Malaria Rapid Diagnostic Tests

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Global efforts to control malaria are more complex than those for other infectious diseases, in part because of vector transmission, the complex clinical presentation of Plasmodium infections, >1 Plasmodium species causing infection, geographic distribution of vectors and infection, and drug resistance. The World Health Organization approach to global malaria control focuses on 2 components: vector control and diagnosis and treatment of clinical malaria. Although microscopy performed on peripheral blood smears remains the most widely used diagnostic test and the standard against which other tests are measured, rapid expansion of diagnostic testing worldwide will require use of other diagnostic approaches. This review will focus on the malaria rapid diagnostic test (MRDT) for detecting malaria parasitemia, both in terms of performance characteristics of MRDTs and how they are used under field conditions. The emphasis will be on the performance and use of MRDTs in regions of endemicity, particularly sub-Saharan Africa, where most malaria-related deaths occur.

The 2011 World Health Organization (WHO) report on malaria shows 106 countries or regions where malaria is endemic, with up to one-half of the worldwide population at risk for acquiring the infection [1]. This report indicates that, during 2010, both the number of cases and deaths continued to decrease, from 225 million cases and 781,000 deaths in 2009 to 216 million cases and 655,000 deaths [1]. Although the most dramatic decrease in both cases and deaths has been in Africa, the African region still accounts for 81% of malaria cases and 91% of malaria-related deaths [1]. Global progress in malaria control is documented by the observations that 8 countries are now in the preelimination phase of malaria control, another 8 countries are in the prevention of reintroduction phase of malaria control, and there were only 176 indigenous cases of Plasmodium falciparum malaria reported in 2010 from the European region [1].

Global malaria control efforts are based on 2 broad components: vector control and improved diagnosis and treatment of patients with clinical malaria. Until recently, conventional diagnosis of malaria has been based on either clinical diagnosis or use of microscopic examination of peripheral blood smears. There now are good data to show that treatment based on symptoms results in overtreatment of patients who do not have clinical malaria [1–5]. This practice exposes patients to needless antimicrobial therapy, wastes resources in areas that cannot afford such a practice, probably contributes to the development of drug resistance, and perpetuates reluctance of providers to base therapy on diagnostic test results. Microscopy remains the most widely used method for detecting Plasmodium parasitemia, with as many as 165 million smears performed globally during 2010 [1]. For malaria control programs, microscopy has 2 main disadvantages. The first is that the method is not easily adapted to many settings, particularly rural settings, where most patients with malaria present for healthcare and where even basic laboratory infrastructure often is unavailable. The second is that the diagnostic sensitivity of microscopy is too low; although sensitivity varies considerably from region to region, by the relative skill of persons reading smears, and by the magnitude of parasitemia in the specimen, it is no better than 75%–90% under the best conditions.
of conditions [5–7]. In some settings, sensitivity may be as low as 50% [7]. Although the analytical sensitivity of microscopy is good, with the magnitude of parasitemia able to be detected at 50 parasites/μL, this level of detection may not be possible when inexperienced persons perform the test.

Because of limitations of microscopy for malaria diagnosis, global malaria control programs have focused on the development of other diagnostic tests that can be used in the field. This review will focus on the technology currently available for a malaria rapid diagnostic test (MRDT), performance characteristics of available MRDTs, and how MRDTs are used in malaria control programs.

**BIOCHEMICAL BASIS OF MRDTs**

Current MRDTs are based on detection of 3 different types of *Plasmodium* antigen [8, 9]. The first is *Plasmodium* histidine-rich protein (HRP) 2 (pHRP-2), which can be specific to *P. falciparum* or *Plasmodium vivax*. The second is to *Plasmodium* lactate dehydrogenase (LDH) (pLDH), which can be specific to *P. falciparum* or *P. vivax* or be a variant that is common to all *Plasmodium* species ( panspecific). The last is *Plasmodium* aldolase, which is panspecific. By combining detection of these 3 antigens on an immunochromatographic strip (ICS) assay, MRDTs have been developed that can be used to detect any malaria species: *P. falciparum* alone, *P. vivax* alone, or any combination thereof (Table 1) [8, 10].

The use of these 3 antigens results in some fundamental diagnostic limitations: (1) none of the 3 antigens is specific for *Plasmodium ovale*, *Plasmodium malariae*, or *Plasmodium knowlesi*; (2) there are variants of *P. falciparum* in South America that do not produce the 2 most common types of HRP (*P. falciparum* HRP [pHRP] 2 and pHRP-3), which means that MRDTs based on detection of those antigens would not be useful in that region [11]; (3) cross-reactions with a pHRP-2 assay have been reported from patients with *Schistosoma mekongi* infection (without cross-reaction with a pLDH assay) [12]; (4) cross-reactions with some assays have been reported for patients with rheumatoid factor or other circulating auto-antibodies [12]; (5) patients with high levels of *P. falciparum* parasitemia may give false-positive results with pLDH assays designed to detect *P. vivax* [13]; (6) unlike microscopy, MRDTs cannot be used to determine the magnitude of parasitemia; and (7) because pHRP-2 is not cleared from blood for up to 30 days after treatment, MRDTs that test for this antigen should not be used to monitor response to therapy [8, 9, 14]. Although pLDH and aldolase are cleared quickly from blood after treatment, gametocytes are not eliminated with standard antimalarial therapy and continue to produce all 3 antigens. As a result, assays testing for these 2 antigens also should not be used to monitor response to therapy [9].

**DESCRIPTION OF MRDT TECHNOLOGY**

Almost all MRDTs are based on ICS technology. At least 200 assays are sold on the market worldwide; although there are many variations of LIS technology, most assays are more similar to each other than different. With ICS assays, a liquid specimen, such as blood, is applied to one end of a nitrocellulose strip. After a brief period during which the specimen mixes with lysing agents, a buffer solution, and labeled anti-*Plasmodium* indicator antibody, the liquid mixture is allowed to migrate down the strip to where capture antibodies are fixed in lines on the strip surface. The capture antibodies are directed against different epitopes on parasite antigens or to the indicator antibodies (already bound to parasite antigens). After capture occurs, the complex of indicator antibodies and parasite antigens (by one of a number of chemical methods) will create a visible line on the ICS to yield a positive test result. The time to yield a test result varies between assays but is generally ≤15 minutes.

**DETERMINING MRDT PERFORMANCE CHARACTERISTICS: CHALLENGES**

Determining diagnostic sensitivity, specificity, and predictive values of MRDTs is challenging because of the (1) use of a reference standard (microscopy), which is not optimal for generating consistent test results in different regions during clinical trials; (2) multiplicity of commercial products (≥200 commercial products from >60 manufacturers [1]); (3) geographic variation in the distribution of *Plasmodium* species; (4) geographic variation in prevalence rates; (5) challenges in conducting research in resource-limited areas; and (6) variation in commercial products through time as manufacturers modify them to improve their performance characteristics [8, 9].

### Table 1. Types of Malaria Rapid Diagnostic Tests

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>HRP-2 (<em>Plasmodium falciparum</em> specific)</td>
</tr>
<tr>
<td>2</td>
<td>HRP-2 (<em>P. falciparum</em> specific) and aldolase (panspecific)</td>
</tr>
<tr>
<td>3</td>
<td>pLDH (<em>P. falciparum</em> specific) and pLDH (panspecific)</td>
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<tr>
<td>4</td>
<td>pLDH (<em>P. falciparum</em> specific) and pLDH (panspecific)</td>
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<tr>
<td>5</td>
<td>pLDH (<em>P. falciparum</em> specific) and pLDH (<em>Plasmodium vivax</em> specific)</td>
</tr>
<tr>
<td>6</td>
<td>HRP-2 (<em>P. falciparum</em> specific), pLDH (panspecific), and pLDH (<em>P. vivax</em> specific)</td>
</tr>
<tr>
<td>7</td>
<td>Aldolase (panspecific)</td>
</tr>
</tbody>
</table>

Modified from reference [14].

Abbreviations: HRP, histidine-rich protein; pLDH, *Plasmodium* lactate dehydrogenase.
the most important challenge is that too many providers base treatment not on test results but rather on clinical diagnosis.

**DETERMINING MRDT PERFORMANCE CHARACTERISTICS: WHO EVALUATIONS**

Because of these factors, the WHO has undertaken an evaluation of MRDTs using donor blood to help determine which commercial products are most likely to yield accurate test results [10]. This evaluation was done in collaboration with the US Centers for Disease Control and Prevention, the Foundation for Innovative New Diagnostics, and the WHO Special Programme for Research and Training in Tropical Diseases.

The WHO evaluation was undertaken in 3 rounds [10]. In each round, assays were selected on the basis of the manufacturer's ability to meet ISO 13485:2003 Quality System Standard manufacturing requirements. The first round involved 41 assays from 21 manufacturers, the second 29 assays from 13 manufacturers, and the third 50 assays from 23 manufacturers. Some of the assays from earlier rounds were modified before inclusion in the third round. Initial testing in each round was with cultured *Plasmodium* isolates, with 118 of the 120 assays progressing to testing with wild (not cultured, but derived from patient isolates) strains of *P. falciparum* and *P. vivax*. Control testing involved use of blood not seeded with parasites. Because of the need for MRDTs to be used in tropical settings and the need for transportation from manufacturing facilities to sites of use, the heat stability of products was also evaluated [10].

The results of round 3 were published recently, showing that commercial MRDTs (1) have widely varying ability to detect parasites at low parasite concentrations, (2) perform better at high parasite concentrations, (3) show marked variability in performance between products, (4) show variability between lots of the same product, and (5) show variable heat stability [10]. It should be noted that the WHO evaluation was not designed or intended to determine the superiority of one product over another, but rather to determine which products might be acceptable for further evaluation in clinical trials and for procurement programs [10]. With these goals in mind, of >200 commercial MRDTs available from ≥60 manufacturers, relatively few merit further evaluation.

**DETERMINING MRDT PERFORMANCE CHARACTERISTICS: CLINICAL TRIALS**

Despite the challenges in conducting clinical trials of MRDTs, many have been undertaken, yielding a large number of published studies. Not surprisingly, most trials have been directed toward detection of *P. falciparum* parasitemia by MRDTs in symptomatic patients living in areas of endemicity; a recent meta-analysis by the Cochrane Collaboration evaluated 74 published studies with that specific objective [14]. As defined in that evaluation, MRDT types 1–3 tested for *p*HRP-2 either alone or in combination with other malarial antigens and MRDT types 4 and 5 tested for *P. falciparum* LDH (*pfLDH*), either alone or in combination with other malaria antigens. The evaluation included comparisons of MRDTs with microscopy, yielding a total of 71 evaluations of type 1 tests, 8 of type 2 tests, 5 of type 3 tests, 17 of type 4 tests, and 3 of type 5 tests [14]. Meta-analytical mean sensitivities and specificities for type 1 tests were 94.8% and 95.2%, 96.0% and 95.3% for type 2 tests, 99.5% and 90.6% for type 3 tests, 91.5% and 98.7% for type 4 tests, and 98.4% and 97.5% for type 5 tests, respectively [14]. Together, assays that test for HRP-2 showed (meta-analytical) mean sensitivity and specificity of 95.0% and 95.2%, respectively; assays that test for LDH showed mean sensitivity and specificity of 93.2% and 98.5%, respectively [14]. Thus, for MRDTs designed to detect *P. falciparum* antigens, those that detect HRP-2 are slightly more sensitive but slightly less specific than MRDTs that test for LDH [14].

Because most malaria-related deaths are caused by *P. falciparum*, emphasis on detection of parasitemia caused by this species is appropriate. Fewer data are available regarding the performance characteristics of MRDTs for detecting parasitemia caused by other malaria species, with minimal data regarding the ability of MRDTs to detect *P. ovale* or *P. malariae* parasitemia and none for *P. knowlesi*. For detection of *P. vivax* parasitemia, diagnostic sensitivity and specificity have been reported to be 84.2% and 96.5% from an area of endemicity in India [15]. A similar sensitivity, 75.8%, was observed in another study, in an area of nonendemicity [16].

**USE OF MRDTs IN AREAS OF ENDEMICITY**

The usefulness of diagnostic tests is determined by whether test results can be and are used in such a way that morbidity and mortality rates are reduced (directly or indirectly) by test results. For malaria, this means that tests must be useful in clinical settings where treatment can be started before patients are lost to follow-up. There has been marked success in these efforts; the number of MRDTs in use has increased substantially during the past few years. The most recent WHO data show that approximately 50 million MRDTs were distributed during 2010: 65% of these in Africa and 30% in Southeast Asia [1]. As noted by the WHO, this is likely to be an underestimate, because only 32 of 44 African countries where malaria is endemic reported data in 2009 [1]. Nonetheless, the rapid increase in MRDT utilization since 2005, when <200,000 kits were delivered, is an impressive accomplishment.
Rapid expansion in MRDT use has helped offset a trend in global decrease in microscopy in several regions, which decreased from 165 million in 2005 to 151 million in 2009, although the overall number of smears performed did increase in 2010, back to 165 million [1]. In the public sector outside the African region, the WHO estimates that >80% of suspected cases of malaria now receive parasitological confirmation by laboratory testing. However, in the African region, only 45% of suspected malaria cases received parasitological confirmation in the reporting countries [1]. The marked increase in use of MRDTs is a result of policy changes that emphasize the need for testing to guide therapy, expansion of testing and treatment programs that are part of the global effort to control malaria, and availability of funding to make these tests available.

Despite the WHO recommendation that malaria treatment should be based on diagnostic test results and the global increase in diagnostic testing for malaria, it is evident that treatment based on testing has yet to gain universal acceptance [1]. Moreover, this recommendation will become more important as the incidence of malaria decreases, because decreasing incidence means that it will become more important to distinguish cases of malaria from other febrile illnesses. The WHO estimates that, in the African region during 2010, the number of courses of artemisinin-combination therapy (ACT) exceeded the total number of malaria diagnostic tests (both microscopy and MRDTs) by a factor of 2, “indicating that many patients receive ACTs without confirmatory diagnosis” [1].

Recent studies however, show that it is possible to improve rates of malaria treatment that are based on diagnostic test results. As examples, a recent study from Tanzania showed that close adherence to treatment protocols is possible after MRDTs were introduced in an area that previously did not have access to microscopy [17]. Similar results were obtained in a recent study from Ghana when MRDTs were introduced; in areas that previously did not have microscopy, introduction of MRDTs was associated with a significant reduction in use of antimalarial drugs [18]. Where microscopy had been in use before introduction of MRDTs, however, only limited improvement was observed [18]. As a last example, with somewhat different and encouraging results, introduction of MRDT in healthcare settings in Dar es Salaam, where microscopy had been in use previously was associated with significant decreases both in the number of patients classified as having malaria and in the number of patients with a negative test result who were treated [19]. The conflicting data on the impact of use of MRDTs on treatment indicate that development of policies regarding use of MRDTs to guide treatment, training of staff, and ongoing audit of compliance are critical factors in malaria diagnosis and treatment programs.

The factors that cause providers to ignore test results are complex and probably vary from region to region. In one study from Ghana, use of laboratory testing was studied using a survey distributed to physicians in a teaching hospital [2]. Both physician and patient attitudes toward testing, as well as cost, were found to be barriers to using laboratory tests [2], whereas availability of testing, quality of testing, and availability of test results were not. When asked about testing for malaria, “64% of physicians said that they frequently or always diagnose malaria without the aid of malaria smear” [2]. Although other studies from Africa have noted that access to laboratory testing is an important barrier to testing, the findings of the Ghana study are similar to those of other studies regarding the use of tests to guide treatment of patients with malaria, as well as the WHO finding of overprescription of antimalarial drugs despite widespread use of diagnostic tests.

USE OF MRDTs IN AREAS OF NONENDEMICITY

Most MRDTs were designed for use in regions of endemicity, where use of diagnostic testing to support malaria control programs needs to expand quickly. As a result, few studies have been conducted to evaluate use of MRDTs in areas of nonendemicity, where few MRDTs are even available. In the United States, only one MRDT has received Food and Drug Administration clearance and is marketed.

One study compared a commercial MRDT with microscopy and a polymerase chain reaction (PCR) assay using 852 consecutive specimens obtained from travelers returning to the United States from areas of endemicity [20]. Of the 852 samples, 103 tested positive for malaria by any method, of which 95 were positive by PCR. Of these 95 samples, malaria was identified by microscopy in 81 (85%), compared with 92 (97%) by the rapid diagnostic test [20]. The rapid diagnostic test had a negative predictive value of 99.6%, compared with 98.2% for microscopy. For detecting *P. falciparum*, the rapid diagnostic test detected 74 (100%) of 74 cases, compared with 65 (88%) by microscopy. For the 21 non-*falciparum* cases, the rapid diagnostic test detected 18 (86%), compared with 16 (76%) by microscopy. The rapid diagnostic test yielded 8 false-positive test results that were not detected with PCR, but 7 of the 8 patients had previously received antimalarial therapy [20]. As noted previously, similar diagnostic sensitivity (75.8%) was observed in another study in an area of nonendemicity [16]. Although these are limited data, the results suggest that at least some MRDTs may be useful in areas of nonendemicity.

MOLECULAR ASSAYS

There are no commercially available molecular assays for malaria diagnosis. A few reference laboratories and government
CONCLUSIONS

The current state of rapid diagnostic testing for malaria can be summarized as follows: (1) commercial MRDTs show wide variability in diagnostic sensitivity and specificity; (2) assays that detect pfHRP-2 are more sensitive but less specific than assays that detect pLDH, but the differences are small; (3) tests that are manufactured with good manufacturing practices and good regulatory oversight perform better; (4) MRDTs should not be used to monitor the outcomes of therapy; (5) recent evidence suggests that strains of *P. falciparum* lacking pfHRP-2 and pfHRP-3 exist in ≥1 region of the world, which may cause false-negative results with tests targeting pfHRP; (6) false-positive test results for *P. vivax* can occur with high levels of *P. falciparum* parasitemia when tested on some assays; (7) none of the existing MRDTs is specific for *P. ovale*, *P. malariae*, or *P. knowlesi*; and (8) there remain serious concerns that, despite widespread use of MRDTs in areas of endemicity, too few providers base treatment on test results.

Keeping these limitations in mind, acceptable MRDTs are available for use in regions of endemicity where global malaria control efforts are most needed. It would be a wiser use of resources to direct future efforts on improving access to these tests and how they are used to guide treatment, rather than pursuing ever-diminishing incremental improvements in diagnostic sensitivity and specificity.

Note

Potential conflicts of interest. Author certifies no potential conflicts of interest.

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