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Editorial
How Do We Best Diagnose Malaria in Africa?

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For many decades, the cornerstone of malaria management in Africa was to treat all febrile children with chloroquine. With high-level resistance to chloroquine and improved means of malaria diagnosis, recommendations for the management of malaria in Africa have changed in two important ways in the last few years. First, recommended therapy for uncomplicated falciparum malaria has moved to highly effective artemisinin-based combination therapies.[1] Second, it is now recommended that the treatment of malaria be confined to parasitologically confirmed cases. This recommendation requires the availability of reliable diagnostic tests. The gold standard test for the diagnosis of malaria is microscopy. Evaluation of Giemsa-stained thick smears, when performed by expert microscopists, provides accurate diagnosis of malaria, although assuring expert slide preparation and reading can be difficult.[2] Indeed, microscopy is often unavailable, especially in rural settings. In this regard, the advent of rapid diagnostic tests (RDTs) for malaria is an important advance. Multiple immunochromatographic tests, incorporating a number of different parasite antigens and produced by many different manufacturers, are now available.[3] At best, these tests offer a simple, fairly inexpensive, and reliable means of diagnosis that can be performed by healthcare workers with limited training. However, concerns with RDTs include potential unreliability because of inconsistent manufacture or poor storage, uncertain supply, and potential misreading of results by unskilled health workers. An additional, generally unappreciated concern when considering RDTs is differences between available tests.

RDTs for malaria are based principally on the detection of one of three antigens, histidine-rich protein-2 (HRP2), lactate dehydrogenase (LDH), and aldolase. These tests have important differences. HRP2 is only expressed by *Plasmodium falciparum*, and therefore, HRP2-based tests necessarily diagnose only falciparum malaria. This limitation has generally not been considered important in Africa, where in most areas, over 95% of episodes of malaria are caused by *P. falciparum*. In addition, HRP2 can circulate for an extended period of time after an infection has been cleared[4] and is also expressed by gametocytes.[5] Thus, false-positive tests may be caused by recent but not current infection. LDH-based RDTs can be designed to recognize only the *P. falciparum* antigen or those antigens produced by all human malaria parasites. LDH tests are generally less sensitive than those tests directed against HRP2. However, they may be more specific, because the antigen circulates only briefly after eradication of infection.[4] In a study from multiple sites in Uganda using microscopy as a gold standard, an HRP2-based assay offered improved sensitivity over an LDH-based assay, and the negative predictive value of the LDH-based test dropped significantly as transmission intensity increased; thus, the HRP2-based test was recommended for areas with medium to high levels of malaria transmission.[6] Genus-specific aldolase-based RDTs are also available; these RDTs are offered with HRP2 detection to provide pan-species diagnosis of malaria and diagnosis of falciparum malaria in a single test.

Surprisingly, the malaria control community seems to pay quite little attention to the type of RDT that is used for malaria diagnosis. World Health Organization (WHO) guidelines state that the tests "have different characteristics, which may affect suitability for use in different situations," but they do not offer specific guidance as to the choice of test.[1] In practice, HRP2-based tests have been most widely used, with the improved sensitivity of these tests considered more important than their potential for loss of specificity because of identification of antigen after prior infections have been cleared. Indeed, there is particular interest in avoiding false negatives, because failure to treat a child with acute falciparum malaria can have disastrous consequences. However, might the HRP2-based RDT also engender concerns regarding sensitivity?

In this issue of the journal, Koita and others[7] identify another limitation of HRP2-based RDTs in Africa. Koita and others[7] evaluated 480 blood samples from Mali that were positive by standard malaria microscopy and found that 26 were negative by HRP2-based RDT. All of these samples were positive for malaria based on repeat microscopy and polymerase chain reaction (PCR) using primers that amplify conserved *P. falciparum* sequences. However, for the 22 evaluable samples, amplification of the HRP2 gene was successful for only 12; 10 samples, thus, seemed to include *P. falciparum* lacking an intact HRP2 gene. All of these samples contained monoclonal infections, whereas most of the HRP2-positive samples were polyclonal, supporting the existence in Mali of a minority of *P. falciparum* parasites that do not express the HRP2 gene. The results do not rule out the possibility that these parasites expressed an altered HRP2 that was not recognized by the RDT and whose gene was not amplified by standard primers. Regardless, these infections were not identified by the RDT, pointing to definitive false negatives for this test. Similarly, failure to amplify the HRP2 gene was recognized previously in multiple *P. falciparum* samples from Peru.[8]

The new work had some limitations.[7] First, it studied samples that were collected in 1996 and then stored for an undisclosed number of years before PCR analysis, potentially increasing the likelihood of false-negative assays for HRP2. It is not clear if parasites from Mali and elsewhere in Africa are more or less likely to be undetectable by HRP2-based RDT at the present time. Second, only one set of primers was used to detect the HRP2 gene in these samples. It is possible that the gene was present in some or all of these samples but that the primers could not detect the gene because of some variation in sequence. Third, all HRP2-negative infections in Mali

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were asymptomatic. Symptomatic infections caused by HRP2-negative parasites were noted in Peru,[8] but it is not clear if such parasites can commonly cause illness among Africans, who generally have high-level antimalarial immunity.

Another limitation of HRP2-based RDTs is the inability of these assays to identify nonfalciparum malaria infections. Although the large majority of malaria infections are caused by *P. falciparum* in sub-Saharan Africa, non-falciparum infections do play an important role. In a recent study of children in an urban cohort in Kampala, Uganda, where parasite species was determined by species-specific PCR, 94% of episodes of symptomatic malaria were caused by *P. falciparum* (including mixed infections), but 4.6% were caused solely by *P. malariae*, 0.8% were caused solely by *P. ovale*, and 0.5% were caused solely by *P. vivax*.[9] Thus, in this setting, about 6% of episodes of malaria would be missed by an HRP2-based RDT. Importantly, the landscape of malaria is changing. Aggressive control efforts are leading to marked decreases in the incidence of malaria and prevalence of malarial infection at a number of locations in Africa.[10] These efforts may lead to improved control of *P. falciparum* infection compared with *P. ovale* and *P. vivax* infections, because the latter species are not eliminated by most antimalarials because of lack of activity against chronic liver stages. Thus, with improving control, more malaria infections may be caused by non-falciparum parasites, and HRP2-based RDTs may have decreasing sensitivity for the diagnosis of malaria.

Considering the limitations of RDTs for malaria, what is the appropriate course of action? One response would be to return to reliance on a clinical diagnosis of malaria. This approach might be reasonable in highly endemic areas, where most fevers in children are caused by malaria,[11] but it will lead to many inappropriate treatments and will clearly be less desirable in areas with low to moderate or decreasing incidence of malaria.[12] Another approach will be to rely fully on microscopy and push this diagnostic modality into rural areas with aggressive implementation and training efforts, which has been successful in some areas.[13] However, it is unlikely that reliable microscopy can be brought to all areas of rural Africa, whereas RDTs can be used in nearly all clinical settings. Therefore, most likely, consistent with WHO guidelines, RDTs will increasingly be used for the diagnosis of malaria in Africa. Which RDT should be used? First, it is important that oversight of test manufacture, care in shipping and storage of tests, and rigorous quality control lead to the consistent availability of high-quality tests. Second, serious consideration of the optimal antigen for RDTs is warranted. The HRP2-based RDT has been considered the best choice for Africa in large part because of its high sensitivity, but increasing prevalence of parasites that do not express HRP2 may challenge this assumption. The sensitivity of HRP2-based RDTs is limited because of their inability to recognize non-falciparum infections, and as described in the work by Koita and others[7] in AJTMH, it also may be limited by the circulation of *P. falciparum* that does not express HRP2. These results suggest that the optimal RDT for Africa may need to be reconsidered. In particular, additional study of the sensitivity of HRP2-based and other RDTs is needed, and if the prevalence of HRP2-negative *P. falciparum* parasites or non-falciparum malaria parasites is increasing, the use of RDTs recognizing antigens other than HRP2 may be advisable.

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REFERENCES

1. World Health Organization, 2010. *Guidelines for the Treatment of Malaria*. Geneva, Switzerland: World Health Organization.
2. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH, 2007. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *Am J Trop Med Hyg 77:* 119–127.
3. Murray CK, Gasser RA Jr, Magill AJ, Miller RS, 2008. Update on rapid diagnostic testing for malaria. *Clin Microbiol Rev 21:* 97–110.
4. Iqbal J, Siddique A, Jameel M, Hira PR, 2004. Persistent histidine-rich protein 2, parasite lactate dehydrogenase, and panmalarial antigen reactivity after clearance of *Plasmodium falciparum* monoinfection. *J Clin Microbiol 42:* 4237–4241.
5. Tjitra E, Suprianto S, McBroom J, Currie BJ, Anstey NM, 2001. 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 39:* 1025–1031.
6. Hopkins H, Bebell L, Kambale W, Dokomajilar C, Rosenthal PJ, Dorsey G, 2008. Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda. *J Infect Dis 197:* 510–518.
7. Koita OA, Doumbo OK, Ouattara A, Tall LK, Konare A, Diakite M, Diallo M, Sagara I, Masinde GL, Doumbo SN, Dolo A, Tounkara A, Traore I, Krogstad DJ, 2012. False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the *hrp2* gene. *Am J Trop Med Hyg 86:* 194–198.
8. Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW, Incardona S, Perkins M, Bell D, McCarthy J, Cheng Q, 2010. A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. *PLoS One 5:* e8091.
9. Clark TD, Njama-Meya D, Nzarubara B, Maiteki-Sebuguzi C, Greenhouse B, Staedke SG, Kamya MR, Dorsey G, Rosenthal PJ, 2010. Incidence of malaria and efficacy of combination antimalarial therapies over 4 years in an urban cohort of Ugandan children. *PLoS One 5:* e11759.
10. O'Meara WP, Mangeni JN, Steketee R, Greenwood B, 2010. Changes in the burden of malaria in sub-Saharan Africa. *Lancet Infect Dis 10:* 545–555.
11. English M, Reyburn H, Goodman C, Snow RW, 2009. Abandoning presumptive antimalarial treatment for febrile children aged less than five years—a case of running before we can walk? *PLoS Med 6:* e1000015.
12. D'Acremont V, Lengeler C, Mshinda H, Mtasiwa D, Tanner M, Genton B, 2009. Time to move from presumptive malaria treatment to laboratory-confirmed diagnosis and treatment in African children with fever. *PLoS Med 6:* e252.
13. Kiggundu M, Nsobya SL, Kamya MR, Filler S, Nasr S, Dorsey G, Yeka A, 2011. Evaluation of a comprehensive refresher training program in malaria microscopy covering four districts of Uganda. *Am J Trop Med Hyg 84:* 820–824.