

THE RAPID MANUAL *ParaSight*TM-F TEST FOR DIAGNOSING *PLASMODIUM FALCIPARUM* MALARIA IN SAUDI ARABIA

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Currently, about 1.6 million people live in areas of Saudi Arabia where malaria is transmitted. In the southwestern region of the Asir lowlands (Tihama), *Plasmodium falciparum* is the predominant species, accounting for over 90% of all malaria infections.¹ The peak of malaria transmission in the Asir lowlands is between October and April, and coincides with the rainy season (550 mm/year).

In the malarious areas of Asir, two categories of population are at risk of contracting the infection: 1) permanent residents of the foothills and lowlands of Tihama and the coastal plain along the Red Sea; and 2) residents of the non-malarious areas in the highlands (Sarawat), who frequently travel for recreational activities to the endemic lowland areas during the transmission season. The latter group is especially prone to severe infection and a high degree of parasitemia, as they have little or no resistance to malaria.

In the rural areas of Saudi Arabia, management of human malaria at the primary health care level is based on clinical diagnosis of febrile patients before institution of chloroquine therapy. The absence of specific diagnostic criteria and supportive laboratory confirmation of parasitemia mostly leads to inaccurate diagnosis and overtreatment. In regional referral hospitals, where appropriate facilities for better diagnosis and light microscopy are present, examination of blood slides for malaria is the cornerstone for early diagnosis and prompt treatment. However, the sensitivity of the standard thick-blood examination and microscopy has many limitations.²

In recent years, the antigen detection *ParaSight*TM-F rapid dipstick capture assay for the diagnosis of *Falciparum malaria* (Beckton Dickson Company, USA) has been extensively evaluated in endemic and non-endemic areas of the world.³ The method detects parasites, with limits of detection equal to or better than those provided by light microscopy, with matching specificity and sensitivity of around 90%.^{3,4}

In the present study, we conducted a laboratory evaluation of the *ParaSight*TM-F test versus the standard thick-blood film examination for malaria diagnosis. The comparison was made between two study populations in the Asir region during periods of high malaria transmission.

Patients and Methods

The *ParaSight*TM-F test was evaluated in a total of 89 subjects during the malaria transmission seasons of 1996-1998. The study population comprised two groups: 1) patients with febrile or other commonly associated malaria symptoms (n=38) who presented to an outpatient clinic at a primary health care center (PHCC) in Maraba, in lowland Tihamat Asir, located 50 km south of Abha, and 2) malaria inpatients admitted to Asir Central Hospital (ACH) in Abha (n=51) and suffering from acute uncomplicated or severe malaria. The study was approved by the College Research Center ethics committee. The demographic characteristics of the populations under study are summarized in Table 1.

From each patient, 2 mL of venous blood was withdrawn in heparinized tubes and transported on ice to the Parasitology Laboratory of the Abha College of Medicine within 4 hours of collection. In the laboratory, two thick and one thin blood films were prepared from each sample. The microscopical examination of the standard Giemsa-stained blood films was performed by a microscopist experienced in malaria diagnosis and checked by a consultant. Smears were examined with 100x magnification under oil-immersion objectives. Parasites were counted against 1000 white blood cells (WBCs) or until a count of 500 asexual parasites (trophozoites) had been reached. The parasite density was estimated assuming 8000 WBC/ μ L of blood.⁵ Parasite species' determination was made by examination of both thick and thin blood smears.

The *ParaSight*TM-F dipstick assay was performed on the day of collection according to the manufacturer's instructions,⁶ although testing was extended for several hours after blood collection for some specimens. Four levels of reactivity were assessed, based on the intensity of the color reaction, as follows: no reaction (0); faint (1);

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TABLE 1. Demographic characteristics of the patient population at the PHCC, Maraba, and at the ACH, Abha, Saudi Arabia.

	PHCC	ACH	Total
Age in years*	32.3±16.0 (7-80)	30.0±16.7 (3-66)	31.0±16.3 (3-80)
M:F ratio	3:1	5:1	3.9:1
Number of subjects	38	51	89
Nationality (Saudi/non-Saudi)**	35/3	46/5	81/8
<i>P. falciparum</i> †	12 (31.6%)	46 (90.2%)	58 (65.2%)

PHCC=Primary Health Care Centre; ACH=Asir Central Hospital; *mean±SD (range in parentheses); **four Yemeni, two Egyptians, one Palestinian, one Indian; †as examined by laboratory thick and thin blood films.

clear (2); and strong (3). The results were read by two investigators. The presence of microscopically detectable parasitemia using thick blood film was taken as the reference standard to determine sensitivity and specificity of the *ParaSight*TM-F test.

The current treatment of uncomplicated *Falciparum malaria* at PHCC is based on presumptive diagnosis and utilizes standard chloroquine regimen as the first-line drug. In ACH, treatment of malarial patients started immediately after positive diagnosis. Acute uncomplicated *P. falciparum* infections were treated with the standard chloroquine regimen (10 mg/kg start, 5 mg/kg after 6 hours, and then 5 mg/kg daily for 2 days),⁵ or a single dose of Fansidar (sulfadoxine + pyrimethamine) for patients who did not respond to chloroquine. Oral quinine (10 mg/kg every 8 hours for 7 days), or parenteral quinine at the same dose, was used in cases of complicated disease, including cerebral malaria.

Data were analyzed using SPSS software (Norusis NJ/SPSS Inc. 1993). Correlation between the intensity of the color reaction of the *ParaSight*TM-F test grade and the level of parasitemia was estimated by applying the Spearman rank correlation coefficient and the significance was considered at 0.05 level. Mann-Whitney rank sum test was used for comparing the mean parasitemia between the two populations studied. Also, the level of agreement between the *ParaSight*TM-F test and thick blood-film examination results was determined by the calculation of the kappa coefficient.

Results

The results of the field study (PHCC) and hospital inpatient (ACH) groups are compared in Table 2. Of the 89 subjects whose blood was examined, 58 (65.2%) were infected with *P. falciparum* and two (2.3%) with *P. vivax*, as revealed by microscopy (Table 1). No mixed infections were recorded. Table 2 shows the number of specimens that were positive by both blood-film examination and *ParaSight*TM-F test. The mean admission parasite density for patients in ACH was significantly higher (75,193±109,037.8) than that of those presenting with fever at the

outpatient clinic of the PHCC (7476.3±13,288) (95% CI for the difference between means 34,512:100,922, with $P=0.0004$). The intensity of the *ParaSight*TM-F test readings was significantly and positively correlated with peripheral blood parasitemia. (Spearman rank correlation coefficient between the grade of the test strip and level of parasitemia is equal to 0.90, $P<0.0001$). At ACH, two *ParaSight*TM-F test false-negative results were due to samples containing *P. vivax* and were excluded from the analysis (Table 2), because the test does not detect antigen of this species.

In the two studies, 55 of the specimens (69.6%) had matching *ParaSight*TM-F test and blood-film results. In the field study, the thick blood film and *ParaSight*TM-F test were discrepant in three cases, with the latter giving three false-negative results. All three false-negatives had relatively low parasite counts of 16-48 parasites per μL (Table 2). With higher levels of parasitemia ($>50/\mu\text{L}$), both the thick blood-film examination and the *ParaSight*TM-F methods were 100% sensitive (Tables 2 and 3). Table 3 shows the distribution of positive thick blood film and *ParaSight*TM-F test results, and the corresponding sensitivities stratified according to parasite level when results from both studies were combined. The sensitivity of the *ParaSight*TM-F test was greater with increasing peripheral parasitemia, giving a sensitivity of 95%-100%, a specificity of 100% and positive and negative predictive values of 100% and 91%, respectively. The kappa index of agreement was 92% between the *ParaSight*TM-F test and the conventional thick blood-film examination.

Of the 38 patients enrolled in the field study (PHCC), all presenting with fever, only 12 (31.6%) had positive blood films, and the *ParaSight*TM-F tests were positive in 9 cases (23.7%), showing a low correlation between the presence of fever and positive diagnosis. The 26 patients who were finally diagnosed as negative by both the thick blood film examination of 1000 WBCs and *ParaSight*TM-F test were unnecessarily treated with chloroquine by the clinic's staff.

Discussion

The *ParaSight*TM-F test is based on the detection of the trophozoite-specific histidine-rich protein II antigen, and has been shown to give a constant specificity and sensitivity of around 90%, compared to the standard thick blood-film examination.³ In our study, we found the threshold parasitemia for detection by the *ParaSight*TM-F test to be 16-48 asexual parasites per μL blood. In previous reports, false-negative results appeared to be associated with parasitemia below 60 parasites per μL .^{3,7,8} In this report, the 95% sensitivity of the *ParaSight*TM-F test was due to three false-negative results observed in patients with parasitemia below 50 parasites per μL , whereas the endpoint sensitivity reached 100% when parasitemia

TABLE 2. Comparison of the ParaSight™-F test with thick blood film examination in the outpatient clinics of the PHCC and ACH.

	ParaSight™-F test grade	# of subjects	# Positive	Blood film examination parasitemia/μL*
PHCC	0	29	3	26±18 (16-48)
	1	3	3	1011±814 (224-1850)
	2	2	2	968±803 (400-1536)
	3	4	4	21,166±16,472 (8791-45,454)
ACH	0	3	0	0
	1	11	11	8949±16,270 (320-55,553)
	2	19	19	37,634±44,683 (800-16,666)
	3	16	16	165,338±139,364 (1280-444,444)

* Mean±SD (range in parentheses).

TABLE 3. Performance of ParaSight™-F test at various levels of *P. falciparum* parasitemia in all subjects tested.

Parasite density/μL blood	Standard thick blood film examination +/-	ParaSight™-F test +/-	Total	Sensitivity (%)
≤50	3/0	0/3	3	95
>50	55/0	55/0	55	100
Negative samples	0/26	0/29	29	
Total	58/26	55/32	87	95

exceeded that level. It has been suggested that malaria cases with relatively low parasite counts may be early infections which may not have produced sufficient antigenemia to be detected by the ParaSight™-F test.^{8,9} The absence of false positives among the study population may merely reflect the fact that patients were clinically symptomatic on admission and had viable parasites in their peripheral blood before treatment. In contrast to previous reports,^{4,10} however, there was a positive correlation between the test strip readings and parasitemia levels ($P<0.0001$).

Parasite density is usually high in non-immune subjects, whereas those living in endemic areas who have acquired high levels of immunity usually have a low-density parasitemia.² The significantly high difference in the degree of parasitemia ($P=0.0004$) between patients of the lowlands (semi-immune) and those in the highlands (non-immune) is probably related to differences in the immune status of the two populations.

In the field study group, only about one-third (31.6%) of persons presumptively diagnosed with malaria as they were admitted to the PHCC and sequentially treated with chloroquine were found to have positive results. The ParaSight™-F test could be of particular use in such situations where, in the absence of microscopical diagnosis, better clinical management of malarial patients can be made, reducing unwanted drug pressure. This is particularly important in Saudi Arabia, where evidence of diminishing sensitivity to chloroquine and clinical resistance to the drug in indigenously transmitted *Falciparum malaria* has been on the rise in recent years.¹¹⁻¹⁴ Apart from autochthonous malaria, the

ParaSight™-F test could be used in the rapid screening of imported cases of multidrug-resistant *P. falciparum* malaria among frequent travelers and highly mobile expatriate workers returning from malarious countries with known resistance.¹⁵

Based on present and previous published reports, the ParaSight™-F test and the standard thick blood-film examination were equal as diagnostic tools for *Falciparum malaria*. The test is simple, quick and can give accurate diagnosis of patients with low as well as high parasitemia under laboratory and field conditions. It is highly recommended to be introduced at the primary health care level, especially in remote endemic areas of the Kingdom, where early diagnosis and treatment of *Falciparum malaria* is needed, and referral to the next level of health facility is not feasible.

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