

## THE PARASIGHT™ - F TEST; USE IN MALARIA EPIDEMIC IN NEPAL

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### ABSTRACT

A field based trial to compare microscopy and a rapid diagnostic antigen capture detection dipstick (ParaSight™-F) to diagnose malaria was conducted in 2 village development committees (VDCs) of Nepal during epidemic (1996/97). 57 and 273 subjects were tested from Parasan and Guthiparsauni VDC respectively yielding 15 (26.3%) and 152 (55.7%) positive by ParaSight™ -F who were given radical treatment on the spot. Compared to thick blood film (TBF), the ParaSight F (PSF) test was 93.8% and 94.4% sensitivity respectively in Parasan and Guthiparsauni for the detection of *P. falciparum* malaria. There were 10 false negative results with the PSF test from the two villages; 6 samples with <100 parasites / ?l, 1 sample with 240 parasite/? l and 3 samples having only gametocytes. In this population, the PSF was found to be highly accurate simple and rapid suggesting that it is suitable to be used in remote areas and/or epidemic situation for prompt treatment. However microscopy is good to prevent transmission of the parasites from gametocyte carriers.

**Key Words:** *Malaria, diagnosis, ParaSight™ - F, An. fluviatilis, epidemic, Nepal.*

### INTRODUCTION

Malaria remains a major public health problem in most countries of Southeast Asia. Many areas are covered by deep forests that serve as site of transmission of *Plasmodium falciparum* (Pf). Epidemics are recurring in areas where transmission had been interrupted and are generally associated with deteriorating social and economic condition.<sup>1</sup> In Nepal, in the years 1978 and 1998 there were 14,212 and 8,498 cases of malaria respectively, having slide positivity rate as 0.9 and 4.88 in respective years.<sup>2</sup> This shows the hidden cases, which cause epidemic almost every year. The detection of malaria parasites in thick blood film has been the reference standard for decades. But at village based health posts (HP) even if microscope and microscopists are available there are technical problems for adequate preparation and reading of slides. In these places in all cases presumptive diagnosis is made and chloroquine is administered. But in Thailand usually

sulfadoxine- pyrimethamine is given as presumptive treatment.<sup>3</sup> In Nepal 1<sup>st</sup> line drug of choice for pf malaria is SP. *An. fluviatilis* (anthropophilic index 30-60%) is one of the important vectors of malaria in Nepal.<sup>2</sup> Thus there is acute need for rapid diagnosis of Pf cases because of the fatal nature of this infection and as often it is not easily distinguishable from other diseases.<sup>4,5</sup> The test should be simple, which can be stored, performed and read easily in malaria endemic and non-endemic areas and decrease the use of microscope. A rapid manual test incorporating a dual antibody immunoassay against the histidine rich protein - II antigen of *P. falciparum* (HRP-II) has been developed. Evaluation of HRP-II dipstick (ParaSight™ -F; Becton Dickinson, Sparks, MD) for rapid detection of *P. falciparum* has shown good sensitivity and specificity in several countries.<sup>3,6-14</sup> Present study was carried out during out breaks of malaria in two VDCs of Nepal with the objective to evaluate the efficacy of ParaSight™ -F (PSF) as a diagnostic test for Pf. The necessity was more emphasized

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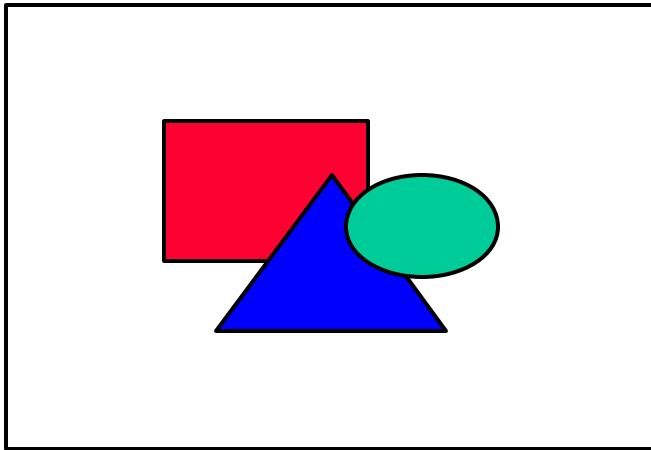
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by the fact that during 1997-98 out of 159,903 blood slides collected only 131,457 (82.2%) slides were examined. Rest of the slides had not been examined due to lack of laboratory facilities, manpower shortage and the collected slides could not be transported to the examination facilities in time.<sup>15</sup>

## MATERIALS AND METHODS

### Study area

Parasan village development committee (VDC) (Fig-1) of Kanchanpur district of far- western region of Nepal is the one of the VDCs where the test was conducted. Basic health services are provided to this VDC by Parasan health post. In 1996 Khadda and Dhansinha village of Parasan VDC had experienced an epidemic during which the test was performed.



Kathahawa village of Guthiparsauni VDC (Fig-1) in Nawalparasi district of western region of Nepal is the another village, where the test was performed. Guthiparsauni VDC is under the catchment area of Pratappur Ilaka health post, which provides health services to a population of 49,233. Kathahawa village is situated at about 40 Km. south east of Parasi (Head quarter of Nawalparasi district). In south of the village at 5 Km is the Indian border town of Jhumbibazar. This village is divided into two pockets Musahar tola and Kathahwa tola with a population of 116 and 457 respectively. These tolas suffered malaria epidemic in 1997.<sup>16</sup> During that period this test was performed. The main occupation of both the villagers is agriculture, wood-cutting and wage labor in road construction work etc. Malaria transmission is perennial with seasonal peaks.

### Histidine rich protein II

ParaSight™-F (PSF) test was used according to manufacturers instruction for detection of histidine rich protein -II (HRP-II). Principles and methods of this test were described by Shiff and others.<sup>3,6</sup> Besides the test materials provided by the manufacturer in the kit, only blood lancets, cotton wool, alcohol swabs and slides for thick blood film (TBF) were needed.

### Sample collection

ParaSight™-F (PSF) test kits and basic materials were provided to the health workers (health post in-charge and laboratory technicians) after training of 2-3 hours (including demonstration and practice). After getting informed consent, a simple form having some information i.e. age, sex and any antimalarial drugs taken within a week, was filled in each case. Blood was obtained from persons having fever and not taken antimalarials for a week, by finger prick for the dipstick PSF assay and thick blood film (TBF). TBF were prepared in order to use as a gold standard to determine reliability of the test system and also to test for possible Pv infection as the PSF having the capacity of detecting only Pf. The blood films were coded and sent to vector borne disease research and training center (VBD RTC), Hetauda, Nepal, where the blood films were stained with 10% Giemsa and examined by senior laboratory technicians under microscope (X1000). All the slides were re-examined by either 1<sup>st</sup> or 2<sup>nd</sup> examiner to correct a false negative result, mixed infections or misidentification of the species. Parasite density in TBF were determined by counting the trophozoites and schizonts in 100 oil immersion fields and multiplying by four, assuming the blood volume of 0.25  $\mu$ l per 100 fields. Under normal circumstances microscopists examine 100 oil immersion fields of TBF. For this study, we had examined 200 fields to declare a slide negative.

### Treatment

Patient, positive by PSF were administered standard Sulfadoxine, Pyrimethamine and Primaquine treatment on the spot. Additional patients, diagnosed after examination of TBF were treated on subsequent dates.

### Analysis

Infection with Pf and mixed infections (Pf + Pv) were included in the analysis, *P. vivax* were not included in the analysis because the test is not able to detect antigen of *P. vivax*.

## RESULTS

A total of 57 and 273 persons had been included in this study from village Parasan and Guthiparsauni respectively and the ratio of the male and female patient was 1:1.4 and 1.3:1 in respective villages. In Parasan mean age of males was 29.6 years (ranged from 5-70 years) while mean age of females was 22.8 years (ranged from 3 to 60 years). But in Guthiparsauni males ranged from 7 months to 80 years (mean age 21.9 years) and females ranged from 1 to 65 years (mean age 23.4 years). Among the patients examined 26.3 % and 55.7 % were positive for Pf, HRP II antigen by PSF but 28% and 61.9% were positive by the examination of TBF respectively from Parasan and Guthiparsauni villages (Table I).

**Table I : Malaria diagnosed by ParaSight™ - F and thick blood film**

Tests	Total examined	<i>P.falciparum</i>	Mixed (Pf+Pv)	<i>P.vivax</i>	Total positive
<b>Village Parasan</b>					
a. ParaSight™-F	57	15	0	0	15
b. TBF	57	16	0	1	17
<b>Village Guthiparsauni</b>					
a. ParaSight™-F	273	152	0	0	152
b. TBF	273	159	2	24	185

**Table II : Efficacy of ParaSight™ - F test excluding all *P.vivax* infection**

A. Village Parasan				B. Village Guthiparsauni			
<i>P.falciparum</i> by microscopy	Diagnosis by ParaSight™-F		Total	<i>P. falciparum</i> by microscopy	Diagnostic by ParaSight™-F		Total
+	15	1	16	+	152	9	161
-	0	40	40	-	0	88	88
<b>Total</b>	<b>15</b>	<b>41</b>	<b>56</b>	<b>Total</b>	<b>152</b>	<b>97</b>	<b>249</b>
Sensitivity = 15/16 = 93.75% PPV = 15/15 = 100%				Sensitivity = 152/161 = 94.4% PPV = 152/152 = 100%			
Specificity = 40/40 = 100% NPV = 40/41 = 97.56%				Specificity = 88/88 = 100% NPV = 88/97 = 90.72%			

**Table III : Stage and number of *P.falciparum* diagnosed by thick blood film and number missed by ParaSight™ - F**

A. Village Parasan				B. Village Guthiparsauni			
Pf stages	No. TBF	No. missed by PSF	% missed	Pf stages	No. TBF	No. missed by PSF	% missed
Trophozoites	11	0	0	Trophozoites	89	2	2.24
Trop. +Game.	4	0	0	Trop.+Game.	62	5	8.06
Gametocyte	1	1	100	Gametocyte	8	2	25.0
Mixed	0	0	0	Mixed	2	0	0

**Table IV : Performance of ParaSight™ - F test at various levels of *P.falciparum* asexual parasitaemia in village Guthiparsauni**

Parasites /?l	No. +ve by microscopy <sup>a</sup>	No +ve by ParaSight™F	Sensitivity %
<48	6	2	33
52-100	9	7	78
104-240	20	19	95
244-1000	26	26	100
1004-10000	38	38	100
>10004	54	54	100
<b>Total</b>	<b>153</b>	<b>146</b>	

*a = Patients having only gametocytes are excluded (n 161-8=153)*

Of the 24 Pv cases positive by TBF none was positive by PSF. As PSF has capacity to detect Pf only and there was no case reactivity of Pv antigen with PSF in our study, we have excluded the *P. vivax* infections from data before calculating the different indicators of the test. In the village Parasan, Table II shows that, sensitivity was 93.8%, specificity was 100 % positive predictive value (PPV) was 100 % and negative predictive value (NPV) was 97.6%. When PSF was tested for bigger sample size in village Guthiparsauni sensitivity slightly increased to 94.4%, specificity and PPV remained 100% but NPV dropped to 90.7%. Table III shows the percentage of different stages of Pf missed by PSF. In Parasan PSF was 100% sensitive for the stages of Pf other than gametocyte stage. In

that village there was a patient having gametocytes only, which was not detected by PSF. But in Guthiparsauni it was found that the PSF was unable to diagnose 2% of the 89 blood with trophozoites, 8% of the 62 blood with trophozoites & gametocytes and 25% of the 8 blood samples having only Pf gametocytes.

The distribution of positive PSF test results and the sensitivities stratified by the parasite density has been demonstrated in table 4. The results shows that when the Pf, asexual, parasitaemia was greater than 240 parasites /?l, the PSF test was 100% sensitive but when there was <40 parasites /?l the PSF detected only 33% of the infections.

## DISCUSSION

ParaSight™ -F (PSF) was evaluated for the first time in an epidemic situation. Initially we tested it in smaller population (Parasan) followed by in bigger population (Guthiparsauni).

PSF test has been evaluated in various settings.<sup>3,6-14</sup> A comparison of sensitivity, specificity PPV and NPV recorded by several authors have been presented by Stephens.<sup>13</sup> Our results indicated that in both villages the PSF was sensitive (94%) and specific (100%) for the diagnosis of Pf malaria in epidemic situation. Sensitivity improved to 95% if the patients harboring only gametocytes were excluded from calculation.

A continuing area of concern with this antigen detection assay remains the occurrence of occasional false negative results (table -4). In this study false negative results were most common in the samples with low parasitaemia (<100 parasites /?l). However one higher parasitaemia (<240 parasites /?l) yielded negative result. False negative PSF test results at higher parasitaemia was reported by others.<sup>9,11</sup> Detection of HRP-2 by PSF depends not only on the number of parasites but also on the length of infection.<sup>17</sup> Other underlying-reasons are unknown. When clinical suspicion of malaria is high and initial PSF results is negative, repeating PSF assays on subsequent days may improve detection.<sup>9</sup> Only one case undetected by PSF test but microscopically positive in village Parasan had gametocytes only. In Guthiparsauni, 9 samples were not detected by PSF test out of them 2 samples had gametocytes only and others had a few trophozoites. Mature circulating gametocytes do not produce HRP-II, which tend to remain in the blood weeks to months after clearing the trophozoites. A very low trophozoite number may not provide sufficient levels of antigen to be detected by the test.

There was no cross-reaction of this test with any of the 24 *P. vivax*.in our study But, PSF have been reported giving false positive reactions (FPR) with individuals without malaria or with *P. vivax* infections.<sup>8,9,11,13</sup> FPR is also reported in rheumatoid factors cited by Bartoloni.<sup>18</sup> False positive interpretations may lead to delayed diagnosis and inappropriate treatment of other causes of fever such as rheumatoid factors. However all the cases in this study that were demonstrated as having sufficient levels of antigen proved to have parasitaemias that would have required prompt treatment to prevent unnecessary suffering.

PSF test based on the use of HPR-II is a valuable adjunct to and replacement for conventional methods for the diagnosis of malaria as mentioned by Perrone.<sup>19</sup> In this study only 9 (1+8) individuals showed the presence of only gametocytes by TBF out of that 6 (66.7) were positive by PSF but it was reported

to detect only 47% of the samples with gametocytes in previous study.<sup>8</sup> We know that the gametocyte of Pf is the stage, which helps transmission of the disease by vector and may cause epidemic. So, treatment of such patients is of immense importance to control the disease transmission. Thus PSF must be supplemented with TBF so that cases missed by PSF may be detected and treated to make the community free of reservoirs.

In fact patients observed the rapid diagnosis without a microscope and received the radical treatment instead of waiting was greatly appreciated by health staff and the patients both. PSF is almost 5 times expensive than TBF.

## CONCLUSION

Microscopic detection of malaria parasite in thick blood film has been the reference standard for malaria diagnosis; however accurate diagnosis by TBF in the field is time consuming and impossible to be performed in remote areas. ParaSight™-F is the simple available tool for rapid and reasonably accurate means of diagnosis of *P. falciparum* malaria ( as compared to thick blood smear) at the village or HP level in epidemic situation. PSF test can be performed on several samples simultaneously, which reduces the time needed for diagnosis. Thus it helps in reduction of mortality and suffering by making it possible to treat a patient radically on the spot, especially, at the time of epidemics. But TBF should supplement it to make it possible to treat miss-diagnosed patients of PV and undiagnosed patients of Pf, especially having gametocyte stage who might help vectors to cause another episode of epidemic.

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