Use of Dipstick Tests for the Rapid Diagnosis of Malaria in Nonimmune Travelers

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Background: Swift diagnosis of falciparum malaria in nonendemic areas is frequently complicated by lack of experience on the side of involved laboratory personnel. Diagnostic tools based on the dipstick principle for the detection of plasmodial histidine-rich protein 2 (HRP-2) (ICT Malaria P.f.®) and parasite-specific lactate dehydrogenase (pLDH) (OptiMal®), respectively, have become available for the qualitative detection of falciparum malaria.

Methods: In order to evaluate currently available assays, a series of studies was conducted: sensitivity and specificity were evaluated by investigation of specimens from 231 febrile returnees from endemic areas, cross-reactivity in patients with rheumatoid factor (RF) was assessed among 92 patients from a rheumatology unit, and the quality of dipstick self-use by febrile travelers was tested in Kenya.

Results: Whereas 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%. Cross-reactions with sera positive for rheumatoid factor occurred in 8.6% with the ICT Malaria P.f.®, and in 3.3% with the OptiMal® test. Only ICT Malaria P.f.® was tested for quality of self-use among travelers. This dipstick assay was performed successfully by 67 patients (68.4%), but 31 (31.6%) were unable to obtain a result.

Conclusion: Dipstick tests have the potential of enhancing speed and accuracy of the diagnosis of falciparum malaria, especially if non-specialized laboratories are involved. However, microscopical testing remains mandatory in every single patient with the possible diagnosis of malaria. Self-use of dipstick tests for malaria diagnosis by travelers should only be recommended after appropriate instruction and training, including a successful performance of the test procedure.

Due to the steadily increasing numbers of international travelers to malarious areas, falciparum malaria is becoming an escalating diagnostic and therapeutic challenge. It has been estimated that 90% of infected travelers do not develop symptoms until after returning home. 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 blood films requires considerable expertise that is not necessarily available at peripheral medical centers in nonendemic countries. Similarly, febrile travelers might experience severe diagnostic problems and delay in therapy when reporting to understaffed and poorly equipped health posts in endemic countries. Thus, the availability of a simple and accurate test could greatly aid the diagnosis of malaria in nonimmune travelers.

Two fast and simple immunochromatographic tests based on the dipstick principle have recently become available for the diagnosis of falciparum malaria. Both tests detect circulating parasite antigen by 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 Plasmodium falciparum whereas OptiMal® (Flow Inc., Portland, Oregon, USA) detects parasite-specific lactate dehydrogenase (pLDH). In several European countries, the ICT Malaria P.f.® test is marketed for self-use by tourists. Travelers to malarious areas are advised to carry the test kit as an emergency tool in their luggage, and to use it themselves in case they develop fever in a situation where they cannot reach adequate professional help within a safe time limit. The utilization of this method by travelers has not been investigated under realistic conditions.

In order to evaluate currently available assays, a series of studies was conducted: in a multicenter, prospective study, sensitivity and specificity were evaluated by investigation of specimens from 231 febrile German returnees from endemic areas, cross-reactivity in patients with rheumatoid factor was assessed among patients from a rheumatology unit, and the quality of dipstick self-use by febrile travelers was tested in Kenya. Sensitivity and specificity were investigated against blood films.

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divergent results occurred, polymerase chain reaction (PCR) was performed for confirmation.

Patients and Methods

Sensitivity and Specificity in Nonimmune Travelers

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 associated with 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 using the following inclusion criteria: they were Germans or residents of Germany for more than 10 years, they had recently traveled to an area endemic for malaria, 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 (1,000x). Parasite density was determined by calculating the percentage of infected erythrocytes in a thin blood film. Patients baseline RBC counts were used to calculate parasitemia (parasites/µL). Both dipstick tests in this survey detect parasite antigen in whole blood by binding to specific antibodies and subsequent color reaction that produces a visible band on the dipstick. Whereas ICT Malaria P.f.® targets plasmodium and to distinguish 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 were dotted on Whatman™ #4 filter paper and air-dried at room temperature. DNA was extracted from the blood spots by soaking in Chelex suspension as previously described. Species identification was performed with species-specific oligoprobes as previously described. 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.

Cross Reactivity with Rheumatoid Factor

All investigated sera were derived from nonimmune German patients with no previous history of malaria, who did not visit endemic regions for at least the past 5 years. Sera stored at −70°C from 79 patients of a rheumatology unit with a variety of immunological disorders, and known to be RF positive, were tested together with negative controls. As standard dipstick test procedures are carried out with full blood, we tested full blood and sera specimens of a further 12 consenting patients concomitantly. P.falciparum positive controls were run in both series. The assay used for quantitative measurement of rheumatoid factor detects autoantibodies of the IgM isotype, reactive with the Fc fragment of IgG by nephelometry, with a cut-off value of 24 IU/mL. Additionally, we screened our samples with the latex agglutination method for qualitative RF detection.

Self-Use of Dipstick Tests by Febrile Travelers

For this study, only the ICT Malaria P.f.® kit was evaluated since this is the only test openly marketed for self-use by travelers. A prospective study among travelers in Kenya was conducted during the period from June 1998 until February 1999. In collaboration with local health care providers in Diani Beach, a holiday resort south of Mombasa, patients reporting with fever (>38°C) were asked to carry out the dipstick test without any assistance other than the manual provided by the manufacturer. Included patients were nonimmune guests or residents of one of the hotels, who had no previous contact with this type of dipstick test. Informed consent was obtained. All participants were supervised and their performance was assessed by a standardized questionnaire that was completed by an attending health professional. Normal diagnostic procedures were not altered, a thick blood film was done in every patient. Microscopical results were used as a “gold standard” for confirmation of malaria diagnosis.

Results

Sensitivity and Specificity in Nonimmune Travelers

Among the 231 patients included in this study, 53 (22.9%) presented with microscopically confirmed falciparum malaria. A further 13 (5.6%) patients were infected with Plasmodium vivax, while 1 (0.4%) presented with Plasmodium ovale and there were 2 (0.9%) patients with quartan malaria. For the detection of falciparum malaria, the results of both dipstick tests in comparison with microscopy are shown in Table 1. In the population of this study, representing nonimmune patients returning from a great variety of endemic locations, ICT Malaria P.f.® performed with a sensitivity of 92.5% (95% confidence interval [CI] 87.2–95.8%) and a specificity of 98.3% (95% CI 97.2–99.8%), as compared to microscopy. The positive predictive value (PPV) of this test was 94.2%, the negative predictive value (NPV) 97.8%. In comparison, OptiMal® showed a sensitivity of 88.7% (95% CI 84.1–91.9%) and a specificity of 99.4% (95% CI
96.4–100%), while PPV was 97.9% and NPV 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, 3 false positive and 4 false negative results were confirmed for the ICT Malaria P.f.®, whereas the OptiMal® assay produced 1 false positive and 6 false negative results (see Table 1). Apart from one sample with a parasitemia of 20,000/µL that was repeatedly negative in both immunochromatographic tests, parasitemia was at 5,000/µL or below in all patients with false negative results, in either of both dipstick tests. In another sample, derived from a patient returning from Tanzania, the ICT Malaria P.f.® was positive, whereas microscopy and the OptiMal® assay were negative. However, on PCR control, this sample was repeatedly positive for P.falciparum DNA. On returning 12 hours later, the patient tested positive for P.falciparum in both dipstick tests and by microscopy, now having 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 sub-sample of our study population (n = 13). Results for the performance of the OptiMal® assay against microscopy are shown in Table 2. As in all discordant tests for 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%, specificity was 100% (PPV 100%, NPV 97.8%).

Cross Reactivity with Rheumatoid Factor

Out of 91 sera screened, 73 samples (80.2%) tested correctly negative in both malaria assays. The rate of false positives was 9/91 (9.9%) in total, with 6/92 (6.6%) for the ICT Malaria P.f.®, and 3/91 (3.3%) for the OptiMal® test, respectively. Only 1 specimen tested false positive in both tests. RF means were 119 IU/mL for all samples, 88 IU/mL for all negative samples, and 234 IU/mL for all positive samples. In the ICT Malaria P.f.® false positives, the mean RF was higher (189 IU/mL), compared to 142 IU/mL in the OptiMal® false positives. All P. falciparum malaria patient controls were correctly positive, all RF negative controls were correctly negative. There was a concordance of 91.7% (11/12) between serum and blood samples tested from the same individuals. Height of RF titers and strength of latex agglutination were not found to be correlated. Neither did we observe significant differences in means of RF titers between falsely positive and correctly negative specimens, nor between positives in the latex agglutination test and the corresponding malaria test results (9 samples tested weakly positive, with one corresponding falsely positive in the ICT Malaria P.f.® test; 2 tested strongly positive, with one corresponding falsely positive in the same test).

Self-Use of Dipstick Tests by Febrile Travelers

Out of 98 patients with fever that were seen during the study period, 52 (53.1%) were male. Eleven patients (11.2%) presented with microscopically confirmed falciparum malaria. The dipstick assay was performed successfully by 67 patients (68.4%), but 31 (31.6%) were unable to obtain a result. The relatively high amount of handling problems was drastically highlighted by the test performance of the 11 patients with falciparum malaria: only 1 was able to obtain a valid test result. Drawing blood by finger prick was the second most frequent problem that hindered the test procedure. This was the only point where patients received limited assistance by the attending health practitioner. Reasons for failure of the dipstick test were noted, and are given in Table 3. If performed by health professionals, there were no differences between dipstick results and microscopical diagnosis.

Discussion

An easily performed, rapid, and accurate test for the detection of plasmodial infections is needed in laboratories lacking trained microscopists not only in malaria endemic, but also in nonendemic areas. It could facilitate early diagnosis, and appropriate therapy, in patients with imported malaria, thereby reducing mortality. The results of our investigations show that both dipstick tests detect falciparum malaria with high specificity and sensitivity. Whereas 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 showed a very high specificity: 99.4 and 98.3% respectively. One
patient, being microscopically negative, was first diagnosed as having falciparum malaria by a positive ICT Malaria P.f. dipstick. Possibly his infection 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.3,7–11 In a study among semi-immune patients in Honduras, the OptiMal® assay was evaluated among a small set of patients with falciparum malaria, showing almost the same sensitivity as in this study. However, our data regarding the detection of P. vivax by OptiMal® (sensitivity 61.5%, specificity 100%) contrast to the results from Honduras: sensitivity was 94% in that study, specificity 100%. Clearly, a larger set of patients with P. vivax infection from various endemic areas needs to be investigated before concluding recommendations for the use of OptiMal® in diagnosing tertian malaria are possible.

An “absence of false positive results” has been proposed for the OptiMal® kit.13 The results of our investigation do not confirm this.14 False positive reactions occurred in samples with and without rheumatoid factor (RF). In our cohort of patients with rheumatoid factor, we found a strong positive correlation between results from full blood and serum samples, thus rendering our results applicable to the field situation. RF positive patients might test falsely positive to a varying extent in all rapid immunochromatographic malaria tests independent of the antigen used for detection. In the smaller collectives recently described, figures of false positives for another immunochromatographic test detecting HRP-2 (Parasight-F®) were even higher than reported here, with 11/16 (68.8%)15 and 13/19 (68.4%)16 cases, respectively. The discrepancy between results obtained with Parasight-F compared with the other techniques might be due to the different RF assays used, the heterogeneity of the antibodies subsumed under the term “rheumatoid factors,” as well as that other autoantibodies such as antinuclear antibodies (ANA), which have been associated with cross-reactions by the authors of both early reports cited. In fact, it is not clear whether RF and ANA are just two out of a larger group of “false reactants,” or whether they are just confounders in individuals harboring another, still unidentified common reacting autoantibody.

Moreover, the mechanisms of how autoantibodies cause false positive results by cross-reacting with test systems based on the capture of specific plasmodial antigens, remain to be elucidated.

In all samples—but one—with false negative results in either of the dipstick tests, parasitemia was at 5,000/µL or below, thus indicating decreased sensitivity in cases with low parasitemia, in both tests. Similar results have been recorded for the use of the OptiMal® assay in semi-immune and nonimmune populations.12,13 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 remain unclear, but similar problems have been recorded before for dipstick assays based on the detection of plasmodial HRP-2.3,9,17

To our knowledge, no previous studies have attempted to document the utilization of a freely marketed dipstick test kit for malaria diagnosis by travelers under field conditions. The discouraging results of our investigation in Kenya (see Table 3) suggest that persons who are inexperienced in laboratory procedures are not likely to develop such skills under conditions of severe stress. The high failure rate among travelers with falciparum malaria (10 out of 11) shows that patients might simply be too sick to obtain diagnosis, and initiate treatment by themselves. Obviously, the assessment situation was artificial for the affected travelers who, while being febrile and waiting for the outcome of their blood film, were asked to perform the dipstick test without assistance. However, it was the closest controlled scenario we were able to create, in order to test how the test kit was used by inexperienced laymen under conditions of severe stress. The use of dipstick tests for malaria diagnosis by travelers should only be recommended after appropriate instruction and training, including a successful performance of the test procedure.

In conclusion, dipstick tests for detection of plasmodial antigens may develop into an important diagnostic tool in nonendemic areas. Both tests are extremely simple and rapid to perform, making it easy to teach the methodology to inexperienced or even untrained persons. However, although at high levels, sensitivity was limited in both tests investigated in this survey. Although a negative dipstick result makes P. falciparum infection with a significant level of parasitemia unlikely, it cannot be ruled

### Table 3 Self-Use of Rapid Tests for Malaria Diagnosis by Tourists: Reasons for Performance Problems (n = 31, Multiple Entries Possible)18

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<thead>
<tr>
<th>Reason</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>Unable to draw blood (finger prick)</td>
<td>22</td>
<td>71.0</td>
</tr>
<tr>
<td>Unable to place the blood drop appropriately on the test kit</td>
<td>8</td>
<td>25.8</td>
</tr>
<tr>
<td>No adherence to the recommended waiting period (8 min)</td>
<td>12</td>
<td>38.7</td>
</tr>
<tr>
<td>Unable to identify the bands indicating the test result</td>
<td>18</td>
<td>58.1</td>
</tr>
<tr>
<td>Unable to interpret the result</td>
<td>27</td>
<td>87.1</td>
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out completely. Other limitations include cross reactions with rheumatoid factor, 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 with the possible diagnosis of malaria is not replaced by the currently available dipstick tests. It should be emphasized that falciparum malaria, a potentially lethal disease, must not be missed because of a false negative dipstick test.

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**References**