Comparative performance of the ParaSight® F test for detection of *Plasmodium falciparum* in malaria-immune and nonimmune populations in Irian Jaya, Indonesia

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A comparison was made of the performance of the ParaSight® F test (F test) for detection of Plasmodium falciparum *in blood from malaria-immune (410 native Irianese) and nonimmune (369 new transmigrants) populations in Irian Jaya, Indonesia, where malaria is hyperendemic and all four species of human malaria occur. There were highly significant differences between populations in the sensitivity (Irianese, 60% versus transmigrants, 84%; P < 0.001) and specificity (Irianese, 97% versus transmigrants, 84%; P < 0.001) of the F test. The test had comparably high levels of sensitivity for Irianese children aged ≤10 years, both age groups of transmigrants (76–85%), but low sensitivity for Irianese aged >10 years (40%), among whom only 7% of parasitaemias <120 per μl and 69% of those >120 per μl were detected. Specificity was comparably high for transmigrant children aged ≤10 years and both age groups of Irianese (93–98%). The low specificity for transmigrants aged >10 years (79%) was due to a preponderance of false positives, frequently identified by microscopy as* P. vivax. *The results suggest that comparison based on microscopy underestimated the performance of the ParaSight® F test and that malaria immune status, irrespective of* P. falciparum *density, may influence the test's sensitivity.*

Introduction

The ParaSight® F test (F test) (Becton-Dickinson Tropical Disease Diagnostics, Sparks, MD, USA) is a novel method for the rapid detection of *Plasmodium falciparum* in blood, using a nonmicroscopic "dipstick" approach. The basis of the F test is the detection of histidine-rich protein II (HRP-II), a species-specific glycoprotein released from infected erythrocytes (*1*, *2*). The test is straightforward to perform, sensitive, and accurate. Also, few skills beyond reading comprehension are required to perform the test and detect *P. falciparum* at a level comparable to that obtained by expert microscopy.

Clinical trials of the F test in a number of malaria-endemic countries have provided fairly consistent results of its performance. At parasitaemias >100 asexual stage parasites per μl of blood, sensitivities >96% were obtained, and the overall sensitivity for parasitaemias of 10–100 asexual stages per μl was 81.3%. The specificity of the test ranges from 81.1% to 99.5%. The lowest levels of sensitivity and specificity are associated with populations living in areas of holoendemic falciparum malaria. Data supporting claims of low cross-reactivity with other species of human malaria are, however, limited (*3*).

Less clear are the parameters of the F test's performance when it is used as a survey tool to gauge the prevalence of malaria among children, whose immunity is less than that of adults, and among malaria-naive adults experiencing their first malaria infection; in both these cases, symptoms of malaria may develop at lower parasitaemia levels than in their respective adult and immune counterparts. To measure the comparative performance of the F test for such groups, it is important to control for variability in the quality of microscopy (the current gold standard), particularly at low parasitaemias, and to eliminate bias that may be created by knowledge of the symptoms associated with the test samples.

This article compares the practical limits of the sensitivity of the F test for detection of *P. falciparum* infections among malaria-immune and nonimmune populations of adults and children. The study was conducted in Irian Jaya, Indonesia, where all four species of human malaria occur, thereby permitting assessment also of the test's specificity and cross-reactivity. Although microscopic evaluation of

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Giemsa-stained thick smears was the gold standard employed for comparison, the polymerase chain reaction (PCR) was also used to amplify *P. falciparum* DNA in samples that yielded discordant results. Appropriate measures were taken to control for technician/microscopist variability and to prevent bias, by testing in a blinded fashion, without knowledge of subject's health status.

Materials and methods

Study sites and subjects

The study was conducted during March, September, and October 1995 in four locations in eastern Irian Jaya, Indonesia. Two of these locations, Arso PIR V and Armopa SP-I, were farming settlements established less than 1 year previously, and yielded a sample of 369 transmigrants, composed primarily of young Javanese and Balinese families that had recently immigrated into the area. Because the incidence of malaria in Java and Bali has been approximately 1 case per 10000 person–years since 1965 (*4*), and the transmigrants in Arso PIR V and Armopa SP-I had resided there for less than 6 months, the population in these locations was considered to be "malaria nonimmune". The two other study locations, Oksibil, in the eastern highlands, and Tarontha, near SP-I, are long-established Irianese villages, which yielded a population of 410 native residents. Because falciparum and vivax malaria were hyperendemic at both of these locations, and there was little or no use of conventional antimalarials, the Irianese population was considered relatively "malaria immune". Enrolment was open to all persons regardless of age, sex, and health status.

Malaria diagnosis

Several drops of blood were taken by fingerprick from each person for preparation of thick/thin blood films and dipstick testing. Giemsa-stained blood films were examined using standard microscopy techniques (oil immersion, ×1000 magnification). A set standard of 200 ocular fields per slide were screened by an expert microscopist and a count was made of all sexual and asexual malaria parasites per 200 white blood cells (WBC). The parasite count was multiplied by 40 to estimate the number of parasites per μl of blood (*5*). Cases that were true positives within the range of 200 ocular fields, but which were too lightly infected to be quantified against 200 WBC, were assigned an arbitrary value of 20 parasites per μl (0.5 parasites per 200 WBC) to permit their inclusion in the statistical analyses. Dipstick testing was conducted in strict accordance with the manufacturer's instructions using 50 μl of blood. Slide reading and dipstick testing were conducted by independent technicians blinded to the clinical status of subjects and the results were kept confidential. To eliminate variability, one microscopist carried out all slide examinations and one technician conducted and interpreted all F tests. Treatment, based on clinical symptoms and microscopy results, was delivered by provincial health care providers. Lysates remaining after dipstick testing were fixed in 8 mol/l guanidine hydrochloride for later PCR-based amplification of parasite DNA. Samples with discordant microscopy/dipstick test results were processed by the filter-paper method (*6*) and amplified using published primer sets for *P. falciparum* (PF-1, PF-2) and *P. vivax* (PV-1, PV-2). The amplified product was viewed following ethidium bromide staining of samples separated in 2% agarose and defined according to its distinctive relative molecular mass and PCR amplification of a known positive control performed in parallel with each assay. The limit of PCR-based detection using these primer pairs was reportedly 1 parasite per μl of blood (*7*).

Data analysis

The results of the microscopy and F testing for each subject were merged in dBase file format and analysed using Epi Info software. Parasite counts per μl were log-transformed to calculate geometric means and these were compared using a one way analysis of variance (ANOVA) or Kruskal–Wallis nonparametric test. Selected groups were compared by appropriate paired or unpaired Student's *t*-test. Proportions were compared using χ^2 tests with Yates' correction or Fisher's exact test. The Irianese and transmigrant populations were arbitrarily subdivided for analysis by age into two groups (those aged ≤10 years and those aged >10 years) to permit comparison of the prevalence of malaria in children and adults and its possible interaction with F test results.

Results

Malaria prevalence

Malaria prevalences, based on microscopy and tabulated by population, age group, and malaria species provided evidence of the relative susceptibility and immunity in the study populations (Table 1). For the transmigrant population there was no significant difference between the younger and older age groups in the prevalence of infection with either *P. falciparum*

Table 1: **Microscopy-based malaria prevalence by population, age group, and parasite species**

	No. of native Irianese					No. of transmigrants		
Age	*n*	*P. falciparum*	*P. vivax*	*P. malariae*	*P. ovale*	*n*	*P. falciparum*	*P. vivax*
≤10 years	157	38 (24.2)[a]	37 (23.6)	4	0	102	48 (47.1)	34 (33.3)
>10 years	253	30 (11.9)	12 (4.7)	3	1	267	131 (49.1)	81 (30.3)
Total	410	68 (16.6)	49 (11.9)	7	1	369	179 (48.5)	115 (31.2)

[a] Figures in parentheses are percentages.

($P = 0.82$) or *P. vivax* ($P = 0.67$), but for the Irianese population, the lower age group had a significantly higher prevalence of patent infection with *P. falciparum* ($P = 0.002$) and *P. vivax* ($P < 0.001$). The prevalence of patent infection with *P. falciparum* was significantly higher in both age groups of transmigrants ($P < 0.001$) than in their respective Irianese cohorts.

Sensitivity and specificity

Tables 2 and 3 compare the sensitivity and specificity of the F test in the transmigrant and Irianese populations. There were highly significant ($P < 0.001$) differences between the two populations in terms of the proportions of true positive cases detected (sensitivity) and in the proportions of true negatives distinguished (specificity). The sensitivity was low for the Irianese, among whom 41 of 68 *P. falciparum* infections were detected, but the specificity was higher in this group because of a significantly lower proportion of false-positive results (Irianese, 3% versus transmigrants, 17%; $P < 0.001$). There was no difference ($P = 0.66$) between populations in terms of the geometric mean parasite count per μl of false-negative F tests (Irianese, 126, 95% confidence interval (CI): 86–185 versus transmigrants, 110, 95% CI: 67–182). However, for transmigrants there were 32 false-positive tests (14 identified by microscopy as *P. vivax* infections) and the geometric mean count per μl of parasites in these false-positives was significantly greater than that of the 66 other *P. vivax* infections that did not elicit false-positive reactions (1406 versus 480, $P = 0.03$). All dipstick test strips produced clearly reactive control lines.

Because of the unusually low sensitivity obtained for the F test in the Irianese sample a second, blinded reading of the slides was carried out by a different microscopist. The results indicated that there was >89% agreement between microscopists for 300 Irianese slides. No significant differences were obtained for either sensitivity (39% versus 31%, $P = 0.61$) or specificity (98% versus 96%, $P =$

Table 2: **Sensitivity, specificity, and predictive values for the ParaSight® F test for detection of *Plasmodium falciparum* in native Irianese with lifelong malaria exposure and nonimmune Indonesian transmigrants**

	Sensitivity (%)	Specificity (%)	PPV (%)[a]	NPV (%)[b]
Native Irianese:				
≤10 years	76.3	95.8	85.3	92.7
>10 years	40.0	97.8	70.6	92.4
Combined	60.3	97.1	80.4	92.5
Transmigrants:				
≤10 years	81.2	94.4	92.8	85.0
>10 years	84.7	79.4	79.8	84.4
Combined	83.8	83.7	82.9	84.5

[a] PPV = positive predictive value.
[b] NPV = negative predictive value.

Table 3: **Detection of *Plasmodium falciparum* in native Irianese with lifelong malaria exposure and non-immune Indonesian transmigrants: malaria microscopy versus the ParaSight® F test**

	Microscopy		
ParaSight® F test:	No. positive	No. negative	Total
Native Irianese:			
No. positive	41	10	51
No. negative	27	332	359
Total	68	342	410
Transmigrants:			
No. positive	150	31	181
No. negative	29	159	188
Total	179	190	369

0.87) for the F test relative to the two readings, and the geometric means of 15 *P. falciparum* parasitaemias identified by both microscopists but negative by the F test were comparable (first, 320 per μl versus second, 530 per μl; $P = 0.14$). An independent expert, contracted by Becton-Dickinson Diagnostics, confirmed the microscopy-based identifications for 19/23 false-negative and 6/7 false-positive cases in this collection. The geometric mean parasitaemia of false negatives calculated by this cross-check (422 per μl) was significantly greater ($P = 0.02$) than that of the original estimate (320 per μl).

P. falciparum DNA was amplified from false-negative cases (7/29 transmigrants; 6/8 Irianese) and false-positive cases (14/31 transmigrant; 2/4 Irianese). These results were used to calculate "corrected" estimates of F test sensitivity and specificity for the entire transmigrant population and an arbitrary selection of 114 Irianese. Test sensitivity in the transmigrants increased from 84% to 94% and in the Irianese subset from 75% to 85%. The specificity of the F test for the transmigrants increased from 84% to 91% and for the Irianese from 95% to 97%. Relative to their "uncorrected" values, the increased proportions of true positives and true negatives were not statistically significant. *P. falciparum* DNA was detected in 10 of 16 false positives that were identified by microscopy as *P. vivax*, and in 6 of 19 false positives considered negative by microscopy.

Age group analyses

Underlying causes for differences between populations in test sensitivity and specificity were explored by making within and between comparisons of the age groups ≤10 years and >10 years. There were no significant differences between F test sensitivities determined for the younger and older age group transmigrants (younger, 81% versus older, 85%; $P = 0.74$) or between the transmigrants and the Irianese for the younger age group (transmigrants, 81% versus Irianese, 76%; $P = 0.60$). Highly significant differences in test sensitivity resulted from comparison between the Irianese younger and older age groups (younger, 76% versus older, 40%; $P < 0.003$) and between the older age groups of the transmigrants and the Irianese (transmigrants, 85% versus Irianese, 40%; $P < 0.001$). Specificity was comparable for both age groups of Irianese studied (younger, 96% versus older, 98%; $P = 0.49$) and for the younger age groups of the Irianese and transmigrants (Irianese, 96% versus transmigrants, 93%; $P = 0.99$). There were significant differences in specificity between the older age groups of transmigrants and Irianese (transmigrants, 80% versus Irianese 98%; $P < 0.001$) and between the younger and older age groups of transmigrants (younger, 93% versus older, 80%; $P = 0.02$). The low specificity of the test among transmigrants aged >10 years was due to a preponderance of false-positive test results (28/267) for this age group — more than three times greater than that for any other age group. PCR confirmations of *P. falciparum* DNA were more frequent among F test "false positives" identified by microscopy as *P. vivax* (8/12) than among those considered negative (5/16) in this age group. *P. falciparum* parasitaemias were comparable for the age groups ≤10 years (548, 95% CI: 297–1012) and >10 years (769, 95% CI: 531–1114) among the transmigrants and for the age group ≤10 years for the Irianese (589, 95% CI: 330–1052). *P. falciparum* parasitaemias for Irianese aged >10 years (197, 95% CI: 124–313) were significantly lower ($P < 0.04$) than for Irianese aged ≤10 years or either age group of transmigrants. Table 4 shows that the endpoint sensitivity of the ParaSight® F test at three ranges of parasitaemia (<40, 40–120, and >120 asexual forms per μl) was comparably high for both transmigrant age groups and for Irianese aged ≤10 years, but consistently low among Irianese aged >10 years. Among Irianese aged >10 years, the test detected 1 of 14 (7.1%) parasitaemias in the range ≤120/μl and 11 of 16 (69%) parasitaemias >120 per μl. Although overall *P. falciparum* parasitaemias for Irianese aged >10 years were significantly lower than for those aged ≤10 years, differences between age groups or populations in the geometric mean parasitemias >120 per μl that were not detected by the F test were not significant ($P = 0.40$) and ranged from 120 to 7280 asexual forms per μl of blood.

Table 4: **Endpoint sensitivity of the ParaSight® F test in native Irianese and transmigrant populations, by age group and parasitaemia**

Population, age group	*Plasmodium falciparum* parasitaemias detected:			
	<40/μl	40–120/μl	>120/μl	Combined
Native Irianese:				
≤10 years	1/1	4/8	24/29	29/38 (76.3)[a]
>10 years	0/2	1/12	11/16	12/30 (40)
All ages	1/3	5/20	35/45	41/68 (60.3)
New transmigrants:				
≤10 yeras	2/4	4/10	33/34	39/48 (81.2)
>10 years	10/12	13/21	88/98	111/131 (84.7)
All ages	12/16	17/31	120/132	150/179 (83.8)

[a] Figures in parentheses are percentages.

Discussion

The results suggest that significant differences in the sensitivity of the ParaSight® F test were independent of the density of *P. falciparum* infections and related to the age-dependent immune status of the population being tested. Sensitivity was low when the test was carried out on an Irianese population with lifelong exposure and clinical immunity to falciparum malaria. Comparison between age groups within this population showed that low sensitivity arose only with older children and adults. The F test had excellent specificity with malaria-immune Irianese and transmigrant children aged ≤10 years, indicating little or no cross-reactivity with other malaria species. Test specificity seemed unaccountably low among older transmigrants, and frequent false-positive reactions were recorded, many of which appeared initially to be cross-reactions with *P. vivax*; however, PCR revealed the presence of *P. falciparum* DNA in many of these cases, suggesting that the detection limit with the F test for nonimmune cases of mixed infection was better than that afforded by microscopy. Low level *P. falciparum* infections appear to have been obscured or more easily overlooked in mixed infections dominated by *P. vivax*.

The test's low sensitivity for the older Irianese age group appears to conflict with other reports of its sensitivity with malaria-immune populations (*8*, *9*), and it is important to discuss the reasons for this. We conducted the tests without regard to the malaria symptoms in populations with differing clinical susceptibility and response to infection, consistently using the same technicians. Strictly standardized techniques were used for Giemsa staining, and for examination of the thick-smear fields. The microscopes were new and had precision, quality optics and were used by an expert with over 30 years of field and laboratory experience. Blinded confirmations were performed by Becton-Dickinson Diagnostics' designated expert microscopist. Various intrinsic factors that may also apply include genetic differences in host expression and metabolism of PfHRP-II, genotypic and phenotypic differences in PfHRP-II structure or production, and possible interference from immune complexes to the various malaria species and genotypes in circulation. Blockage of PfHRP-II binding sites by host immune complexes, and the resultant insufficiency of capture and detection by the test reagents (mouse monoclonal and rabbit polyclonal antibodies), is a possibility that could be investigated straightforwardly by quantitative removal of immune complexes from sample lysates prior to testing for PfHRP-II antigen. If occlusion of binding sites does occur in clinically immune patients, test sensitivity might be enhanced by simple physical/chemical changes in the lysing reagent such as an alteration of its pH and/or salt concentration.

The results we obtained for children and nonimmune adults were as consistent, if not more so, as those obtained by other workers who measured test performance on clinical malaria cases, and with extended microscopy times. However, in terms of applying the test as an epidemiological tool to gauge the prevalence of *P. falciparum* in different populations, our results suggest that exposure history and age-dependent immunity may figure significantly in the test outcomes.

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Résumé

Efficacité comparée du test ParaSight® F pour le dépistage de *Plasmodium falciparum* dans des populations de l'Irian Jaya (Indonésie) immunes et non immunes vis-à-vis du paludisme

Cette étude compare l'efficacité du test ParaSight® F (test F) pour le dépistage sanguin de *Plasmodium falciparum* chez des populations de l'Irian Jaya (Indonésie) immunes (410 sujets autochtones) et non immunes (369 sujets récemment immigrés) vis-à-vis du paludisme. Dans cette région, le paludisme est hyperendémique et les quatre espèces de parasites sont présentes. On a pris comme référence l'examen microscopique de gouttes épaisses colorées au Giemsa, avec confirmation par PCR (amplification génique) en cas de désaccord. Les évaluations ont tenu compte de la variabilité imputable au technicien/microscopiste et ont été effectuées en aveugle, sans connaissance du statut du sujet. Le test F a montré des différences hautement significatives entre les populations en ce qui concerne la sensibilité (60% chez les autochtones contre 84% chez les migrants; $p < 0,001$) et la spécificité (97% contre 84%; $p < 0,001$). Une amplification de l'ADN génomique de *P. falciparum* a été réalisée sur une partie des faux positifs et des faux négatifs de chacune des populations. Les estimations «corrigées» de la sensibilité et de la spécificité du test, tenant compte des cas confirmés par PCR, étaient améliorées, mais de façon non significative. Des analyses comparatives entre groupes d'âges et entre populations n'ont montré aucune différence au niveau de la numération parasitaire moyenne par µl chez les faux négatifs du test F ($p = 0,66$). La sensibilité du test était élevée chez les enfants irianais de 0 à 10 ans et chez les migrants de tous âges (76–85%), mais était faible chez les Irianais de plus de 10 ans (40%), chez lesquels le test ne détectait que 7% des parasitémies inférieures à 120 par µl et 69% des parasitémies supérieures à 120 par µl. La spécificité était élevée chez les migrants de 0 à 10 ans et chez les Irianais de tous âges (93–98%). La faible spécificité observée chez les migrants de plus de 10 ans (79%) était due à une prépondérance des faux positifs, fréquement reconnus comme cas à *P. vivax* à l'examen microscopique. L'examen par PCR a montré que les co-infections par *P. vivax* masquaient souvent les faibles parasitémies à *P. falciparum*, qui échappaient alors à l'examen microscopique. Les résultats indiquent qu'une comparaison fondée sur l'examen microscopique sous-estime l'efficacité du test ParaSight® F et que le statut immunologique vis-à-vis du paludisme, quelle que soit la densité de *P. falciparum*, peut influencer la sensibilité du test.

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