Evaluation of the Makromed Dipstick Assay versus PCR for Diagnosis of *Plasmodium falciparum* Malaria in Returned Travelers

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Microscopy has been the traditional reference standard for malaria diagnosis. However, difficulty in maintaining the required technical skills, particularly in areas where malaria is not endemic, has prompted the development of rapid nonmicroscopic diagnostic assays based on the detection of malaria parasite antigen in whole blood. In this study, we evaluated the performance of one such device, the Makromed dipstick test, blindly compared to PCR and microscopy for the diagnosis of *Plasmodium falciparum* malaria in 200 febrile returned travelers. The Makromed assay detects the presence of *P. falciparum*-specific histidine-rich protein II with an antigen capture immunochromatographic strip format. Compared to PCR as the reference standard, the dipstick assay had a sensitivity of 97.0% and a specificity of 96.0%. The positive and negative predictive values were 81.2% and 99.5%, respectively. Rapid malaria diagnostic devices may provide a useful diagnostic adjunct in a clinical setting.

Malaria imported into industrialized countries has become increasingly common due to dramatic increases in international travel and migration and the development and spread of drug resistance in malaria parasites (6, 14, 15). Malaria caused by *Plasmodium falciparum* can be rapidly fatal. Prompt and accurate diagnosis of infection and the initiation of appropriate treatment are essential to improve outcome (12, 13, 19). Microscopic detection of parasites on Giemsa-stained blood smears has been the reference standard for malaria diagnosis for more than a century. However, diagnostic microscopy is a time-, labor-, and equipment-intensive procedure that even in expert hands is occasionally associated with false-positive and -negative results (20).

Establishing and maintaining competent diagnostic microscopy represents a considerable obstacle to achieving reliable malaria diagnosis, particularly in areas where malaria is not endemic (12, 13). Difficulty in maintaining the required technical skills has prompted the development of nonmicroscopic malaria-diagnostic devices based on the detection of malaria parasite antigen in whole blood. The World Health Organization has recognized the need to overcome problems with diagnostic microscopy and supports the development of nonmicroscopic alternatives (1, 2). Unfortunately, while several recently evaluated rapid diagnostic products, based on antigen capture immunoaassays with immunochromatographic strip technology, appeared promising (3, 5, 7–10, 16, 18, 21, 22, 24, 25), many are no longer being marketed.

The Makromed malaria test is a new, rapid immunochromatographic test for the diagnosis of *P. falciparum* malaria. The objective of this study was to determine the test characteristics of the Makromed dipstick for the diagnosis of *P. falciparum* malaria among febrile travelers returning from malarial areas.

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**MATERIALS AND METHODS**

**Patients.** Patients presenting to the Tropical Disease Unit of the Toronto General Hospital in Canada from January 1995 to July 1997 with a history of fever and travel to a malaria-endemic area were eligible for inclusion in the study. All patients with blood films containing malaria parasites during the study period were enrolled. All patients with repeatedly negative blood films during the first 2 months of the study were enrolled to provide a comparable control group. The prevalence of *P. falciparum* malaria in this population during the study period was 15%.

Whole blood samples (pretreatment) were collected from all patients for thick and thin blood film preparation, PCR, dipstick tests, and complete blood count. An expert microscopist who was blinded to the results of additional diagnostic testing examined the blood films. Smears were considered negative if no parasite was seen in 500 oil immersion fields (1,000×) on a thick blood film. Parasite concentration was calculated by determining the number of parasites per 200 to 500 white blood cells in a thick blood film. Baseline white blood cell counts were used to calculate parasitemia (parasites per microliter). All specimens were coded, divided into aliquots, and frozen at −70°C for further testing by PCR and the dipstick test. Sample storage at −70°C has not resulted in changes in assay performance (unpublished observations). All PCR amplification, species identification, and dipstick tests were performed in a blinded fashion. This study was reviewed and approved by the Ethical Review Committee of the Toronto General Hospital.

**PCR.** PCR detection and malaria species identification were performed as previously described (22, 23, 26). Briefly, genomic DNA was extracted from whole blood samples on Qiagen columns (Qiagen, Chatsworth, Calif.) following the manufacturer’s instructions. A 3-μl aliquot of the DNA extract was used in a nested PCR assay to amplify a segment of the *Plasmodium* 18S rRNA gene. The resulting PCR product was analyzed on a 2% agarose gel stained with ethidium bromide as described before (23).

**Dipstick assay.** The Makromed dipstick test (Makro Medical Pty. Ltd., Johannesburg, Republic of South Africa) is a rapid immunochromatographic assay for the detection of *P. falciparum* in whole blood. The test cassettes contain antibodies specific for the *P. falciparum* histidine-rich protein II antigen. The kits were performed in parallel according to the manufacturer’s instructions with previously frozen whole blood samples collected in EDTA. Dipsticks were independently examined and interpreted by three observers blinded to the microscopic and PCR results. The results with each dipstick were recorded as either negative or positive based on the majority agreement.

**Data analysis.** The sensitivity and specificity of the dipstick test were calculated with the PCR results as the reference standard. Positive and negative predictive values were calculated based on the prevalence of *P. falciparum* in all patients presenting to the Tropical Disease Unit during the study period. The K statistic was used to measure agreement among the three blinded observers.
The parasitemia was less than 500 parasites/μl of blood. Positive dipstick results occurred, and in each of these cases, PCR was confirmed to be positive for P. falciparum. All four samples were confirmed to be P. falciparum by PCR. The PCR and blood smear negative. Patients infected with P. falciparum did not differ significantly from other patients with respect to age, sex, or duration of illness. Only 5% of malaria-infected individuals reported adherence to regimens with chemoprophylactic agents. The results of the PCR-based diagnosis compared with the dipstick assay result are shown in Table 1. Table 2 summarizes the results of the microscopic diagnosis compared with the dipstick assay.

The sensitivity of the dipstick assay for diagnosing P. falciparum malaria was 97% and the specificity was 96% when PCR was used as the reference standard. At the time of enrollment for the study, the prevalence of P. falciparum malaria was 15%. Therefore, the positive and negative predictive values of this test were 81.2% and 99.5%, respectively. There was excellent agreement between independent observers, with a K value of 0.98 (standard error, 0.01). When microscopy was used as the reference, the corresponding sensitivity and specificity of the dipstick assay were 98.9% and 92.5%, respectively. There were four positive dipsticks that were identified as Plasmodium species by microscopy (the parasitemia was too low to permit reliable identification to the species level). All four samples were confirmed to be P. falciparum by PCR.

Compared to PCR as the reference standard, three false-negative dipstick results occurred, and in each of these cases, the parasitemia was less than 500 parasites/μl of blood. The sensitivity of the dipstick test was 98.9%. A K value of >0.81 indicates almost perfect agreement between observers. Discrepancies between readers occurred mainly when the test was weakly positive, most frequently when the sample had a low level of parasitemia.

Diostick assays may be most useful when an expert microscopist is not available. Although expert microscopists can detect as few as 10 parasites per μl of blood, the average microscopist is likely to achieve a sensitivity closer to 100 parasites per μl or higher (1). In this investigation, the sensitivity of this dipstick kit was 99% for the detection of P. falciparum in samples with >100 parasites per μl. With parasitemias of <100 parasites per μl, the sensitivity decreased to 71%. False-negative assays for P. falciparum malaria are concerning, and three false-negatives did occur; however, all three occurred in individuals in whom the parasitemia was low (<0.01% or <500 parasites/μl). Thus, when the clinical suspicion of malaria remains high and dipstick tests or smears are initially negative, they should be repeated 12 to 24 h after the negative result (22).

There were four false-positive dipstick assays in this study. Occasional false-positives due to the presence of rheumatoid factor have previously been reported with diagnostic devices based on the detection of histidine-rich protein II (8). Furthermore, detection of antigen may persist for up to 28 days after cure of infection (7). Whether a clinician treats a patient based on a positive dipstick assay will be in part a function of the pretest probability of infection (95%). Therefore, the positive and negative predictive values of this test were 81.2% and 99.5%, respectively. There was excellent agreement between independent observers, with a K value of 0.98. A K value of >0.81 indicates almost perfect agreement between observers. Discrepancies between readers occurred mainly when the test was weakly positive, most frequently when the sample had a low level of parasitemia.


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probability of *P. falciparum* malaria for a given patient. With a pretest probability of 50%, the positive predictive value would be 96%, but if the pretest probability is only 5%, the positive predictive value would fall to 56%.

Our results and those of others indicate that rapid malaria diagnostic devices could provide a useful diagnostic adjunct in a clinical setting, both in malaria-endemic areas and areas where malaria is not endemic. Since laboratories in areas where malaria is not endemic frequently lack expertise in diagnostic microscopy, a dipstick assay could provide a quick and accurate diagnosis while definitive results are sought from a reference laboratory. Importantly, a negative test essentially rules out *P. falciparum* malaria, with a negative predictive value of 99.5%. However, microscopy is still needed to confirm species identification and calculation of parasitemia for positive assays and as a backup to exclude false-negative assays.

In conclusion, the Makremod malaria test is rapid and easy to use and can provide an accurate diagnosis of *P. falciparum* malaria in returned travelers. Despite its apparent high level of diagnostic accuracy, one obvious limitation to its use is that it only detects one species of malaria-causing organism, and therefore, even with a negative dipstick test, the patient may still have non-*P. falciparum* malaria. In order to reach a high level of clinical utility, the development of nonmicroscopic malaria-diagnostic products capable of detecting multiple *Plasmodium* species is required.

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