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Malaria rapid diagnostic tests in elimination settings—can they find the last parasite?

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Abstract

Rapid diagnostic tests (RDTs) for malaria have improved the availability of parasite-based diagnosis throughout the malaria-endemic world. Accurate malaria diagnosis is essential for malaria case management, surveillance, and elimination. RDTs are inexpensive, simple to perform, and provide results in 15–20 min. Despite high sensitivity and specificity for *Plasmodium falciparum* infections, RDTs have several limitations that may reduce their utility in low-transmission settings: they do not reliably detect low-density parasitaemia (< 200 parasites/ μ L), many are less sensitive for *Plasmodium vivax* infections, and their ability to detect *Plasmodium ovale* and *Plasmodium malariae* is unknown. Therefore, in elimination settings, alternative tools with higher sensitivity for low-density infections (e.g. nucleic acid-based tests) are required to complement field diagnostics, and new highly sensitive and specific field-appropriate tests must be developed to ensure accurate diagnosis of symptomatic and asymptomatic carriers. As malaria transmission declines, the proportion of low-density infections among symptomatic and asymptomatic persons is likely to increase, which may limit the utility of RDTs. Monitoring malaria in elimination settings will probably depend on the use of more than one diagnostic tool in clinical-care and surveillance activities, and the combination of tools utilized will need to be informed by regular monitoring of test performance through effective quality assurance.

Keywords

Diagnosis; elimination; malaria; rapid diagnostic tests; surveillance

Introduction

The diagnosis of malaria has evolved rapidly in the last 10 years, largely because of the introduction of rapid diagnostic tests (RDTs) for malaria. As malaria transmission declines throughout the world, accurate diagnosis is becoming increasingly important both for

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individual case management and for disease surveillance. In early 2010, the WHO recommended that all suspected malaria cases should be confirmed with a parasite-based diagnostic assay [1]. This new policy stands in stark contrast to prior WHO recommendations, which supported universal treatment of febrile children <5 years of age in malaria-endemic areas, and empirical treatment of older children and adults in areas where laboratory testing was unavailable. Currently 78 (91%) of the 86 countries where *Plasmodium falciparum* is endemic have adopted policies to confirm malaria with a laboratory test prior to treatment in all age groups [2]. However, despite the rapid change of policy, many countries are still struggling to implement a comprehensive diagnostic programme for malaria. In 2009, 21 (50%) of 42 reporting countries in the WHO Africa Region reported that fewer than 20% of suspected malaria cases were laboratory-confirmed [2]. Fortunately, the Africa Region is increasing the proportion of laboratory-confirmed cases, and this positive trend is being noted in nearly all WHO Regions.

Prompt, reliable diagnosis is essential for the effective management of malaria. Clinical signs and symptoms are not specific for malaria infection, and result in overdiagnosis of malaria and inappropriate treatment of non-malarial illness with antimalarial drugs [3–5]. Misdiagnosis of hospitalized patients with non-malarial febrile illness can be associated with higher mortality than malaria infection in malaria-endemic countries [6,7], and inappropriate use of antimalarial drugs contributes to the development of antimalarial drug resistance [8,9]. Expansion of the diagnostic capacity for malaria has the potential to reduce inappropriate use of antimalarial drugs. However, in many settings, providers neither test patients meeting the suspected malaria case definition nor adhere to test results when diagnosing and treating malaria [10–15]. There are many potential reasons for poor adherence to test results, including the lack of tests for other diseases, poor understanding of the epidemiology of febrile disease in malaria-endemic settings, patient expectations, unclear policies on managing negative results [16], low confidence in laboratory results, and the legacy of treatment based on clinical symptoms. Despite these challenges, several malaria-endemic countries have successfully introduced RDTs at the most peripheral levels of the health system, in some instances even down to the community health worker [17–19]. The combination of early and accurate diagnosis by RDT and early treatment with artemisinin-based combination therapy by community health workers has also demonstrated the ability to reduce malaria morbidity and mortality in low-transmission settings [20,21].

Advantages and Limitations of RDTs

Malaria RDTs are immunochromatographic tests that detect parasite antigens in whole blood samples. RDTs offer several advantages over clinical diagnosis or microscopy. In field trials, they have demonstrated 90% sensitivity and specificity for *P. falciparum* infection with 200 parasites/ μ L [22–24]. They are simple in comparison with other malaria diagnostic tests, because they do not require electricity or expensive equipment. They provide results in 15–20 min, and they are relatively inexpensive, at \$0.60–1.20 per test. RDTs are simple to use, and clinicians or community health workers can be taught to perform them with a half day or full day of didactic and practical training. Correct interpretation of RDTs is less subjective than that of microscopy—the test line is either present or absent. Currently available RDT products detect one or more of three target antigens. Histidine-rich protein 2

(HRP2)-detecting tests are highly sensitive and specific for *P. falciparum*. *Plasmodium* lactate dehydrogenase-based tests can be species-specific for *P. falciparum* or *Plasmodium vivax* or detect all malaria species (pan-specific). A minority of commercially available RDT products also use *Plasmodium* aldolase as a pan-species target antigen. RDTs typically have a shelf-life of 18–24 months, allowing sufficient time for delivery, distribution, and use in most settings. Because of their ease of use and accuracy, RDTs have an increased capacity to provide malaria diagnosis in nearly all healthcare settings, an essential component of accurate disease surveillance in an elimination setting.

However, RDTs do have some disadvantages. RDTs, especially HRP2-based tests, are highly sensitive for *P. falciparum* infections above 100–200 parasites/ μL , but presently do not reliably detect lower-density parasitaemia. Results for the detection of *P. vivax* are more variable at densities of 100–200 parasites/ μL or even at higher parasite densities, depending on the target antigen and the product [25]. Of the 45 products tested in recent WHO/FIND/CDC evaluations, only nine had panel detection scores of $\geq 90\%$ when tested against clinical samples of *P. vivax* at 200 parasites/ μL [26]. Detection rates for *Plasmodium ovale* and *Plasmodium malariae* were not included in the WHO/FIND/CDC product testing, and there are limited data from field trials, which do suggest that there is considerable unreliability in detecting infections with these two species. Quantification of malaria parasitaemia is an essential part of managing severe malaria and monitoring treatment response, but is not possible with RDTs that give qualitative results only. RDTs cannot distinguish current from recently treated infections, especially those that detect HRP2, which may remain positive for several weeks after treatment. Persistent positivity of RDTs may be incorrectly interpreted by health workers as treatment failure, reducing confidence in antimalarial drug effectiveness. There has also been considerable variation in the manufacturing quality of RDTs. Some RDT products are consistently poor performers, with considerable inter-lot variability, whereas others have only minor inter-lot variability [26]. The monoclonal antibodies that are used to bind antigen and produce a positive test result are also sensitive to a combination of heat and humidity, especially those used in *Plasmodium* lactate dehydrogenase-based tests, and may degrade in the tropical conditions common to many malaria-endemic countries when not protected by appropriate packaging. There is also recent evidence that HRP2-based RDTs may miss *P. falciparum* infections in regions where the *hrp2* genes are deleted from a large proportion of the parasite population, or at low parasitaemia densities, when there are fewer repeated epitopes in the HRP2 product [27,28], but the frequency of such deletions and lower repeat epitope copy numbers remains poorly defined in most settings. Finally, quality control of RDTs in remote locations is challenging, because there are currently few available positive controls or other tools for determining the reliability of RDTs in use in field locations. Therefore, quality control is frequently limited to supervisory visits or review of blood smears at locations away from the point of use. Despite these challenges, RDTs remain a useful and widely deployed diagnostic tool for malaria surveillance and control.

Alternatives for Malaria Diagnosis

Other methods of malaria diagnosis require significantly greater investments in equipment, reagents, and training. The historical reference standard for malaria diagnosis is light

microscopy. It is highly sensitive and specific when used by a highly competent microscopist working with an optimally functioning microscope and good reagents, and can reliably detect as few as 10 parasites/ μL . However, because microscopy requires extensive training, quality materials, and several years of experience to attain and maintain proficiency, routine diagnosis by microscopy is of variable quality, and lower-density infections are frequently not identified. Nucleic acid-based tests (NATs) have consistently demonstrated superiority to RDTs and microscopy in detecting infections at levels below the detection limits of a competent microscopist [29–34]. As countries progress towards malaria elimination, the need to detect submicroscopic infections is becoming increasingly important, as reservoirs of infected persons may sustain transmission even without this manifesting in clinical illness. Therefore, it may be necessary to incorporate more sensitive NATs into elimination programmes.

NATs differ in their sensitivity, ease of use, and other requirements, such as DNA targets, primers and probes, and instrumentation. The most commonly used NATs for parasite detection are nested-PCR amplification and quantitative nucleic acid sequence-based amplification PCR. Other NATs, such as loop-mediated isothermal amplification [35–38] and fluorescent microbead technology [32,39,40], have also been described. Increased sensitivity is achieved with these tests by repeated amplification of an initially low level of parasite genetic material and subsequent detection by specific fluorescent tags. In addition, primers and probes can be designed for different *Plasmodium* species and, possibly, variants within a species. Because the critical steps of amplification and detection are automated, test results are less subjective than those of microscopy and RDTs. When appropriately calibrated and targeted, some real-time NATs have the additional advantage of parasite quantification.

The currently available NATs are not suitable for routine malaria diagnosis, because they require expensive equipment and expensive reagents that require refrigeration, along with highly qualified technicians. They are best suited for specialized laboratories that rarely exist in clinical-care settings in most malaria-endemic countries. However, these drawbacks do not necessarily preclude their use in elimination settings as adjuncts to microscopy and RDTs. Indeed, their amenability to high throughput means that samples from multiple locations can be collected and sent to a central laboratory for analyses, which is more cost-effective. Further cost savings can be realized by DNA sample pooling [41,42] in elimination settings, where most samples are expected to be parasite-negative. Expected innovations, such as making NATs less costly, easier to perform, and field ready, will allow their use in a variety of elimination settings and vastly improve the quality of surveillance in low-transmission settings.

Another surveillance tool that may be useful in elimination and near-elimination settings is malaria serology. Population-level antibody responses to certain *Plasmodium* parasite antigens can be used to determine transmission trends over a period of months to years [43–45]. The choice of the specific antigen targeted for serological monitoring will depend on characteristics such as immunogenicity and seroconversion rates, persistence of the specific antibody (decay rate), and limited variation in the target antigen. Serological testing could provide useful population-level data with which to measure progress in control and

elimination programmes, but is unsuitable for individual case management in endemic settings.

The diagnostic tools mentioned above will probably be deployed in combination in elimination settings. The Malaria Eradication Research Agenda Consultative Group on Diagnosis and Diagnostics recommended that, as countries shift from control to eradication, the emphasis may shift from light microscopy and RDTs to greater reliance on appropriate NATs and serology [46]. Specific programme requirements and malaria transmission dynamics will determine which test combinations are deployed for individual clinical diagnosis at each level of the health system for passive case detection, for active case detection, and for population-level surveillance. Identification and treatment of individuals with asymptomatic malaria infections is critical to the success of elimination programmes. Therefore, the use of RDTs for case management and surveillance in elimination settings requires an understanding of the limitations of these tests, in order to adjust diagnostic strategies when necessary and also to put test results in the appropriate perspective. Until tests with greater sensitivity are able to be performed in peripheral health centres and/or by community health workers, RDTs remain the best option for laboratory-confirmed diagnosis of malaria in remote locations. However, population-level surveillance will probably require more sensitive tests to estimate the true burden of malaria infection.

Parasite Density and RDTs in Surveillance

Making informed decisions about the utility of RDTs in elimination settings requires knowledge of the proportion of low-density infections in symptomatic and asymptomatic persons. The proportion of such infections is likely to be influenced by a variety of factors, including disease endemicity, parasite species and strain virulence, host factors such as age, race, prior malaria exposure, haemoglobinopathies, and others [47–51]. Recent studies using PCR in low-transmission settings have demonstrated a high proportion of low-density parasitaemias that were not detected by microscopy or RDT, and revealed that a high proportion of infected individuals were asymptomatic [29,52,53]. Traditionally, it is to be expected that many asymptomatic infections will be found in areas of high malaria prevalence, because of acquired immunity resulting from repeated infections. Conversely, because areas of low or sporadic malaria transmission provide less malaria exposure, the convention is to expect to find few asymptomatic infections. However, in areas where malaria transmission has decreased rapidly over a relatively short period of time, there may be different effects of waning immunity on the proportion of asymptomatic infections than in areas where low or sporadic malaria transmission has been sustained for decades. These new data suggest the potential limited utility of RDTs for monitoring disease or parasite prevalence in some elimination settings. However, parasite densities associated with asymptomatic infections are not static, and may need to be periodically monitored to determine whether RDT use remains appropriate.

Malaria Indicator Surveys (MISs) may provide a useful means of monitoring low-density infection prevalence among symptomatic and asymptomatic children. The proportion of low-density *P. falciparum* infections (<200 parasites/ μ L) in asymptomatic and symptomatic children was highly variable in Kenya, Mozambique, Senegal and Zambia MISs, ranging

from 2.6% to 29.7% of children surveyed with positive blood smears (Fig. 1). Despite the variability in the proportion of low-density infections, the overall sensitivity of RDTs remained relatively high in all four surveys, ranging from 80.5% to 97.7% (Table 1). The sensitivity for low-density infections (<200 parasites/ μL) was less consistent, ranging from 71.7% to 100% (data not shown). The specificity remained over 90% in all surveys except for that in Mozambique (74.6%). Higher parasite prevalence and recently treated infections may have led to a higher proportion of false-positive RDT results because of ongoing HRP2 circulation reducing specificity in Mozambique. The parasite prevalence measured by RDTs was consistently higher than that measured by microscopy in all four surveys, which may represent continued HRP2 antigenaemia following treatment, submicroscopic infection, or human error in labelling, interpreting or recording test results. The Roll Back Malaria Monitoring and Evaluation Reference Group is considering recommending the use of RDTs alone for future MISs, which could limit the ability to monitor parasite density, and gametocytaemia, and will have unknown effects on the monitoring of *P. ovale* and *P. malariae* infections.

Studies of the pyrogenic threshold for *P. falciparum* malaria in immunonaïve individuals have demonstrated a wide spectrum of parasitaemias at fever onset, ranging from 10 to 200 000 parasites/ μL [54]. Data from several malaria-endemic countries indicate that the proportion of low-density (<200 parasites/ μL) infections in symptomatic persons is higher in low-transmission than in high-transmission areas, and also higher in *P. vivax* than in *P. falciparum* infections [55]. This suggests that a larger proportion of symptomatic cases may be missed in low-transmission (i.e. elimination) settings and for *P. vivax* infections if RDTs are the sole means of parasitological confirmation. However, these studies were conducted with confirmation by microscopy alone, and the true burden of submicroscopic infection may be much higher when determined by PCR [56]. Irrespective of the parasite density, all malaria infections, if not identified and treated, represent parasite reservoirs with the potential to infect mosquitoes [31,34,57]. Active case detection will be an essential component of any elimination plan. Malaria control programmes will also need to actively monitor the sensitivity of RDTs and microscopy in detecting low-density parasitaemia in symptomatic patients presenting to health facilities and in population-based surveys to capture asymptomatic infections with more sensitive NATs. The development of more sensitive RDTs would be ideal, but there will be limits to detection with immunochromatographic tests without an amplification step to increase parasite detection.

Malaria Surveillance in Elimination Settings

Nearly all malaria-endemic areas experience some degree of seasonality in malaria transmission associated with periods of increased rainfall. The proportion of fevers attributable to malaria illness will rise in the high-transmission season and fall in the low-transmission season. Likewise, the proportion of fevers attributed to malaria will decrease as malaria elimination progresses [58], while other febrile diseases may remain relatively stable in the population and appear to be relatively more important. A reduction in the proportion of fever cases positive for malaria may have consequences for the use of RDTs and malaria case management. First, there could be an erroneous perception by health workers that negative test results are frequently false-negative results, and this may reduce confidence in

the tests [59]. Second, a high prevalence of non-malarial fevers may discourage testing for malaria, resulting in missed malaria cases. Therefore, the use of RDTs in elimination settings may require substantial efforts to maintain regular testing and quality case management. However, doing this means that resources will be channelled into testing for a very low-prevalence disease that could be used for managing non-malaria fevers. Without the ability to test for and diagnose other febrile illnesses, health practitioners may revert to treating all fevers as malaria or to injudiciously prescribing antibiotics for a broader range of symptoms associated with fever. Dissatisfaction with a 'not malaria' diagnosis may arise as patients become more informed health consumers. Improving diagnostic resources and training for other causes of severe and non-severe febrile illness will be an essential component of malaria elimination, to encourage accurate testing and reporting of malaria cases.

High-quality malaria surveillance through rigorous testing of symptomatic persons presenting to health facilities will help to target other malaria control interventions in an elimination setting. Early identification of localized areas of high malaria activity will allow targeted action to eliminate pockets of local transmission, and may serve as a useful intervention tool to reduce the further spread of malaria beyond the identified zone. Quality malaria surveillance, including active case detection in travellers from malaria-endemic areas, will also be an essential component of sustaining malaria elimination for countries that share borders with malaria-endemic nations and those with frequent importation of malaria from travellers. The infrastructure developed to improve malaria diagnosis during elimination may also be utilized to expand the diagnostic capacity for other diseases, including tuberculosis and human immunodeficiency virus disease: an individual trained to perform malaria microscopy can be easily trained to perform tuberculosis microscopy, and those trained to perform malaria RDTs would probably be capable of performing human immunodeficiency virus rapid testing as well. Epidemiological data regarding outcomes of non-malarial febrile disease will also be useful to assist clinicians in determining the best course of treatment for non-malarial febrile illness and to reduce overprescription of antimalarial drugs and antibiotics.

Hypnozoites and Elimination of *P. vivax* and *P. ovale*

Elimination of *P. vivax* and *P. ovale* malaria will be further complicated by the presence of liver hypnozoites and the inability of current tests to detect them. *Plasmodium* hypnozoites are not eliminated by most antimalarial drug combinations, and frequently cause relapse in patients who are not treated with 8-aminoquinoline drugs (primaquine, tafenoquine, and pamaquine). Incorrect speciation or failure to identify mixed infections with *P. vivax* or *P. ovale* may result in continued transmission of malaria from asymptomatic carriage of hypnozoites that later mature and produce blood-stage infections. Treatment with primaquine and other drugs in this class can produce severe haemolytic anaemia in persons with glucose-6-phosphate dehydrogenase (G6PD) deficiency, an inherited enzymatic defect that is common in much of the malaria-endemic world [60]. The currently available rapid G6PD screening tests are relatively expensive and have not been widely implemented. Identification of alternative drugs for eliminating hypnozoites or more affordable, rapid tests

for G6PD deficiency will be an essential component in safely eliminating *P. vivax* and *P. ovale* in malaria-endemic areas.

Conclusion

The currently available RDTs are useful tests for expanding the availability of malaria diagnosis in a variety of field settings. They are highly sensitive for *P. falciparum* infections with parasite densities of > 200 parasites/ μL , and a few products achieve similarly high sensitivity for *P. vivax* infections. However, there is increasing evidence that low-density and microscopically subpatent infections may be more common than previously recognized in near-elimination settings. Therefore, unless RDTs are able to improve sensitivity measures at low densities of parasitaemia without sacrificing specificity, newer diagnostic tools will be required for malaria elimination. An ideal diagnostic test for malaria would have high sensitivity and specificity at any density of parasitaemia, be portable, and be easily and accurately used in field settings. In addition, the characteristics of an ideal test are likely to differ for programmes at differing stages along the road from malaria control to pre-elimination and elimination [46]. NATs already achieve high sensitivity, but are not currently portable or simple enough for widespread use. Future research and development in malaria diagnostics should emphasize: highly sensitive detection of low-density infections; detection of liver-stage and sexual-stage parasites; multiplex systems to detect malaria and non-malarial causes of illness or severity markers; and detection of G6PD deficiency and other factors that may help to guide treatment. Monitoring malaria in elimination settings will probably depend on the use of more than one diagnostic tool in clinical-care and surveillance activities, and the combination of tools utilized will need to be informed by regular monitoring of test performance through effective quality assurance. Investments in improving malaria surveillance may seem costly, but will probably be beneficial in improving the diagnosis of other diseases and preventing the re-introduction of malaria.

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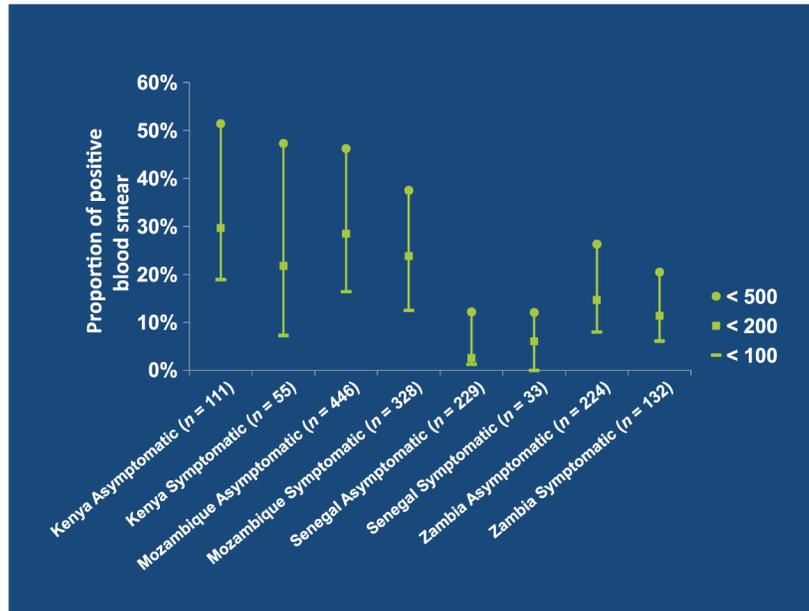


FIG. 1. Proportion of positive blood smears with *Plasmodium falciparum* parasite density <100, <200 and <500 parasites/ μL in symptomatic and asymptomatic children 6–59 months of age surveyed during Malaria Indicator Surveys, 2007–2009.

TABLE 1

Microscopy and rapid diagnostic test results from Malaria Indicator Surveys that collected the results of both malaria tests, 2007–2009

	Kenya 2007	Mozambique 2007	Senegal 2008–2009	Zambia 2008
Number with blood smear results	4598	3238	4139	3656
Malaria parasite prevalence by microscopy (%) ^a	3.6	31.7	6.3	9.7
RDT product	ICT Malaria Pf (ICT Diagnostics)	ICT Malaria Pf (ICT Diagnostics)	Paracheck Pf (Orchid Biomedical)	ICT Malaria Pf (ICT Diagnostics)
Number with RDT results (positive/negative)	5117	3876	4032	3652
Malaria prevalence by RDT (%) ^a	8.1	43.8	12.0	16.3
Number with RDT and BS results	4582	2438	3960	3652
RDT sensitivity (%) ^b	94.0	87.0	80.5	97.7
RDT specificity (%) ^b	95.6	74.6	92.8	92.5

BS, blood smear; RDT, rapid diagnostic test.

^aPrevalence estimates not weight-adjusted.

^bMicroscopy considered to be the reference standard.