Abstract. The Parasight®-F test based on the detection of a soluble antigen specific for Plasmodium falciparum is designed for the immediate diagnosis of malaria infection. We evaluated its use by clinicians during consultations. This prospective study of its diagnostic utility in febrile patients consulting a travel clinic on their return from areas endemic for malaria was conducted between May 1996 and May 1997. The Parasight®-F test was performed by the clinician with confirmation by means of standard microscopic examination of venous blood. One-hundred and forty patients were enrolled. Forty-three (31%) cases of malaria were identified by microscopic examination. Thirty-eight were due to P. falciparum. The Parasight®-F tests yielded 6 false-positive and 3 false-negative results compared to the microscopic findings. The specificity and sensitivity for the diagnosis of P. falciparum malaria were 94% and 92%. These results show that the Parasight®-F test alone cannot replace microscopic diagnosis of malaria in travel clinics.

INTRODUCTION

The annual number of cases of clinical malaria worldwide is estimated to be 300 and 500 million, with 1.5 to 2.7 million deaths.1 Plasmodium falciparum malaria, which is potentially fatal if not diagnosed and treated rapidly, is the most frequent form of imported malaria. In western countries, 10,000 cases of malaria are reported each year, with 4,000 to 5,000 cases in France.2,3 The diagnosis of malaria is based on microscopic examination of a stained blood sample (thin and thick blood films) or fluorochrome labelling (QBC® Malaria System test [QBC® test], Becton Dickinson Europe, Meylan, France).4 Rapid qualitative manual tests applicable to whole blood have recently been developed, including Parasight®-F (Becton Dickinson Europe), ICT Malaria® Pf. (Laboratoires Funouze, Levallois-Perret, France), and OptiMAL® (Flow Inc., Portland, OR). Parasight®-F and ICT Malaria® Pf. detect a soluble exoantigen specific for Plasmodium falciparum, known as histidine-rich protein 2.5,6 This antigen can be also detected by an enzyme-linked immunosorbent assay (ELISA) test (Malaria-Ag, Celisa, Cel labs, Sydney, Australia). Tests based on the detection of histidine-rich protein 2 cannot detect species other than P. falciparum. Lactate dehydrogenase (pLDH), an enzyme produced by live Plasmodium, is detected by OptiMAL®7 Antibody detection tests based on immunofluorescence and ELISA technology are also available. Because antibodies appear several days after the onset of symptoms, such tests are appropriate for the emergency setting.

The diagnosis of malaria is often difficult in non-immune travellers who have not taken chemoprophylaxis correctly or who have taken presumptive treatment. Microscopic examination remains the primary diagnostic method but can be negative when parasite viability is impaired by chemotherapy or when parasitemia is low (below 5 × 107 per total blood volume). Antigen testing may thus be useful for confirming P. falciparum infection. Furthermore, in outpatient travel clinics, it takes one to two hours from the time of sampling blood to obtain the results of microscopic examination. This delay warrants the use of a more rapid diagnostic test. Our study was undertaken to assess the performance of the Parasight®-F test for the diagnosis of P. fal-

ciparum malaria in patients with a history and clinical findings compatible with malaria. It was evaluated for making decisions regarding therapy before microscopic confirmation.

MATERIALS AND METHODS

This prospective study was conducted between May 1996 and May 1997. It involved subjects consulting the travel clinic of a university hospital in Paris on their return from an endemic area with symptoms compatible with malaria (fever, headache, chills, diarrhea and digestive disorders). A standard interview and physical examination were done by the clinician. With the patient’s informed consent, the Parasight®-F test was applied to a fingertip capillary blood sample during the consultation. The Parasight®-F test was done as recommended by the manufacturer.7 The test was judged positive if the reaction band became pink and the control band was visible (Figure 1, strip C). The response was scored according to staining intensity; when the investigator doing the Parasight®-F test found a weak reaction, he/she recorded it as weakly positive. Three experienced physicians specifically trained by the parasitology laboratory staff did the tests.

Five mL of venous blood were collected simultaneously in an ethylenediaminetetraacetic acid tube and 5 mL in a dry tube. Both tubes were sent to the parasitology laboratory where the following tests were done blindly to confirm the result of the Parasight®-F test for each patient:

1. A thin blood film stained with Diff-Quick® (Dade, Mau repas, France), and scored as negative if no parasites were seen in 200 microscope fields.8
2. A thick blood film stained with 5% Giemsa (Réactifs RAL, Paris, France), and scored as negative if no parasites were seen after counting 1,000 white blood cells. Asexual malaria parasites were counted per 1,000 white blood cells, and the parasite count was multiplied by 8 to estimate the number of parasites per µL blood.10
3. A quantitativeuffy coat test (QBC® test), done as recom mended by the manufacturer.11
4. Antibodies were measured by indirect immunofluores-
The Parasight®-F test was positive in 41 cases. Thirty-five corresponded to *P. falciparum* identification by microscopic examination. In 10 cases the Parasight®-F test was weakly positive. Conflicting results were obtained between the Parasight®-F test and microscopic examination in nine cases out of the total of 140 patients. Six false-positive Parasight®-F tests were found. Five of these were weakly positive. The clinical details, IFAT, and rheumatoid factor tests in these cases are described in Table 2.

With respect to the diagnosis of *P. falciparum* malaria, three false-negative results were obtained in which microscopic examination showed 16, 24, and 48 parasites/μL (the density lowest associated with a positive Parasight®-F test was 8/μL). The positive and negative predictive values of the Parasight®-F test for the diagnosis of malaria, relative to thin/thick blood films were 85% and 92%. The negative predictive value was 97%. The QBC® test gave 40 positive results, yielding 93% concordance with microscopic examination. *Plasmodium falciparum* was identified in the three false-negative QBC® tests with parasitemia values of 8, 48, and 112/μL.

The patients reported no immediate or long-term local adverse effects after the pinprick sampling procedure. In 30% of cases, the physician had to prick the finger twice to obtain enough blood to fill the 50 μL capillary tube.

**DISCUSSION**

The prevalence of malaria in patients with a compatible history and clinical presentation consulting on their return from an endemic area was 31%. Eighty-eight percent were *P. falciparum*, a proportion explained by the fact that most patients were returning from sub-Saharan Africa. This figure is in keeping with published data on imported malaria in

**TABLE 2**

Clinical and biological settings of the 6 cases of false-positive Parasight®-F tests *

<table>
<thead>
<tr>
<th>Clinical features</th>
<th><em>Pf</em> specific antibodies</th>
<th>Rheumatoid factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexplained fever</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Resident in malarial endemic region</td>
<td>0</td>
<td>Absent</td>
</tr>
<tr>
<td>Lyme disease</td>
<td>0</td>
<td>Present</td>
</tr>
<tr>
<td>Recent <em>P. falciparum</em> infection (gametocytemia)</td>
<td>1,024</td>
<td>Absent</td>
</tr>
<tr>
<td>Resident in malaria endemic region</td>
<td>64</td>
<td>ND</td>
</tr>
<tr>
<td>Flu-like syndrome 19 days after a malaria attack</td>
<td>64</td>
<td>ND</td>
</tr>
</tbody>
</table>

* *Pf* = Plasmodium falciparum, ND = not done.
France. A diagnosis of Loa loa filariasis and malaria co-infection was made in one case, underlining the value of microscopy for the identification of blood parasites other than *Plasmodium*.

In our experience, the pink band on the Parasight®-F strips was still present after 2 years, even for only slightly-positive results. QBC® test tubes can only be kept for a few days at +4°C. Thin and thick blood films can be kept indefinitely.

If the Parasight®-F test had been used alone for decision-making, 18.6% of the patients with acute malaria (n = 8; 3 with *P. falciparum*) would not have been treated. This could have had severe clinical consequences. On the other hand, six febrile patients with false-positive Parasight®-F test results would have inappropriately been treated with anti-malarial drugs. Malarial serology was positive in three of these six cases. One patient was a resident of an African country; one who visited the clinic for fever of viral origin after presumptive treatment of a malaria attack while travelling had *P. falciparum* gametocytes; and one patient had a flu-like syndrome 3 weeks after a malaria attack that had been treated and cured. In all three situations, the Parasight®-F test positivity may have been due to residual antigen. Undetectable asexual *P. falciparum* parasitemia seems improbable as the patient had not been treated at the time of the test and no malaria attacks occurred in the following four weeks. Polymerase chain reaction amplification of *Plasmodium* DNA, a method more sensitive than microscopy, might have been able to discriminate circulating antigen from very low level of parasitemia.

Previous studies of the Parasight®-F test have reported false-positive results associated with hepatitis or phlebitis, and with the presence of rheumatoid factor in the sample. Rheumatoid factor was screened-for in three of the six patients with false-positive Parasight®-F tests, and found in one patient with Lyme disease. To our knowledge, this is the first report of a false-positive Parasight®-F test result due to Lyme disease.

The positive and negative predictive values of the Parasight®-F test for the diagnosis of malaria taking optical microscopy as the standard were 85% and 92%, but the negative predictive value was 97% for the diagnosis of *P. falciparum* (92% sensitivity and 94% specificity). These values are similar to those described in a review from the World Health Organization (84.2% to 93.9% sensitivity and 81.1% to 99.5% specificity). The performance of the Parasight®-F test in the current report is similar to that described by Van den Ende and others for imported malaria.

*Plasmodium falciparum* parasitemia was low in the three patients with false-negative Parasight®-F tests. This is consistent with previous studies showing that the Parasight®-F test detects only 70% to 81% of positive samples with parasitemia values between 11 and 60/μL. A negative Parasight®-F test in a clinical setting compatible with malaria should therefore be considered with care given the possibility of false-negative results when parasitemia is near the limit of detection of the thick-blood film. Of the three samples positive by the thick film and negative by thin film, two contained *P. falciparum*. The Parasight®-F test was negative for one (parasitemia 8/μL) and positive for the other (parasitemia 48/μL). The third case was due to *P. malariae* (negative Parasight®-F test). Of the five cases due to *Plasmodium* species other than *falciparum*, the Parasight®-F test was negative as would be expected given the specificity of histidine-rich protein 2 for *P. falciparum*. Another test, OptiMAL® (Flow Incorporated, Portland, OR), based on a strip reacting with a malarial enzyme, lactic dehydrogenase, can detect and discriminate between infection by the four species pathogenic for humans.

In the two cases of concomitant *P. falciparum* and *P. ovale* infection, the Parasight®-F test was positive in one and negative in the other; *P. falciparum* parasitemia values were 35,000/μL and 250/μL. The QBC® test was falsely negative on 3 samples from patients with *Plasmodium falciparum* malaria, whereas the Parasight®-F test gave positive results. The specificity and sensitivity values in this study (100% and 95%) were similar to those reported in the literature. The thin-film and QBC® test are as rapid to perform as the Parasight®-F test (each taking respectively 10, 15, and 15 minutes). In contrast the thick-film method takes at least 90 minutes to perform. The slides must then be inspected microscopically. Diagnostic difficulties must then be resolved. The Parasight®-F test is unaffected by such factors.

The Parasight®-F test is easy to perform. Nevertheless, the physician had to re-prick the patient’s finger in 30% of cases to obtain enough blood to fill the capillary tube. The relative rapidity of the Parasight®-F test (15 minutes on average) is an additional factor favoring its use outside the laboratory setting.

One difficulty in interpreting the Parasight®-F test is that deposition of the histidine-rich protein-2 antigen band leaves a ghost image on some strips. This may incorrectly be considered as a weakly positive result (Figure 1, strip E). Among the ten weakly positive tests, only four corresponded to proven *P. falciparum* infection and none to other species (Figure 1, strip B). Furthermore, it is impossible to distinguish a true-positive from a false-positive result (Figure 1, strips C and D).

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