ASSESSING THE PARASIGHT®-F TEST IN NORTHEASTERN PAPUA, INDONESIA, AN AREA OF MIXED PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX TRANSMISSION

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Abstract. User-friendly, reliable, and inexpensive methods for diagnosing malaria are needed at the primary health care level. During a randomized treatment trial, the Parasight®-F test was assessed on days 0, 3, 7, and 28 against standard light microscopy of Giemsa-stained thick blood smears for diagnosing Plasmodium falciparum parasitemia in patients with P. falciparum (n = 84) or P. vivax (n = 59) malaria. The median P. falciparum parasite count on day 0 was 2,373/μL (range = 20–74,432/μL). At the start of treatment, the Parasight®-F test had a sensitivity of 95.2% (80 of 84; 95% confidence interval [CI] = 88.2–98.7), and a specificity of 94.9% (56 of 59; 95% CI = 85.8–98.9). On day 7, this test showed false-positive results in 17 (16.3%) of 104 patients (95% CI = 9.8–24.9). The Parasight®-F test performed well when compared with light microscopy in detecting P. falciparum parasitemia in patients presenting with clinical malaria. However, the high false-positive rate on day 7 limits its use for patient follow-up.

INTRODUCTION

Plasmodium falciparum is a global problem resulting in considerable morbidity and mortality.1,2 Plasmodium vivax also causes appreciable morbidity and has become chloroquine-resistant in some endemic areas.3–5 Therefore, the accurate diagnosis of malaria in febrile patients is essential for optimal patient management and the rational use of antimalarial drugs in malaria control programs. However, because standard light microscopy is often lacking in malaria-endemic areas, malaria is diagnosed clinically, a practice that is known to be inaccurate.6–7 Overdiagnosis of malaria and the excessive use of antimalarial drugs are common in the tropics and result in inappropriate treatment of non-malaria fevers, wasted resources, potentially avoidable drug toxicity, and drug pressure that promotes the development of drug-resistant P. falciparum and P. vivax.5–10

The Parasight®-F test (Becton Dickinson, Sparks, MD) is an immunochromatographic dipstick test that specifically detects P. falciparum histidine-rich protein II (PF HRP II) in whole blood by antibody agglutination, resulting in the appearance of a red band on the dipstick.11 In previous trials of patients from areas of transmission of P. falciparum, this test has generally demonstrated high sensitivity and specificity when compared with light microscopy, the current gold standard for diagnosing malaria.12–24 High sensitivity and specificity values have also been documented for detecting P. falciparum malaria in areas of mixed P. falciparum/P. vivax transmission in Brazil (91% and 97%, respectively), Sri Lanka (90.2% and 99.1%, respectively), and Sumba Island in eastern Indonesia (95.5% and 89.8%, respectively).25–27 However, in northeastern Papua, Indonesia, the sensitivity of the Parasight®-F test in detecting P. falciparum in asymptomatic, malaria-immune Papuans was only 60.3% (41 of 68).28

We report the results of the Parasight®-F test in patients with confirmed P. falciparum or P. vivax malaria acquired in northeastern Papua, Indonesia.

MATERIALS AND METHODS

The Parasight®-F test was assessed during a randomized clinical trial comparing chloroquine alone (25 mg/kg over a 48-hour period), doxycycline alone (100 mg twice a day for seven days), or chloroquine/doxycycline for treating P. vivax or uncomplicated P. falciparum malaria acquired in the town of Jayapura, Papua, or its rural areas. This area has moderate transmission of drug-resistant P. falciparum malaria (two infections/person-year) and chloroquine-resistant P. vivax malaria (one infection/person-year).4,29–33 Patients were either indigenous Papuans or immigrants from other parts of Indonesia (transmigrants). All enrolled patients had confirmed P. vivax or P. falciparum parasitemia and were monitored for 28 days. Study end points were the parasitologic end points of the World Health Organization (WHO) 28-day in vivo test: parasite sensitivity (S = complete and sustained clearance of asexual parasitemia by day 7 to day 28) or resistance (R = complete asexual parasite clearance by day 7 but recrudescence within 28 days; RI = marked reduction [≥75%] of asexual parasitemia within 48 hours but no clearance by day 7; and RII = no marked reduction of asexual parasitemia by 48 hours).34 Thick and thin malaria blood smears were stained with Giemsa, read, and reported according to standard methods.35 Parasite counts were quantified using the measured total white blood cell count (Beckman Coulter, Inc., Fullerton, CA or the quantitative buffy coat method; Becton Dickinson); if not measured, a white blood cell count of 8,000/μL was assumed.

The Parasight®-F test was performed on days 0, 3, 7, and 28. Data were analysed (chi-square test for proportional data and Mann-Whitney U test for continuous data) using Epi Info 6.04b (Centers for Disease Control and Prevention, Atlanta, GA). Standard diagnostic test values (sensitivity and specificity) were calculated, as well as the test efficiency (the proportion of correct test results): TP + TN/TP + FP + TN + FN and Youden’s misclassification index (a measure of test reliability): 1 – (α + β), where TP = true positive, TN = true negative, FP = false positive, FN = false negative, α =
probability of a false-positive result (FP/TP + FP), and $\beta =$ probability of a false-negative result (FN/TN + FN).

Written informed consent was obtained from all patients. The study was conducted according to the Indonesian Ministry of Health, the Indonesian Navy, and the United States Navy and Army regulations governing the protection of human subjects in medical research.

RESULTS

There were 152 enrolled patients with either *P. falciparum* (n = 89) or *P. vivax* (n = 63) parasitemia; all had symptoms or signs consistent with malaria at presentation. Of these, 143 (94%) had Parasight*-F* testing done, and 142 (93.4%) had measured total white blood cell counts (median = 6,300/µL, range = 2,100–13,600/µL). Day 0 parasite counts ranged from 20 to 74,432/µL (median = 2,375/µL) for patients infected with *P. falciparum* and from 54 to 14,124/µL (median = 2,567/µL) for patients infected with *P. vivax*. Papuan and transmigrants had similar day 0 median *P. falciparum* parasite counts (2,359/µL versus 2,856/µL, respectively; $P = 0.7$), but transmigrants had a higher day 0 median *P. vivax* parasite counts (3,168/µL versus 703.5/µL; $P = 0.05$).

The sensitivity and specificity of the Parasight*-F* test on days 0, 3, and 7 are shown in Table 1. On days 0, 3, 7, and 28, the respective proportions of false-positive tests were 3 of 59 (5.1%); 95% confidence interval [CI] = 1.1–14.1%), 14 of 83 (16.9%; 95% CI = 9.5–26.7]), 17 of 104 (16.3%; 95% CI = 9.8–24.9), and 3 of 54 (5.5%; 95% CI = 1.2–15.4). The three false-positive results on day 0 occurred in patients with microscopically confirmed *P. vivax* infections. The four patients with false-negative Parasight*-F* test results on day 0 had low *P. falciparum* parasite counts (20–175/µL). Papuan and transmigrant patients had similar day 0 test results (Table 2).

At follow-up, the proportion of patients infected with *P. falciparum* who had a positive Parasight*-F* test result decreased with time; these proportions were similar in cases of sensitive or RI resistant parasitemia (Table 3). The median day 0 *P. falciparum* counts were higher in patients with positive Parasight*-F* test results on day 3 (3,904.5/µL versus 693.75/µL; $P = 0.02$) and day 7 (6,084/µL versus 1,989/µL; $P = 0.013$). Patients with day 0 *P. falciparum* parasitemia > 2,373/µL (the median) were significantly more likely to be test positive on day 3 (33 of 40 [82.5%] versus 17 of 37 [45.6%], relative risk [RR] = 1.8, 95% CI = 1.2–2.6, $P = 0.007$), but not on day 7 (14 of 35 [40%] versus 7 of 33 [21.2%], RR = 1.9, 95% CI = 0.9–4.0, $P = 0.09$).

DISCUSSION

This study has shown that the Parasight*-F* test performed well when compared with light microscopy for detecting and identifying *P. falciparum* infections in symptomatic Indonesian adults with low to moderate parasitemias at the time of disease presentation. The results of this test and those of light microscopy were discordant on days 3, 7, and 28. There were no differences in the day 0 test values between Papuan patients and transmigrants.

Our data are broadly consistent with the results of a growing number of clinical studies conducted in epidemiologically diverse malaria-endemic regions and in travelers, but contrast with those of Fryauff and others, who assessed the Parasight*-F* test as an epidemiologic tool for the detection of asymptomatic infections in children and adults with life-long exposure to malaria. They found that the Parasight*-F* test had an overall sensitivity of 60.3% (41 of 68) in Papuans of all ages, but in patients less than 10 years old the sensitivity was only 40% (12 of 30). They postulated that the high degree of malaria immunity of these individuals may have blocked the reaction between the Pf HRP II protein and the monoclonal antibody on the dipstick. They also found a low specificity of 79% (108 of 136) in transmigrants more than 10 years old; the false-positive test results (21%) were predominantly associated with microscopic diagnoses of *P. vivax*.

The high specificity (95%) we obtained is comparable with results from other areas of significant *P. vivax* transmission. We had false-positive test results that were associated with *P. vivax* parasitemia on day 0, and negative slides on days 3, 7, and 28. *Plasmodium falciparum* parasitemia may be missed if *P. vivax* is the predominant species. The negative slide results are consistent with continuing production of Pf HRP II (asexual forms/young gametocytes) or persistence after parasitologic cure. *Plasmodium falciparum* HRP II has been detected for up to four weeks in patients and travelers following parasitologic clearance. We found that a positive day 7 test result was not an early predictor of recrudescence parasitemia, although the number of RI cases was low (n = 9). In Thailand, one study showed that test positivity on day 14 was predictive of recrudescence, while another did not. A small number of our patients with low *P. falciparum* parasitemias had false-negative test results, a finding consistent with other studies that have documented sensitivities of 7–40% in patients with parasitemias < 120/µL.

### Table 1

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>95.2% (80/84) [88.2–98.7]</td>
<td>86% (37/43) [72.1–94.7]</td>
</tr>
<tr>
<td>Specificity</td>
<td>94.9% (56/59) [85.8–98.9]</td>
<td>83.1% (69/83) [73.3–90.5]</td>
</tr>
<tr>
<td>Efficiency</td>
<td>95.1% (136/143) [90.2–98]</td>
<td>84.1% (106/126) [76.5–90]</td>
</tr>
<tr>
<td>Youden’s index</td>
<td>90.1% [89.3–91]</td>
<td>69.2% [67.6–70.7]</td>
</tr>
</tbody>
</table>

* Values in brackets are 95% confidence intervals.

### Table 2

Comparison of day 0 Parasight*F*-F test results between indigenous Papuan and transmigrant patients from other parts of Indonesia

<table>
<thead>
<tr>
<th>Parasite count*</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papuans</td>
<td>2,359.5 (20–64,998)</td>
<td>93.9% (31/33)</td>
</tr>
<tr>
<td>Transmigrants</td>
<td>2,856 (38–74,432)</td>
<td>96.1% (49/51)</td>
</tr>
</tbody>
</table>

* Values are the day 0 median (range) *Plasmodium falciparum* counts per microliter.
The Parasight®-F test and other immunochromatographic tests have clear advantages over light microscopy. They are low technology, user friendly, and yield rapid results. Clinicians should be aware that the results obtained must be interpreted in light of the symptoms and signs of the patients, and the epidemiologic context of patient presentation. A positive Parasight®-F test result in high transmission areas merely indicates the presence of *P. falciparum* parasitemia and may not explain the patient’s illness. Cost is a major impediment for the widespread deployment in malaria control programs.19,23,24 There may be utility in the Integrated Management of Childhood Illnesses, a UNICEF/WHO clinical system designed to assist health care workers in differentiating and managing serious febrile illnesses in children less than five years old. Limited operational experience exists with the pannmalarial antigen immunochromatographic test for detecting non-falciparum parasitemia; they may have advantages over rapid tests that only detect *P. falciparum* in specific settings.25,38,39

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### Table 3

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Sensitive no. (%)</th>
<th>R1 resistance no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Day 0</td>
<td>37 (92.5)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>3</td>
<td>23 (59.0)</td>
<td>16 (41.0)</td>
</tr>
<tr>
<td>7</td>
<td>10 (25.6)</td>
<td>29 (74.4)</td>
</tr>
<tr>
<td>28</td>
<td>3 (8.1)</td>
<td>34 (91.9)</td>
</tr>
</tbody>
</table>

REFERENCES

20. Watson PA, Laidoueu AB, Kacou E, Koudou G, Traore M, 1998. Comparison of a rapid dipstick test and thick blood films for detecting parasites of *Plasmodium falciparum* used under typi-


