MALARIAN DIAGNOSIS BY DIPSTICK ASSAY IN A HONDURAN POPULATION WITH COENDEMIC PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX

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Abstract. A Plasmodium lactate dehydrogenase dipstick designed to separately detect P. falciparum and P. vivax malaria was evaluated in two Honduran populations where both species are endemic. The dipstick was compared to thick film microscopy; the polymerase chain reaction (PCR) was used to analyze discordant results. The dipstick had a sensitivity of 100% and a specificity of 95% compared with microscopy in the diagnosis of Plasmodium infections in a hospital population; the mean parasite density was approximately 590/mm³. In a field sample of mostly asymptomatic volunteers, the sensitivity of the dipstick for Plasmodium infection varied with parasite density. Additionally, the sensitivity and specificity of the dipstick was similar to thick film microscopy in the diagnosis of vivax malaria compared with the PCR. The dipstick was unable to detect P. vivax in the presence of P. falciparum because of cross-reactivity in the pan-specific band. Accurate species identification in mixed infections remains a problem in malaria diagnosis.

The advent of immunochromatographic wicking dipstick assays for malaria diagnosis has provided a rapid, user-friendly technique useable in remote areas. However, for dipsticks to effectively replace microscopy, they must provide reliable species diagnosis. Of the four Plasmodium species that naturally infect humans, P. falciparum causes the most severe disease. Despite its obligate requirement for reticulocytes, P. vivax, does not always cause benign infections. Reports of significant morbidity, mortality and drug resistance in P. vivax infections are generating new interest in this Plasmodium. The need to clear the hepatic hypnozoite stage of patients with P. vivax infections is another reason to document vivax infections. Most routinely administered antimalarial agents are ineffective against the exo-erythrocytic stages of Plasmodia. Thus, a tissue schizontocidal drug must be added to the treatment regimen for vivax malaria to preclude an unpredictable relapse of the disease.

Plasmodium species diagnosis in mixed infections can be difficult to make. Discrimination by microscopy is based on morphologic characteristics of the various asexual stages and the erythrocytes that they inhabit. Experienced microscopists are increasingly rare, especially at hospitals and clinics in endemic areas. The uncommon presence of mature gametocytes on a stained blood film is diagnostic for falciparum malaria. Moreover, P. falciparum asexual parasites undergo deep tissue schizogony and unlike P. vivax, only the ring stage parasite is found on the blood smear. Primers exist for the reliable identification of the human malarias by polymerase chain reaction (PCR) analysis; however, this is largely a research tool unsuited for routine clinical laboratory or field use.

The Plasmodium lactate dehydrogenase (pLDH) assay derives from the unique ability of Plasmodia to use 3-acetylpyridine adenine dinucleotide (APAD) instead of NAD as a cofactor in the interconversion of lactate to pyruvate. The pLDH dipstick assay has a dipstick format (OptiMAL®) with a falciparum-specific band and a pan-specific band. It purports to distinguish between P. falciparum and P. vivax infections. This assay captures pLDH as an antigen and does not measure enzyme activity. The effectiveness of OptiMAL® was evaluated in Honduras.

MATERIALS AND METHODS

The pLDH dipstick assay was evaluated in a Honduran hospital and field population. Malaria is a cause of morbidity and mortality in both populations. The study protocol used was approved by the Walter Reed Army Institute of Research Human Use Committee and the Honduran Ministry of Health (HMOH). The hospital study was conducted in May 1997 at the HMOH hospital in Trujillo, a port town located approximately 180 km east of La Ceiba, Altanitida. The clinical laboratory at the hospital in Trujillo evaluates between 80 and 200 patients a week for malaria using thick film microscopy. Approximately 25% of these patients have positive blood smears. Consenting patients referred to the hospital laboratory with a presumptive diagnosis of malaria were recruited into the study.

In the field study, blood samples were collected from residents of La Ceibita, a small village located approximately 10 km south of Tocoa, Colon. Every home in the village was visited in November 1996, and blood taken from all consenting individuals, regardless of symptoms. A previous study in June 1996 found 18% of the 550 residents of La Ceibita infected with P. vivax and 5% with P. falciparum (Quintana M, unpublished data). Blood was obtained from the same fingerstick for the thick blood film, pLDH dipstick assay, and PCR analysis. Heparinized blood for the pLDH dipstick assay was stored on wet ice until the assay was performed later that same day.

Thick film microscopy. Thick films were coded and stained with Giemsa. Parasitemias were determined by counting the number of parasites in 100 oil-immersion fields (100 high-powered fields) 1,000× magnification. Parasitemias were then expressed as the number of parasites counted per/mm³.

Dipstick pLDH assay. The immunochromatographic test referred to as OptiMAL® was provided by Flow, Inc. (Portland, OR). This assay detects the presence of pLDH antigen in lysed whole blood. Aside from a control antibody reaction zone at the top of the test strip, the OptiMAL® assay contains two test lines or reaction zones. The first encountered...
Comparison of the Plasmodium lactate dehydrogenase (pLDH) dipstick assay, polymerase chain reaction (PCR), and thick film microscopy in the diagnosis of Plasmodium falciparum and P. vivax infection (hospital sample)

<table>
<thead>
<tr>
<th></th>
<th>PCR</th>
<th>pLDH Dipstick</th>
<th>Microscopy</th>
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<tbody>
<tr>
<td>P. vivax</td>
<td>14</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>11</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Mixed infections</td>
<td>5</td>
<td>NA*</td>
<td>1</td>
</tr>
<tr>
<td>Total positive</td>
<td>30/84 (35%)</td>
<td>27/84 (32%)</td>
<td>24/84 (29%)</td>
</tr>
</tbody>
</table>

* NA = not applicable.

Comparison of the plLDH dipstick assay, PCR, and thick film microscopy in the diagnosis of Plasmodium falciparum and P. vivax infections (field samples)*

<table>
<thead>
<tr>
<th></th>
<th>PCR</th>
<th>pLDH Dipstick</th>
<th>Microscopy</th>
</tr>
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<tbody>
<tr>
<td>P. vivax</td>
<td>47</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>3</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Mixed infections</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total positive</td>
<td>52/68 (77%)</td>
<td>50/68 (74%)</td>
<td>41/68 (60%)</td>
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</tbody>
</table>

* For definitions of abbreviations, see Table 1.

In contrast to the hospital samples, the PCR was done on only the field samples that were positive by either dipstick or microscopy.

RESULTS

Hospital samples. Twenty-four (28.6%) of 84 patients had positive thick films, with P. falciparum comprising eight (33%) of 24, P. vivax 15 (62.5%) of 24, and mixed infections one (4.1%) of 24. The average parasitemia was approximately 591/μm³. The pLDH assay detected malaria infections in 27 (32.1%) of 84 patients: P. falciparum 14 (51.9%) of 27 and P. vivax 13 (48.1%) of 27 (Table 1). Concordance between microscopy and the pLDH assay for malaria infection was 96.4% (81 of 84). The sensitivity of the pLDH assay for malaria diagnosis was 100% (24 of 24) and the specificity was 95.0% (57 of 60) compared with microscopy. The pLDH dipsticks detected all 24 patients that were positive for malaria infection by microscopy, plus three additional cases. Two of the three additional cases were confirmed by PCR analysis.

Field samples. Blood was obtained from 319 (58%) of the 550 residents of La Ceibita. Only three (0.9%) of 319 participants had symptoms consistent with malaria at the time their blood was taken. Microscopic examination of the thick films detected 46 P. vivax-positive slides and one P. falciparum-positive slide, with no mixed infections (Table 2). The average parasite density was approximately 167 parasites/μm³ and 50% of the positive slides had less than 57 parasites/μm³. The pLDH assay detected malaria parasites in 53 samples: 43 P. vivax and 10 P. falciparum (Table 2).

Concordance between microscopy and the PLDH assay for malaria infection was 89.7% (285 of 319). Further evaluation of 68 samples positive by either microscopy or the PLDH assay by PCR analysis yielded 52 positive samples (Table 3). The pLDH assay was positive for 73.5% (50 of 68) of the samples and microscopy was positive for 60.3% (41 of 68). All except two of the 11 samples that were positive for malaria by either PCR or microscopy but negative by PLDH had parasite densities < 44 parasites/μm³. In one of the two, the PCR result was negative. The sensitivity of the pLDH assay was 100% for parasite densities > 88 parasites/μm³ but decreased to 52.9% for parasite densities between four and 40 parasites/μm³ (Table 4).

<table>
<thead>
<tr>
<th>Parasites per mm³</th>
<th>Positive by PCR</th>
<th>Positive by Dipstick (sensitivity)</th>
<th>Positive by thick film</th>
</tr>
</thead>
<tbody>
<tr>
<td>4–44</td>
<td>15</td>
<td>9 (52.9%)</td>
<td>17</td>
</tr>
<tr>
<td>45–88</td>
<td>8</td>
<td>7 (77.8%)</td>
<td>9</td>
</tr>
<tr>
<td>89–1,320</td>
<td>15</td>
<td>15 (100.0%)</td>
<td>15</td>
</tr>
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</table>

* For definitions of abbreviations, see Table 1.
**Parasite speciation.** All 13 *P. vivax*-positive hospital cases by the pLDH dipstick were also *P. vivax* positive by PCR and microscopy (Table 1). One vivax-positive case and one falciparum-positive case by PCR was negative by both the dipstick and microscopy. The pLDH dipstick assay cannot distinguish *P. falciparum* from mixed infections because the former will react with both the falciparum and vivax bands. Therefore, mixed infections would be indistinguishable from *P. falciparum* in the pLDH assay. Discordant species results between the dipstick and PCR (Table 1) were noted in 7.1% (1 of 14) of the *P. vivax* cases and 27.3% (3 of 11) of the *P. falciparum* cases. The agreement between the PCR and the dipstick assay 75% (3 of 4) was better than that with microscopy 4% (0 of 4) for the falciparum cases. In the five mixed samples, the PCR agreed with microscopy in 20% (1 of 5) of the cases.

Regarding the field samples where half of the volunteers had parasitemias < 57/mm³, the sensitivity and specificity of the dipstick (53% and 50%) was similar to microscopy (50% and 50%) when compared with the PCR for the detection of *P. vivax*. Six falciparum cases were detected by the pLDH dipstick that were negative by microscopy. The PCR showed parasites in 33.3% (2 of 6) of these cases. The number of falciparum and mixed infection cases was too few to determine the sensitivity and specificity of the tests.

**DISCUSSION**

These data show that the pLDH dipstick assay is as sensitive as thick film microscopy in the diagnosis of malaria when the parasite density exceeds 88/mm³. In a clinical laboratory serving a hospital in a malaria-endemic area, the parasite densities of symptomatic patients were high enough for the dipstick to perform at a sensitivity of 100% and a specificity of 95%. If this observation is validated in larger multicenter clinical studies, the pLDH dipstick may prove to be a reliable alternative for the diagnosis of *P. falciparum* or *P. vivax* malaria in that setting. The dipstick format is less prone to examiner bias and more dependable in remote areas without electricity.²,³ It is also more suited for batch testing and the results are easier to store for future reference. Even though these features are shared with other wicking immunochromatographic dipstick assays, the pLDH OptiMAL® assay offers yet another advantage. In contrast to dipsticks based on the histidine-rich proteins, ¹⁷ the pLDH assay measures the enzyme levels of intact viable parasites. Consequently, the decrease in parasite LDH enzyme levels 48–72 hr after the initiation of effective chemotherapy can be used to monitor response to specific treatment.¹³

The incorporation of falciparum and vivax bands on the dipsticks allows for separate detection of *P. vivax* and *P. falciparum* infections with the same product. Arguably, some clinical utility exists in this capacity to rule out falciparum malaria in a population with coendemic, falciparum/vivax malaria. For example, drug resistance is less prevalent in vivax malaria than falciparum malaria infections and chloroquine should be the drug of choice in most cases of vivax malaria.⁴ However, the present inability to distinguish mixed from *P. falciparum* infections with this dipstick mutes the clinical utility of this added feature. Although it may curb the use of more expensive and less familiar drugs in the treatment of vivax malaria, it complicates the treatment of falciparum malaria cases. A tissue schizontocidal drug must still be added to the treatment course of all falciparum malaria cases diagnosed either by this dipstick assay or microscopy to exclude the possibility of a relapse from a missed concomitant *P. vivax* infection.

In general, our data seems to underscore the difficulty in making the correct species diagnosis in populations endemic for more than one *Plasmodium* species. The under-representation of mixed infections in populations coendemic for *vivax* and falciparum malaria has been previously reported.⁸,¹⁰ Species identification is done on a thin smear by a microscopist in an endemic area with mixed infections. However, it is unclear if this paucity of mixed cases stems largely from an inherent difficulty in the detection of coinfecting plasmodial organisms by microscopy or a biological interaction between the species.¹⁸ Nonetheless, the finding of far fewer mixed infections by microscopy than by the PCR in our study suggests that observer bias may be a contributing factor. In a test slide with a preponderance of vivax parasites, compelling evidence such as the presence of falciparum gametocytes may be needed for a diagnosis of mixed infection to be made by microscopy. Additionally, the explanation for the negative PCR results in four cases positive by the pLDH dipstick assay is unknown. This dilemma of mixed infections, with its therapeutic implications, has not been resolved by the present pLDH dipstick assay. Its final resolution must await a *P. vivax*-specific antibody that does not cross-react with *P. falciparum*. In the interim, however, distinguishing falciparum from non-falciparum malaria will serve as a pragmatic short-term clinical goal.

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**REFERENCES**


