DIAGNOSIS OF *PLASMODIUM FALCIPARUM* INFECTION USING *PARA*SIGHT[R]-F TEST IN BLOOD AND URINE OF PAPUA NEW GUINEAN CHILDREN

Blaise Genton[1,2], Sarah Paget[3], Hans-Peter Beck[2], Nicky Gibson[1], Michael P Alpers[1] and Jeffrey Hii

[1]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea; [2]Swiss Tropical Institute, Basel, Switzerland; [3]Department of Public Health and Tropical Medicine, James Cook University of North Queensland, Townsville, Australia

Abstract. Rapid, simple, accurate and cheap methods are required for the diagnosis of malaria in endemic areas. The *Para*Sight[R]-F test, which is based on qualitative detection by monoclonal antibody of the *Plasmodium falciparum* (Pf) histidin-rich protein-II in the blood, showed promising results. As some antigens of Pf have been detected in the urine, we assessed the efficiency of the *Para*Sight[R]-F test in the whole blood and in the urine using microscopy and PCR as gold standards. One hundred and twelve children living in the East Sepik Province of Papua New Guinea (PNG) were recruited during a cross-sectional community survey. When using microscopy as reference, the *Para*Sight[R]-F test applied to whole blood had 84% sensitivity and 77% specificity. The semi-quantitative assessement showed that the intensity of the color on the wick correlated with parasite density. The *Para*Sight[R]-F test performed on urine had 81% sensitivity but only 26% specificity. Very similar results for blood and urine were obtained when using PCR as reference. The present evaluation of the *Para*Sight[R]-F test applied to blood compares well with findings in endemic areas of Africa or Asia, and confirms its usefulness to diagnose Pf infection in endemic areas of the South Pacific. Because of the lack of specificity, the *Para*Sight[R]-F test performed on urine cannot be recommended.

INTRODUCTION

Rapid, simple, accurate and cheap methods are required for the diagnosis of malaria. The gold standard at present is microscopic examination of thick films. This technic requires a well-maintained equipment, a reliable source of electricity and a prolonged training, all essentials which are rarely available in rural malaria endemic areas. A simplified detection assay that uses a dipstick has recently been developed (Schiff *et al*, 1994). This test is based on the detection by a monoclonal antibody of the histidin-rich protein II (HRP-II), a water soluble antigen released by blood-stages of *Plasmodium falciparum* (Pf) from many geographical areas (Rock *et al*, 1987; Parra *et al*, 1991). Presented as a test drip, *Para*Sight[R]-F requires no special equipment for its performance or interpretation. In a malaria-endemic area of Tanzania, the test gave a sensitivity of 89% and a specificity of 88% (Schiff *et al*, 1993); in Kenya, the sensitivity was 75% and specificity 88% (Beadle *et al*, 1994). In clinical cases of Sri Lanka, the sensitivity was 90% and the specificity 99% (Kodisinghe *et al*, 1997). In all instances, the sensitivity increased with increasing parasite densities. All the studies up until the present one used whole blood as described by the manufacturer. Since some *Plasmodium* antigens have been found in the urine of parasitemic individuals (Valle *et al*, 1991), we evaluated the test drip in the urine of 57 adults living in a malaria-endemic area of Papua New Guinea (PNG) (Genton *et al*, 1996). Results of this pilot study showed that the HRP-II can be detected by the *Para*Sight[R]-F test in the urine of infected individuals. We describe here the results of a more extensive study which was aimed at evaluating the performance of the *Para*Sight[R]-F test using whole blood or urine. Also we were interested to assess the performance of the test in the South Pacific region, where no study had been conducted. Since the evaluation of a new test depends on the performance of the gold standard, we used both microscopic examination and polymerase chain reaction as references.

Correspondence: Dr Blaise Genton, Policlinique Médicale, Universitaire César-Roux 19, 1005 Lausanne, Switzerland.
Tel: 41-21-345-2222, Fax: 41-21-345-2323, Email: Blaise.genton@chuv.hospvd.ch

MATERIALS AND METHODS

Subjects and field procedures

The dipstick was evaluated in children aged 0.5 to 5 years during a baseline cross-sectional survey done in July 1995, prior to vitamin A supplementation. The assessment was done during the dry season, when Pf prevalence is expected to be around 50% in this age group (for details of the malaria epidemiology in the area, see Genton *et al*, 1995a, b). Blood samples were collected by venepuncture for parasitological and immunological assessment. The *Para*Sight^R-F test was performed within 10 minutes of sample collection by two different persons, one working on the blood and one working on the urine.

*Para*Sight^R-F test

The test was performed according to the standard procedure (Schiff *et al*, 1994). Briefly, the dipstick, which was pretreated with a mouse monoclonal antibody against HRP-II, was placed in a drop of blood previously hemolysed with a detergent. Following this a drop of developing reagent was applied to the base of the dipstick. When the reagent had been absorbed, two drops of clearing reagents were applied. Positive cases leave a thin pink line across the wick with a broken line above it as reagent control. In negative cases, only the control broken line is seen. The same procedure was used for urine (with a volume of 150 μl), except the application of the hemolysing detergent (Reagent 1).

A quantitative assessment was done using the reagent control broken line as reference (negative = sample line absent, weak = pink paler than the control, intermediate = pink as intense as the control, strong = pink more intense than the control).

Microscopy and parasite density determination

Thick films were stained with 4% Giemsa and examined at magnification of 1,000. Slides were read for 400 fields prior to be declared negative. Densities were recorded as the number of parasite per 200 white blood cells (WBC) and converted to parasite per μl using the individual WBC count.

Polymerase chain reaction (PCR)

DNA was extracted using the blood from a quarter of the thin film (Edoh *et al*, 1997). PCR amplification for Pf MSP-2 was performed with 1/3 of isolated DNA (10 μl) as decribed previously (Felger *et al*, 1993). PCR products were visualized on 12% polyacrylamide gels. All Pf negative PCR reactions were repeated with DNA from a second quarter of the thin smear. In order to test the quality of the DNA, all remaining Pf negative samples were amplified for the human TNF-α promoter using the previously described primers and amplification profile (McGuire *et al*, 1994). Ten μl of amplified product was analysed on a 1.5% agarose gel.

All tests (dipstick in blood, dipstick in urine, microscopy and PCR) were done by independent persons. The assessors were all blind to the results obtained with the other technics.

Data analysis

Sensitivity, specificity and predictive values were calculated using microscopy or PCR as gold standards.

RESULTS

Microscopic examination, PCR and *Para*Sight^R-F test in blood and urine were assessed in 112 children. 91/112 (81%) were found parasitemic by microscopic examination, 73 (65%) with *Plasmodium falciparum* (Pf), 38 (34%) with *P. vivax* (Pv) and 7 (6%) with *P. malariae* (Pm). 70 (63%) had a positive *Para*Sight^R-F test in the blood and 88 (79%) in the urine. Of the 73 which were Pf positive by microscopy, 61 were also positive by dipstick in the blood and 59 in the urine. Among the 39 which were negative by microscopy, 30 were also negative by dipstick in the blood but only 10 in the urine (Table 1). When using the microscopic examination as a gold standard, the *Para*Sight^R-F test in the blood gave a sensitivity of 83.6% (95% confidence intervals 73-91), a specificity of 76.9% (60-89), a positive predictive value (PPV) of 87.1% (77-94) and a negative predictive value (NPV) of 71.4% (55-84). The *Para*Sight^R-F test in the urine gave a sensitivity of 80.8% (70-89), a specificity of 25.6% (14-42), a positive predictive value (PPV) of

Table 1

Comparison of the *Para*Sight^R-F test in blood and urine with results of microscopy.

Microscopy result	*Para*Sight^R-F test in blood: No. positive	in blood: No. negative	in urine: No. positive	in urine: No. negative	Total
No. Pf* positive	61	12	59	14	73
No. Pf negative	9	30	29	10	39
Total	70	42	88	24	112

* *Plasmodium falciparum*

67% (52-77) and a negative predictive value (NPV) of 41.7% (23-63). Sensitivities increased with increasing densities, both for blood and urine (Table 2). Of the 13 children with a single Pv infection, 3 had a *Para*Sight^R-F test positive in the blood and 10 in the urine. Of the 4 children with a single Pm infection, the same 2 had a *Para*Sight^R-F test positive in the blood and in the urine. The quantitative assessment showed that the intensity of the color of the *Para*Sight^R-F line from the whole blood was related to the parasite density [median Pf density of 0 (range 0-8) in the 'negative' ones, 1.5 (0-16) in the 'weak' 6 (0-345) in the 'intermediate' and 98 (0-40,000) in the strong ones]. The line of all the samples which were positive in the urine were weak.

For the comparative assessment of the *Para*Sight^R-F test with PCR we included only 78 samples since we were not able to amplify human TNF in 11 samples, and in another 23 the signal was too weak to be reliable. 55/78 (71%) samples were positive by PCR. 56 (72%) were Pf positive by microscopy, 52 (67%) were positive by dipstick in the blood and 62 (79%) by dipstick in the urine. Of the 55 positive by PCR, 47 were also positive by dipstick in the blood and 45 in the urine. Among the 23 which were negative by PCR, 18 were also negative by dipstick in the blood but only 6 in the urine (Table 3). When using PCR as a gold standard, the *Para*Sight^R-F test in the blood gave a sensitivity of 85.5% (73-93) and a specificity of 78.3% (56-92). The *Para*Sight^R-F test in the urine gave a sensitivity of 81.8% (69-91) and a specificity of 26.1% (11-49).

Table 2

Comparison of the *Para*Sight^R-F test in blood and urine with results of microscopy.

Parasites/µl	Samples positive by microscopy	Samples positive by *Para*Sight^R-F test: in blood	in urine	Sensitivity (%): in the blood	in the urine
<40	12	4	7	33	58
41-80	8	5	8	63	100
81-200	5	5	4	100	80
201-2,000	26	25	21	96	81
>2,000	22	22	19	100	86
Any Pf* positive	73	61	59	84	81

* *Plasmodium falciparum*

Table 3

Comparison of the *Para*Sight[R]-F test in blood and urine with PCR results.

PCR result	*Para*Sight[R]-F test in blood: No. positive	in blood: No. negative	in urine: No. positive	in urine: No. negative	Total
No. positive	47	8	45	10	55
No. negative	5	18	17	6	23
Total	52	26	62	16	78

DISCUSSION

The prevalence rate of *P. falciparum* infections detected by microscopy (65%) was higher than expected from previous studies (Genton *et al*, 1995a). This may be due partly to temporal variation, but certainly also to the way the blood films have been examined. Indeed 400 fields were read prior to declare a slide negative, instead of the usual 100 fields, in order to increase the accuracy of the assessment of sensitivity and specificity. This may explain why the prevalence of Pf detected by PCR (71%) was almost the same as the one found by microscopy (72%) in the same 78 children. Since we used only half of the thin film for PCR assessment, the volume of blood was about the same than the one screened by microscopic examination, and so the likelihood of having at least one parasite in the sample was similar.

The *Para*Sight[R]-F test performed on whole blood gave a sensitivity of 84% when using microscopy as gold standard, and 86% when using PCR. These results are similar to the one estimated by Beadle *et al* (1994) in the group of children in Kenya (86%), using blood films only for comparison. It is slightly lower than the sensitivity obtained in Tanzania (89%) by Shiff *et al* (1993), but the subjects of the latter study were clinical cases who are more likely to have high parasite densities and therefore to be detected as positive. Indeed we know from all previous studies that the *Para*Sight[R]-F test performs better when densities are high and this was confirmed in the present study. The high sensitivity which was found when the test was compared to PCR was less expected, but it could be explained by the fact that the *Para*Sight[R]-F test detects circulating antigenemia, and not parasites as does PCR. The end-product assessed is different but the parasite load required for a test to be positive with either method may be the same. Similar results were reported in a study conducted in Thailand where sensitivity was 93% using microscopy as reference and 92% using PCR (Banchongaksorn *et al*, 1996).

When done on the whole blood, the *Para*Sight[R]-F test had a specificity of 77%, using microscopy as reference, and 78% using PCR. The assessment of specificity is difficult in an area highly endemic for malaria, because one can never be sure that an individual with a negative thick film is truly parasite-free. We decided to include a comparative evaluation with PCR as gold standard to overcome this problem. Possible explanations for the lower specificity found in the present study when compared to the 87% reported by Schiff *et al* (1993) and Beadle *et al* (1994) are i) higher level of cross-reaction with one of the protein rich in histidine normally present in the serum (Morgan, 1978), ii) lower performance of microscopists (parasite or species identification), iii) higher prevalence of children recently treated for malaria. The latter explanation is the most likely as previous studies (Schiff *et al*, 1993; Uguen *et al*, 1995) observed positive tests two to four weeks after clearance of parasitemia; in the same area we also found that antigenemia persists up to 28 days after treatment initiation (Genton *et al*, unpublished data); Humar *et al* (1997) reported that 27% of non-immune patients had still a positive test at day 28. The fact that the specificity was not increased when PCR was used as reference indicates that parasites have truely disappeared after treatment, in contrary to circulating antigens, which may be detected much

longer afterwards. Alternatively, DNA preparation was done on a too small volume of blood under suboptimal conditions using fixed and stained microscopic slides.

The *Para*Sight[R]-F test done in the urine gave good results in terms of sensitivity, both with microscopy and PCR as gold standards (81% and 82% respectively). They confirm the findings of our pilot-study in adults (Genton *et al*, 1996) which showed that the HRP-II is present and can be detected in the urine. The concentration of the protein is likely to be lower in the urine than in the blood, which could explain the differences of sensitivity obtained. Moreover, since all the samples were collected between 08.00 and 14.00 hours, only few of the urines must have been the first micturition after sleep, decreasing even more the concentration of antigen.

The important draw-back with the *Para*Sight[R]-F test done in the urine is the very low specificity (26%) obtained, whatever gold standard was used. This poor specificity may be related to difficulties in the interpretation of the *Para*Sight[R]-F in the urine. All positive sample bands in the urine were pale pink, and most of them were weaker than the weakest in the blood, so the determination for positivity or negativity in the urine was not as straightforwad as in the blood. Because the dipsticks were read by one person only, no inter-rater agreement was assessed. False positive may also be related to the persistance of antigens in the blood after parasite clearance and/or delay in excreting all the products of degradation of the parasites, but the most likely explanation for the low specificity is cross-reactions with other histidin-rich proteins or any other products present in the urine.

The present study confirms the efficiency of the *Para*Sight[R]-F test done in the blood to diagnose *P. falciparum* infection. It allows this test to be used with confidence in PNG and probably in all the South Pacific region. Previous authors have underlined its limited usefulness for determining the cause of fever in highly endemic areas (where the prevalence of *P. falciparum* is high) because this test is not quantitative. This is true in epidemiological or intervention studies, when the aim is to know the malaria attributable fraction of all febrile episodes. In the management of clinical cases however, this limitation is marginal as all patients with fever associated with *P. falciparum* infection should be given antimalarials, whatever density they have. Also, we have shown in the present study that the intensity of the sample line color correlate to some extent to the parasite density. To our view the only important limitation of the *Para*Sight[R]-F test is that it only detects *P. falciparum* infection. In areas where prevalences of other species are high, such as in PNG, the test will not add to the decision whether chloroquine must be given. On the other hand, with increasing levels of chloroquine-resistant *P. falciparum*, 'second-line' drugs may be required in many countries. If this is to happen, it may be necessary to have an appropriate diagnosis before treatment since these drugs are more expensive than the 'first-line' ones and may lead to resistance more rapidly. The *Para*Sight[R]-F test may serve this need.

In the view of our findings, the *Para*Signt[R]-F test in the urine cannot be recommended as a diagnostic tool to diagnose Pf infection, due to its low efficiency. It is however encouraging to note that antigens of *Plasmodium* parasites can be detected in the urine using a dipstick only and further research should be focused on such methods.

ACKNOWLEDGEMENTS

We thank the children and parents who participated in this study, A Shankar for allowing the samples to be tested, the Papua New Guinea Institute of Medical Research field staff in Maprik and the microscopists in Madang. This work received financial support from the UNDP/World Bank/ WHO Special Program for Research and Training in Tropical Diseases and the United States Agency for International Development.

REFERENCES

Banchongaksorn T, Yomoktul P, Panyim S, Rooney W, Vickers P. A field trial of the *Para*Sight[TM]-F test for the diagnosis of *Plasmodium falciparum* infection. *Trans R Soc Trop Med Hyg* 1996; 90 : 244-5.

Beadle C, Long GW, Weiss WR, *et al*. Diagnosis of malaria by detection of *Plasmodium falciparum* HRP-2 antigen with a rapid dipstick antigen-capture assay. *Lancet* 1994; 343: 564-8.

Edoh D, Steiger S, Genton B, Beck H-P. PCR amplification from fixed and stained malaria thick and thin films. *Trans R Soc Trop Med Hyg* 1997; 91 : 361-3.

Felger I, Tavul L, Beck HP. *Plasmodium falciparum*: A rapid technique for genotyping the merozoite surface protein 2. *Exp Parasitol* 1993; 77 : 372-5.

Genton B, Hii J, Paget S, Alpers M. Rapid manual diagnosis of *Plasmodium falciparum* malaria using *Para*Sight[R]-F dipsticks applied to human blood and urine. *J Trav Med* 1996; 3; 172-3.

Genton B, Al-Yaman F, Beck H-P, *et al.* The epidemiology of malaria in the Wosera area, East Sepik Province of Papua New Guinea, in preparation for a vaccine trials. I. Malariometric indices and immunity. *Ann Trop Med Parasitol* 1995a; 89 : 359-76.

Genton B, Al-Yaman F, Beck H-p, *et al.* The epidemiology of malaria in the Wosera area, East Sepik Province of Papua New Guinea, in preparation for vaccine trials. II. Mortality and morbidity. *Ann Trop Med Parasitol* 1995b; 89 : 377-90.

Humar A, Ohrt C, Harrington MA, Pillai D, Kain KC. *Para*Sight[R]-F test compared with the polymerase chain reaction and microscopy for the diagnosis of *Plasmodium falciparum* malaria in travellers. *Am J Trop Med Hyg* 1997; 56 : 44-48.

Kodisinghe HM, Perera KLRL, Premanwansa S, de S Naotunne T, Wickramasinghe AR, Mendis KN. The *Para*Sight[TM]-F dipstick test as a routine diagnostic tool for malaria in Sri Lanka. *Trans R Soc Trop Med Hyg* 1997; 91 : 398-402.

McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNFα promoter region associated with susceptibility to cerebral malaria. *Nature* 1994; 371 : 508-11.

Morgan WT. Human serum histidine-rich glycoprotein: interaction with heme, metal ions and organic ligands. *Biochim Biophys Acta* 1978; 535 : 319-33.

Parra ME, Evans CB, Taylor DW. Identification of *Plasmodium falciparum* histidine-rich protein 2 in the plasma of humans with malaria. *J Clin Microbiol* 1991; 29 : 1629-34.

Rock EP, Marsh K, Saul AJ, *et al.* Comparative analysis of the *Plasmodium falciparum* histidine-rich proteins HRP-1 HRP-2 and HRP-3 in malaria parasites of diverse origin. *Parasitology* 1987; 95 : 209-27.

Shiff CJ, Minjas JN, Premji Z. The *Para*Sight[R]-F test: a simple rapid manual disptick test to detect *Plasmodium falciparum* infection. *Parasitol Today*, 1994; 10 : 494-5.

Shiff CJ, Premji Z, Minjas JN. The rapid manual *Para*Sight[R]-F test. A new diagnostic tool for *Plasmodium falciparum* infection. *Trans R Soc Trop Med Hyg* 1993; 87 : 646-8.

Uguen C, Rabodonirina M, De Pina JJ, *et al. Para*Sight[R]-F rapid manual diagnostic test of *Plasmodium falciparum* infection. *Bull WHO* 1995; 73 : 643-9.

Valle MR, Quakyi IA, Amuesi J, Quaye JT, Nkrumah FK, Taylor DW. Detection of antigens and antibodies in the urine of humans with *Plasmodium falciparum* malaria. *J Clin Microbiol* 1991; 29 : 1236-42.