INTRODUCTION

Early diagnosis and treatment of parasitemia is vital for the control of malaria, one of the most prevalent parasitic diseases worldwide. Microscopic examination of thick blood film is currently the standard method for malaria diagnosis. This method is relatively simple and has low direct costs, but its reliability and cost-effectiveness are questionable, particularly at low levels of parasitemia, i.e., density and in the interpretation of mixed infection.1,2 Recently, three rapid non-microscopical assays for the detection of Plasmodium falciparum infection have been developed. These tests are based on the detection of antigen(s) released from parasitized red blood cells. Two of the assays, ParaSight F (Becton Dickinson, Cockeysville, MD) and the ICT Malaria Pf (ICT Diagnostics, Sydney, Australia), detect Plasmodium falciparum histidine-rich protein-2 (PfHRP-2).3-5 Plasmodium falciparum histidine-rich protein is a water-soluble antigen expressed by trophozoites of P. falciparum and by immature gametocytes. However, since PfHRP-2 is only produced by P. falciparum, these tests cannot detect infections with Plasmodium vivax, Plasmodium ovale, or Plasmodium malariae. The third test, pLDH assay (OptiMAL) (Flow Inc., Portland, OR) is relatively new and detects a different malarial antigen, Plasmodium-specific lactate dehydrogenase (pLDH).6 Unlike the PfHRP-2 assays, the pLDH assay can be used to detect infections with any of the Plasmodium species that infect humans. Both ICT and ParaSight F tests have been evaluated for their sensitivity in detecting malaria infection at various epidemiological settings; however, these tests have not been compared and evaluated for their sensitivity against pLDH assay to detect P. falciparum infection.

The present study examined the performance of the PfHRP-2 assay (ICT Malaria) and pLDH assay (OptiMAL) for detecting imported malaria infection in patients returning to Kuwait from countries endemic for malaria. Both assays were compared with the microscopy of thick blood film and with each other.

MATERIALS AND METHODS

From January 1997 to June 1998, blood specimens were collected from 515 individuals who presented with fever at Mubarek Al-Kabeer Teaching Hospital, and at the Malaria Laboratory, Kuwait. The majority of these individuals were immigrants from tropical countries where malaria infection is endemic. These individuals had arrived in Kuwait in the previous 2–6 months.

Information about recent treatment for malaria was collected from all of the patients, and a urine specimen was taken for the detection of 4-aminoquinolines (Dill-Glazko) and sulphonamides (lignin). The tests were performed either at the Malaria Laboratory, Ministry of Health, or at the Faculty of Medicine, Kuwait University. Both the PfHRP-2 and pLDH assays were performed blind. Informed consent was taken from all the patients included in the study. The study was approved by the ethical committee of the Faculty of Medicine, University of Kuwait.

Microscopy of Giemsa-stained blood films. Thick and thin blood films were stained with 10% Giemsa stain for 10 minutes and examined by two experienced microscopists who had no knowledge of patient disease status or nationality to avoid any bias in blood-film readings. The microscopist counted a minimum of 200 consecutive fields of P. falciparum and by immature gametocytes. From January 1997 to June 1998, blood specimens were collected from 515 individuals who presented with fever at Mubarek Al-Kabeer Teaching Hospital, and at the Malaria Laboratory, Kuwait. The majority of these individuals were immigrants from tropical countries where malaria infection is endemic. These individuals had arrived in Kuwait in the previous 2–6 months. Information about recent treatment for malaria was collected from all of the patients, and a urine specimen was taken for the detection of 4-aminoquinolines (Dill-Glazko) and sulphonamides (lignin). The tests were performed either at the Malaria Laboratory, Ministry of Health, or at the Faculty of Medicine, Kuwait University. Both the PfHRP-2 and pLDH assays were performed blind. Informed consent was taken from all the patients included in the study. The study was approved by the ethical committee of the Faculty of Medicine, University of Kuwait.

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were calculated as the proportion of true-positive results obtained among samples whose Trophozoites; the specificity was calculated as the proportion of negative results obtained among samples containing P. falciparum and P. vivax. Nine cases had P. falciparum gametocytes only (Table 1).

The PfHRP-2 assay detected PfHRP-2 antigen in 53 P. falciparum patients and in 1 case with P. vivax infection; however, it was unable to detect 3 patients with mixed infections and 5 patients with gametocytes only (Table 1). Considering P. falciparum trophozoites only, the PfHRP-2 assay detected PfHRP-2 antigen in 58 patients, but failed to detect infection in 2 patients with high parasitemia (970 and 1,800 parasites/µL) and in 6 patients with parasitemia of <100/µL (Table 1). The pLDH assay detected 56 P. falciparum infections but failed to detect mixed infections in 4 patients and in 6 patients with gametocytes only (Table 1). Considering P. falciparum trophozoites only, the pLDH assay detected infection in 61 patients; however, it failed to detect infection in 1 patient with high parasitemia (1,450 parasites/µL) and in 4 patients with parasitemia <100/µL (Table 1). The data presented suggest that both PfHRP-2 and pLDH assays are useful in detecting single infections, but failed to detect majority (>70%) of mixed P. falciparum and P. vivax infections.

Comparing PfHRP-2 and pLDH assays with the microscopy for the detection of P. falciparum trophozoites only, both the PfHRP-2 and pLDH assays failed to detect malaria antigen in 12 and 7 microscopy-positive cases, respectively; the majority of these cases had parasitemia <40/µL (Table 2). There was one PfHRP-2 positive but pLDH negative case. However, there were three cases which were positive with pLDH assay but negative with the PfHRP-2 assay; two of these three cases had parasitemia <40/µL. The PfHRP-2 assay detected malaria infection in 4 cases that were negative by microscopy; 2 of these 4 cases were also positive with pLDH assay. Three of these 4 cases reported treatment with chloroquine in the precious 3–5 weeks. The sensitivity and specificity of both the PfHRP-2 and pLDH assays were comparable to that of microscopy which was used as the standard test. The sensitivity of the PfHRP-2 assay was 82%, the specificity 99%, the positive predictive value 93%, and
TABLE 3

<table>
<thead>
<tr>
<th>Microscopy†</th>
<th>Positive</th>
<th>Negative</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive‡ (66)</td>
<td>12</td>
<td>54</td>
<td>7</td>
<td>59</td>
</tr>
<tr>
<td>Negative‡ (449)</td>
<td>445</td>
<td>4</td>
<td>447</td>
<td>2</td>
</tr>
<tr>
<td>Total tested (515)</td>
<td>457</td>
<td>58</td>
<td>454</td>
<td>61</td>
</tr>
</tbody>
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* For definitions of abbreviations, see Table 1.
† Sensitivity, specificity, and positive and negative predictive values were calculated using microscopy as the standard test as described in the Materials and Methods.
‡ A minimum of 200 consecutive fields in the thick blood film were examined.

The negative predictive value was 97%. The sensitivity of the pLDH assay was 89%, specificity 99.5%, positive predictive value 97%, and negative predictive value was 98% (Tables 2 and 3).

DISCUSSION

Each year more than 800 cases of imported malaria are detected in Kuwait among the immigrant population who visit their home countries in southeast Asia (India, Bangladesh, Sri Lanka, Pakistan) to spend holidays. Both *P. falciparum* and *P. vivax* infections are frequently encountered in Kuwait. In this study 87 of the 163 malaria cases had *P. vivax* infection and 12 cases had mixed infections of *P. falciparum* and *P. vivax*. As expected, the PHRP-2 assay which detects PfHRP-2 antigen failed to detect all but one case of *P. vivax* infection because PfHRP-2 antigen is released by *P. falciparum*-infected red blood cells only. In most malaria-endemic areas, especially in West Africa, malaria species other than *P. falciparum* amount to less than 5% of infections, but in imported malaria, as in Kuwait, it is important to detect all species of malaria. Sixty-one percent (99 of 163) of all our malaria cases were infected with *P. vivax*. The sensitivity of the PHRP-2 assay to detect all malaria infections was only 39%. Considering *P. falciparum* trophozoites, the sensitivity of PHRP-2 assay was only 82% with a specificity of 99%, which is comparable to most earlier studies done to detect *P. falciparum* infections.5,10

The pLDH assay was more suited to our setting because it can detect both *P. falciparum* and *P. vivax* infections and can differentiate between them. This differentiation is clinically relevant for the proper management of the patients because of the need to initiate radical treatment to avoid relapse of infection. The pLDH assay detected 77 of the 87 *P. vivax* infections and 56 of the 63 *P. falciparum* infections with an overall sensitivity of 87%. Considering *P. falciparum* trophozoites only, the sensitivity of pLDH assay was 89% with a specificity of 99.5% in our study; this was comparable to an earlier study which reported a sensitivity of 94% in detecting *P. falciparum* infection.5 The performance of both the PHRP-2 and pLDH assays was comparable to that of *P. falciparum* infection at parasitemia < 100/μL; however, the PHRP-2 assay failed to detect, as expected, all but one *P. vivax* infection. The performance of both the PHRP-2 and pLDH assays was greatly influenced by the level of parasitemia in peripheral blood. The sensitivity of both the assays was ≥ 95% at parasitemia > 100/μL; however, the sensitivity dropped to 84% for the PHRP-2 assay and to 76% for pLDH assay at parasitemia < 100/μL. In contrast to endemic countries, in imported malaria low parasitemias are frequently responsible for clinical disease. In this study 38% of the patients with *P. falciparum* infection had parasitemia < 100/μL. Both the PHRP-2 and pLDH assays missed 2 and 1 *falciparum* malaria cases, respectively with high parasitemia > 900/μL. False-negative dipstick test results at higher parasitemias have been noted by others, but the underlying reason is not known.5,10–14 We also could not explain the failure to detect some cases with gametocytes only.

Only a small proportion of individuals with negative blood film results were diagnosed as positive by the PHRP-2 assay (4 cases) and pLDH assay (2 cases). Examination of fever history revealed that 3 had a history of recent treatment with chloroquine. Thus a false positive reaction may occur in individuals who have been recently treated for malaria as reported earlier.5,10–16 The preliminary data show that PHRP-2 antigen, which is detected by the PHRP-2 assay, may persist for up to 7–10 days after asexual parasite clearance,5,15 whereas circulating pLDH activity, which is detected by pLDH assay, drops profoundly immediately after the parasites are cleared from the peripheral blood.17 Thus, the pLDH assay may provide the potential to monitor the effectiveness of antimalarial therapy and thus assist in the detection of drug-resistant infections. We are presently investigating this particular role of pLDH assay in the patient management.

In conclusion, the performance of both the PHRP-2 and pLDH assays is comparable to that of microscopy to detect *P. falciparum* infection at parasitemia > 100/μL; however, the pLDH assay offers an advantage over PHRP-2 assay as samples infected with *P. vivax* are easily distinguished from those infected with *P. falciparum* and also for the detection of mixed infections of *P. falciparum* and *P. vivax*. In addition, the pLDH assay holds promise for clinical monitoring during the course of malaria infection and treatment. However, thick blood-film examination is still the standard method for diagnosing malaria because it detects all *Plasmodium* species and offers the clear distinction between parasite growth stages which is essential for making therapeutic decisions.

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