COMPARISON OF A PARASITE LACTATE DEHYDROGENASE–BASED IMMUNOCHROMATOGRAPHIC ANTIGEN DETECTION ASSAY (OPTIMAL®) WITH MICROSCOPY FOR THE DETECTION OF MALARIA PARASITES IN HUMAN BLOOD SAMPLES

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Abstract. Microscopic examination of blood smears remains the gold standard for malaria diagnosis, but is labor-intensive and requires skilled operators. Rapid dipstick technology provides a potential alternative. A study was conducted in The Gambia to compare the performance of OptiMAL®, an immunochromatographic antigen detection assay for the diagnosis of malaria using parasite lactate dehydrogenase, against standard microscopy in patients with suspected malaria. For initial diagnosis of Plasmodium falciparum, irrespective of stage, this assay had a sensitivity of 91.3%, a specificity of 92%, a positive predictive value of 87.2%, and a negative predictive value of 94.7%. The sensitivity of the test decreased markedly at parasitemias < 0.01%. This assay can be used for the diagnosis of malaria in areas where microscopy is not available and for urgent malaria diagnosis at night and at weekends, when routine laboratories are closed and when relatively inexperienced microscopists may be on duty.

Microscopic examination of thick blood smears is the most widely used routine method for determining malaria infection in humans and remains the gold standard for malaria diagnosis. However, microscopic examination is labor-intensive and individuals that examine slides need to be skilled and experienced to differentiate parasites from artifacts. Also, the many variations in staining and microscopic techniques between laboratories make comparison of results between centers difficult. A centrally standardized dipstick test could overcome these problems. Furthermore, the vast majority of malaria cases occur in areas where there is little or no access to reference laboratories and in many areas, microscopy is not available.

These limitations justify the development and implementation of simple to use dipstick antigen-capture assays. One such test (ParaSight®-F test; Becton Dickinson, Meylan, France) detects Plasmodium falciparum histidine-rich-protein 2 ( PfHRP-2) in peripheral blood. This assay can be done quickly and easily, but can only detect P. falciparum infections. Another problem with the assay is that the circulating antigen is detectable even several days after viable parasites have been eliminated from the peripheral blood. This makes it difficult for health providers to assess accurately the effectiveness of drug therapy. The ICT malaria Pf test (ICT, Sydney, New South Wales, Australia) is an alternative card-based test for the immunochromatographic detection of PfHRP-2. Measurement of an abundant malarial enzyme, Plasmodium lactate dehydrogenase (pLDH), provides another means for non-microscopic detection of malaria parasites. Makler and others have shown that Plasmodium parasites can be accurately detected by the unique ability of the parasite lactate dehydrogenase (pLDH) to use 3-acetyl pyridine adenine dinucleotide (APAD) as a cofactor. They have also shown that pLDH can be detected by immune capture.

The pLDH assay stick (OptiMAL®, Flow, Inc., Portland, OR) detects malaria parasites by using antibody capture to determine the presence of the pLDH antigen in lysed whole blood. The assay stick detects the presence of parasites in 10 µL of fresh, frozen, or dried whole blood samples (fingerstick or venipuncture) collected in EDTA. The pLDH first binds to a gold-labeled antibody particle. This complex then migrates up the test strip where it is captured by an immobilized secondary antibody. A visual antibody-antigen-antibody complex is then formed at the reaction site. The new immunochromatographic test was found to be effective as a diagnostic test for malaria when used in a Specialist Parasitology Laboratory at the Hospital for Tropical Diseases (HTD) (London, United Kingdom) (Moody AH, Hunt Cooke A, Chiodini PL, unpublished data). Therefore, the performance of this assay was assessed in The Gambia during the season of high malaria transmission.

MATERIALS AND METHODS

This study was approved by the Gambian Government–Medical Research Council (MRC) Ethical Committee. Patients more than one year of age attending the Outpatients’ Clinic, MRC (Fajara, The Gambia) during November 1996 with fever or history of fever and a suspected diagnosis of malaria were recruited into the study. Samples were collected from persons solely as required to confirm or exclude the presence of malaria parasites. Verbal consent was obtained to use the sample for further testing, but no additional samples were taken for the purposes of this study. Fingerprick blood samples (maximum volume = 200 µL) were collected from 409 individuals. This sample was used to prepare two thick films on one slide, which was labeled with the laboratory number. One of these films was then stained with Field’s stain and 10 oil-immersion fields at 1,000× magnification were examined by the clinic microscopist, as is the standard practice at this clinic. The result of the blood film was reported to the clinician. These results were used for comparison with the OptiMAL® test after each day’s tests. The second blood film was left unstained and returned to the main laboratory for possible later comparison by the second local expert technician if necessary. After the films were made, the remainder of the blood sample was collected into a tube containing potassium EDTA and labeled with the...
study number. This was matched to the laboratory number only after both the blood film examination and the OptiMAL® test had been completed. The EDTA sample was taken to the main laboratory and divided as follows: 1) a standard thick/thin film was made and kept for further examination, scanning 500 high-power fields; 2) an additional thick film was made and stained with Giemsa in the MRC laboratory for later assessment at the HTD; and 3) 10 μl was used for the OptiMAL® assay and the remainder was stored at −20°C at the MRC.

Dipstick assay. Whole blood from each donor was used with the OptiMAL® assay. Test strips and reagents were obtained from Flow, Inc. A member of the study team (AHC) was trained by Flow, Inc in use of the assay and was present to advise and, if necessary, perform the assays and record the results. Each sample was tested with the dipstick on the day of collection and the results were recorded immediately after the test was completed. A negative control sample, taken from an individual who had not been exposed to malaria for three years, was also tested. Two drops of reagent A (30 μl of colloid/buffer solution) were added to a test well on a configured well plate. Four drops of reagent B (80 μl of clearing solution) were added to a second test well. Ten microliters of blood were then placed into the first test well with gentle mixing. The assay test strip was then placed into the first test well with its wick at the top and the sample was allowed to run up the test strip. After 8 min, the assay test strip was moved to the second test well, which contained the clearing buffer, for an additional 5–10 min. Interpretation of the assay results was performed immediately after completion of the clearing step; approximately 15–20 min after the test was initiated. A negative control sample was included with each batch tested. In the OptiMAL® assay, there are two diagnostic zones of reaction containing different antibodies. A monospecific antibody that recognizes only *P. falciparum* is present in the bottom reaction zone. A second pan-specific antibody is present immediately above this zone. This monoclonal antibody recognizes the pLDH isofrom of *P. vivax*. A third reaction zone is present at the top of the immunochromatographic test strip where there is an antibody that captures the excess colloid conjugate and serves as a positive control for the assay. The colloid conjugate is coupled with a third monoclonal antibody that is pan-specific.

The interpretation of the assay test strip results is as follows: 1) positive—*P. falciparum*: one control band plus two test bands; 2) positive—*P. vivax*: one control band plus one test band; 3) negative—one control band at the top of the test strip. Examples are shown in Figure 1.

Treatment. All patients with slide-positive malaria as determined by standard operating procedures of the clinic were treated according to accepted and standard guidelines for the management of malaria. Information regarding the result of the pLDH-based dipstick assay was not made available to the attending physician and was not used to guide treatment. Individuals who had positive blood smears using the established standard procedure were treated with chloroquine and Fansidar® (F. Hoffmann-La Roche, Basel, Switzerland) in accordance with the then current Gambian National guidelines for the management of malaria. If there was disagreement between the OptiMAL® test and blood film results, the film was re-examined. If the blood film result was then found to be different, the revised blood film result, but not the OptiMAL® test result, was issued to the clinicians and the nursing sister in charge of the clinic.

RESULTS

Four hundred nine samples were examined. Eight were excluded from analysis: seven because the original results were not recorded in the clinic results book and one because the test strip failed to give a result. In comparison with the results from the MRC Outpatients’ Clinic (Table 1). The OptiMAL® test detected *P. falciparum* (trophozoites or trophozoites plus gametocytes) with a sensitivity of 92% and a specificity of 92%. For initial diagnosis of *P. falciparum*, irrespective of stage, the sensitivity was 91.3%, the specificity was 92%, the positive predictive value was 87.2%, and negative predictive value was 94.7%. In seven instances in which the blood film showed trophozoites or trophozoites plus gametocytes of *P. falciparum*, the OptiMAL® test correctly detected the presence of malaria parasites, but indicated the absence of *P. vivax*. If these and the two results for *P. malariae* are included, the sensitivity for the detection of malaria (all stages, unspeciated) is 95.4%. At the HTD (Table 2), the OptiMAL® test detected *P. falciparum* (trophozoites or trophozoites plus gametocytes) with a sensitivity of 91.5%, and a specificity of 94.9%. For initial diagnosis of *P. falciparum*, irrespective of stage, the sensitivity was 90.3%, the specificity was 94.9%, the positive predictive value was 92.1%, and the negative predictive value was 93.7%. As in the MRC Clinic results, there were seven instances where the OptiMAL® test incorrectly indicated the presence of *P. vivax* in samples that contained *P. falciparum* tropho-

![Figure 1. OptiMAL® test strips showing a negative result and positive results for *Plasmodium vivax* and for *P. falciparum.*](image-url)
zoites or trophozoites plus gametocytes. If these and the other species are included, the sensitivity for the detection of malaria (all stages, unspeciated) on the Gambian samples tested becomes 92%.

*Plasmodium ovale* was not detected in the MRC Clinic Laboratory, but was reported in four Giemsa-stained blood films examined at the HTD. The OptiMAL® test correctly detected the presence of malaria in only one of these samples, reporting the presence of *P. vivax*; the remaining three showed negative results in this test. *Plasmodium malariae* was detected in two Field’s stained thick films examined in the MRC Clinic. The OptiMAL® detected *P. falciparum* in one of these samples (328, which was later found to contain *P. falciparum* and *P. malariae*) and showed a negative result in the other sample. The HTD results showed *P. malariae* in three blood films. One of these samples was reported as *P. vivax* by the OptiMAL® test; the other two showed negative results. Mixed infections were reported in three blood films examined at the HTD and in none of the films read at the MRC Laboratory. Sample 045 showed *P. falciparum* (gametocytes only) plus *P. ovale*. The OptiMAL® test result indicated the presence of *P. vivax*. Sample 049 showed trophozoites of *P. falciparum* (0.001% parasitemia) plus *P. ovale*. The OptiMAL® test result was *P. vivax*. Sample 328 showed *P. falciparum* (> 1% parasitemia) plus *P. malariae*. The OptiMAL® test result was *P. falciparum*. None of the samples was found to contain *P. vivax*.

**DISCUSSION**

Since all of the microscopists in this study had substantial experience in the laboratory diagnosis of malaria, the OptiMAL® test was subjected to a stringent comparison with the established gold standard (microscopy). The OptiMAL® test readings were compared with the microscopic results both before and after expert review of the blood films to ensure that this comparison was as rigorous as possible. Overall sensitivity for the diagnosis of *P. falciparum* was good, both at the MRC Outpatients’ Clinic and when smearers were re-read at the HTD, especially at parasitemias > 0.01%. The sensitivity of the OptiMAL® test decreased markedly below a parasitemia of 0.01% (500 parasites/µL) (Table 3). In the current study, 89.6% of the samples from unselected patients with fever who were found to have *P. falciparum* malaria showed parasitemias ≥ 0.01%, so the OptiMAL® test would still have detected a high proportion of clinically relevant parasitemias with a good specificity in this setting. In nonendemic areas, where a sizeable proportion of the malaria cases occur in nonimmune individuals, the sensitivity of non-microscopic methods for diagnosis should be as high as possible. In an in-house study at the HTD, 75.9% of the patients presenting with *P. falciparum* malaria had an asexual parasitemia ≥ 0.01% (Gabbett E, Moody AH, Hunt Cooke A, Chiodini PL, unpublished data), so although the lack of sensitivity of the OptiMAL® test below this level is a disadvantage in nonimmune patients, the test would still perform well in a high proportion of malaria cases presenting in the temperate zone.

There are currently two immunochromatographic tests marketed for the diagnosis of *P. falciparum*: the Parasight®-F test1 and the ICT malaria Pf test.2 In a United Kingdom–based study, the Parasight®-F test detected *P. falciparum* with a sensitivity of 92% and a specificity of 98%.4 Sensitivity at parasitemias ≥ 0.01% was 100% and remained good at lower levels of parasitemia (87.5% at 0.001–0.009% parasitemias). In a field evaluation in the Solomon Islands,2 the ICT test had a sensitivity of 100% and a specificity of 96.2%, compared to thick blood film examination, for the diagnosis of *P. falciparum* malaria. The OptiMAL® test was compared with the Parasight®-F test and the ICT malaria Pf test for the diagnosis of malaria in samples from Honduras.5 The OptiMAL® test had a sensitivity of 94% for the detection of *P. vivax*. The sensitivities for diagnosis of *P. falciparum* were OptiMAL® test = 88%, Parasight®-F test = 65%, and the ICT malaria Pf test = 65%. The lower sensitivity of the HRP-2-based assays in this versus other studies is so far unexplained.

The OptiMAL® test was designed to diagnose *P. falciparum* and *P. vivax* malaria and to differentiate between them. This differentiation is clinically relevant since the salient feature of malaria diagnosis is to determine whether a malarial infection is positive or negative for *P. falciparum*. None of the patients in this study was found to have *P. vivax* and this parasite is not endemic in West Africa. Evaluation of the OptiMAL® test on samples from the HTD has shown a sensitivity of 91% (60 of 66) for the detection of *P. vivax* (Moody AH, Hunt Cooke A, Chiodini PL, unpublished data). In 1996, 2,500 cases of imported malaria were reported in the United Kingdom: 1,283 *P. falciparum* and 1,014 *P. vivax* (Warhurst DC, unpublished data). Thus, the OptiMAL® test is capable of diagnosing a high proportion of the two malaria parasites that are numerically most imp-

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### Table 2

Comparison of the OptiMAL® test with the Hospital for Tropical Diseases slide readings

<table>
<thead>
<tr>
<th>Blood film</th>
<th>OptiMAL®</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trophozoites or trophozoites</td>
<td>130</td>
<td>7</td>
</tr>
<tr>
<td>plus gametocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gametocytes only</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><em>Plasmodium ovale</em></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>Plasmodium malariae</em></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>224</td>
</tr>
</tbody>
</table>

### Table 3

Sensitivity of the OptiMAL® test at different levels of parasitemia

<table>
<thead>
<tr>
<th>Blood film</th>
<th>OptiMAL® result</th>
<th>% parasitemia*</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1</td>
<td>69/69</td>
<td>100</td>
<td>43/43</td>
<td>100</td>
</tr>
<tr>
<td>0.1–0.9</td>
<td>15/17</td>
<td>88.2</td>
<td>17/17</td>
<td>100</td>
</tr>
<tr>
<td>0.01–0.09</td>
<td><em>Plasmodium falciparum</em></td>
<td>16/16</td>
<td>6/7</td>
<td>50</td>
</tr>
<tr>
<td>0.001–0.0009</td>
<td>Unspecified malaria</td>
<td>3/7</td>
<td>5/7</td>
<td>71.4</td>
</tr>
<tr>
<td>0.00001–0.00009</td>
<td><em>P. falciparum</em></td>
<td>100</td>
<td>0/2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Parasitemia was assessed by examining 500 fields of the thick blood film using a 100× objective. Unspecified malaria includes those OptiMAL® results that identified *P. falciparum* as *P. vivax*, in addition to those where it correctly identified *P. falciparum*. 

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portant. Further work is in progress to make the OptiMAL® assay capable of detecting P. malariae and P. ovale.

False-positive OptiMAL® test results were reported with 20 samples compared with MRC Clinic film results, but with only 12 samples compared with the HTD results. Of the eight discrepant results, five showed P. falciparum at low parasitemias, which had become apparent as a result of the longer time allowed for microscopic examination at the HTD. One had P. falciparum gametocytes only, one had P. ovale only, and one had P. falciparum gametocytes plus P. ovale. A difference in detection rates is to be expected since at the MRC Outpatients’ Clinic 10 fields of a thick blood film scanned with a 100× objective are examined for the routine diagnosis of malaria, whereas examination of a minimum of 500 fields is done at the HTD. Mixed infections were detected in three samples. The configuration of the strip and the detecting antibodies used are such that a pure infection with P. vivax would show one line plus the control line, while two lines plus the control line could indicate a pure infection with P. falciparum or a mixed infection with P. falciparum plus P. vivax. Although mixed infections are uncommon (1.3% of the reported cases in the United Kingdom in 1996), it would be desirable for mixed infections containing P. falciparum always to be read as that species rather than another species, given the more serious nature of P. falciparum infections.

The OptiMAL® test was evaluated in an air-conditioned laboratory, rather than in field conditions. Its stability at ambient temperatures in malarious parts of the tropics remains to be determined.

Medical technicians and biomedical scientists in both laboratories have found that the OptiMAL® test is easy to use. There was only one strip failure in which the test control line failed to appear. Sensitive, rapid diagnostic tests for malaria that can be read easily by individuals with minimal training in a rural clinic setting, or during the night in hospitals remote from diagnostic laboratories, are highly desirable. The cost of the strips will clearly influence the extent to which they are deployed in any setting, but would be offset by reducing the morbidity and expenditure on unnecessary treatment that occur when an inaccurate diagnosis is made. The OptiMAL® test is likely to play an important part in urgent malaria diagnosis at night and at weekends, when routine laboratories are closed and when relatively inexperienced microscopists may be on duty.

The current configuration of the OptiMAL® assay potentially offers two advantages over currently available rapid tests based on the detection of HRP-2. First, the test follows the course of P. falciparum infection, since preliminary data show that a profound decrease in circulating pLDH activity occurs immediately after parasites are cleared from the peripheral blood (Piper R, University of Iowa, Iowa City, IA, unpublished data). Second, samples infected with P. vivax are clearly and easily distinguished from those infected with P. falciparum.

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