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Can *ParaSight-F* be used for rapid diagnosis of Falciparum malaria in Nepal ?

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ABSTRACT

The dipstick, *ParaSight-F*, is a commercial dignostic test for *Plasmodium falciparum*, based on antigen capture. This test was used in a survey in rural endemic areas of Southern Nepal compared to thick blood film examination. In 101 *P. falciparum* thick film positive samples, 94 (93.1%) were positive with *ParaSight-F*. However, in 122 *P. vivax* blood film positive samples, 32 (26.2%) were also positive by *ParaSight-F* test and there was 15.1% false *ParaSight-F* positivity where no parasites could be found by careful examination of thick blood films. While this study confirms that *ParaSight-F* is easy to perform in field conditions, the unpredictable cross-reactivity with *P. vivax* may render it difficult to use in situations where *P. vivax* is the predominant species. However, the possibility of an early diagnosis of Falciparum malaria and the possibility of preventing the development of severe malaria may outweigh this disadvantage.

Keywords: Falciparum malaria; malaria diagnosis; ParaSight-F test; Nepal.

INTRODUCTION

Malaria is still a global problem and improved diagnostic methods are constantly being sought. One of the objectives of the World Health Organization Global Malaria Control Strategy (WHO, 1992) is the development of rapid diagnosis of malaria at the village and district level to enable effective treatment to be administered promptly to reduce morbidity and mortality. The need for rapid diagnosis is particularly acute for Falciparum malaria because of the severe nature of this infection and its non-specific symptoms.

While the examination of thick blood films remains the gold standard, microscopy is not always available. In village-based health-posts and clinics, presumptive diagnosis is made

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and the available drug, chloroquine, is dispensed. As long as chloroquine was 100% effective, this approach was adequate. Since the appearance of chloroquine resistance to *P. falciparum* in most parts of the world, policies have changed to second line antimalarial drugs but, since such compounds are more toxic and costly, a definitive diagnosis at the village level has become more important. Even though a microscope and a competent microscopist are available at the rural health post, there are still technical problems for adequate preparation and reading of slides. There must be a constant supply of Giemsa stain and buffered water, a clean environment for the preparation and reading of slides, a reliable source of electricity to illuminate the microscope and a microscope in good working order. The microscopical diagnosis of malaria is time-consuming and technically demanding, and when results are finally available they may be sent back to the patient too late to be of any real value.

There is, therefore, a need for a simple rapid and accurate diagnostic test. Such a test should be cheap, not requiring microscopy, be simple to perform and to read and the reagents should have a long shelf life; if the test is to be useful for patient management, it should also be quantitative.

A capture ELISA antigen detection test has been developed to detect a *P. falciparum* specific protein, the Histidine Rich Protein-2 (HRP-2) (Parra *et al*., 1991; Taylor & Voller, 1993; Namsiripongpun *et al*., 1993). This assay was found to detect parasitaemia as low as 0.002% (i.e. almost as good as the microscopical examination of a thick blood film performed in average conditions). However, the ELISA test format still requires sophisticated laboratory equipment. The rapid manual dipstick, *ParaSight-F*, test was developed by Shiff *et al*. (1994) and appears to have most of the features required of a field test.

The present study is to evaluate the commercial *ParaSight-F* test against the two prevalent malaria species, in different clinical situations and in varied geographical areas of Southern Nepal with different species prevalence rates.

MATERIALS AND METHODS

Study samples and area

The dipstick antigen-capture assay (*ParaSight-F* test) was performed in six groups of subjects. A total of 377 blood samples from different regions of Southern Nepal and from different patient groups (hospital, health post and village survey) were tested (see Table I). Selectively collected samples, previously checked by microscope, were used in preference to random sampling because of the high cost of the test.

Table I: Origin of samples used in the study.

Group	*Geographical origin*	*Total No.*	*Thick film +ve*
1	Southeast (Beldangi village)	73	34
2	Southern Central (Tulaschichauda village)	126	86
3	Southwest (Kailali and Kanchanpur villages)	70	30
4	Hospital (Janakpur Zonal Hospital)	27	22

5	Dhalkebar Health Post	53	25
6	Chisapani village	28	28

ParaSight-F test method

The test is dipstick chromatography technique, where the HRP-2 antigen present in malaria-infected red cells (and released after lysis of the cells) is captured by a monoclonal antibody against *P. falciparum* HRP-2 and then revealed using a sulpho-rhodamine B-labelled rabbit polyclonal antibody against HRP-2.

The test was performed according to manufacturer's instructions (Beckton Dickinson Co.) and as briefly as follows. The dipstick consists of a preparation of nitrocellulose and glass fibre on which two reagents are applied as a horizontal line. The first line is a mouse monoclonal antibody (mAb) against HRP-2 (applied 1 cm from the base of the dipstick); the HRP-2 antigen is applied as a dotted line about 2-3 mm above the line of mAb, as a control reagent.

Approximately 50 µl of blood is placed on a plastic plate and haemolysed with a drop of detergent. The dipstick is placed in the haemolysed blood, which is rapidly absorbed and starts migrating up the dipstick by chromatography; a drop of developing reagent (a suspension of micelles containing sulpho-rhodamine B coupled with rabbit anti-HRP-2 antibody) is then applied to the base of the dipstick followed by two drops of clearing reagent. In positive cases, there will be a thin red line across the wick with a broken line above it. In negative cases, only the broken control line is seen.

Sensitivity, specificity and accuracy of *ParaSight*-F test

The sensitivity, specificity and accuracy of *ParaSight*-F test for the diagnosis of malaria was computed using the following method, based on Burton & Diana (1992).

Table II: Interpretation of results.

Microscopical examination	*ParaSight-F test*		*Total*
	+ve	*-ve*	
+ve	a	b	a+b
-ve	c	d	c+d
Total	a+c	b+d	a+b+c+d

Sensitivity (percent) = 100% (a/a+c)
Specificity (percent) = 100* (d/b+d);
Accuracy (percent) = 100* (a+d/a+b+c+d)
Positive predictive value = a/(a+b)
Negative predictive value = d/(c+d);
+ve = test positive
-ve = test negative

RESULTS

The overall result for the six groups of samples was as follows:

Out of 225 thick smear positive samples, 128 were positive by *ParaSight-F*. There was a 15.1% false positivity. The overall performance of *ParaSight-F* test compared to microscopy is shown in Table III. The sensitivity, specificity, positive and negative predictive values are shown in Table IV, showing specific differences between the six groups.

Table III: Overall correlation of the *ParaSight-F* test with microscopically positive test for malaria from different groups of study samples.

Microscopical	*ParaSight-F*	*Total*

examination	*+ve*	*-ve*	
+ve *P. falciparum*	94 (93.1%)	7	101
-ve *P. vivax*	32 (26.2%)	90	122
+ve *P.f./P.v.*	2 (100%)	0	2
-ve	23 (15.1%)	129	152
Total	151	226	377

Table IV: Sensitivity, specificity, positive predictive value and negative predictive value of *ParaSight-F* in the 6 groups of samples.

Categories	*Sensitivity*	*Specificity*	*Positive predictive value*	*Negative predictive value*
Group 1	80	71	60.7	73.3
Group 2	86.9	42.5	46.5	85
Group 3	83.3	71.2	50	92.5
Group 4	94.1	40	72.7	80
Group 5	83.3	82.7	80	85.7
Group 6	91.6	50	57.9	88.9
Overall	**85.0**	**57.1**	**67.1**	**84.9**

DISCUSSION AND CONCLUSIONS

New methods for the diagnosis of malaria that complement or replace microscopical examination of stained blood films would be of great use in the diagnosis of uncomplicated and severe malaria, in epidemiological studies and for the follow-up of patients after chemotherapy (Oaks *et al*., 1991). The problem with new assays is that they have to compete with the gold standard of thick blood film which is cheap, very sensitive, highly specific (it detects a parasitaemia of 0.001 to 0.0001%) and quantitative. In contrast, the detection of malaria antigens offers the theoretical possibility of an accurate detection of a current infection, without the need for microscopy or skilled microscopists. *Para-Sight-F* (Schiff *et al*., 1993) is a commercial detection test in dipstick format, in which a monoclonal antibody captures a specific antigen of *P. falciparum* (PfHRP-2, a molecule present in the parasite throughout the erythrocyte cycle); if antigen is present, the positivity of the test is visualized by a second anti-HRP2 antibody labelled with a coloured marker, which produces a visible line on the dipstick. The whole test only takes 10 minutes and gives a sensitivity almost comparable to that of thick films. This simple, robust assay requires no equipment and can be taught to village health workers, since its reading is a straight-forward positive/negative assessment (Premji *et al*., 1994; Uguen *et al*., 1995; WHO, 1996). This suggests that this dipstick antigen-capture assay maybe useful in many situations to diagnose of *P. falciparum* malaria.

As in other published studies, the results described here confirm that this simple test for *P. falciparum* antigenaemia is effective in

identifying infections and the results compare well with the blood film examination in individuals infected with *P. falciparum* (sensitivity 93.1%, specificity 94.9%). This test was readily performed at rural health-posts, making the diagnosis of malaria possible without the need for microscopy. The situation is complicated by a degree of cross reactivity of the test with *P. vivax*, which had previously observed in a study in Brazil (Dietze, 1993) and more recently in India, Thailand and Venezuela (WHO, 1996). In the present study, this cross-reactivity resulted in an overall sensitivity of 85% but with a specificity of only 57.1% and a positive predictive value of 84.9%. The false positive reactions were mostly observed in individuals with high antibody levels as measured by IFAT or ELISA (data not shown) and is more likely explained by the persistence of antigenaemia after treatment than by a higher sensitivity of *ParaSight*-F compared to microscopy; other studies have indicated that antigenaemia may persist for up to 3 weeks after clearance of parasites.

In Nepal, *P. vivax* is the predominant species in most of the malarious areas by a factor of 10:1 and a test which mainly targets *P. falciparum* but has a degree of cross-reactivity with *P. vivax*, will of necessity appear to have an inadequate degree of specificity. However, *ParaSight-F* test is a very promising test, which in certain circumstances would be helpful for ruling out the most dangerous malaria species at the first patient encounter and prevent the development of severe Falciparum malaria; in such a situation even a 50% false positivity would be acceptable and would ensure that a majority of cases of *P. falciparum* are given appropriate treatment.

Since this study was performed, two new antigen detection tests have been developed: OptiMal which detects the parasite lactate dehydrogenase (Makler *et al*., 1998) and a version of the test for HRP-2 adapted to recognise *P. vivax* HRP-2; both tests are not able only to recognise malaria regardless of the species involved but also to differentiate between infections with *P. falciparum* and other species. Such tests would presumably be more adapted for malaria diagnosis in a country like Nepal, where *P. vivax* is the predominant species, but have not so far been available for field testing.

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