The Sensitivity of the OptiMAL Rapid Diagnostic Test to the Presence of *Plasmodium falciparum* Gametocytes Compromises Its Ability To Monitor Treatment Outcomes in an Area of Papua New Guinea in which Malaria Is Endemic

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Received 17 April 2006/Returned for modification 27 May 2006/Accepted 21 November 2006

Using in vivo samples from treatment failure malaria cases, we demonstrate the high sensitivity of the parasite lactase dehydrogenase (pLDH)-based OptiMAL rapid diagnostic test in the detection of *P. falciparum* gametocytes. This high sensitivity limits the use of pLDH-based tests in the monitoring of treatment outcomes in circumstances where gametocytemia is common.

In many countries where malaria is endemic, the capacity for accurate diagnosis of malaria cases at the peripheral health facility level is often limited by the lack of reliable malaria microscopy. As a possible solution to this problem, rapid diagnostic tests kits that are simple to perform have been advocated. Several such tests, based on a variety of methodologies, are currently on the market, and most have shown good sensitivity and specificity for the diagnosis of malaria (2, 4, 10, 13, 17), even when used in field conditions (5, 7, 9, 21). However, the monitoring of treatment success is complicated by very low levels of parasitemia after treatment, leading to false negatives. Furthermore, the persistence of some of the antigens used in these tests for more than 2 weeks after an infection has been cleared (e.g., HRP-2 [histidine-rich protein 2]) (14) potentially leads to false positives.

The OptiMAL dipstick test (DiaMed, Cressier, Switzerland) is an immunochromatographic assay based on the detection of *Plasmodium*-specific lactate dehydrogenase (pLDH) in whole blood. Positive results are indicated by the presence of colored (red) bands on the dipstick. The test contains an internal control band (top), a pan-*Plasmodium*-specific band (middle), and a *P. falciparum*-specific band (bottom). pLDH is produced only by living malaria parasites and has a short half-life in the blood (11, 12). This has led to the recommendation of OptiMAL as a suitable test for treatment monitoring (2, 5, 15, 16, 18, 19, 21, 22). However, pLDH is also produced by gametocytes (16), which are not affected by many antimalarial drugs and may thus be present long after asexual parasitemia has been cleared and the patient has recovered. The presence of gametocytes could therefore greatly reduce the utility of pLDH-based tests (23). While the ability of pLDH-based tests to monitor the clearance of parasites has been demonstrated by several studies in large referral centers in areas in which malaria is not endemic (15, 19, 22) or after treatment with fast-acting or gametocidal drugs (5, 16, 21), there is a lack of large studies with patients from highly malaria-endemic areas with high levels of gametocytemia.

Recently, we conducted a clinical trial comparing a novel antimalaria drug combination treatment (cotrifazid, i.e., cotrimoxazole-rifampin-isoniazid), mefloquine (Lariam), and quinine plus sulfadoxine-pyrimethamine in Madang, Papua New Guinea. As part of the trial, OptiMAL tests were performed at enrollment and on days 2, 7, and 14. Additionally, thick smears were performed on days 0, 1, 2, 3, 7, and 14 (3).

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>No. of patients assesseda</th>
<th>Light microscopy</th>
<th>OptiMAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Trophozoites only</td>
<td>Trophozoites and gametocytes</td>
</tr>
<tr>
<td>Day 2</td>
<td>223</td>
<td>42 (18.8)</td>
<td>20 (9.0)</td>
</tr>
<tr>
<td>Day 7</td>
<td>235</td>
<td>5 (2.1)</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Day 14</td>
<td>240</td>
<td>6 (2.5)</td>
<td>3 (1.3)</td>
</tr>
</tbody>
</table>

a Number of patients available for assessment at each time point.

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Published ahead of print on 29 November 2006.
TABLE 2. Performance of OptiMAL in detecting persistent blood-stage infections compared to standard light microscopy: tabulation of paired OptiMAL and microscopy results.

<table>
<thead>
<tr>
<th>Day 7 (n = 223)</th>
<th>Microscopy result</th>
<th>OptiMAL result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. falciparum</td>
<td>Non- P. falciparum</td>
</tr>
<tr>
<td>Non-negative</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>49.3%</td>
<td>66.7%</td>
</tr>
<tr>
<td>Specificity</td>
<td>49.3%</td>
<td>66.7%</td>
</tr>
<tr>
<td>PPV</td>
<td>35.1%</td>
<td>13.8%</td>
</tr>
</tbody>
</table>

- As the presence of asexual blood-stage parasites only indicates treatment failure, slides with gametocytes only were scored as negative.
- Following informed consent, the OptiMAL test were performed with finger prick blood from the same lancet wound as the malaria smears, collected into an EDTA microtube. All tests were performed according to the manufacturer’s instructions, by the same specifically trained technician (M.G.), and within 2 h of collection. All test kits were used within their expiration dates. Blood smears were read by two independent expert microscopists without reference to the result of the OptiMAL test. A total of 200 fields were examined before a slide was counted negative. Densities of asexual-stage parasites and gametocytes were calculated under the assumption of a total white cell count of 8,000/µl.

It is well known that the sensitivity of the OptiMAL test depends on the density of parasitemia (1, 6, 8). Given the generally very low parasitemia observed after treatment, test bands were often extremely light. For the test evaluation, we thus scored OptiMAL tests as true positives even if they indicated a species different from that identified in the thick smear.

A total of 255 patients were available for the evaluation of OptiMAL performance. Of these, 215 had had a confirmed antimalarial treatment in the 4 weeks prior to enrollment; 235 patients had single infections with P. falciparum, and 20 had mixed infections (P. falciparum and P. vivax). P. falciparum gametocytes were present in 68 patients (26.7%) at the time of enrollment. At this pretreatment stage, most infections were heavy; 57%, ≥10,000 parasites/µl; 29%, 1,000 to 9,999/µl; 15%, <1,000/µl. At enrollment, all patients tested positive by OptiMAL (entry criterion).

After initiation of treatment, asexual P. falciparum parasites were rapidly cleared, with trophozoites persisting in only 2.9% of patients on day 7 and 3.8% on day 14 (Table 1) Microscopic parasite counts were generally very low. A similar, although less pronounced, decrease in positivity was observed in the OptiMAL test results. Gametocytemia, however, increased following treatment, and gametocytes were found in 42.0% of samples at day 7. In many cases, gametocytemia persisted until day 14.

On day 2, the OptiMAL results corresponded rather poorly with microscopy, with only 51.6% (115/223) of samples positive or negative for both (Table 2) (P = 0.14 [Fisher’s exact test]). Furthermore, in the identification of the species, the two methods differed in 10/34 cases. The two methods showed better correspondence on days 7 (P = 0.004) and 14 (P = 0.001), in particular due to the high number of true-negative OptiMAL tests, resulting in markedly improved specificity (77.6% and 92.1%, respectively) and negative predictive values (97.7% and 96.3%, respectively). However, sensitivity and positive predictive values remained very low. P. falciparum gametocytes were found in the thick smears of 41/50 (82.0%) false-positive OptiMAL results on day 7 and in 12/18 (66.7%) of those on day 14. All four false negatives on day 7 and six of eight on day 14 had asexual-stage parasitemia of less than 100/µl.

Positive OptiMAL test results recorded in the presence of only P. falciparum gametocytes were strongly dependent on the density of gametocytemia (Table 3): while only 20.5% of tests gave a positive result in samples with <200 gametocytes/µl, 72.1% were positive in samples with ≥500 gametocytes/µl. This effect was observed throughout the follow-up period, even though the overall ability of OptiMAL to detect gametocytemia decreased with time.
The present study clearly demonstrates the high sensitivity of the OptiMAL test in detecting *P. falciparum* gametocytes. This severely limits its use for monitoring treatment outcomes in areas where malaria is highly endemic or in treatment failure malaria cases, where gametocytes are a common feature. Under such circumstances, a positive OptiMAL test is clearly insufficient proof of treatment failure, and additional methods, such as microscopy, are needed for confirmation.

The present sample set constitutes an extreme-case scenario, with a high number of gametocytes both at baseline and during follow-up. The fact that most patients had failed earlier treatment means that the present cohort had a longer duration of illness than that observed in most uncomplicated cases. Moreover, unsuccessful drug treatment (with chloroquine and in some cases sulfadoxine-pyrimethamine) has been observed to increase gametocytemia (20). Also, treatment with cotrimoxazole has been shown to be unsuccessful in preventing gametocyte production (20). It could therefore be expected that the OptiMAL test might perform better at monitoring treatment success in nonresistant cases or following treatment with a gametocidal drug. The performance of the OptiMAL test for treatment monitoring appeared to be substantially better in previous studies conducted either in large referral centers in countries where malaria is not endemic (15, 19, 22), where early and highly effective treatment reduces the risk of gametocytemia (20), or when fast-acting and/or gametocidal drugs were used as part of the treatment regimen (5, 16, 21).

The study presented here clearly indicates that the effectiveness of the OptiMAL test for monitoring treatment is highly dependent on both the treatment circumstances and the type of drug used, particularly the ability of that drug to kill gametocytes and/or to prevent gametocyte multiplication.

### REFERENCES