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Performance of Rapid Diagnostic Testing in Patients with Suspected Malaria in Cambodia, a Low-Endemicity Country Aiming for Malaria Elimination

To the Editor—We read with interest the article by Ranadive et al [1] assessing the performance of malaria rapid diagnostic testing (RDT) vs polymerase chain reaction (PCR) in Swaziland, a low-transmission country aiming at elimination. Through a large regional data set collected from 37 health facilities over 2 years, they demonstrated the poor sensitivity of RDT (First Response Malaria Ag P. falciparum HRP-2 Detection Rapid Card Test, Premier Medical) for *Plasmodium falciparum* (*Pf*) diagnosis (51.7%), due to a high proportion of low-density infections among symptomatic subjects (54/162 [33.3%]), along with a low positive predictive value (PPV) (67.3% for all samples and 62.3% for ≥100 parasites/μL samples), due to the high proportion of false positivity (32.4%). To overcome some of the limitations of the study (eg, the decision to include only 10% of negative RDTs samples), the authors called for more inclusive analyses.

We would like to share our ongoing experience in Chey Saen district (population 22 499, 27 villages), Preah Vihear province, Cambodia [2]. The district is served by 3 health centers, 2 health posts, and 28 village malaria workers. In 2014, the *Pf* prevalence detected by PCR was estimated at 0.73‰ [3]. The incidence of symptomatic *Pf* infections in 2016 was 3.6‰. Since 2014, a network of malaria RDT providers has been supported and trained by Médecins Sans Frontières, in providing national guidelines treatment and in the RDT use (SD FK80 p.f/P.v Malaria Antigen Rapid Test, Standard Diagnostics). Since October 2015, the network is routinely collecting filter paper blood spots for subsequent qualitative and quantitative (using parasite density-calibrated controls) real-time PCR diagnosis (Institut Pasteur in Cambodia) [2, 4].

We conducted an overall analysis of the data collected between October 2015 and March 2017. A total of 4382 patients with suspected malaria were tested with both RDT and PCR. Of the 168 PCR-positive *Pf* samples, 23.8% (40/168) had a parasite density <100/μL.

Table 1 displays all RDT and PCR results either including (n = 4382) or excluding samples with parasitemia <100/μL (n = 4342). The false-positive and false-negative rates were 11.0% (15/136) and 1.1% (47/4246), respectively. The sensitivity of RDT (vs PCR) was 72.0% (95% confidence interval [CI], 64.5%–78.5%), compared to 90.6% (95% CI, 83.8%–94.8%) after exclusion of low parasitemia samples. The negative predictive value increased from 98.9% to 99.7% when low-density samples were excluded. In both analyses, specificity was 99.7%, and the PPV scored 89.0% and 88.5%, respectively. Low parasitemia was the main reason for false-negative RDT

Table 1. Comparison of Rapid Diagnostic Test (RDT) and Polymerase Chain Reaction (PCR) Results Among All Samples and Samples From High Density (≥100 Parasites/μL) Infections—Diagnostic accuracy of RDTs Versus PCR as Gold Standard

	PCR Positive, No.	PCR Negative, No.	Total, No.	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
All samples							
RDT positive	121	15	136				
RDT negative	47	4199	4246				
Total	168	4214	4382				
RDT accuracy				72.0 (64.5–78.5)	99.7 (99.4–99.8)	89.0 (82.2–93.5)	98.9 (98.5–99.2)
Excluding samples with parasite density <100/μL							
RDT positive	116	15	131				
RDT negative	12	4199	4211				
Total	128	4214	4342				
RDT accuracy				90.6 (83.8–94.8)	99.7 (99.4–99.8)	88.5 (81.5–93.2)	99.7 (99.5–99.8)

Abbreviations: CI, confidence interval; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; RDT, rapid diagnostic test.

results (35/47 [74.5%]). For the remaining 25.5%, although rare in Asia [5], the deletions of *pfhrp2* and/or *pfhrp3* genes are currently investigated.

In conclusion, the present study complements the previous findings by Ranadive [1]. In particular, it grants more accuracy to the RDT in terms of PPV (89.0% vs 67.3%). Moreover, it confirms that the sensitivity of RDT, although higher than previously calculated (72.0% vs 51.7%), remains insufficient to meaningfully detect *Pf* infection in low-transmission, preelimination areas.

Note

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Reply to Rossi et al

To the Editor—We thank Rossi and colleagues for sharing their findings from Cambodia [1], which complement our recent article reporting limitations of rapid diagnostic testing in patients with suspected malaria from Swaziland, a low-endemic country in southern Africa aiming to eliminate malaria [2]. Using polymerase chain reaction (PCR) as gold standard, they performed a diagnostic accuracy evaluation of rapid diagnostic testing (RDT) to diagnose *Plasmodium falciparum* in subjects with suspected malaria. Sensitivity was low at 72% (compared to 52% in our study). Low-density infection, defined as <100 parasites/μL, explained 75% of false-negative results (compared to 76% in our study). With the large sample size of 4382 patients, sampling of all RDT negatives (vs selective sampling employed in our study), and use of quantitative PCR, the study is a useful addition to the few published studies on performance of RDT to assess symptomatic malaria in low-transmission settings [1, 3, 4].

As malaria transmission declines, the proportion of low-density infection among symptomatic as well as asymptomatic individuals increases [5–7]. It is generally assumed that symptomatic individuals will present with high-density infection; however, low-density infections accounted for 24% of all PCR-positive cases, compared to 22% in our study (taking into accounting the sampling of RDT negatives). Given the low prevalence of infection in these settings [8, 9], the unexpectedly high proportion of low-density infection cannot solely be explained by background parasitemia. Rather, patients in low-endemic settings may have a lower pyrogenic threshold for malaria due to decreased immunity, other host factors, or virulence of the parasite [10]. Interestingly, *Plasmodium falciparum* strains from Cambodia have been associated with a lower pyrogenic threshold than some African and American strains [10]. Early access to care, before the parasite has undergone multiple cycles of replication, would be facilitated by village malaria workers in the Rossi et al study and may also explain the low parasite densities observed.

Missed low-density infections represent missed opportunities to prevent further transmission. They also represent missed opportunities for transmission reduction activities in the community, as passively identified cases may trigger targeted interventions such as active case detection and vector control. On the flip side, overdiagnosis is also a problem. We would like to note that the false-positivity rates, or the percentage of healthy individuals who incorrectly receive a positive test result, were incorrectly reported in both studies. The correct false-positive rates were low at 5.9% in Swaziland and 0.3% in Cambodia (not 32% and 11%, respectively). However, due to the low prevalence of malaria, positive predictive values (PPVs) were compromised. Rossi et al report a higher PPV than our study (89%, compared to 67% in Swaziland), but a PPV of 89% still equates to overtreatment in roughly one-tenth of patients, and potential "overintervention" in the communities where activities were triggered by passively detected cases. A new RDT with reported sensitivity 10 times higher than current RDTs has recently been launched. While its use has potential to reduce transmission [11], there may be compromises in specificity due to the fact that the target antigen can persist in the bloodstream for several weeks, despite clearance of infection. Confirmatory testing with a highly specific test, as is done with human immunodeficiency virus testing, may be one solution. Certainly, as alternative diagnostic approaches are being considered for malaria, the balance of predictive values, sensitivity, specificity, as well as impact at individual and community levels, will need to be thoughfully considered.

Notes

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