Sensitivity and Specificity of Dipstick Tests for Rapid Diagnosis of Malaria in Nonimmune Travelers

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Swift diagnosis of Plasmodium falciparum malaria in areas where the disease is not endemic is frequently complicated by the lack of experience on the side of involved laboratory personal. Diagnostic tools based on the dipstick principle for the detection of plasmodial histidine-rich protein 2 (HRP-2) and parasite-specific lactate dehydrogenase (pLDH), respectively, have become available for the qualitative detection of P. falciparum malaria. In order to evaluate two of the currently available assays, specimens from 231 patients were screened during a prospective multicenter study. Among the screened specimens, samples from 53 patients (22.9%) were positive for P. falciparum malaria by microscopy and/or PCR. While the test kit based on the detection of HRP-2 performed with a sensitivity of 92.5% and a specificity of 98.3%, the kit for the detection of pLDH showed a sensitivity of 88.5% and a specificity of 99.4%. Dipstick tests have the potential of enhancing speed and accuracy of the diagnosis of P. falciparum malaria, especially if nonspecialized laboratories are involved.

Due to the steadily increasing numbers of international travelers to malarious areas, imported malaria is an escalating problem in many countries. It has been estimated that 90% of infected travelers do not develop symptoms until after returning home (8). Accurate and timely treatment of imported malaria requires fast and reliable diagnosis. Microscopic examination of stained blood films still remains the mainstay of diagnostic methods. However, correct interpretation of the blood films requires considerable expertise that is not necessarily available at peripheral medical centers in countries where the disease is not endemic (7). The availability of a simple and accurate test could greatly aid in the diagnosis of malaria in nonimmune travelers returning to their home countries.

Two fast and simple immunochromatographic tests based on the dipstick principle have recently become available for the diagnosis of Plasmodium falciparum malaria. Both tests detect circulating parasite antigen by the use of specific antibodies which are bound to a membrane: ICT Malaria P.f. (ICT Diagnostics, Sydney, Australia) targets histidine-rich protein 2 (HRP-2) of P. falciparum, whereas OptiMAL (Flow, Inc., Portland, Oreg.) detects parasite-specific lactate dehydrogenase (pLDH). In order to evaluate the potential impact these new methods might have on diagnosing malaria in nonimmune patients, a multicenter, prospective study was performed among febrile German travelers returning from malarious areas. The sensitivity and specificity of both tests were investigated by using blood films. If divergent results occurred, PCR was performed for confirmation.

MATERIALS AND METHODS

Patients. During a prospective study that involved outpatient clinics and their laboratory facilities at three different sites (Department of Infectious Diseases and Tropical Medicine and Central University Hospital, both of the University of Munich; and the Infectious Diseases Department of the Medical Clinic at Campus Virchow Hospital, Humboldt University, Berlin), patients presenting with fever (>37.5°C) were selected by consideration of the following inclusion criteria: they were Germans or residents of Germany for more than 10 years, they had recently traveled to an area where malaria is endemic, and they gave informed consent to participate in the survey. Before treatment was initiated, a whole-blood sample was derived from each patient for thin and thick blood film, complete blood count, dipstick tests, and PCR, where applicable. Blood films were considered negative if no parasites were seen in 200 oil-immersion fields (1,000×). The parasite density was determined by calculating the percentage of infected erythrocytes in a thin blood film. The baseline erythrocyte counts of patients were used to calculate the parasitemia (parasites/μl).

Methods. Both dipstick tests in this survey detect parasite antigen in whole blood by binding to specific antibodies, and a subsequent color reaction produces a visible band on the dipstick. While ICT Malaria P.f. targets HRP-2 and therefore detects exclusively infections with P. falciparum, OptiMAL is designed to detect infections with both P. falciparum and P. vivax and to distinguish between them by the binding of species-specific pLDH. Manufacturer’s instructions were followed strictly in both tests. Individual investigators were blinded to the results of microscopy and the other test line. If discordant results between microscopy and one of the dipstick tests occurred, a PCR method was used to confirm the presence of plasmodial DNA. For this, approximately 10 μl of peripheral blood was placed on Whatman number 4 filter paper and then air dried at room temperature. DNA was extracted from the blood spots by soaking in Chelex suspension as previously described (5). Species identification was performed with species-specific oligoprobes as previously described (10). In short, a nested-PCR protocol combining a first primer pair for identification of plasmodial infection in general and a second primer pair for species identification was followed.

RESULTS

Among the 231 patients included in this study, 53 (22.9%) presented with microscopically confirmed P. falciparum malaria. A further 13 (5.6%) patients were infected with P. vivax, while 1 (0.4%) patient presented with P. ovale and 2 (0.9%) patients were infected with quartan malaria. For the detection of P. falciparum malaria, the results of both dipstick tests compared to microscopy are shown in Table 1. In the population of this study of nonimmune patients returning from a great variety of areas where malaria is endemic, ICT Malaria P.f. performed with a sensitivity of 92.5% (95% confidence interval [CI]; range, 87.2 to 95.8%) and a specificity of 98.3% (95% CI; range, 97.2 to 99.8%) compared to microscopy. The positive predictive value (PPV) of this test was 94.2%, and the negative
TABLE 1. Detection of *P. falciparum* infection by OptiMAL, ICT Malaria P.f., blood films and PCR (n = 231)

<table>
<thead>
<tr>
<th>Dipstick test</th>
<th>Blood film result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
</tr>
<tr>
<td>OptiMAL</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>47</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
</tr>
<tr>
<td>ICT Malaria P.f.</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>49</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
</tr>
</tbody>
</table>

predictive value (NPV) 97.8%. In comparison, OptiMAL showed a sensitivity of 88.7% (95% CI; range, 84.1 to 91.9%) and a specificity of 99.4% (95% CI; range, 96.4 to 100%), while the PPV was 97.9% and the NPV was 96.7%. All samples producing discordant results between microscopy and dipstick tests were evaluated by PCR for the detection of plasmodial DNA. By this method, three false-positive and four false-negative results were confirmed for the ICT Malaria P.f. test, while the OptiMAL assay produced one false-positive and six false-negative results (Table 1). Apart from one sample with a parasitemia of 20,000/µl that was repeatedly negative in both immunochromatographic tests, parasitemia was ≤5,000/µl in all patients with false-negative results in either of the dipstick tests. In another sample, derived from a patient returning from Tanzania, the ICT Malaria P.f. test was positive, while microscopy and the OptiMAL assay were negative. However, on the PCR control, this sample was repeatedly positive for *P. falciparum* DNA. The patient tested positive, on returning 12 h later, for *P. falciparum* in both dipstick tests and by microscopy and now had a parasitemia of 0.5%. In both dipstick methods, cross-reactions with other plasmodial species did not occur.

Apart from reacting positive on contact with *P. falciparum*-specific pLDH, the OptiMAL assay is designed to detect *P. vivax* as well. Infection with *P. vivax* did occur in a small subsample of our study population (n = 13). Results for the performance of the OptiMal assay against microscopy were as follows. Of 231 samples, 8 were positive and 218 were negative by both OptiMAL and blood film analysis. Another 5 were found to be negative by OptiMAL but positive by blood film analysis. No samples were found to be negative by blood film analysis. As in all discordant tests for *P. falciparum* malaria, species identification by PCR was used as the confirmatory method. The sensitivity of OptiMAL for the detection of *P. vivax* was 61.5%, and the specificity was 100% (PPV, 100%; NPV, 97.8%).

**DISCUSSION**

An easily performed, rapid, and accurate test for the detection of plasmodial infections is needed in laboratories lacking trained microscopists not only where malaria is endemic but also where it is not endemic. Such a test could facilitate the early diagnosis and an appropriate therapy in patients with imported malaria, thereby reducing mortality. The results of this study show that both dipstick tests detected *P. falciparum* malaria with high specificity and sensitivity. While OptiMAL detected *P. falciparum* infections with a sensitivity of 88.5%, ICT Malaria P.f. did so with a sensitivity of 92.5%. Both tests also showed a very high specificity: 99.4 and 98.3%, respectively. One patient, found to be negative on microscopy, was first diagnosed as having *P. falciparum* malaria as a result of a positive ICT Malaria P.f. dipstick. Possibly his infections would have been overlooked until much later without the use of this test kit. Similar results for sensitivity and specificity have been recorded for other test kits detecting HRP-2 antigen (2, 3, 4, 6). In a study among semimmune patients in Honduras, the OptiMAL assay was evaluated among a small set of patients with *P. falciparum* malaria and showed almost the same sensitivity as in this study (9). However, our data regarding the detection of *P. vivax* by OptiMAL (sensitivity, 61.5%; specificity, 100%) is in contrast to the results from Honduras: i.e., a sensitivity of 94% and a specificity of 100%. Clearly, a larger set of patients with *P. vivax* infections from various areas where it is endemic needs to be investigated before concluding recommendations for the use of OptiMAL in diagnosing tertian malaria are possible.

In all of the samples except one (with false-negative results in both of the dipstick tests), parasitemia was ≥5,000/µl, indicating a decreased sensitivity in cases with low parasitemia in both tests. Similar results have been recorded for the use of the OptiMAL assay in semimmune populations (9). However, the observation that one patient with a parasitemia of 20,000/µl was repeatedly negative in both tests is of concern. The reasons for this finding remain unclear, but similar problems have been recorded before for dipstick assays based on the detection of plasmodial HRP-2 (1, 4, 6).

In conclusion, dipstick tests for the detection of plasmodial antigens may develop into an important diagnostic tool for areas where malaria is not endemic. Both tests are extremely simple and rapid to perform, making it easy to teach the methodology to inexperienced or even untrained persons. However, although sensitivity was at high levels, it was limited in both tests investigated in this survey. Even though a negative dipstick result makes *P. falciparum* infection with a significant level of parasitemia unlikely, it cannot be ruled out completely. Other limitations of these tests include the inability to provide information about the level of parasitemia and the lack of reliable discrimination between mixed infections and those with *P. falciparum* alone. The necessity for obtaining blood films for microscopical examination in every single patient who may have malaria is not replaced by the currently available dipstick tests. It should be emphasized that *P. falciparum* malaria, a potentially lethal disease, must not be missed because of a false-negative dipstick test.

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**REFERENCES**


