: round 1 (2008). Geneva, Switzerland: WHO, 2009. Available at: http://www.finddiagnostics.org/resource-centre/reports_brochures/malaria-diagnostics-report-2009.html Accessed 14 November 2010.
11. World Health Organization (WHO). Malaria rapid diagnostic test performance. Results of WHO product testing of malaria RDTs: round 2 (2009). Geneva, Switzerland: WHO, 2010. Available at: http://www.finddiagnostics.org/resource-centre/reports_brochures/malaria-diagnostic-test-report-round2.html. Accessed 14 November 2010.
12. Birku Y, Welday D, Ayele D, Shepherd A. Rapid diagnosis of severe malaria based on the detection of Pf-Hrp-2 antigen. *Ethiop Med J* **1999**; 37:173–9.
13. Maxwell CA, Chambo W, Mwaimu M, Magogo F, Carneiro IA, Curtis CF. Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. *Malar J* **2003**; 2:28.
14. Nadjm B, Amos B, Mtove G, et al. WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense *Plasmodium falciparum* transmission: prospective study. *BMJ* **2010**; 340:c1350.
15. Alonso PL, Sacarlal J, Aponte JJ, et al. Duration of protection with RTS, S/AS02A malaria vaccine in prevention of *Plasmodium falciparum* disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. *Lancet* **2005**; 366:2012–2018.
16. Brentlinger PE, Behrens CB, Kublin JG. Challenges in the prevention, diagnosis, and treatment of malaria in human immunodeficiency virus infected adults in sub-Saharan Africa. *Arch Intern Med* **2007**; 167:1827–36.
17. World Health Organization (WHO). Severe and complicated malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2000**; 94(Suppl. 1):S1–S90.
18. World Health Organization (WHO). Basic malaria microscopy. Geneva, Switzerland: WHO, 1991. ISBN 9241544309.
19. Newcombe RG. Simultaneous comparison of sensitivity and specificity of two tests in the paired design: a straightforward graphical approach. *Stat Med* **2001**; 20:907–15.
20. World Health Organization (WHO). Malaria rapid diagnosis: Making it work. Meeting report 20–23 January. Geneva, Switzerland: WHO,

2003. Available at: <http://who.int/malaria/publications/atoz/rdt2/en> Accessed 19 April 2010.
21. Gwer S, Newton CR, Berkley JA. Over-diagnosis and co-morbidity of severe malaria in African children: a guide for clinicians. *Am J Trop Med Hyg* **2007**; 77(6 Suppl):6–13.
 22. Berkley JA, Bejon P, Mwangi T, et al. HIV infection, malnutrition, and invasive bacterial infection among children with severe malaria. *Clin Infect Dis* **2009**; 49:336–43.
 23. D'Acromont V, Lengeler C, Genton B. Reduction in the proportion of fevers associated with *Plasmodium falciparum* parasitaemia in Africa: a systematic review. *Malar J* **2010**; 9:240.
 24. Dondorp AM, Lee SJ, Faiz MA, et al. The relationship between age and the manifestations of and mortality associated with severe malaria. *Clin Infect Dis* **2008**; 47:151–7.
 25. Snow RW, Omumbo JA, Lowe B, et al. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet* **1997**; 349:1650–4.
 26. Shujatullah F, Malik A, Khan HM, Malik A. Comparison of different diagnostic techniques in *Plasmodium falciparum* cerebral malaria. *J Vector Borne Dis* **2006**; 43:186–90.
 27. World Health Organization (WHO). Information note on interim selection criteria for procurement of malaria rapid diagnostic tests (RDTs). Geneva, Switzerland: WHO, 2010. Available at: http://www.who.int/malaria/diagnosis_treatment/diagnosis/RDT_selection_criteria.pdf Accessed 14 November 2010.
 28. Bojang KA. The diagnosis of *Plasmodium falciparum* infection in Gambian children, by field staff using the rapid, manual, ParaSight-F test. *Ann Trop Med Parasitol* **1999**; 93:685–7.
 29. Kyabayinze DJ, Tibenderana JK, Odong GW, Rwakimari JB, Counihan H. Operational accuracy and comparative persistent antigenicity of HRP2 rapid diagnostic tests for *Plasmodium falciparum* malaria in a hyperendemic region of Uganda. *Malar J* **2008**; 7:221.
 30. Mawili-Mboumba DP, Bouyou Akotet MK, Ngoungou EB, Kombila M. Evaluation of rapid diagnostic tests for malaria case management in Gabon. *Diagn Microbiol Infect Dis* **2009**; 66:162–8.
 31. Ashley EA, Touabi M, Ahrer M, et al. Evaluation of three parasite lactate dehydrogenase-based rapid diagnostic tests for the diagnosis of falciparum and vivax malaria. *Malar J* **2009**; 8:241.
 32. Ochola LB, Vounatsou P, Smith T, Mabaso ML, Newton CR. The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. *Lancet Infect Dis* **2006**; 6:582–8.
 33. Hopkins H, Kambale W, Kamya MR, Staedke SG, Dorsey G, Rosenthal PJ. Comparison of HRP2- and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda. *Am J Trop Med Hyg* **2007**; 76:1092–7.
 34. Mayxay M, Pukrittayakamee S, Chotivanich K, Looareesuwan S, White NJ. Persistence of *Plasmodium falciparum* HRP-2 in successfully treated acute falciparum malaria. *Trans R Soc Trop Med Hyg* **2001**; 95:179–82.
 35. Iqbal J, Siddique A, Jameel M, Hira PR. Persistent histidine-rich protein 2, parasite lactate dehydrogenase, and panmalarial antigen reactivity after clearance of *Plasmodium falciparum* mono-infection. *J Clin Microbiol* **2004**; 42:4237–41.
 36. Swarthout TD, Counihan H, Senga RK, van den Broek I. Paracheck-Pf accuracy and recently treated *Plasmodium falciparum* infections: is there a risk of over-diagnosis? *Malar J* **2007**; 6:58.
 37. Oduola AM, Omitowoju GO, Sowunmi A, et al. *Plasmodium falciparum*: evaluation of lactate dehydrogenase in monitoring therapeutic responses to standard antimalarial drugs in Nigeria. *Exp Parasitol* **1997**; 87:283–9.
 38. Hayward RE, Sullivan DJ, Day KP. *Plasmodium falciparum*: histidine-rich protein II is expressed during gametocyte development. *Exp Parasitol* **2000**; 96:139–46.
 39. Tjitra E, Suprianto S, McBroom J, Currie BJ, Anstey NM. Persistent ICT malaria P.f/P.v panmalarial and HRP2 antigen reactivity after treatment of *Plasmodium falciparum* malaria is associated with gametocytemia and results in false-positive diagnoses of *Plasmodium vivax* in convalescence. *J Clin Microbiol* **2001**; 39:1025–31.
 40. Gillet P, Mori M, Van Esbroeck M, Van den Ende J, Jacobs J. Assessment of the prozone effect in malaria rapid diagnostic tests. *Malar J* **2009**; 8:271.
 41. Lee N, Baker J, Andrews KT, et al. Effect of sequence variation in *Plasmodium falciparum* histidine-rich protein 2 on binding of specific monoclonal antibodies: implications for rapid diagnostic tests for malaria. *J Clin Microbiol* **2006**; 44:2773–8.
 42. Baker J, McCarthy J, Gatton M, et al. Genetic diversity of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. *J Infect Dis* **2005**; 192:870–7.
 43. Mariette N, Barnadas C, Bouchier C, Tichit M, Menard D. Country-wide assessment of the genetic polymorphism in *Plasmodium falciparum* and *Plasmodium vivax* antigens detected with rapid diagnostic tests for malaria. *Malar J* **2008**; 7:219.
 44. de Oliveira AM, Skarbinski J, Ouma PO, et al. Performance of malaria rapid diagnostic tests as part of routine malaria case management in Kenya. *Am J Trop Med Hyg* **2009**; 80:470–4.