Diagnostic Performance of Rapid Diagnostic Tests versus Blood Smears for Malaria in US Clinical Practice

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Background. Approximately 4 million US travelers to developing countries are ill enough to seek health care, with 1500 malaria cases reported in the United States annually. The diagnosis of malaria is frequently delayed because of the time required to prepare malaria blood films and lack of technical expertise. An easy, reliable rapid diagnostic test (RDT) with high sensitivity and negative predictive value (NPV), particularly for Plasmodium falciparum, would be clinically useful. The objective of this study was to determine the diagnostic performance of a RDT approved by the US Food and Drug Administration compared with traditional thick and thin blood smears for malaria diagnosis.

Methods. This prospective study tested 852 consecutive blood samples that underwent thick and thin smears and blinded malaria RDTs at 3 hospital laboratories during 2003–2006. Polymerase chain reaction verified positive test results and discordant results.

Results. Malaria was noted in 95 (11%) of the 852 samples. The RDT had superior performance than the standard Giemsa thick blood smear (P = .003). The RDT’s sensitivity for all malaria was 97% (92 of 95 samples), compared with 85% (81 of 95) for the blood smear, and the RDT had a superior NPV of 99.6%, compared with 98.2% for the blood smear (P = .001). The P. falciparum performance was excellent, with 100% rapid test sensitivity, compared with only 88% (65 of 74) by blood smear (P = .003).

Conclusions. This operational study demonstrates that the US Food and Drug Administration–approved RDT for malaria is superior to a single set of blood smears performed under routine US clinical laboratory conditions. The most valuable clinical role of the RDT is in the rapid diagnosis or the exclusion of P. falciparum malaria, which is particularly useful in outpatient settings when evaluating febrile travelers.

An estimated 4 million returning US travelers are ill enough to seek health care annually, with >1500 cases of malaria reported in the United States [1–3]. Of travelers who contract malaria, 90%–95% will not become ill until after they return home, with 85% developing symptomatic disease within 30 days after return [4, 5]. Because malaria is unusual in the United States, patients frequently present to physicians who have no tropical medicine expertise and to primary health care facilities that lack expert diagnostic capabilities. Kain et al [4] found 59% of malaria cases were initially misdiagnosed in nonendemic North American settings. In addition, they found that 64% of community-based microscopic diagnoses provided incorrect species identification. Furthermore, Kain and colleagues reported that the mean time from the ordering of a blood smear until the laboratory diagnosis of malaria was 2.5 days, which was partially responsible for an average time from symptom onset to malaria diagnosis of 7.6 days for Plasmodium falciparum. Even where malaria is more
frequently encountered, delay is common because of the necessary time for formal Giemsa-stained thick blood smear preparation and reading (6–8 h).

Of individuals developing *P. falciparum* infection in the United States, ~1% will die [4, 5]. Yet an estimated 80% of deaths are preventable, with a significant proportion due to diagnostic delay or error [6]. An easily performed test that is sensitive and reliable would be a desirable tool that could augment malaria diagnosis. A rapid diagnostic test (RDT) with high sensitivity and a negative predictive value (NPV) for *P. falciparum* would be of particular use in the acute care setting, where the decision for hospitalization is being made. Lastly, it also may benefit severely ill patients by confirming or excluding a malaria diagnosis rapidly and facilitating prompt intervention.

The rapid antigen capture assay (NOW Malaria Test; Binax, Inverness Medical Professional Diagnostics) received US Food and Drug Administration (FDA) approval in June 2007 for diagnosis of symptomatic malaria. Antigens detected by monoclonal antibodies in this rapid antigen capture assay include the histidine-rich protein for specific identification of *P. falciparum* and *Plasmodium* aldolase for identification of all malaria species. Reasonable sensitivity and specificity have been reported in clinical research trials outside the United States, mainly in endemic areas where malaria dynamics and environmental conditions differ greatly from those in the United States [7, 8]. Few studies have investigated RDTs in settings where malaria is not endemic, and no study has evaluated the operational use of the current FDA-approved version of the test in the United States. This study investigated the diagnostic performance of a single blood smear versus the rapid antigen capture assay (NOW Malaria Test) in detecting symptomatic malaria to determine the strengths and limitations of this test in real-world US clinical use.

**METHODS**

*Setting and participants.* This prospective, operational laboratory study was conducted at the 3 hospital-based laboratories with the highest incidence of malaria in Minnesota from 1 March 2003 through 28 February 2006. All consecutive samples sent to the laboratories for thick and thin blood smears to test for malaria were included. Participants were returning travelers, most of whom were visiting friends and relatives.

*Methods of measurement.* Thick and thin Giemsa-stain blood smears were prepared from EDTA-anticoagulated venous blood. Smears were examined for the presence of malarial parasites by trained technologists or hematopathologists per individual laboratory protocol with a minimum of 500 high-powered fields examined. An expert pathologist or hematopathologist confirmed all positive smears. The rapid antigen capture assay was performed per the manufacturer’s instructions by technologists masked to the blood smear results. The rapid antigen capture assay is a lateral flow immunochromatographic test, similar to a pregnancy test, with a visual result in <15 min.

A nested case-control study verified all positive and discrepant results by polymerase chain reaction (PCR) in a separate laboratory, as previously described [9]. For each positive sample, 2 samples that yielded negative results by both blood smear and RDT served as masked, negative controls for PCR. Finally, unsual or discrepant PCR results were tested at separate laboratory by a second PCR method using different primers [10] and using a second, independent DNA extraction to prevent contamination. For verifying discrepant results between RDT and blood smear, PCR served as the reference standard for validation. All personnel were masked to other test results, and no person performed more than 1 test type.

*Outcome measures and data analysis.* The primary outcome measure was the test performance of the RDT and traditional blood smear. Specifically, we were primarily interested in the sensitivity for malaria diagnosis and the NPV to exclude malaria of each diagnostic modality. Differences between the diagnostic performances were analyzed by using the 2-tailed McNemar test to compare the paired, nominal data. The STARD (STAndards for the Reporting of Diagnostic accuracy studies) checklist for the reporting of studies of diagnostic accuracy was used [11]. This study was approved as exempt by the institutional review boards of all 5 institutions involved. The only patient data collected were deidentified data provided to the laboratory at the time of the thick or thin blood smear order, as would occur in routine clinical practice.

**RESULTS**

A total of 103 individual specimens (12%) tested positive for malaria by either blood smear or RDT, with 95 results (11%) confirmed by PCR, among 852 specimens tested (Figure 1). Fifty-six percent of patients with malaria were male, and 44% were female. The mean age (± standard deviation) was 33 ± 1.9 years (range, 18 months to 67 years).

Overall, in all species of malaria, the RDT was superior to traditional blood smear examination (Table 1), with a better sensitivity of 97% (92 of 95 specimens), compared with 85% (81 of 95) for traditional Giemsa blood smear (*P* = .003), and a better NPV (99.6% vs 98.2%; *P* = .001). The largest discrepancy in performance was for the diagnosis of *P. falciparum*, with 100% sensitivity (74 of 74 specimens) for the RDT, compared with only 88% (65 of 74) for blood smear (*P* = .003).

For nonfalciparum malaria (ie, malaria due to *Plasmodium vivax*, *Plasmodium ovale*, or *Plasmodium malariae*), the sensitivity of the RDT was lower, at 86% (18 of 21 specimens), which was comparable to the 76% sensitivity (16 of 21) for
blood smear ($P = .63$). However, even with nonfalciparum the RDT had a high NPV ($>98\%$). In addition, although there were few cases involving $P$. ovale ($n = 5$), both the RDT and blood smear had poor specificity ($60\%$). One noteworthy anomaly was the false-positive results of the RDT, which occurred in 8 (1%) of the 757 specimens that yielded negative results by smear and PCR; however, in 7 of the 8 cases, recent, documented antimalarial treatment had been given. Because the RDT is an antigen-based test, antigen may persist in the blood long beyond viable $Plasmodium$ parasites. An example of the range of RDT test results for $P$. falciparum and non–$P$. falciparum malaria is provided in Figure 2.

 specificity by thin blood smear was excellent (100%), with correct malaria speciation in 68 (84%) of 81 specimens as referenced by PCR. However, the inability by blood smear to identify the species occurred in 10 (14%) of 74 $P$. falciparum specimens and 2 mixed $P$. ovale and $P$. malariae specimens, and there was 1 misidentification of $P$. malariae as $P$. ovale. The larger clinical concern was the 9 (12%) of 74 $P$. falciparum cases that read as negative on blood smear but positive by both RDT and PCR.

PCR was used to validate all positive samples, confirming the malaria species $P$. falciparum ($n = 74$), $P$. vivax ($n = 8$), $P$. ovale ($n = 5$), $P$. malariae ($n = 6$), and mixed $P$. malariae and $P$. ovale ($n = 2$). The parasite percentage ranged from $<0.1\%$ to 18%. Malaria cases were exposed in multiple regions, including Africa (93%), Asia/Pacific (6%), and Latin America (1%). No malaria cases occurred in newly arrived refugees. This finding was likely due to the institution of predeparture, presumptive antimalarial therapy for migrating refugees by the Centers for Disease Control and Prevention in 1999 [12].

## DISCUSSION

Malaria is the leading infectious cause of mortality among travelers, yet most returning febrile travelers will not have malaria [2]. Thus, the diagnosis is frequently delayed or missed in regions where it is not endemic. It is likely that a sensitive and reliable RDT would improve malaria diagnosis and treatment in the United States. This study compared the rapid antigen capture assay (NOW Malaria Test) with the current clinical method of Giemsa-stained blood smears in returning travelers. This was an operational study to compare the performance of each test in a typical US setting to assess the utility of the RDT in clinical practice. Thus, we believe the results are generalizable to other US acute care settings.

There are many rapid tests available on the global market, and although they have shown variable results in the past, the newer-generation assays have been refined, particularly for

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**Table 1. Test Performance Characteristics of the Rapid Diagnostic Test (RDT) versus Blood Smear for Malaria Diagnosis**

<table>
<thead>
<tr>
<th>Species, test</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
<th>Positive LR</th>
<th>Negative LR</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All malaria ($n = 95$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Malaria RDT</td>
<td>97 (99.3–91.0)</td>
<td>99</td>
<td>92</td>
<td>98</td>
<td>24</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Blood smear</td>
<td>85 (91.7–76.5)</td>
<td>100$^b$</td>
<td>99</td>
<td>93</td>
<td>160</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>$Plasmodium falciparum$ ($n = 74$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Malaria RDT</td>
<td>100 (100–95.1)</td>
<td>99</td>
<td>90</td>
<td>100</td>
<td>9.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Blood smear</td>
<td>88 (94.3–78.2)</td>
<td>100$^b$</td>
<td>100</td>
<td>95</td>
<td>166</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>$Plasmodium vivax$, $Plasmodium ovale$, and $Plasmodium malariae$ ($n = 21$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td>Malaria RDT</td>
<td>86 (97.0–63.7)</td>
<td>99</td>
<td>69</td>
<td>99</td>
<td>21</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Blood smear</td>
<td>76 (91.8–52.8)</td>
<td>100$^b$</td>
<td>100</td>
<td>98.5</td>
<td>144</td>
<td>0.33</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Blood smear, Giemsa-stained thick and thin blood smear; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; RDT, rapid diagnostic test.

$^a$ $P$ value is for sensitivity via the McNemar test.

$^b$ Inability to correctly speciate occurred in 10 (14%) of 74 $P$. falciparum and 3 (14%) of 21 nonfalciparum specimens.
acute *P. falciparum* infections [7, 8]. Because *P. falciparum* malaria is the most common life-threatening infection in travelers, and because the health of infected persons may deteriorate rapidly [2, 13], outpatient physicians must be able to reasonably exclude *P. falciparum* before allowing outpatient treatment in nonimmune patients. Therefore, a rapid test with a high NPV would be of particular use in treating outpatients if a physician must decide in a timely manner if a nontoxic-appearing, nonimmune patient with exposure to *P. falciparum* should be hospitalized. Such a test with a high NPV may fundamentally alter the current approach to treating the febrile returning traveler as an outpatient.

A pivotal clinical trial evaluating the performance of the rapid antigen capture assay in areas of endemicity among >4000 patients was recently presented at the American Society of Tropical Medicine and Hygiene annual conference [14]. For *P. falciparum*, the sensitivity was 95% and the specificity was 94% overall. In those with higher parasitemia (parasite count, >5000/μL), the sensitivity was 99.7%. Travelers presenting in regions where malaria is not endemic to acute care centers have relatively high malaria parasite loads, typically with a parasite count on the order of >10,000/μL (0.2% parasite percentage) [15]. Thus, the slightly better RDT performance in our study is most likely due to 2 facts. First, the study population consisted of nonimmune, returning US travelers who typically have higher parasite loads than other studies’ populations in malaria-endemic areas. Second, we compared the RDT to single blood smears performed under real-world conditions (rather than the gold standard of 3 tests read by expert malariologists).

Few other studies evaluating this newer-generation RDT assay have been presented or published. Farcas et al [16], in a laboratory research protocol, evaluated 256 ill Canadian travelers and found comparable results, with a sensitivity of 95.5% for *P. falciparum*, 87% for *P. vivax*, and 83.5% for all nonfalciparum malaria. In another study of returning French travelers, the sensitivities were 98.8% for *P. falciparum* and 80% for *P. vivax* [17]. In an Italian study of 145 persons with malaria from among 171 immigrants and 136 returning travelers, 100% *P. falciparum* sensitivity was reported [18]. All studies have reported excellent specificity (>98%) for all species [14–18].

In our study, there were false-positive results for *P. falciparum* detected in the RDT specimens obtained from 8 patients, of whom 7 had known recent treatment. These false-positive results are usually due to the persistence of the histidine-rich protein antigen after treatment, although rheumatoid factor can also yield false-positive results due to binding immunoglobulin G [19].

This study had some interesting results and observations. The RDT is particularly insensitive for detection of *P. ovale* infection (60%), which is consistent with other studies [16, 20]. In fact, 2 of the 5 cases in this study were missed by both microscopy and RDT and were detected in negative samples sent for control purposes. These cases were independently confirmed by 2 distinct PCR techniques in separate laboratories. Another interesting case was a mixed infection with *P. ovale* and *P. malariae*—a rare combination—on 2 separate samples from the same patient, which was confirmed by PCR at both laboratories.

**Clinical implications.** We believe that, in point-of-care settings, the rapid antigen capture assay serves as a rapid and accurate test for reasonably excluding *P. falciparum* (high NPV) and a valuable adjunctive test in rapidly confirming the diagnosis of both *P. falciparum* and nonfalciparum malaria. An ill patient who tests positive for *P. falciparum* should receive immediate therapy with either intravenous quinidine for severe cases or atovaquone-proguanil (Malarone; GlaxoSmithKline), artemether-lumefantrine (Coartem; Novartis), mefloquine (Lariam; Roche), quinine-doxycline, or quinine-clindamycin for nonsevere cases. In severe cases if quinidine is unavailable, artesunate may be acquired from the Centers for Disease Control and Prevention. For *P. vivax* and *P. ovale* malaria, chloroquine is typically the drug of choice, although there are increasing areas of resistance, and primaquine therapy is required after glucose-6-phosphate dehydrogenase testing. Refer to the Centers for Disease Control and Prevention’s Web site on malaria (http://www.cdc.gov/malaria) for current treatment recommendations. Follow-up consultation with a physician knowledgeable in tropical medicine should occur; however, initiation of therapy should not be delayed. When in doubt, treat for the worst case scenario of chloroquine-resistant *P. falciparum*,...
pending the results of additional tests. Persons returning from Southeast Asia—in particular, from the forested regions of Malaysia—may be at risk of Plasmodium knowlesi infection, a primate malaria for which humans act as an unintentional host. No published data are available on the performance of RDT for the detection of P. knowlesi, and traditional smears should be performed in all suspect cases.

We believe that traditional blood smear testing remains valuable, particularly for estimating the level of parasitemia and malaria speciation. In most cases, blood specimens for thick and thin smears should be collected simultaneously with performance of RDT. The package insert and the FDA state that the RDT result, if negative, must be confirmed by thick or thin blood smear. We believe this guidance is particularly valuable when nonfalciparum malaria and mixed infections are being considered. Clinical judgment must be exercised. Even if an RDT result is negative, in the case of high pretest probability, patient care should be conservative. Similarly, regardless of test results, all toxic-appearing returning travelers require hospitalization and appropriate assistance from trained tropical medicine and/or infectious disease specialists.

Study limitations. This study was designed to evaluate the performance of the rapid antigen capture assay during routine practice in US hospital and clinic settings. In keeping with the operational design, although 3 thick and thin blood smears are recommended to exclude malaria, in reality clinical decisions need to be made in a timely manner in the acute care setting. Therefore, the analysis was performed comparing each specimen separately rather than by case. Because of current US Health Insurance Portability and Accountability Act regulations, as a laboratory-based study, no personal identifiable data were collected and comparison by case was not possible. The smears were performed under normal operational circumstances in each laboratory setting. Therefore, this study reflects the RDT’s use in actual US clinical practice and should be broadly generalizable to other US institutions. Furthermore, because of cost constraints, not all samples with negative results were tested by PCR. Conceivably, some additional low-level malaria infections may have been missed by both microscopy and RDT, which would decrease the overall sensitivity of both tests. However, although this is a possibility, even if present, this would not alter the results because it would decrease the sensitivity of both testing techniques equally.

Caution exists with any negative malaria test result, either by RDT or blood smear. A negative test result does not fully exclude infection with malaria. This is particularly true at low parasite levels and with nonfalciparum. Subpatent malaria infection (which is not yet detectable) is possible with early testing soon after the onset of symptoms. In Canada, persons who receive a diagnosis of malaria presented to health care institutions after an mean of 3.5 days of symptoms with initial thick blood smears being diagnostic in 99% but not all [4]. In febrile travelers recently returned from a malaria-endemic region with unexplained febrile illness, physicians should arrange clinical follow-up within 24 h and consider repeated malaria tests.

Conclusions. This operative study comparing traditional blood smear to rapid antigen capture test demonstrates that the RDT, as performed in a routine clinical setting, is superior to a single set of Giemsa-stained blood smears for quickly evaluating a patient for malaria. Importantly, the rapid antigen capture assay had a 100% NPV for P. falciparum malaria. Although further clinical experience with this test is necessary, this FDA-approved test appears to be an ideal tool for timely decision making when P. falciparum must be considered for patient disposition. This is especially true when reliable, experienced microscopy is not immediately available, such as after hours or in small or rural settings with physicians without expertise in reading malaria blood smears. Although we believe that blood smears and expert microscopy remain essential, the overall RDT performance provides physicians with a valuable adjunctive diagnostic tool in the timely evaluation of the ill returning traveler.

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