ParaSight®-F rapid manual diagnostic test of Plasmodium falciparum infection

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The ParaSight®-F test is a qualitative diagnostic test of Plasmodium falciparum, which is based on the detection by a monoclonal antibody of a species-specific soluble antigen (histidine-rich protein (HRP-II)) in whole blood and which can be performed without special equipment. A visual reading is given by a polyclonal antibody coupled with dye-loaded liposomes; when positive, a pink line appears. The test has been compared with microscopic examination of thin blood smears and with the Quantitative Buffy Coat malaria test (QBC®) in a single-blind study.

A total of 358 patients who had returned to France from malarial areas and consulted their doctor with symptoms or for a routine examination were enrolled in the study; 33 of them were found to have a falciparum malaria infection by the diagnostic test. On the day of consultation, the specificity of the ParaSight®-F test was 99% and its sensitivity 94%. The follow-up of infected patients after treatment showed that the test became negative later than the other reference tests. There was no correlation between antigen persistence and the intensity of the ParaSight®-F signal or circulating parasitaemia. No cross-reaction was noted for seven malaria cases due to other Plasmodium species. The test was performed quickly (10 tests in 20 minutes), was easy to read, and required minimal space. For cases of imported malaria, the test’s specificity and low threshold for detection could make it a valuable adjunct test. However, in its present form, it cannot replace microscopic techniques which are species-specific and quantitative. In endemic areas, the test seems to be very promising by its results and ease of use according to published field studies.

Introduction

The WHO-sponsored Ministerial Conference on Malaria in Amsterdam in October 1992 stressed the need for control because of the evolution of this disease which now threatens more than 40% of the world population. One of the main priorities in the global strategy is accurate diagnosis of the disease. Current diagnostic methods are based on microscopic examination which takes 5–10 minutes for staining and up to 20 minutes for reading (in the case of negative samples), and requires a well-maintained microscope and an experienced microscopist. New techniques, such as hybridization with DNA probes, are too sophisticated for routine use in the field. There is therefore an urgent need for a field test which is simple, rapid and accurate, and can be performed without equipment.

Recently Shiff et al. (1) and Beadle et al. (2) reported their experience in malaria-endemic areas with a rapid manual diagnostic test for Plasmodium falciparum, the ParaSight®-F test.® This test is based on the detection in whole blood of a soluble antigen, histidine-rich protein II (HRP-II), a specific glycoprotein of P. falciparum (3) which is secreted during the parasite’s erythrocytic cycle, with a peak during schizont rupture (4). HRP-II has been isolated from P. falciparum in Africa, Asia, and South America (5) and appears to be a suitable marker of infection. Presented as a test strip, ParaSight®-F requires no special equipment for its performance or interpretation. In this study, the test was compared with examination of thin blood smears and the Quantitative Buffy Coat (QBC®) malaria diagnosis system® on patients in a European hospital to assess both performance and usefulness in a non-endemic area.

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Patients and methods

The study was conducted in the Tropical Medicine Department of the Hôpital de la Croix rousse in Lyon, and in the Hôpital Alphonse Laveran in Marseille, France. A control group was made up of randomly selected inpatients with no history of malaria exposure, while the study group included all travelers returning from endemic malarial areas who presented themselves at either hospital because of clinical symptoms (fever or diarrhoea) or for a routine check-up.

All malarial infections were followed until recovery, as indicated by apyrexia and negative parasitaemia. Falciparum malaria patients were treated with either intravenous quinine or two doses of halofantrine; patients with other plasmodial species were treated with chloroquine. Treatment was started immediately after the test results were known to be positive. Blood samples were collected in Vacutainer® EDTA tubes. The ParaSight®-F test was run using this blood sample.

The reference tests were the QBC® malaria diagnosis system and by examination of thin blood smears. The former was performed following the recommended technique (6) and used as a screening tool. They were read for 20 minutes to confirm that the QBC® results were negative; when positive, the parasitaemia was expressed as a percentage of parasitized erythrocytes. The thin blood smears were stained with Diff-Quick™ — thiazine blue and eosine red — and examined with ×1000 magnification.

The ParaSight®-F test is a capillary immunoassay for the direct qualitative detection of \textit{P. falciparum} HRP-II antigen in whole blood. Mouse monoclonal antibody raised against the peptide AHH (AHHAAD)\textsubscript{2} is immobilized on a nitrocellulose-membrane test strip. A 50-μl whole-blood sample collected in a capillary tube was lysed in a test tube by the addition of 3 drops of lysing reagent; the sample was then dispensed through a filter into a reaction well. The test strip was placed in the lysed blood sample and antigen in the sample binds to the immobilized antibody as it soaks up the test strip (Fig. 1).

A drop of liposome detector particles containing a red dye and coated with rabbit antibodies, which were also raised against the peptide sequence AHH (AHHAAD)\textsubscript{2}, was added next. These particles were bound to the captured antigen as they swept past, resulting in a distinct solid pink line in the case of a positive reaction (Fig. 2). Two drops of the wash reagent were added to the strip to clear the unbound detector. If there was no antigen in the sample, the liposomes did not bind and no pink line was formed. A dashed control line appeared above the reaction line in both positive and negative tests to indicate that the detection reagent had been added and that the test strip and detection reagent were working properly (Fig. 2). The intensity of the reaction line may vary depending on the amount of antigen present in the sample. Both reagents and tubes, including a positive serum and a negative serum control set, were supplied in the test kit ready for use. The kits we used were stored at ambient temperature for 5 months (duration of the study).

In our single blind study, the reference diagnostic techniques (thin blood smears and QBC®) were interpreted by a technician while the ParaSight®-F test was read by two independent investigators. After a period of familiarization with the test, the time necessary for its performance (preparation and interpretation) was determined. Where discrepancies were observed, the QBC® system and the thin blood films were examined again and the ParaSight®-F test was performed a second time.

Results

The study was conducted from 15 June to 24 October 1992. The control group included 50 randomly selected inpatients with no history of malaria exposure; average age, 42 years (range, 18–91 years); 46% were females. All the tests were negative.

The study group comprised 358 patients; average age, 34 years (range, 10 months to 79 years); 43% were females. On their first visit, 33 patients had \textit{P. falciparum} infection, 32 of them having returned from Africa and 1 from India/Pakistan; 5 other patients had \textit{P. vivax} infection, 1 had \textit{P. ovale} and 1 had \textit{P. malariae}. No mixed infections were recorded.

There was no discordance between the two reference test results, i.e., between thin blood films and the QBC® diagnosis. Table 1 shows the comparison between these reference tests and the ParaSight®-F test for the detection of \textit{P. falciparum}. The specificity and sensitivity of the latter were 99% and 94% respectively; the positive and negative predictive values were 89% and 99% respectively. The kappa index of agreement was 0.89 between the ParaSight®-F test and the two reference tests.

Fig. 3 shows the follow-up of six patients with signs of falciparum malaria. There was no correlation between the persistence and intensity of the ParaSight®-F signal and the circulating parasite densities observed in thin blood films.

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Fig. 1. Last step in the ParaSight®-F test, the chronometer showing the time elapsed since the beginning of the test.

Fig. 2. Appearance of results in a positive and a negative test.
The samples taken from seven patients showing infection with other plasmodial species were negative. The ParaSight®-F test performed on blood from mice parasitized by P. berghei and from dogs parasitized by Babesia canis also gave negative results. No cross-reaction was observed for Loa loa (2 cases), Dipetalonema perstans (1 case), Schistosoma mansoni (2 cases) and Entamoeba histolytica (1 case) infections.

In routine testing, the different investigators could perform one ParaSight®-F test in 7 minutes and 10 tests in 20 minutes. The QBC® malaria diagnosis method requires 6–10 minutes and examination of thin blood smears takes 10–40 minutes. At the time of submission of this paper, the ParaSight®-F test signal has remained stable for 24 months with test strips stored in the dark. This could represent an advantage over microscopy for certain research applications.

**Discussion**

Microscopic techniques for the diagnosis of P. falciparum infections present technical constraints and can only detect circulating parasites, with no indication as to the number of parasitized erythrocytes which cytoadhere on the endothelial cells. It has been clearly established that the latter plays a major part in the pathogenicity of the disease (7) and that their number is not correlated to the number of circulating parasites (8, 9). The detection of antigaenaemia could therefore be of great importance in diagnosing low levels of infection.

The 94% sensitivity of the ParaSight®-F test was due to the two false-negative results observed in patients with a proven P. falciparum parasitaemia (the scarce parasites were seen on thin blood smears/ QBC®). The possibility of a manipulation problem was discarded because the integrated controls were positive. Three assumptions were therefore considered:

- insufficient concentration of HRP-II, although positive results were obtained with patients having low parasitaemias;
- binding of HRP-II to antibodies; the possible interference with naturally acquired anti-HRP-II antibodies must be investigated (so far, we do not know if immune complexes interfere with the test); and
- a mutant colony cultivated in vitro, which does not secrete HRP-II has been described (10), and large-scale field studies will be required to show whether such mutations exist naturally.

The specificity and negative predictive values of 99% were excellent. Four confirmed false positives, on the day of consultation, were observed: one case each of phlebitis and hepatitis, one patient returning from Burkina-Faso who had treated himself with antimalarial drugs during a febrile episode one month previously, and one semi-immune Comorian patient with abdominal pain whose thin blood film and QBC® test were found to be positive 48 hours later. The last case was considered a false positive, but the parasitological findings exclude this; the last two cases support the absence of antigen in the absence of parasites. The first two cases could be due to a cross-reaction with one of the proteins rich in histidine normally present in the serum (11), although in the pathological context this could have been specific to the patients with phlebitis and hepatitis. In the case of the Comorian patient, the lag-time between the reference tests and the positive ParaSight®-F test would indicate a lower detection threshold than with the reference tests used.

In the follow-up of patients, the ParaSight®-F test which became negative later than the reference tests also supports this hypothesis; in one case, the test remained positive for 24 days after the negative parasitaemia by the reference tests. The kinetics of HRP-II during the decline of the malarial attack are not yet well-known and other parameters such as the influence of treatment and the patient's immune status will also need to be considered. Previous studies reported great variability in the kinetics of HRP-II. According to Shiff et al. (1), antigaenaemia may have persisted for up to two weeks after the clearance of parasites in five individuals treated with chloroquine; on the other hand, Beadle et al. (2) reported that “HRP-II is no longer detectable in blood 6 days after starting curative chemotherapy”. Interestingly in our study, one case of cerebral malaria, who had negative reference tests immediately after exchange transfusion, remained HRP-II-positive for 9 days.

No problems were encountered in the performance or interpretation of the ParaSight®-F test. In our hands, the test proved to be 94% sensitive and
Fig. 3. **Follow-up of six patients showing percentage parasitaemia (as estimated on thin blood films) against ParaSight®-F test results.** Stippled columns indicate a positive test.
99% specific, and was simple and rapid to perform. It is the first diagnostic test for *P. falciparum* which requires no special equipment. The disposables take up minimal space.

For the diagnosis of imported malaria, however, the ParaSight®-F test cannot replace microscopic techniques because it is qualitative and specific to *P. falciparum*. In this study, 17.5% of the cases were not falciparum malaria. Although we did not observe cross-reaction among species, the sample size prevents us from drawing conclusions. The ParaSight®-F test can, however, be used in conjunction with other techniques because its sensitivity makes it a rapid adjunct test. For patients who, before consulting the doctor, had treated themselves, which may cause the usual tests to be negative or show results that are difficult to interpret, the ParaSight®-F test permits retrospective diagnosis and, when necessary, complementary treatment. Indeed, the ParaSight®-F test was positive for two patients studied outside the test protocol who presented themselves after self-medication. The clinical improvement of these patients with antimalarial treatment and their positive serologies (greater than 1/2560 in indirect immunofluorescence) were strongly in favour of the diagnosis of a malarial attack. The test is also useful in cases of low parasitaemia which is a frequent finding among travellers with malaria; in this study, 13 of the 31 patients (42%) had a parasitaemia below 0.1% on admission (data not shown).

In malaria-endemic areas, the ParaSight®-F test should improve the diagnosis of *P. falciparum* malaria because of its accuracy, simplicity, rapidity and other advantages (2). In addition, the accurate diagnosis of *P. falciparum* is needed in chloroquine-resistant areas in order to prescribe correctly the new antimalarial drugs which are often expensive and toxic. Indeed, some interesting results have already been published (1, 2).

The effectiveness of the ParaSight®-F test has been globally assessed in endemic and non-endemic areas. As underlined by Beadle et al. (2), its usefulness is limited when determining the cause of fever in holoendemic areas (where the prevalence of *P. falciparum* is very high) because these test results are not quantitative. Further evaluation should be focused on the role of this dipstick antigen-capture assay in managing malarial patients in different situations of *P. falciparum* transmission.

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**Résumé**

Le ParaSight®-F, test manuel rapide de diagnostic de l'infection à *Plasmodium falciparum*

Le test ParaSight®-F est le premier test diagnostique du paludisme à *Plasmodium falciparum* qui ne nécessite aucun équipement spécial. Cette technique qualitative est basée sur la détection par un anticoag mononclonal d'un antigène solubile spécifique d'espèce, l'histidine-rich protein (HRP II) dans le sang total. Un signal visuel est donné par un anticoag polyclonal couplé à un pigment recouvrant des liposomes. En cas de positivité, une ligne rose apparaît, visible à l'œil nu.

Le ParaSight®-F a été comparé avec les techniques de référence (frottis mince/microscope et Quantitative Buffy Coat Malaria test (QBC®)) lors d'une étude en simple aveugle sur 358 sujets, de retour en France de zones d'endémie paludéenne, qui ont consulté soit pour des troubles variés soit pour un examen de routine. Trente-trois sujets présentaient une infection à *P. falciparum* confirmée par les techniques de référence. À l'admission, la spécificité et la sensibilité du ParaSight®-F étaient respectivement de 99% et 94%. Le suivi des sujets impaludés pendant et après le traitement a montré que le test devenait négatif aprés les techniques de référence. Il n'y avait aucune corrélation entre la persistance de l'antigène et l'intensité du signal du ParaSight®-F ou la parasitémie circulante. Aucune réaction croisée n'a été observée parmi les 7 cas de paludisme dus à des espèces autres que *P. falciparum*. Le test était rapide à réaliser (10 tests en 20 minutes) et facilement interprétable. Le signal est resté stable au moins 2 ans, le test étant conservé à l'abri de la lumière.

Les réactifs étaient peu encombrants. Pour le paludisme d'importation, la spécificité du ParaSight®-F ainsi que son seuil de détection bas pourraient en faire un test d'appoint intéressant. Ne détectant que *P. falciparum* et n'étant pas quantitatif, le ParaSight®-F ne peut cependant remplacer l'examen microscopique. En zones d'endémie, ce test pourrait jouer un rôle important, d'après les résultats de 2 études de terrain, de par ses qualités analytiques et ergonomiques.

**References**

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